A TAXONOMIC STUDY OF THE GENUS STREPTOCOCCUS

A thesis submitted for the degree of Doctor of Philosophy

in the University of Leicester.

by

Paul Dennis Bridge

October 1981.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

STATEMENT

This thesis, submitted for the degree of Doctor of Philosophy, is based on original work carried out by the author in the Department of Microbiology at the University of Leicester in the period October 1978 to October 1981. None of the work has been submitted for another degree in this or any other university.

Signed .....

Date 23.10.81.....

LIST OF CONTENTS

SUMMARY

Two-hundred and two strains of streptococci and related organisms were used in a numerical taxonomy. Ten major phenons containing twenty-seven subphenons and one loosely linked subphenon were found using Gower's coefficient and UPGMA methods. The Simple Matching coefficient and the Pattern difference were also used and these gave findings in broad agreement with those from Gower's coefficient.

Nine of the ten phenons contained streptococci, the tenth containing representatives of Leuconostoc and Gemella. Strains of Pediococcus appeared only distantly related to the streptococci, clustering as the loosely linked subphenon. Overlap statistics were performed on the subphenons and with few exceptions they proved distinct.

An identification matrix was made from the taxonomy and tested. This matrix was also used to construct a further dendrogram, based solely on the sixty characters in the matrix. This dendrogram was similar to those seen earlier. A further identification matrix was constructed using both tests from this study and from the literature. Both of the matrices were tested for overlap, the matrix based solely on this study giving more distinct groups.

Further work was undertaken on representative strains from the subphenons. This involved the determination of DNA base ratios, detection of esterases in polyacrylamide gels and the

numerical analysis of protein traces in polyacrylamide gels. This further work failed to group any of the organisms at anything other than species level. However, the results did not directly contradict the numerical taxonomy, and the groups from this were retained. These groups consisted of eight species-groups. These were, enterococci, viridans, pyogenic, para-viridans, para-pyogenic, S. thermophilus, S. pneumoniae and lactic. The strains received as aerococci did not form a distinct cluster within the numerical taxonomy and did not appear different from the streptococci in the other work. They showed properties similar to both Streptococcus and Pediococcus and may be intermediate between the two genera.

Page

LIST OF TABLES

Page

LIST OF FIGURES

ACKNOWLEDGEMENTS

The author would like to express his sincere gratitude towards the following without whom this work would not have been completed.

Professor P.H.A. Sneath for his supervision, guidance and enthusiasm throughout this project.

Dr. R.K.A. Feltham for providing many of the cultures used and for his advice and encouragement.

Dr. D.Jones for her informative encouragement.

Mr. M.J.Sackin and Mr.R.Key for their assistance and advice on the computing methods used.

The many people not mentioned by name who provided cultures or advice and encouragement.

INTRODUCTION

1.1 General introduction to the Streptococcaceae.

The family Streptococcaceae is divided by Diebel & Seeley in the most recent (Eighth) edition of Bergey's Manual, (Buchanan & Gibbons, 1974) into five genera. These are Streptococcus Rosenbach 1884, Leuconostoc van Tiegham 1879, Pediococcus Balcke 1884, Aerococcus Williams, Hirch & Cowan 1953 and Gemella Berger 1961. The family is described in the following way: "Cells spherical or oval, in pairs or chains of varying length or in tetrads. Non motile or rarely motile. Endospores not formed. Gram-positive. Chemoorganotrophs. Metabolism fermentative; lactic, acetic and formic acids, and ethanol and carbon dioxide formed from carbohydrates. Nutritional requirements complex and variable. Catalase test is variable, benzidine negative. Facultatively anaerobic. The G+C content of DNA ranges from 33-44 moles %."

The different genera are characterised by their plane of cell division, their method of fermentation and the optical activity of any lactic acid formed, as shown in Table 1.1.a. However, these characters are not always reliable and these genera may be defined rather too artificially (Jones, 1978).

The five genera are described in Bergey's Manual as being catalase negative except for Pediococcus and Aerococcus, which are considered to be catalase variable. Some strains of Streptococcus are capable of producing a pseudo-catalase and a haem-dependant catalase from blood and other haem-containing media (Whittenbury, 1964). All of these genera are also considered to lack cytochromes.

Table 1.1.a. Differentiation of the genera of the family *Streptococcaceae*.

Fermentation	Lactic acid	Cell division	Genus
Homofermentative	Dextrorotatory	One plane	*Streptococcus*
Heterofermentative	Laevorotatory	One plane	*Leuconostoc*
Homofermentative	Inactive	Two planes	*Pediococcus*
Homofermentative	Dextrorotatory	Two planes	*Aerococcus*
Not known	Not known	One plane	*Gemella*

However it has been found that some strains, mainly of serological group D, are capable of producing cytochromes on haem-containing media (Bryan-Jones & Whittenbury, 1969; Pritchard & Wimpenny, 1978).

The genus Gemella is considered to be gram-variable although it has been shown to have a gram-positive type cell wall (Reyn et al., 1970).

These organisms are all described as facultative anaerobes. There are some similar organisms that are obligate anaerobes. However, Rogosa (1974), and Holdeman & Moore (1974) have said that anaerobic gram positive cocci that occur in chains and pairs, and produce lactic acid could be considered as Streptococci. At the present time however, these organisms are classified separately in the genus Peptostreptococcus Kluyver & van Niel 1936 (Diebel & Seeley, 1974), and not with the other Streptococcus species that will grow aerobically.

The Streptococcaceae form an homogenous group of organisms that are of great importance in both the medical and industrial fields. They have been known and studied since the turn of the century although their taxonomy has been, and in some cases still remains, rather confused.

1.2 Early taxonomy

The term streptococcus was first used by Billroth in 1874 to describe spherical to oval cells appearing in a formation of chains and pairs (Jones 1978). Streptococcus was proposed as a genus name by Rosenbach in 1884, and in 1889 de Toni & Trevisan proposed Streptococcus Rosenbach 1884 as the type genus of the family Streptococcaceae (Wilson & Miles, 1975).

At the turn of the century, the streptococci were considered to be among the most important of the pathogenic bacteria. However the main criteria then for the differentiation of bacteria were the morphology of the colonies and the individual cells. This method failed to differentiate many different groups, one of these being the Streptococcaceae (Wilson & Miles, 1975).

In 1891 von Lingelsheim considered the chain length of the streptococci as a differential character. He proposed the terms "Streptococcus brevis" for the short chain varieties and "S. longus" for the long chain varieties. He noted that the most virulent organisms were found in the latter.

In 1903 Schotmüller introduced the character of haemolysis on solid media containing washed human blood. He proposed three groups of streptococci; "S. pyogenes vel. erysipelatos" which gave a clear zone of haemolysis, "S. mitior vel. viridans" which gave greening and "S. mucosus" which gave viscid growth and no change in blood (Wilson & Miles, 1975).

Gordon (1905) first differentiated the streptococci by their metabolic reactions. He proposed nine tests as being useful and reliable, and these became known as "Gordon's tests". These were the production of acid from sucrose, raffinose, lactose, inulin, salicin, coniferin and mannitol, the clotting of milk and the anaerobic reduction of neutral red. In 1906 Andrewes & Horder undertook a study of the streptococci pathogenic to man. They considered von Lingelsheim's criterion of chain length and Schotmüller's criterion of haemolysis as valid, but proposed that they should be considered in relation to other tests as they were not

themselves fundamental enough. Andrewes & Horder also considered that cultural characters and mode of growth were too variable to be of any use, but that characters such as temperature limits of growth could be used. As a result, they used Gordon's tests as well as growth temperatures, haemolysis, pathogenicity and chain length to differentiate two hundred strains of streptococci. They proposed a classification consisting of seven groups. These were:

(1) S. equinus, a group of saprophytic organisms derived from herbivores.

(2) S. mitis, a group of saprophytic but occasionally pathogenic organisms.

(3) S. pyogenes, the major group of pathogens.

(4) S. salivarius, a group of the normal flora found in saliva.

(5) S. anginosus, a long chain variety of S. salivarius.

(6) S. faecalis, a group of human faecal organisms.

(7) Pneumococcus, a group containing the diplococci.

The last group was treated as distinct from the others although Andrewes & Horder were not certain of the wisdom of this, considering them very closely related. There were some similarities between these groups and those of Schotmüller. However, Andrewes & Horder also found many variants within their groups and a number of intermediate strains between them.

The next significant advance in the taxonomy came when Orla-Jensen (1919) produced a comprehensive study of the lactic acid bacteria. Orla-Jensen, unlike some of his predecessors, paid more attention to the composition of culture media, and by using basic biochemical tests such as acid production from carbohydrates, produced a group classification made up of ten groups.

These were:

(1) S. cremoris, isolated from milk and cream.
(2) S. lactis, isolated from milk and cream.
(3) "S. mastiditis", the causative organisms of mastitis.
(4) S. thermophilus, from dairy products but very heat tolerant.
(5) S. bovis, isolated from cattle faeces.
(6) "S. inulinaceus", a group of organisms that split inulin.
(7) S. faecium, a group of human faecal organisms.
(8) "S. glycerinaceus", isolated from human faeces.
(9) "S. liquefaciens", human faecal organisms that liquefy gelatin.
(10) S. pyogenes, haemolytic, pathogenic organisms

There were several interesting points in this classification. Orla-Jensen observed the formation of tetrads in S. cremoris, which had not been reported before. He believed S. lactis was identical to "Bacterium lactis" Lister. "S. mastiditis was thought to correspond to the organism called S. agalactiae by Lehmann & Neumann in 1896. Orla-Jensen considered his S. faecium to be different to Andrewes & Horder's S. faecalis. He noted that "S. liquefaciens" differed from "S. glycerinaceus" only in its ability to liquefy gelatin. He also noted that there was a group of saprophytic streptococci which did not fit in with any of his species groupings, but were not sufficiently similar to each other to justify them as a group on their own.

From this time on, many new tests were devised for the streptococci, which resulted in many new species and groups being proposed. Between the years 1919 and 1937, sixty-three new species were proposed (Buchanan, Holt & Lessel, 1966). The taxonomic confusion at this time caused a step to be taken that in the light

of later work proved to be an extreme one. The following passage was inserted as a note in the second edition of Bergey's Manual (Bergey et al., 1926). "Attempts to classify streptococci on the basis of their powers to ferment different carbohydrates gives no concordant results with serological tests in the form of agglutination reactions and absorption of agglutinins. For these reasons, the species of streptococci of human and animal origin are here grouped on the basis of action in blood agar; serological reactions; and only secondarily on the basis of carbohydrate fermentation".

Lancefield (1933; 1934), following up the discovery of the specific cell wall antigen by Hitchcock (1924), found that it was group specific and used it to classify the streptococci into various groups.

Shortly after this, Sherman (1937), produced a taxonomy based on physiological and biochemical techniques. He used the Lancefield group antigen as a taxonomic character in this and obtained a good correlation between the two taxonomies. Sherman's work can be considered as the first systematic taxonomy as he ordered strains into groups as well as species.

He excluded the anaerobic streptococci and the pneumococci from the genus Streptococcus. He used nine primary tests to separate the organisms into four divisions; he then used twenty-three secondary tests to subdivide the divisions into seventeen species or groups:

Division	Species or group
Pyogenic	*S. pyogenes*
	"*S. mastiditis*"
	S. equi
	Animal pyogenes
	Human group C
	Group G strains
	Group E strains
	Group H strains
Lactic	*S. lactis*
	S. cremoris
Viridans	*S. salivarius*
	S. equinus
	S. bovis
	S. bovis variants
	S. thermophilus
Entero-	*S. faecalis*
coccus	"*S. liquefaciens*"
	"*S. zymogenes*"
	"*S. durans*"

Sherman showed that all of the strains in the enterococcus division possessed the group D antigen described by Lancefield. However, he did not consider this to mean that all group D strains were enterococci. *S. bovis* gave a positive group D reaction but not all strains gave it. *S. equinus* also gave a group D reaction but the majority of strains only gave a weak reaction.

In his later work on the enterococci (1938), Sherman considered that the S. faecalis of Andrewes & Horder was the same group of strains as the S. faecium of Orla-Jensen because he considered the differences were insufficient to justify separation. Sherman did however suggest that "S. liquefaciens" and "S. zymogenes" could be considered as varieties of S. faecalis, designated as "S. faecalis var. liquefaciens" and "S. faecalis var. zymogenes" respectively.

Abd-el-Malek & Gibson (1948), studied the streptococci of milk. They used the tests that Sherman described and from these they divided these streptococci into five groups. These were:

(1) a mastitis group

(2) a viridans group

(3) a lactic group

(4) a heterofermentative group

(5) a faecal group

Abd-el-Malek used six further tests to differentiate these groups. However, because only milk and milk products were being observed a comparatively small number of species were found. It is interesting to note that an enterococcus group was found under these circumstances. The heterofermentative streptococci were not easily identified except by their ability to produce carbon dioxide from glucose. They were identified mainly as "S. citrovorus" and "S. kefir".

1.3 Present day taxonomy

This resumé of some of the recent work is not intended as a full and exhaustive list of all work but to give an overall representation of the major fields in which recent work has been conducted.

There have been several major attempts to revise the classification of the genus Streptococcus, for example, Colobert & Blondeau (1962) used numerical methods with strains of S. faecalis. Raj & Colwell (1965) extended this to a numerical taxonomy of the enterococci, Colman (1968) produced a wide ranging numerical taxonomy of the Streptococci, as did Seyfried (1968). On the more restricted side, Carlsson (1968) published a numerical taxonomy of some oral streptococci. Some strains of streptococci have been used in taxonomies involving bacteria of many different genera. Two numerical examples of this are Beers et al. (1962) and Lockhart & Hartman (1963). Davies et al. (1969) made a numerical taxonomy of streptococci, Listeria and other related bacteria. Drucker & Melville (1969) produced a classification of the oral streptococci which was in some ways similar to that of Carlsson. Jones et al. (1972) produced a numerical taxonomy of the group D streptococci; they included in this some serological group Q organisms, as they had been found to contain the group D antigen in addition to the group Q antigen (Smith & Shattock, 1964).

However a large amount of the recent work on streptococci has been based on methods other than numerical ones.

Physiological and biochemical tests used on small groups of the streptococci have been the most common method used in their classification. Medrek & Barnes (1962) studied the physiology and serology of strains resembling S. bovis, Diebel et al. (1963) studied the physiology of the enterococci as related to their taxonomy. Whittenbury (1965a; 1965b) studied the relationships of the faecal streptococci to each other and to Aerococcus and Pediococcus. Nowlan & Diebel (1967a; 1967b) characterised the serological group Q, "S. avium" group of organisms on the basis of their biochemical properties. de Moor & Thal (1968) observed the biochemical and physiological characters of some β-haemolytic organisms. Facklam (1972) and Facklam & Moody (1970) proposed physiological and biochemical tests for the recognition of group D organisms; similarly for viridans organisms (Facklam, 1977) and organisms of groups A, B and D (Facklam et al., 1974)

Serology has been used in many different studies in conjunction with other methods. The grouping of organisms solely on the basis of their antigenic structure is now less prevalent than it was. However, new serological groups are still being proposed. An example of one of these is the serological group V proposed by Jélinková & Kubín (1974). The presence of a surface protein antigen was used by Lütticken (1978) to confirm numerical taxonomic groups. The use of enzyme linked immunosorbent assay gives results in agreement with conventional methods for serological grouping (Cumming et al., 1980).

One area where there has been renewed interest in observing streptococcal relationships is enzymology. This has advantages

in the fact that it is now relatively quick and simple, and it is used regularly in the dairy industry, where the specific bacterial enzymes are of economic importance. Lund (1965; 1967) examined the esterase enzymes of faecal streptococci by gel electrophoresis. Sørhaug & Solbert (1973) have worked on the fractionation of dipeptidase activities. London & Kline (1973) and London et al. (1975) have published work on the aldolase enzyme systems as an evolutionary marker, and on the immunological relationships of the enzyme in different strains. Garvie & Bramley (1979a; 1979b) have used the lactate dehydrogenase enzyme type in the classification of S.uberis and S.bovis. Law (1979) and Cliffe & Law (1979) have studied the peptidases in starter streptococci.

A recent advance in bacterial classification is the comparison between the DNA of different organisms. This has been studied in some streptococci. Roop et al. (1974) carried out DNA hybridisation studies on group D and N streptococci. Coykendall (1977) used the mole % G+C ratio to propose new subspecies of S. mutans. Vaughn et al. (1979) used both mole % G+C ratios and DNA/DNA hybridisation techniques in a study of yellow, motile group D streptococci. Many other recent studies have included data on the DNA of the organisms, as an additional criterion to the more common biochemical and physiological ones. Garvie (1978) used only data on the DNA and the lactate dehydrogenases to propose S.raffinolactis as a new species. Weissman et al. (1966) have published findings for some strains of streptococci from DNA/RNA hybridisation studies.

Another new technique which has found applications in taxonomy in recent years is pyrolysis gas liquid chromatography. Carlsson (1973) noted this as a useful technique. Amstein & Hartman (1972) used this method to differentiate some members of the enterococci. Stack et al. (1978) used a similar technique with a group of oral organisms and they also performed various experiments to show the high level of repeatability of this method. However, recent work by Gutteridge & Norris (1979) suggests that the method is better suited as a diagnostic tool than as a classification method, although the reasons for this are not yet clear.

As with many other species of bacteria, cell wall studies have been made on the streptococci. This has now become a powerful taxonomic tool. Jones & Shattock (1960) studied the cell wall structure of group D organisms in relation to the location of the group antigen. Slade & Slamp (1962; 1972) also published similar work to this. Classification and identification methods have been proposed that are based on the cell wall antigens. The M,T and R proteins and the serum opacity factor have all been found to have some degree of specificity (Rotta, 1978). Colman & Williams (1965) made a comprehensive study of the cell walls of some two hundred strains of streptococci by paper chromatography. Kandler et al.(1968) used cell wall structure to differentiate S. faecalis from S. faecium. More recently Slade & Slamp (1972) have looked at the different peptidoglycan structure in some streptococcal groups. Other chemical constituents of the cell walls also show promise.

Collins & Jones (1979) have studied the isoprenoid quinone constituents of group D and group N organisms and have found that their chemical findings correlate well with other more traditional methods.

The Streptococcaceae are considered not to have cytochromes, but Whittenbury (1964) found that when strains of S. faecalis were grown in media containing haem compounds, cytochromes were formed. This was also confirmed by Bryan-Jones & Whittenbury (1969) and Pritchard & Wimpenny (1978). A survey of this cytochrome activity showed that this ability was limited to S. faecalis, "S. faecalis subsp. liquefaciens" and "S. faecalis subsp. zymogenes" in the group D organisms, and to S. lactis and "S. lactis subsp. diacetylactis" in the group N organisms (Ritchey & Seeley, 1976).

The antibiotic resistance of the streptococci is of particular importance, apart from its taxonomic implications, because streptococci are implicated in many diseases of both humans and animals.

Susceptibility to bacitracin has been used as a taxonomic character by Facklam (1974). Jokipii & Jokipii (1979) used antibiotic sensitivity as a presumptive test in the isolation of group B organisms.

The study of the antibiotic resistances of the streptococci is related to their genetics. Clewall et al. (1974) showed that resistance to erythromycin in one strain of S. faecalis was plasmid mediated. Resistance to drugs in general was shown to involve plasmids by Dunney & Clewall (1975). Extrachromosomal elements in group N streptococci have been studied by Cords et al. (1974) and these studies confirmed the presence of plasmids. Some strains

of S.lactis produce the antibiotic nisin; this has been shown by Kozak et al. (1974) and Fuchs et al. (1975) to be due to a plasmid. Genetic transfer has been shown to take place in the streptococci by McKay et al. (1973). They showed the occurrence of phage mediated transduction in some strains of S. lactis. Jones & Sneath (1970) considered the effects of genetic transfer on bacterial taxonomy.

The production of bacteriocins and bacteriolysins has been reported as an identification procedure for some streptococci (Brandis, 1978).

A further recent innovation for the grouping of streptococci is phage-typing. This has yet to be fully standardised but appears to have some uses for group D and group B strains (Jélinková & Rotta, 1978).

Despite all of this work and these new techniques, the taxonomy of the streptococci is still not very clear in some areas. In general, the grouping of the streptococci given in "Topley and Wilson's Principles of Bacteriology and Immunology" (Wilson & Miles, 1975) is used. This and the identification scheme given in "A Manual for the Identification of Medical Bacteria" (Cowan and Steel, 1974) are based on the classifications of Sherman (1937). They also give a lot of importance to the serological grouping of the streptococci devised by Lancefield in 1934. Serological grouping is used for routine medical identification and this has been partly responsible for the proliferation of serological groups.

Less work has been done on the classification of Aerococcus and Pediococcus. Aerococcus viridans and some strains of pediococci were differentiated from the enterococci by biochemical and physi-

ological tests by Whittenbury (1965b). Dolezeil & Kirsop (1977) characterised the pediococci with the API 50 Lactobacilli system. Williams et al. proposed the genus Aerococcus in 1953. They considered them to be purely free living streptococci and they have been poorly studied since then. The genus Gemella is considered by Reyn (1974) to consist of only one species, Gemella haemolysans. This was previously described as a member of the gram-negative family of Neisseriaceae but was transferred to the Streptococcaceae when it was found to give an indeterminate gram-stain reaction and have a gram-positive type of cell wall (Reyn et al., 1970).

The genus of Leuconostoc was somewhat neglected taxonomically until the work of Garvie in 1960. She effectively classified it as it now stands, consisting of six species. These were initially defined by biochemical and physiological tests and later, DNA data was included (Garvie, 1969; 1974).

The eighth edition of Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974) describes the genus Streptococcus as containing twenty-one defined species and several species incertae sedis. Since that date new species have been proposed. These include Streptococcus raffinolactis, a group N organism (Garvie, 1978) and Streptococcus iniae isolated from the mouths of freshwater dolphins (Pier & Madin, 1976; Pier et al., 1978).

Clarke (1924) originally proposed the term S. mutans. This has been readopted as a species by Coykendall (1974). Coykendall (1977) also characterised S. mutans as containing five subspecies on the basis of their biochemical reactions and DNA level. These were

termed "S. mutans subsp. mutans", "S. mutans subsp. rattus", "S. mutans subsp. cricetus", "S. mutans subsp. ferus" and "S. mutans subsp. sobrinus". However, the Approved Lists of Bacterial Names (Skerman et al., 1980) gives only the species S. mutans, S. rattus and S. cricetus.

1.4 The major groups of the streptococci

The streptococci are at present divided into six groups on the basis of serological and physiological tests. Table 1.4.a shows these groups and the organisms assigned to them. This table is based on information contained in Wilson & Miles (1975) and Jones (1978).

Table 1.4.a The major groupings of the streptococci

PYOGENIC ORGANISMS

S. pyogenes Rosenbach 1884

S. equi Sand & Jensen 1888

"S. zooepidemicus" Frost & Englebrecht 1936

"S. equisimilis" Frost & Englebrecht 1936

"S. dysgalactiae" Diernhofer 1932

S. agalactiae Lehman & Neumann 1896

"S. suis" Elliot 1966

S. anginosus Andrewes & Horder 1906

Large colony type group G

Serological group E

"Minute" haemolytic streptococci

FAECAL ORGANISMS

S. faecalis Andrewes & Horder 1906

S. faecium Orla-Jensen 1919

"S. avium" Nowlan & Diebel 1967

S. bovis Orla-Jensen 1919

S. equinus Andrewes & Horder 1906

LACTIC ORGANISMS

S. lactis (Lister 1873) Lohnis 1909

S. cremoris Orla-Jensen 1919

S. raffinolactis Orla-Jensen & Hansen 1932

PNEUMOCOCCI

S. pneumoniae Klein 1884

ORAL ORGANISMS

S. salivarius Andrewes & Horder 1906

"S. milleri" Guthof 1956

S. mitis Andrewes & Horder 1906

S. sanguis White & Niven 1946

S. mutans Clarke 1924

S. cricetus Coykendall 1977

S. rattus Coykendall 1977

OTHER STREPTOCOCCI

S. uberis Diernhofer 1932

S. thermophilus Orla-Jensen 1919

S. acidominimus Ayers & Mudge 1922

S. iniae Pier & Madin 1976

1.4.1 The pyogenic streptococci

This group contains the majority of the major medically important organisms. They are nearly all human or animal pathogens. This group used to be differentiated as the β-haemolytic organisms, although "S. dysgalactiae" is α-haemolytic and S. agalactiae, "S. suis" and S. anginosus may also show α-haemolysis (Diebel & Seeley, 1974). The placing of "S. suis" in this group may be open to criticism as will be shown later. S. pyogenes is the most common member of the Lancefield group A organisms and is the most widely studied. It is the type species of the genus and it used to be considered the most common pathogen among this group (Andrewes & Horder, 1906). However, this may have been due to the identification methods used at that time. It is the causative agent of Scarlet fever, Puerperal sepsis and sometimes respiratory tract infections or a generalised septicaemia (Cruickshank et al., 1968).

Streptococcus agalactiae possesses the Lancefield group B antigen. Group B organisms are becoming more widely implicated in human infections (Jokipii & Jokipii, 1979). It has been known for many years as the causative organism of bovine mastitis and as such it has been relatively well studied. However, the Lancefield group antigen has been taken as referring only to this species, although recent work indicates that the human and animal group B pathogens may form two separate groups (Jones, 1978). The problems in isolating group B strains from human samples has been illustrated by Jokipii & Jokipii (1979) and this may result in some group B infections remaining either undiscovered or being wrongly diagnosed.

Streptococcus equi, "S. equisimilis", "S. zooepidemicus" and "S. dysgalactiae" are all members of the Lancefield group C. All of these organisms are β-haemolytic and well studied, with the exception of "S. dysgalactiae". This appears to be taxonomically similar to "S. zooepidemicus" (Feltham, 1979) and its cell wall structure has been reported as being identical to that of "S. equisimilis" (Colman & Williams, 1965). The aldolase types found by London & Kline (1973) indicated that "S. dysgalactiae" was very similar in this respect with S. equi. However, of these four names only S. equi appears in the Approved Lists of Bacterial Names (Skerman et al., 1980).

"Streptococcus suis" is a species that is normally associated with diseases in pigs. Its taxonomic position is rather uncertain as the numerical study of Colman (1968) in some ways contradicted that of the proposer of the species, Elliot (1966). Elliot found "S. suis" to be related to the enterococci. Wilson & Miles (1975) did not consider it as an independant species, although Colman (1968) considered it as a pyogenic organism.

The Lancefield group E organisms have become a taxonomic problem that as yet has not been resolved. Moreira-Jacob (1956) detected two physiological types. One of these he designated as "S. infrequens" and the other as "S. subacidus". Diebel & Seeley (1974), use only the term group E streptococci and detail some of the differences between strains of bovine and porcine origin.

Members of the Lancefield group F streptococci are grouped with the small colony type group G organisms as the "minute" haemolytic streptococci (Wilson & Miles, 1975). They are inhabitants of the human throat and may be responsible for sepsis in the upper respiratory tract.

Large colony type group G organisms are grouped separately from the small colony type. They are physiologically similar to some strains of S. pyogenes and have been found to cause infections in humans and other animals (Wilson & Miles, 1975). Diebel & Seeley (1974) suggested that more than one physiological type may be present among them, although Colman & Williams (1965) found that they all had the same unique cell wall structure.

Streptococcus anginosus is considered by Diebel & Seeley (1974) to consist of the "minute" β-haemolytic organisms and the β-haemolytic strains of the organism known as "Streptococcus MG". This in turn is considered similar to some strains of Lancefield group O (Facklam, 1977). S. anginosus reacts with both group F and group G antisera and so possesses both of these specific antigens. However this species is not considered by Wilson & Miles (1975) and they place these organisms within their group of "other streptococci", while suggesting that they may be related to "S. milleri".

Other streptococci that have been assigned to the pyogenic group of organisms include representatitives of the Lancefield serological groups H, K, L, M, O, R, S, T and V (Jones, 1978). None of these appear to be homogenous groups, the majority may be divided into several different biotypes (Wilson & Miles, 1975).

1.4.2 The pneumococci

The pneumococci or diplococci, a group of the streptococci, consist of one species, S. pneumoniae. This organism is now considered to be a member of the Streptococcaceae although at one time it was placed in a separate family (Andrewes & Horder, 1906). The species is characterised by the possession of "draughtsman-shaped" colonies and its solubility in bile, as well as strong ∝-haemolysis and sensitivity to optochonin. The cells occur in pairs and virulent strains possess a capsule. The species does not possess a specific group antigen. The cell wall structure however is distinct from that of other species of streptococci (Colman & Williams, 1965).

The pneumococci have received a lot of attention on account of their pathogenicity to man. The pneumococci cause lobar pneumonia, secondary broncho-pneumonia and meningitis. However, the organisms may be isolated from healthy individuals in the absence of any disease. It is commonly a secondary invader, often following a viral illness (Cruickshank et al., 1968).

1.4.3 The faecal streptococci

The faecal or enterococcal group of streptococci are the organisms that are normally isolated from the faeces of humans or other animals. They are considered to consist of five separate species. These are: S. faecalis, S. faecium, "S. avium", S. bovis and S. equinus. They all possess the Lancefield group D antigen and they have been studied in isolation many times (Sherman, 1938; Colobert & Blondeau, 1962; Diebel, 1964; Whittenbury, 1965a; Jones

et al., 1972; Jélinková & Rotta, 1978).

Streptococcus faecalis is the most common streptococcus species isolated from human faeces. It is conventionally divided into four subspecies by Diebel & Seeley (1974). These are: "S. faecalis subsp. faecalis", "S. faecalis subsp. liquefaciens", "S. faecalis subsp. zymogenes" and "S. faecalis subsp. malodoratus". "S. faecalis subsp. malodoratus" appears to be taxonomically distinct from the other subspecies (Jones et al., 1972; Collins & Jones, 1979). It may not therefore be closely related to the others. "S. faecalis subsp. liquefaciens" and "S. faecalis subsp. zymogenes" are both proteolytic, being the only streptococci known to liquefy gelatin. "S. faecalis subsp. zymogenes" - unlike the other subspecies may show β-haemolysis (Diebel & Seeley, 1974). Diebel (1964) and Jones et al. (1972) have questioned the validity of these subspecies, proposing that they should be considered as atypical strains of S. faecalis. This view is supported by the cell wall information of Collins & Jones (1979).

Streptococcus faecium was proposed by Orla-Jensen (1919) but it was not recognised as being distinct from S. faecalis until the report of Skadhauge in 1950. Since then many tests have been described that separate the two species (Whittenbury, 1965a; Kandler et al., 1968). Diebel & Seeley (1974), Wilson & Miles (1975) and Jones (1978) consider them to be distinct. S. faecium has not been found to produce cytochromes from haem, unlike S. faecalis (Ritchey & Seeley, 1976). Its esterase enzymes have been shown to be different from those of S. faecalis (Lund , 1965) and its cell wall and membrane constituents also differ (Colman & Williams, 1965; Collins & Jones, 1979).

Streptococcus faecium has been divided into three subspecies; "S. faecium subsp. faecium", "S. faecium subsp. casseliflavus" and "S. faecium subsp. mobilis". The latter two are similar in that they are both yellow pigmented and motile; their cell membrane constituents are quite distinct from those of "S. faecium subsp. faecium" (Collins & Jones, 1979) and this is supported by pyrograms from gas chromatography of fatty acids (Amstein & Hartman, 1972) DNA/DNA hybridisation (Roop et al., 1974) and their esterase enzymes (Lund , 1967). Both subspecies have been isolated from plants, (Mundt, 1963b). It has recently been suggested that these two subspecies form between them a distinct species (Roop et al., 1974; Jones, 1978).

Also similar to S. faecium is the group of organisms known as "S. durans". "S. durans" was once considered to be distinct from S. faecium (Sherman, 1938). However recent work (Diebel, 1964; Jones et al., 1972; Collins & Jones, 1979) suggests that it is taxonomically indistinguishable from S. faecium and appears to form a group within them. Many atypical strains of S. faecium and S. faecalis have been isolated from animals and vegetation associated with them (Mundt, 1963a; 1963b; Barnes et al., 1978).

"Streptococcus avium" was proposed by Nowlan & Diebel (1967a). It consists of streptococci that at first appear to be closely related to both S. faecalis and S. faecium. "S. avium" possesses both the Lancefield group D antigen and a specific antigen, Q (Smith & Shattock, 1964; Nowlan & Diebel, 1967a). Nowlan & Diebel suggested that this group of organisms may represent the intermediate atypical strains of S. faecalis and S. faecium, but they now appear to be distinct (Jones, 1978; Feltham, 1979).

Streptococcus bovis is physiologically quite distinct from the previously mentioned enterococci, and it is considered by Jones (1978) not to be a true member of the enterococci. S. bovis has many atypical strains (Barnes, et al., 1961;Medrek & Barnes, 1962; Kiel & Skadhauge, 1973). There are possibly two biotypes, characterised by the fermentation of mannitol and the formation of dextran from sucrose (Medrek & Barnes, 1962; Kiel & Skadhauge, 1973). Garvie & Bramley (1979b) used DNA/DNA hybridisation studies and lactate dehydrogenase types in an attempt to classify strains of S. bovis. They reported that S. bovis formed a variable species with no apparent subgroups. This conclusion was also reached by Jones et al., (1972).

Streptococcus equinus is physiologically very similar to S. bovis and it is characteristic of the intestines of horses (Sherman, 1938). S. equinus, like S. bovis gives only a weak reaction with group D antisera. It was suggested by Seeley & Dain (1960) that they should be considered as varieties of one species on the basis of their similar physiological properties. This view is however opposed by Smith & Shattock (1962); Jones et al. (1972); Diebel & Seeley (1974) and Wilson & Miles (1975) as well as by many other workers. Their aldolases have been reported as being different (London & Kline, 1973).

As can be seen, despite all of the studies that have been undertaken on the enterococci, their taxonomy is still confused. Kalina (1970) has proposed that S. faecalis and S. faecium should both be placed into a genus Enterococcus; however this has not been accepted by other workers.

1.4.4 The lactic streptococci

The lactic group of streptococci is considered to consist of three species; these are S. lactis, S. cremoris and S. raffinolactis. All of these possess the Lancefield group N antigen. They are normally found in raw milk and as a result they are important in the dairy industry. Because of their souring action on milk they are used as "starter" cultures in the production of cheese and yoghurt.

Streptococcus lactis is a well studied and defined species (Diebel & Seeley, 1974). It was reported in the early literature under a variety of names. It was also in some cases confused with S. faecalis, with which it shares some properties such as growth at 10°C and tolerance of bile (Sherman, 1937). It is however physiologically distinct from S. faecalis. There is one puzzling similarity; some strains of both species have been shown to produce cytochromes on media containing haem compounds (Ritchey & Seeley, 1976). Some strains of S. lactis produce the antibiotic nisin. It is thought that this ability may be plasmid-controlled (Kozak et al., 1974).

"Streptococcus lactis subsp. diacetylactis" is a subspecies that is really distinguished from "S. lactis subsp. lactis" only by its ability to produce diacetyl, acetoin and carbon dioxide from citrate (Diebel & Seeley, 1974). Some strains of "S. lactis subsp. diacetylactis" also produce cytochromes under the same conditions as "S. lactis subsp. lactis" and S. faecalis. It may be that these are more like atypical strains than actual subspecies (Jones, 1978).

Streptococcus cremoris was separated from S. lactis by Orla-Jensen (1919) and further characterised by Yawger & Sherman (1937a; 1937b). There is now an increasing belief that they may both be variants of the same species (Jones, 1978; Collins & Jones, 1979).

The biochemical properties of the two are very similar and the cell wall structures are almost exactly the same, S. cremoris having one extra type of cross bridge (Schleifer & Kandler, 1972). Their menaquinone profiles are identical (Collins & Jones, 1979). A subspecies "S. cremoris subsp. alactosus" has been described, but again it may simply represent atypical strains.

Streptococcus raffinolactis has only recently been accepted as a distinct species (Garvie, 1978) although it was proposed as long ago as 1932 (Orla-Jensen & Hansen, 1932). It is considered as a distinct species now on the basis of its lactate dehydrogenases, the mol % G+C values and DNA/DNA homology as well as its biochemical properties (Garvie, 1978). From the work of Collins & Jones (1979), it appears that the cell wall structure of S. raffinolactis is distinct from that found in S. lactis and S. cremoris.

1.4.5 The oral streptococci

The oral group of streptococci are those that are commonly found in the mouth and they are often implicated in dental caries of both humans and other animals (Carlsson, 1968; Dent et al., 1978; Beighton et al., 1979). They form an integral part of the flora of the mouth, but they are found with other organisms, mainly members of the Lactobacillaceae. Recent work by Beighton et al. (1979) indicates that the streptococcal concentration and distribution in dental plaque is affected by the diet of the animal, as is the structure of the overall population. The oral organisms contain many of the organisms that have previously been described as the "viridans" group. This is a name that used to be given to the α-haemolytic organisms that caused a strong greening reaction on blood agar.

Streptococcus salivarius is perhaps the most predominant of the oral streptococci. As stated earlier, this was initially isolated from human saliva by Andrewes & Horder (1906). It was later found to possess the Lancefield group K antigen. However, many other strains that do not correspond to S. salivarius have been found to possess this antigen (Williams, 1956). Many strains produce a levan from sucrose. The main habitat of S. salivarius is the oropharanyx, rather than the teeth and gums (Gibbons et al., 1964).

Streptococcus mutans is another species found in the mouth. This group was originally proposed to contain all the non-haemolytic streptococci isolated from carious teeth (Clarke, 1924). Facklam (1974) found no differences between oral strains of S. mutans and others isolated from blood. Facklam found some forms of S. mutans to be different but considered these as atypical strains. However, Coykendall (1977) defined five subgroups, three of which; S. mutans, S. cricetus and S. rattus are included in the Approved Lists of Bacterial Names (Skerman et al., 1980). S. mutans may form a dextran from sucrose. It has been found experimentally to be caries-inducing and its main habitat appears to be the surface of the teeth (Drucker & Melville, 1969; Carlsson 1968).

Streptococcus sanguis is another species found in the oral cavity and is also associated with dental plaque. Like S. mutans some strains may occur in the blood giving rise to sub-acute endocarditis. S. sanguis possesses the Lancefield group H antigen. However, over recent years strains conforming to the physiological characteristics of S. sanguis have been noted that do not possess this antigen (Colman & Williams, 1972). Colman & Williams (1965) noted several different cell wall patterns, and Carlsson (1968) found two distinct clusters of these organisms

in his numerical taxonomy; this was also found by Facklam (1977). He correlated some of the nomenclature used for these strains by himself, Colman & Williams (1972) and Carlsson (1968). From this, while it appeared that different workers were in agreement that there was more than one type of S. sanguis; the different groups found often appeared to be similar to S. mitis. S. sanguis usually produces a dextran from sucrose, but Cole & Kolstad (1974) have found some strains which do not do this. These strains are also different from other strains of S. sanguis in their pattern of antibiotic resistance.

"Streptococcus mitior" and S. mitis are two different names for the same loose group of organisms. Only the name S. mitis appears in the Approved Lists of Bacterial Names (Skerman et al., 1980). It is considered that this species replaces most closely the older term "S. viridans". Facklam (1977) considered that S. sanguis and S. mitis may be closely related. S. mitis does not possess a specific group antigen. Wilson & Miles (1975) consider that some strains of S. mitis may be closely related to streptococci of serological groups O and M.

"Streptococcus milleri" is another oral species which is similar to some of the "minute" streptococci of serological groups, A, C, F and G. Wilson & Miles (1975) consider that the species is made up of non-β-haemolytic strains of the organism "Streptococcus MG" and some of the "minute" organisms. Diebel & Seeley (1974) do not consider it as an independant species. The "minute" organisms are usually grouped with the pyogenic organisms. "S. milleri" is

found almost exclusively in the mouth although it has been known to cause brain abcesses. It does not possess a specific group antigen and is reported as being non-haemolytic (Wilson & Miles, 1975). As was stated earlier, there may be some relationship between S. anginosus and "S. milleri" and further work is required to clarify this and any relationships with the "minute" organisms and "Streptococcus MG" (Jones, 1978).

1.4.6 Other streptococci

There are various streptococci that do not fit into any of the previously mentioned groups. Four of these are S. uberis, S. acidominimus, S. thermophilus and S. iniae.

Streptococcus uberis is a species of streptococci associated with cows, it is found in faeces, milk, the throat and on the skin. It causes a form of bovine mastitis. It is non-haemolytic and serologically it reacts with a number of the Lancefield group antisera, particularly those of groups E and P (Roguinsky, 1971). Although S. uberis has been reported as giving a positive reaction to the CAMP test, usually characteristic of Lancefield group B organisms, it has not been reported as reacting with this antiserum (Jones, 1978).

Streptococcus acidominimus is considered by Diebel & Seeley (1974) to be a distinct species, although they point out that it could be considered as a variant of S. uberis. From the physiological properties that they provide this seems most likely.

Streptococcus thermophilus was first described by Orla-Jensen (1919) and was isolated from milk and other dairy products. Abd-el-Malek & Gibson (1948) isolated it from pasteurised milk. S. thermophilus is unique among streptococci in that it survives heating to 65°C (Jones, 1978). Diebel & Seeley (1974) have reported that it will give α-haemolysis on blood agar. This is however not clear, as Sherman (1937) failed to obtain haemolysis and other workers (e.g. Abd-el-Malek & Gibson, 1948; Jones, 1978) failed to get growth on blood agar. It is only rarely isolated from milk today because new pasteurisation methods use higher temperature. It is used as a "starter" culture in the dairy industry. Its aldolase types resemble those of S. lactis (London & Kline, 1973) and its cell wall structure was reported by Schleifer & Kandler (1967) to be similar to that of S. faecalis.

Some other species of streptococci have been reported, but few are sufficiently characterised to warrant inclusion here. S. iniae was reported by Pier & Madin (1976) as a β-haemolytic streptococcus isolated from the oral abcesses of freshwater dolphins. It is recognisable by being distinct from the known Lancefield groups, but physiologically it appears similar to some of the organisms of serological group E. This may represent a species that is transitional between the pyogenic and the oral groups of organisms.

1.4.7 Conclusions on the review of the taxonomy

The taxonomy of the streptococci and related organisms can be seen to be confused. It is clear that there are some distinct groups within these organisms (e.g. S. faecalis / S. faecium and S. thermophilus) but in other groups there is a lot of variation (e.g. S. bovis and S. sanguis). Much of the existing taxonomy relies on divisions and groups determined by only a few characters. Some organisms have consequently been assigned in the past to groups where they may not belong (e.g. S. equinus in the enterococcus group).

The streptococci are in many ways similar to one another, and it may be that they do not form such clear cut species as other bacteria. There seem to be a number of intermediate groupings between the major ones (e.g. S. iniae and "S. milleri").

The genetic information available for the streptococci is less than for many other genera. Williams drew attention to this recently (1979). In comparison with other genera a large amount of information is available on the habitat and pathogenicity of these organisms. In routine medical and public health laboratories, the minimum of well established tests are performed on clinical isolates. These in practice tend to consist of haemolysis, serological groupings and antibiotic sensitivity. As a result of this it is clear that any new information on taxonomy would have to be able to relate to these practical situations where time is at a premium. The genus Streptococcus has only in the last few years been considered in depth in its relationship to dental caries. As a result, one of

the major groups, S. mutans, subdivided by Coykendall (1977), is a resurrection of a group first proposed in 1924 (Clarke, 1924). Much of the early work on the pathogenicity of streptococci was in many ways geared to finding a vaccine against them (Andrewes & Horder, 1906), and this has recently been revised as a possible method for the prevention of tooth decay.

1.5 Numerical taxonomy

Numerical taxonomy is the use of numerical methods in the classification of organisms. It may be defined as the grouping by numerical methods of taxonomic units into taxa on the basis of their character states. In practice the numerical taxonomy of a genus involves undertaking a large number of tests on representative strains, and from this computing the similarity of any one strain to any other. This is followed by the grouping of the strains into clusters on the basis of the similarities. Although the process is relatively new scientifically, its concepts have been known for many years. A taxonomy that uses these methods may be called an "Adansonian" taxonomy, because the concepts involved may be traced back to a French botanist called Michel Adanson (1727-1806).

In 1898 Heincke used a measure of phenetic differences to distinguish between different herring populations. Many other workers between 1900 and 1955 used measures of phenotypic distances and correlation coefficients. It was not however until the late 1950's that the first methods and theories of numerical taxonomy were developed (Sneath, 1957; Michener & Sokal, 1957). Many new methods have been developed since then. These have in turn been used for studies on a large number of different organisms, including over two

hundred bacterial classifications (Sneath & Sokal, 1973).

There are seven major principles of numerical taxonomy. These are:

1. The greater the content of the information in the taxa of a classification and the more characters on which it is based, the better a given classification will be.

2. A priori, every character is of equal weight in creating natural taxa.

3. Overall similarity between any two entities is a function of their individual similarities in each of the many characters in which they are being compared.

4. Distinct taxa can be recognised because correlations of characters differ in the groups of organisms under study.

5. Phylogenetic inferences can be made from the taxonomic structures of a group and from character correlations, given certain assumptions about evolutionary pathways and mechanisms.

6. Taxonomy is viewed and practised as an empirical science.

7. Classifications are based on phenetic similarity.

The aims of numerical taxonomy are repeatability and objectivity. It is hoped that by using numerical taxonomic methods, similar relationships between organisms may be found by different workers in different laboratories at different times (Sneath & Sokal, 1973).

When performing a numerical taxonomy a table of the character states of t organisms against n characters is first prepared. Each organism is compared over all of the n characters to all others in the study and this gives a similarity coefficient for each possible pair. The result of this is a table or matrix of similarities of each organism against each other. This is called a t x n matrix. From this it is usual to cluster the organisms. This means that the most similar organisms are grouped together and the least similar further apart. As a result of this a dendrogram may be drawn to show the different clusters of organisms and their relationships to one another. Beers & Lockhart (1962) proposed the use of a measure of taxonomic distance (d) rather than similarity. This method of representation, as a dendrogram, is two dimensional. However, other methods may be used. Thus, the organisms may be represented in a three dimensional space. In this the separate organisms are points in space, at various distances apart. Unlike a dendrogram, such three dimensional models include direction and elevation. It is possible by computer techniques to obtain a two or three dimensional representation of similarity from the n dimensional space represented by n characters. Finally, from the clusters the worker can tabulate the best characters for use in an identification system, many of which are often numerical in nature.

There are many advantages of numerical taxonomy.

1. Numerical taxonomy has the power to integrate data from a variety of sources, such as morphology, physiology, chemistry, affinities between DNA strands, amino acid sequences of proteins and so on. This is very difficult to do by conventional taxonomy.

2. Through the automation of large portions of the taxonomic process, greater efficiency is promoted. Thus, much taxonomic work can be done by less highly skilled workers or automata.

3. The data after coding in numerical form can be integrated with existing electronic data processing systems in taxonomic institutions and used for the creation of descriptions, keys, catalogues and maps as well as other documents.

4. Being qualitative, the methods provide greater discrimination along the spectrum of taxonomic differences and are more sensitive in delimiting taxa. Thus they should give better classifications and keys than can be obtained by conventional methods.

5. The creation of explicit data tables for numerical taxonomy has already forced workers in this field to use more and better described characters. This necessarily will improve the quality of conventional taxonomy as well.

6. A fundamental advantage of numerical taxonomy has been the re-examination of the principles of taxonomy and the purposes of classification.

7. Numerical taxonomy has led to re-interpretation of a number of biological concepts and to the posing of new biological and evolutionary question.

There are other advantages to numerical taxonomy, but some of these are not as simple as they appear. If two organisms share a large number of similar characters, then the similarity between them is large. A greater number of characters enables the similarity to be observed over a greater number of properties, so the greater the number of characters, the clearer the classification. The relationship between accuracy of similarity and the number of characters is asymptotic. So, just as there is a point at which a classification breaks down due to too few characters, there is also a point above which it may not be worth going due to the small change that would result from it. It is best in general to try to use a moderate number of good tests, usually between one and two hundred, rather than adding less reproducible or superfluous tests.

1.6 Mole % G+C introduction

The base composition of bacterial deoxyribonucleic acid (DNA) varies between 25 and 75 moles percent guanine plus cytosine (mol % G+C) (Belozersky & Spirin, 1960). This range of values may be used as an aid to bacterial classification (Hill, 1968). While some genera such as Aerobacter are very homogenous in DNA base composition, others, such as Micrococcus are heterogenous or even discontinuous.

A relationship between the mol % G+C value and the denaturation temperature (T_M) was proposed by Marmur & Doty (1962), a higher guanine and cytosine content conferring a higher degree of thermal stability. The denaturation temperature is taken as being the mid-point of the increase in absorbance at 260 nm. This

increase is brought about by the disassociation of the bonds between the double stranded DNA molecule (Kreig & Lockhart, 1970). A relationship between this temperature and the mol % G+C of the molecule has been derived by De Ley (1970) on the basis of original formulae from Marmur & Doty (1962) and Schildkraut & Lifson (1965). This is given as:

$$\text{Mol \% G+C} = 2.44\ (T_M - 69.4)$$

This equation is only true for a solvent containing 0.15 M NaCl. This relationship is linear but it is affected by the concentration of the cation in the buffer used. This is due to the ionic strength of the solution affecting the disassociation of the bonds in the DNA molecule. As a result the equation relating to the mol % G+C and the T_M may be written with respect to the cation concentration. This takes the form:

$$\text{Mol \% G+C} = 2.44\ (T_M - 81.5 - 16.6 \log M)$$

Where M is the concentration of the cation present (Schildkraut & Lifson, 1965). A high salt concentration increases the thermal stability and so the T_M values are elevated.

Because of its ease in determination and the high degree of reproducibility of the results, the thermal transition profile has generally become the favoured method of determining the mol % G+C value of DNA. The use of UV absorbance can be extended to give a quantitative measurement of the amount of DNA and protein present in a sample (as shown in Section 2.12).

The mol % G+C values for many bacterial groups have been determined (Marmur & Doty, 1962; Hill, 1966). Likewise the mol % G+C values of the family Streptococcaceae have been observed by many different workers (Marmur & Doty, 1962; Coykendall, 1974; Roop et al., 1974; Garvie, 1978; Garvie & Bramley, 1979a; 1979b). These studies in general have concentrated on particular groups or species, such as the group D organisms (Roop et al., 1974), the group N organisms (Garvie, 1978) and Leuconostoc (Garvie, 1976). The overall range of the mol % G+C values for the family Streptococcaceae is given as being 33 to 44, whereas the range for the genus Streptococcus is only slightly narrower at 33 to 42 (Diebel & Seeley, 1974).

The method of preparation of the DNA is important to the denaturation temperature procedure. Damaged or broken DNA may give a different value from the intact molecule as the base sequences present in the fragments may not represent the overall composition (Krieg & Lockhart, 1970). Similarly, if the DNA should become partly or wholly denatured in the extraction or purification procedure then the effects of the thermal denaturation will be altered, as will the T_M. The lysis of the organisms must therefore avoid any undue shearing of the DNA. Gram-positive organisms are difficult to lyse, many requiring both an enzymic agent such as lysozyme and a detergent (Marmur, 1961). This process can be helped by the inclusion in the media of an agent which will produce defective cell walls during growth. However, easier lysis of some of the serological group D organisms has been obtained by the altering of salt concentration during the treatment of lysis (Metcalf & Diebel, 1969). In some cases the T_M of partly purified DNA has

been found to be similar to that obtained from pure DNA and this has been proposed as an alternative in cases where incomplete lysis results in low yields of DNA (Owen & Lapage, 1976).

1.7 Protein electrophoresis

Electrophoresis involves the movement of particles through an electric field in a medium buffered to a particular pH value. As a result, different particles move by differing amounts depending upon their size and charge. Electrophoresis may be carried out in a system whereby the solution containing the ions to be separated is supported in a more or less inert medium, such as paper, starch or poly-acrylamide. Few, if any, classes of charged biological material have not been separated by electrophoresis, and proteins are no exception (Sargent, 1971). Proteins are ampholytes and so act as acid or bases depending upon the pH of the surrounding medium. They are electrolytes and will migrate in an electrical field, the direction of migration being determined by their overall charge and the distance by the size of the molecule (Nozaki & Hayaishi, 1971).

A bacterial strain growing under the same standardised conditions will always produce the same set of proteins. Their structure, molecular weight and charge, as well as the number of copies produced are determined by the genetic material of the organism (Kersters & De Ley, 1980). Electrophoresis of a mixture of these proteins, under standardised conditions, produces protein banding patterns (electropherograms) which are considered as fingerprints for the bacterial strain being considered. These fingerprints may be realised by staining with a dye specific for proteins such as Napthalene black 10 B or Page blue after electrophoresis. However,

starch gels may shrink on staining, although they do produce better separation than paper gels. This problem has been largely eliminated by polyacrylamide which is not subject to such shrinkage (Gordon, 1969).

At first protein patterns were compared by visual methods (Lund , 1965). The first major attempt to quantitate them involved the measurement of $\underline{E_F}$ values (Fowler, 1963). This is a measure of how far a band has migrated represented as a percentage of the furthest distance moved by the bands. A further measure of quantitating protein patterns involves the use of a densitometer. This uses a light beam to scan along the gel, recording the position and intensity of all the bands. The resulting trace may then be converted into numerical values. These values may be compared between traces by statistical methods such as those used in numerical taxonomy, and a dendrogram and similarity matrix may be constructed (Kersters & De Ley, 1975). As a result of the storage facilities offered by computers, large numbers of traces may be compared together easily.

In order to achieve reproducible results, densitometer traces may be standardised by the inclusion of known marker proteins such as ovalbumin. One of these may be used as an end marker when quantitiating traces (Kersters & De Ley, 1980). Similarly, traces obtained from the same strain may be compared in order to take into account variation within the method.

A further application of computer methods is the formation of identification matrices. These allow for the identification of unknown traces against a reference collection (Feltham & Sneath, 1979).

The use of protein gel electrophoresis in microbial systematics has been well established for many years. The technique has produced criteria for both taxonomy and identification and appears to be a useful tool in both of these fields (Rouatt et al., 1970; Morris, 1973). Particularly useful is the ability to often identify problematical strains (Lund , 1965; Kersters & De Ley, 1980).

Protein gel electrophoresis within the Streptococcaceae has been confined to particular areas, both taxonomic and practical. One of the earliest works was that of Lund (1965). In this protein and esterase patterns were compared visually for some serological group D organisms. A further study (Lund , 1967) again used serological group D strains but on this occasion distances of esterase bands from the origin were measured. This method of measuring the migration of esterase bands had previously been used in the taxonomy of Bacillus thuringiensis, by Norris (1964).

The electrophoresis of particular enzymes has been used to study some groups of streptococci. Glucose 6-phosphatase dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase enzymes have been studied in S. faecalis (Williams & Bowden, 1968). Exracellular sucrose metabolising enzymes in S. mutans were studied by Osborn et al., (1976). The lactate dehydrogenase enzymes of some streptococci have been studied electrophoretically, particularly S. raffinolactis, S. bovis, S. uberis and Leuconostoc sp. (Garvie, 1978; Garvie & Bramley, 1979a; 1979b; Garvie, 1969). Peptidase

enzymes have been studied in S. lactis and S. cremoris (Cliffe & Law, 1979). The membrane proteins of S. mutans and related organisms have been studied by isoelectric focusing in polyacrylamide gels (Hamada & Mizuno, 1974). The proteins present in cell free extracts of serological group A streptococci have been studied by Hess & Slade (1965).

MATERIALS AND METHODS

2.1 Strain list

The two-hundred and two strains used in the numerical study are listed in numerical order in Table 2.1.a. The strain details are given, where known.

2.2 Routine procedures

2.2.1 Maintenance of cultures and basal media.

On receipt cultures were grown on basal medium agar with the addition of 7½% (v/v) horse blood (Difco). The cultures were subcultured once a week.

Basal medium agar

This was Blood agar base No. 2 (Difco) and is referred to as BMA; it contained no blood unless stated.

Basal medium broth

This is referred to as BMB and consisted of the following:

Proteose peptone (Difco)	15g
Sodium chloride	5g
Yeast extract (Difco)	5g
Liver digest (Oxoid)	2.5g
Distilled water to	1000ml

Long term maintenance of the cultures was obtained by freezing them on glass beads at -80°C. The method of Feltham et al. (1978) was used, with glycerol as a cryoprotectant. The suspending broth consisted of BMB with the addition of 15% (v/v) glycerol.

2.2.2 Incubation temperatures

Unless otherwise stated all strains were incubated at 35°C. The inoculum was a loop of a 16 h broth culture. However the strains of the genera Gemella and Leuconostoc (PB 161-167) were incubated at 25°C.

2.2.3 Sterilisation procedure

Unless otherwise stated all sterilisation was performed by autoclaving at 121°C for 15 min.

2.3 Initial tests

On receipt all strains were subjected to five initial tests.

Gram stain.

This was performed as described by Cowan & Steel (1974).

Cell shape and aggregation

These were observed on the gram stained samples. The different arrangements were recorded as single cocci, cocci in short chains and pairs, cocci in chains and cocci in tetrads.

Table 2.1.a. Strain list.

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 1	*Streptococcus faecalis*	D			DJ	W 1
PB 2	*Streptococcus faecalis*	D	TAL*		DJ	W 48 = NCTC 775
PB 3	"*Streptococcus faecalis* subsp. *liquefaciens*"	D			DJ	W 2
PB 4	"*Streptococcus faecalis* subsp. *zymogenes*"	D			DJ	W 3
PB 5	*Streptococcus faecium*	D			DJ	W 4
PB 6	*Streptococcus faecium*	D			DJ	W 5
PB 7	*Streptococcus bovis*	D	Capsulated		DJ	W 7
PB 8	*Streptococcus bovis*	D			DJ	W 9
PB 9	*Streptococcus salivarius*	K	TAL		RKAF	K 485 = NCTC 8618
PB 10	"*Streptococcus durans*"	D	Capsulated		DJ	W 11
PB 11	*Streptococcus equinus*	D			DJ	W 14
PB 12	*Streptococcus equinus*	D			DJ	W 15
PB 13	*Streptococcus equinus*	D			DJ	W 16
PB 14	"*Streptococcus avium*"	Q			DJ	W 32

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 15	"Streptococcus avium"	Q			DJ	W 33
PB 16	"Streptococcus avium"	Q			DJ	W 34
PB 17	"Streptococcus avium"	Q			DJ	W 35
PB 18	Streptococcus sp.	D		Chicken	EB	F 87/283
PB 19	Streptococcus sp.	D		Chicken	EB	F 87/274
PB 20	Streptococcus sp.	D		Chicken	EB	F 87/275
PB 21	Streptococcus sp.	D		Chicken	EB	F 87/276
PB 22	Streptococcus sp.	D		Chicken	EB	F 87/277
PB 23	Streptococcus sp.	D		Chicken	EB	F 87/278
PB 24	Streptococcus sp.	D		Chicken	EB	F 87/279
PB 25	Streptococcus sp.	D		Chicken	EB	F 87/296
PB 26	Streptococcus sp.	D		Chicken	EB	F 87/307
PB 27	Streptococcus sp.	D		Chicken	EB	F 87/311
PB 28	Streptococcus sp.	D		Chicken	EB	F 87/344
FB 29	Streptococcus sp.	D		Chicken	EB	F 87/358
FB 30	Streptococcus sp.	D		Chicken	EB	F 87/361

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 31	Streptococcus sp.	D		Chicken	EB	F 87/362
PB 32	Streptococcus sp.	D		Chicken	EB	F 87/364
PB 33	Streptococcus sp.	D		Chicken	EB	F 87/365
PB 34	Streptococcus sp.	D		Chicken	EB	F 87/366
PB 35	Streptococcus thermophilus				DJ	W 36
PB 36	Streptococcus thermophilus				DJ	W 37
PB 37	Streptococcus thermophilus				DJ	W 38
PB 38	Streptococcus thermophilus				DJ	W 39
PB 39	Streptococcus thermophilus				DJ	W 40
PB 40	Streptococcus agalactiae	B	TAL		DJ	W 44 = NCTC 8181
PB 41	Streptococcus equi	C	TAL		DJ	W 45 = NCTC 9682
PB 42	"Streptococcus infrequens"	E	Low acid producer		DJ	W 52 = NCTC 5385
PB 43	Streptococcus sp.	F			DJ	W 53 = NCTC 5389
PB 44	Streptococcus sp.	G		Human	DJ	W 54 = NCTC 8547
PB 45	Streptococcus sp.	H			DJ	W 55 = NCTC 7868

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments	Source.	Donor.	Donor number.
PB 46	*Streptococcus* sp.	K			DJ	W 56
PB 47	*Streptococcus* sp.	M			DJ	W 58
PB 48	*Streptococcus* sp.	N			DJ	W 59
PB 49	*Streptococcus* sp.	O			DJ	W 60
PB 50	*Streptococcus uberis*				DJ	W 62
PB 51	"*Streptococcus faecium* subsp. *casseliflavus*"	D		Plant	DJ	W 86 = ATCC 25788
PB 52	"*Streptococcus faecium* subsp. *casseliflavus*"	D		Plant	DJ	W 87 = ATCC 25789
PB 53	"*Streptococcus faecium* subsp. *mobilis*"	D			DJ	W 88 = NCIB 9645
PB 54	*Streptococcus pyogenes*	A	TAL		RKAF	K 400 = NCTC 8198
PB 55	*Streptococcus pyogenes*	A		Human	RKAF	K 403 = NCTC 8191
PB 56	"*Streptococcus milleri*"				RKAF	K 476 = NCTC 10708
PB 57	"*Streptococcus milleri*"				RKAF	K 477 = NCTC 10709
PB 58	*Streptococcus rattus*		TAL		RKAF	K 592 = ATCC 19645

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 59	"Streptococcus sobrinus"				RKAF	K 597
PB 60	"Streptococcus sobrinus"				RKAF	K 598
PB 61	Streptococcus pyogenes	A			RKAF	K 401 = NCTC 2218
PB 62	Streptococcus pyogenes	A		Human throat	RKAF	K 405 = NCTC 831
PB 63	Streptococcus equi	C			RKAF	K 406
PB 64	Streptococcus pyogenes	A			RKAF	K 407 = NCTC 4001
PB 65	Streptococcus pyogenes	A		Human throat	RKAF	K 410 = NCTC 8302
PB 66	Streptococcus sanguis	H	Atypical[1]		RKAF	K 411 = NCTC 7864
PB 67	Streptococcus sanguis	H	TAL		RKAF	K 412 = NCTC 7863
PB 68	Streptococcus sanguis	H			RKAF	K 413 = NCTC 7865
PB 69	"Streptococcus faecalis subsp. malodoratus"	D		Dutch cheese	RKAF	K 415 = NCDO 847
PB 70	Streptococcus pneumoniae		TAL		RKAF	K 416 = NCTC 7465
PB 71	Streptococcus sp.	N		Frozen peas	RKAF	K 424 = NCDO 1871
PB 72	Streptococcus agalactiae	B			RKAF	K 426
PB 73	Streptococcus agalactiae	B			RKAF	K 428 = NCDO 1350

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 74	Streptococcus agalactiae	B			RKAF	K 429 = NCDO 1334
PB 75	Streptococcus agalactiae	B			RKAF	K 430 = NCDO 1520
PB 76	Streptococcus mutans		TAL	Carious dentine	RKAF	K 431 = NCTC 10449
PB 77	Streptococcus faecalis	D			RKAF	K 432 = NCIB 6783
PB 78	Streptococcus bovis	D			RKAF	K 433 = NCDO 964
PB 79	"Streptococcus faecalis subsp. zymogenes"	D			RKAF	K 489 = NCDO 1595
PB 80	Streptococcus bovis	D			RKAF	K 434 = NCDO 1251
PB 81	Streptococcus bovis	D		Babies' faeces	RKAF	K 439 = NCDO 599
PB 82	Streptococcus equinus	D			RKAF	K 441 = NCDO 1090
PB 83	Streptococcus equinus	D	TAL	Horse faeces	RKAF	K 443 = NCTC 1037
PB 84	Streptococcus thermophilus		TAL	Pasteurised milk	RKAF	K 446 = NCDO 573
PB 85	"Streptococcus faecalis subsp. liquefaciens"	D		Babies' faeces	RKAF	K 454 = NCDO 611
PB 86	"Streptococcus durans"	D	Type 2		RKAF	K 456 = NCDO 596
PB 87	Streptococcus faecium	D	TAL		RKAF	K 457 = NCDO 492

Table 2.1.a. continued

Strain number.	Received as	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 88	"Streptococcus avium"	Q	Guthof strain		RKAF	K 461 = NCTC 9938
PB 89	Streptococcus sp.			Human sputum	RKAF	K 490 = NCTC 3166
PB 90	Streptococcus sp.	O		Human	RKAF	K 462 = NCTC 8029
PB 91	Streptococcus sp.	O		Human	RKAF	K 464 = NCTC 8033
PB 92	"Streptococcus sp. (MG)"				RKAF	K 465 = NCTC 8037
PB 93	Streptococcus lactis	N	TAL		RKAF	K 466 = NCDO 604
PB 94	Streptococcus lactis	N			RKAF	K 467 = NCDO 496
PB 95	Streptococcus cremoris	N	TAL		RKAF	K 471 = NCDO 607
PB 96	Streptococcus cremoris	N			RKAF	K 472 = NCDO 500
PB 97	"Streptococcus durans"	D			RKAF	K 478 = NCDO 502
PB 98	Streptococcus mitis			Human sputum	RKAF	K 491 = NCTC 10712
PB 99	Streptococcus salivarius	K			RKAF	K 483 = NCTC 7366
PB 100	Streptococcus salivarius	K			RKAF	K 484 = NCTC 8606
PB 101	Streptococcus mitis				RKAF	K 492
PB 102	Streptococcus sp.			Human tonsil	RKAF	K 495 = NCTC 1080
PB 103	"Streptococcus equisimilis"	C			RKAF	K 496 = NCTC 5371
PB 104	"Streptococcus equisimilis"	C			RKAF	K 499 = NCTC 7136

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 105	"Streptococcus equisimilis"	C		Vagina	RKAF	K 500 = NCTC 8543
PB 106	"Streptococcus dysgalactiae"	C			RKAF	K 501 = NCDO 1356
PB 107	Aerococcus viridans				NCIB	NCIB 9206
PB 108	Pediococcus pentosaceus				NCIB	NCIB 8106
PB 109	"Aerococcus catalyticus"			Medicine bottles	NCIB	NCIB 9643
PB 110	"Aerococcus catalyticus"			Medicine bottles	NCIB	NCIB 9642
PB 111	Pediococcus acidilacti				NCIB	NCIB 6990
PB 112	Aerococcus viridans				NCIB	NCIB 9208
PB 113	Pediococcus halophilus				NCIB	NCIB 9477
PB 114	Pediococcus damnosus		Slime producer		NCIB	NCIB 10563
PB 115	"Streptococcus suis"				LUM	D 1029
PB 116	"Streptococcus dysgalactiae"	C			RKAF	K 503 = NCDO 1358
PB 117	"Streptococcus dysgalactiae"	C		Mastitis	RKAF	K 504 = NCTC 4669
PB 118	Streptococcus uberis		TAL		RKAF	K 506 = NCTC 3858
PB 119	Streptococcus uberis			Mastitis	RKAF	K 510 = NCTC 4674
PB 120	"Streptococcus zooepidemicus"	C		Mouse	RKAF	K 511 = NCTC 6176

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 121	"Streptococcus zooepidemicus"	C			RKAF	K 514 = NCTC 7023
PB 122	Streptococcus sp.	E		Pig	RKAF	K 519 = NCDO 603
PB 123	"Streptococcus suis"				RKAF	K 520
PB 124	"Streptococcus cremoris subsp. alactosus"	N	Lactose negative		RKAF	K 521 = NCDO 1032
PB 125	Streptococcus faecalis	D			RKAF	K 523
PB 126	"Streptococcus faecalis subsp. zymogenes"	D		Danish blue cheese	RKAF	K 525 = NCTC 8176
PB 127	Streptococcus sp.	B		Human cervix	RKAF	K 528 = NCTC 9412
PB 128	"Streptococcus faecalis subsp. liquefaciens"	D		Babies' faeces	RKAF	K 534 = NCDO 591
PB 129	Streptococcus faecium	D			RKAF	K 537 = NCDO 583
PB 130	"Streptococcus lactis subsp. diacetylactis"	N			RKAF	K 545 = NCDO 184
PB 131	"Streptococcus lactis subsp. diacetylactis"	N			RKAF	K 547 = NCDO 1007
PB 132	"Streptococcus equisimilis"	C		Horse	RKAF	K 549 = NCTC 6179
PB 133	Streptococcus sp.	B		Human	RKAF	K 550

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 134	Streptococcus sp.	B		Human	RKAF	K 551
PB 135	Streptococcus sp.	B		Human	RKAF	K 558
PB 136	Streptococcus sp.	B		Human	RKAF	K 560
PB 137	Streptococcus sp.	B		Human	RKAF	K 563
PB 138	Streptococcus sp.	B		Human	RKAF	K 567
PB 139	Streptococcus sp.	B		Human	RKAF	K 573
PB 140	Streptococcus sp.	D		Human	RKAF	K 576
PB 141	Streptococcus sp.	B		Human	RKAF	K 578
PB 142	Streptococcus sp.	G		Human	RKAF	K 579
PB 143	Streptococcus salivarius	K		Human	RKAF	K 582
PB 144	"Streptococcus milleri"			Human	RKAF	K 585
PB 145	"Streptococcus mitior"			Human	RKAF	K 587
PB 146	Streptococcus sanguis	H			RKAF	K 589
PB 147	Streptococcus sp.	B			RKAF	K 590
PB 148	Streptococcus sp.				LUM	T 58
PB 149	Streptococcus sp.				LUM	T 128

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 150	*Streptococcus* sp.				LUM	T 132
PB 151	"*Streptococcus mutans* subsp. *mutans*"				RKAF	K 591
PB 152	*Streptococcus* sp.			Rat eye	LUM	——
PB 153	*Streptococcus* sp.	D	Motile		NCTC	NCTC 4725
PB 154	*Streptococcus* sp.	K			NCTC	NCTC 10232
PB 155	*Streptococcus* sp.	L	Type 3		NCTC	NCTC 10321
PB 156	*Streptococcus* sp.	L		Dog	NCTC	NCTC 10238
PB 157	*Streptococcus* sp.	M		Dog	NCTC	NCTC 10235
PB 158	*Streptococcus* sp.	P			NCTC	NCTC 9824
PB 159	*Streptococcus* sp.	R		Pig	NCTC	NCTC 10234
PB 160	*Streptococcus* sp.	S		Pig	NCTC	NCTC 10237
PB 161	*Gemella haemolysans*				NCTC	NCTC 10243
PB 162	*Leuconostoc paramesenteroides*		TAL		NCDO	NCDO 803
PB 163	*Leuconostoc oenos*		TAL	Wine	NCDO	NCDO 1674
PB 164	*Leuconostoc cremoris*		TAL		NCDO	NCDO 543
PB 165	*Leuconostoc lactis*		TAL		NCDO	NCDO 535

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 166	Leuconostoc dextranicum				NCIB	NCIB 2706
PB 167	Leuconostoc mesenteroides				NCIB	NCIB 3351
PB 168	Pediococcus halophilus				NCIB	NCIB 9735
PB 169	Pediococcus acidilacti				NCIB	NCIB 7881
PB 170	Streptococcus sp.			Human	LGH	MR6/79
PB 171	Streptococcus sp.		Group II	Human oral	CU	OPK1
PB 172	Streptococcus sp.		Group II	Human oral	CU	KPSK2
PB 173	Streptococcus sp.		Group II	Human oral	CU	HPK1
PB 174	Streptococcus sp.	K	Group III	Human oral	CU	OTS1
PB 175	Streptococcus sp.		Group III	Human oral	CU	MSS2
PB 176	Streptococcus sp.	K	Group III	Human oral	CU	KTS1
PB 177	Streptococcus sp.		Group IB	Human oral	CU	KPE2
PB 178	Streptococcus sp.		Group IB	Human oral	CU	MVE1
PB 179	Streptococcus sp.		Group IB	Human oral	CU	KPE1
PB 180	Streptococcus sp.		Group IA	Human oral	CU	LPA1
PB 181	Streptococcus sp.		Group IA	Human oral	CU	MPA1

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 182	*Streptococcus* sp.		Group IA	Human oral	CU	LVG1
PB 183	*Streptococcus* sp.		Group VA	Human oral	CU	OS51
PB 184	*Streptococcus* sp.		Group VA	Human oral	CU	LV51
PB 185	*Streptococcus* sp.		Group VA	Human oral	CU	NS51
PB 186	*Streptococcus* sp.		Group VA	Human oral	CU	OP51
PB 187	*Streptococcus* sp.	G	Group VB	Human oral	CU	NT61
PB 188	*Streptococcus* sp.		Group VB	Human oral	CU	PT51
PB 189	*Streptococcus* sp.		Group IV	Human oral	CU	LV71
PB 190	*Streptococcus* sp.		Group IV	Human oral	CU	LV81
PB 191	*Streptococcus* sp.	M		Dog	NCTC	NCTC 10233
PB 192	*Streptococcus* sp.	T		Pig brain	NCTC	NCTC 10446
PB 193	"*Streptococcus lactis* subsp. *diacetylactis*"	N	Type 4		NCDO	NCDO 176
PB 194	"*Streptococcus lactis* subsp. *diacetylactis*"	N			NCDO	NCDO 802
PB 195	"*Streptococcus lactis* subsp. *diacetylactis*"	N			LUM	C 615
PB 196	*Streptococcus lactis*	N			NCIB	NCIB 10769

Table 2.1.a. continued

Strain number.	Received as.	Serological group.	Comments.	Source.	Donor.	Donor number.
PB 197	Streptococcus cremoris	N			LUM	C 616
PB 198	Streptococcus sp.				NCDO	NCDO 2113
PB 199	Streptococcus raffinolactis	N	TAL		NCDO	NCDO 617
PB 200	Streptococcus raffinolactis	N			NCDO	NCDO 619
PB 201	Streptococcus sp.				NCDO	NCDO 2128
PB 202	Streptococcus sp.				NCDO	NCDO 2112

*, TAL = Type strain as defined in the Approved Lists of Bacterial Names (Skerman et al., 1980).

1, Cole & Kolstad (1974).

2, Type strain designated (Sneath & Skerman, 1966).

3, Listed as type strain in NCTC catalogue, 1972.

4, Listed as type strain in NCDO catalogue, 1974.

Donor abbreviations.

DJ	Dr.Dorothy Jones. Department of Microbiology, Leicester University.
EB	Dr. Ella Barnes. Norwich.
RKAF	Dr. R.K.A. Feltham. Department of Microbiology, Leicester University.
NCIB	National Collection of Industrial Bacteria. Torry Research Station, Aberdeen.
LUM	Microbiology Department, Leicester University.
NCTC	National Collection of Type Cultures. Public Health Laboratory Service, Colindale.
LGH	General Hospital. Leicester.
NCDO	National Collection of Dairy Organisms. Reading.
CU	Prof. J. Carlsson. Umea, Sweden.
ATCC	American Type Culture Collection. Maryland, USA.

Oxidase test

This was performed by spotting inoculum from a single colony on BMA incubated for 16 h, onto a filter paper saturated with Kovacs' reagent. The appearance of a deep purple colour within 15 sec was considered a positive reaction. The time limit was imposed as the reagent is oxidised by the air. Pseudomonas aeruginosa was used as a positive control.

Kovács' oxidase reagent (Cowan & Steel, 1974). 1% (w/v) tetramethyl-p-phenylenediamine in aqueous solution.

Catalase test

Method one of Cowan & Steel (1974) was used. Cultures were grown on BMA and then spotted with 3% (v/v) hydrogen peroxide. A positive result was the effervescence from the production of oxygen. The hydrogen peroxide was agitated before use to remove any dissolved oxygen. Escherichia coli was used as a positive control.

Haemolysis

Haemolysis was obversed on BMA containing 7½% (v/v) horse blood. This was recorded after 16 h incubation. Cultures were subcultured from beads once before the haemolysis was recorded. Results were scored as; α-haemolysis (greening of red cells with partial lysis); β-haemolysis (complete lysis of the red cells); and γ-haemolysis (no change in surrounding medium).

2.4 Morphological tests

Colonial morphology

The texture, edge, colour, elevation and colony diameter were observed on 16 h old cultures on BMA with 7½% (v/v) horse blood, and confirmed by use of a plate microscope. These were

scored in the following categories; texture (rough or smooth, dry or mucoid); edge (entire or broken); colour (grey/white or yellow); and elevation (convex or flat). Colony diameter was measured by recording the mean diameters of five colonies, the results were scored in three categories. These were: less than 0.2 mm, between 0.2 and 0.4 mm and above 0.4 mm.

2.5 Physiological tests

Growth temperatures

Cultures were grown in BMB at varying temperatures in order to see if growth was initiated. A positive result was recorded when the turbidity of the broth was equal to or exceeded No. 1 on the MacFarland scale. This was found by plate counts to be approximately equivalent to 10^5 organisms ml^{-1}. The results for plate counts performed for the MacFarland scale are given in Table 2.5.a. Cultures were examined after the following times; 4°C, 24 d; 10°C, 10 d; 25°C, 24 h; 45°C, 16 h.

Growth at pH 9.6

The method of Chesbro & Evans (1959) was used. The following solutions were made up:

Solution A.

Nutrient broth No.2 (Oxoid)		25g
D (+) glucose		10g
Tween 80		0.5ml
Distilled water	to	1000ml

Solution B.

Potassium hydrogen phosphate		6g
Sodium carbonate		6g
Distilled water	to	1000ml

Solution C.

Distilled water	250ml

These solutions were autoclaved separately for 10-12 min at 121°C. They were then mixed and left over-night at 4°C to equilibriate. 3ml of the sterile solution was then aseptically transferred to bijoux bottles. These were inoculated from a 16 h broth culture and incubated for 24 h. A bottle was recorded as positive if initiated growth was greater than No. 1 on the MacFarland scale.

Final pH in broth

Cultures were grown in universal bottles containing 15 ml of BMB with an added 5g l^{-1} D (+) glucose and 5g l^{-1} potassium hydrogen phosphate. They were incubated for 1 week and the pH was then measured using a digital pH meter. The final pH values were divided into three categories for scoring; below 4.25; between 4.25 and 4.75; above 4.75

2.6 Resistance and tolerance tests

Resistance to 60°C.

0.1ml amounts of a thick (MacFarland No.4) broth culture were added to 9.9ml of BMB heated in a 60°C water bath.

Table 2.5.a. Number of organisms expressed as organisms ml^{-1} as estimated by plate counts compared against the MacFarland scale of density.

MacFarland tube.	Organisms ml^{-1}
0	0
1	1×10^5
2	9×10^7
3	1.2×10^8

These broths were plated out after 1, 15, 60 and 120 min onto BMA with 7½% (v/v) horse blood. These plates were then incubated; resistance to heating after a given time was scored if visible growth was seen on the plates.

Growth in salt solutions

Sodium chloride was added to BMB to produce final concentrations of 3, 4 and 6.5% (w/v). These were dispensed in 3ml aliquots into screw-topped bijoux bottles before autoclaving. One loop of inoculum was used from a 16 h plate culture. Growth greater than tube No. 1 on the MacFarland scale was scored as a positive result.

Growth with inhibitory compounds

BMA was used with the addition of 7½% (v/v) horse blood for the following four compounds.

Ox bile.

10 and 40% (w/v) concentrations of dried ox bile were autoclaved in the media.

Crystal violet.

Two concentrations of crystal violet were used. These were 2ml of a 0.1% (w/v) solution of aqueous crystal violet and 4ml of the same solution, both in 1l of medium. These gave final concentrations in the media of 0.0002% and 0.0004% (w/v). The crystal violet solutions were filter-sterilised before being added to the media.

Sodium azide.

10 ml of a filter-sterilised 10% (w/v) aqueous solution of sodium azide was added to each litre of medium.

Thallous acetate.

10ml of a filter-sterilised 10% (w/v) aqueous solution of thallous acetate was added to each litre of medium.

In each case tolerance was indicated by visible growth after 24 h incubation.

Bile solubility

Cultures were grown in bijoux bottles in 3ml aliquots in BMB for 24 h. 0.5ml of sterile 10% (w/v) bile solution was added to the bottles which were shaken and left at room temperature for 2 h. They were then examined for clearing due to the lysis of cells. All of the samples were then stored at 4°C overnight and examined again.

The bile solution consisted of:

Sodium chloride	0.85g
Dried Ox bile (Oxoid)	10g
Distilled water to	1000ml

Acetic acid-acetate agar

The method of Whittenbury (1965b) was used. The following media was prepared:

D (+) glucose	10g
Peptone (Difco)	5g
Yeast extract (Difco)	15g
Lab-lemco (Oxoid)	5g

Tween 80 0.5ml

Bacto agar (Difco) 20g

Distilled water to 1000ml

This medium was adjusted to pH 5.4 after autoclaving at 115°C for 20 min. On cooling, 100ml of a filter sterilised 1(M) solution of acetic acid-acetate buffer was added. Plates were poured and growth observed at a low pH in the presence of acetate. Visible growth was scored as a positive result. The buffer solution was:

1(M) acetic acid 9.6ml

1.64% (w/v) sodium acetate 80.4ml

Growth on MacConkey agar

Growth on MacConkey agar (Oxoid) was observed after overnight incubation. Acid from lactose, apparent as pink colonies was also scored.

Growth on TCBS agar

Growth was observed after overnight incubation on thiosulphate cysteine bile salts sucrose agar (Oxoid). Acid from sucrose, apparent as yellow colonies, was also scored.

Antibiotic resistance

Multodiscs (Oxoid) were used. These were placed on plates of BMA containing 7½% (v/v) horse blood that had been spread with 0.1ml of bacterial suspension equivalent to No. 4 on the MacFarland scale.

Sensitivity as a clear zone around the antibiotics was scored as a positive character. Three discs containing a total of 24 antibiotics were used.

Antibiotic	Amount
Penicillin G	5 units
Sulphafuraxole	500 µg
Ampicillin	25 µg
Cloxacillin	5 µg
Erythromycin	10 µg
Methicillin	10 µg
Novobiocin	30 µg
Oleandomycin	10 µg
Furazolidone	100 µg
Carbenicillin	10 µg
Colistin sulphate	10 µg
Gentamicin	10 µg
Kanamycin	30 µg
Nalidixic acid	30 µg
Nitrofurantoin	200 µg
Polymixin B	300 µg
Tetracycline	50 µg
Cephaloridine	25 µg
Chloramphenicol	50 µg
Chlor tetracycline	50 µg
Neomycin	10 µg
Oxytetracycline	50 µg
Streptomycin	25 µg
Sulphamethoxazole and Trimethoprim	25 µg (in total)

2.7 Biochemical methods

Reduction of nitrate and gas production

The method of Cowan & Steel (1974) was used. 15ml aliquots of BMB containing 0.1% (w/v) potassium nitrate were inoculated with a loop from a 16 h culture and incubated in universal bottles for 5 d. 1 ml of nitrate solution A was added to each bottle followed by 1ml of nitrate solution B. A red colour indicated the presence of nitrite and was considered a positive result. A few grains of powdered zinc were added to all the bottles remaining colourless. This reduces any residual nitrate to nitrite, so the appearance of a red colour in these bottles indicated no reduction of nitrate. Bottles remaining colourless at this stage contained neither nitrate nor nitrite and this was scored as a positive result because the original nitrate had been reduced beyond nitrite, possibly to nitrogen.

Any gas formation from the nitrate was noted in a Durham tube placed in each bottle before the media was added.

Nitrate solution A.

0.8% (w/v) Sulphanilic acid in 5(M) acetic acid.

Nitrate solution B

0.6% (w/v) Di-methyl-α-napthol in 5(M) acetic acid.

Reduction of nitrite and gas production

The method of Cowan & Steel (1974) was used. 15ml aliquots of BMB containing 0.001% (w/v) sodium nitrite were inoculated and incubated in universal bottles for 7d. After this incubation the nitrate reagents were added as for nitrate reduction. The red colour seen indicates unreduced nitrite, so those tubes remaining colourless were scored as positive.

Gas production from the nitrite was noted in Durham tubes placed in the bottles before the media was added.

Reduction of tellurite

16ml of a filter-sterilised 2% (w/v) aqueous solution of potassium tellurite was added to 1l of BMA. The agar plates were inoculated and incubated for 48h. Reduction of tellurite was shown by the appearance of black colonies.

Reduction of tetrazolium

10ml of a filter-sterilised 10% (w/v) aqueous solution of 2, 3, 5, triphenyltetrazolium chloride was added to 1l of BMA, which also contained 0.5% (w/v) glucose. The plates were inoculated and incubated for 48 h. Reduction of tetrazolium was shown by the appearance of red colonies.

Reduction of methylene blue milk

10ml of a filter-sterilised 2% (w/v) aqueous solution of methylene blue was added to 1l of skim milk medium (Difco). The milk medium had been sterilised by autoclaving at 115°C for 10 min. 9ml of sterile media was dispensed aseptically into 15 x 150mm test-tubes. These were inoculated and incubated for 16 h. A decolourisation of the medium indicated a positive result.

Reduction of Janus green milk

The method of Cooper & Ramadan (1955) was used. 8ml of a filter-sterilised 1% (w/v) aqueous solution of Janus green B was added to 1l of Skim milk medium (Difco). This had been sterilised by autoclaving at 115°C for 10 min. This was dispensed aseptically as for the methylene blue test. The tubes were inoculated and incubated for 16 h. Reduction of the dye to its final form was shown by a red colouration.

Reduction of litmus milk

Litmus milk medium (Oxoid) was used. This was sterilised at 115°C for 10 min. 9 ml aliquots were dispensed aseptically into 15 x 150mm tubes and these were inoculated and incubated for 7 d. Readings were taken after 24 h, 48 h and 7 d. Clotting of the milk before acid production was also scored.

Reduction of selenite

10ml of a filter-sterilised 1% (w/v) aqueous solution of sodium selenite was added to 1l of BMA. The plates were inoculated and incubated for 48 h. Reduction of selenite was shown by the appearance of red colonies.

Arginine hydrolysis

The broth method of Cowan & Steel (1974) was used. 9ml of arginine broth in 15 x 150mm tubes was sterilised and inoculated before incubation for 2 d. The hydrolysis of arginine released ammonia and so the detection of this is a positive result. Nessler's reagent was used for this, an immediate brown colour indicating ammonia.

Arginine broth consisted of:

Yeast extract (Difco)	5g
Bacto tryptone (Difco)	3g
di-potassium hydrogen phosphate	2g
D (+) glucose	0.5g
D arginine hydrochloride	3g

Hydrolysis of starch

The method of Cowan & Steel (1974) was used. BMA was used containing 0.5% (w/v) soluble starch. This was made up initially in 100ml of boiling water before it was added to the agar. Plates were incubated for 7 d and then flooded with Gram's iodine. This turned the remaining starch in the medium dark blue. Hydrolysis was indicated by clear zones around colonies.

Hydrolysis of aesculin

BMA was made up using 7½% (v/v) horse blood. Added to this was 0.1% (w/v) aesculin and 0.05% (w/v) ferric citrate. These were autoclaved in the medium. Plates were incubated and aesculin hydrolysis was indicated by blackening in and around the colonies.

Hydrolysis of hippurate

Method one of Cowan & Steel (1974) was used. 9ml of BMB in 15 x 150mm tubes was used. The BMB contained 1% (w/v) sodium hippurate. These were inoculated, two being left blank. After 4 d incubation acidic ferric chloride solution was added to one of the uninoculated control tubes until a precipitate formed and excess solution was then added until the precipitate just dissolved. This final volume of ferric chloride was added to each sample. The final insoluble precipitate of an iron salt resulting from the hydrolysis of hippurate to benzoate indicated a positive reaction.

Acid ferric chloride solution consisted of:

$FeCl_3.6H_2O$	12g
37% (w/v) HCl	2.5ml
Distilled water to	1000ml

Catalase from haemin

The catalse test was performed on colonies grown for 24 h on BMA containing 0.007% (w/v) haemin. This was added as 10 ml of a filter-sterilised 0.7% (w/v) aqueous solution in 1l of medium.

ONPG

The method of Cowan & Steel (1974) was used. 750ml of sterile peptone water was added to 250ml of a filter sterilised ONPG solution. This was dispensed in 9ml amounts, inoculated and incubated. A yellow colour was a positive reaction.

Peptone water:

Peptone (Difco)	7.5g
Sodium chloride	3.75g
Distilled water to	1000ml

ONPG solution:

O-nitrophenol-β-D-galactopyranoside	1.5g
Sodium di-hydrogen phosphate	0.37g
Distilled water to	1000ml

Production of H_2O_2

The method of Whittenbury (1965b) was used. BMA was used containing 7½% (v/v) horse blood. This was heated to 54°C and 10ml of a 10% (w/v) o-dianisidine aqueous solution was filter-sterilised and added to 1l. After streak inoculation and incubation, the production of H_2O_2 was indicated by a black colouration in and around the colonies.

Production of H_2S

The method of Feltham (1975) was used. This entailed the use of an H_2S agar which progressively blackened with the production of H_2S over 5 d incubation.

H_2S agar consisted of;

Beef extract (Difco)	3g
Bacto tryptone (Difco)	30g
Bacto agar (Difco)	5g
Sodium thiosulphate	0.005g
Cysteine hydrochloride	0.2g
Sodium chloride	5g
Distilled water to	1000ml

Production of levan

The method of Cowan & Steel (1974) was used. The production of levans was observed on colonies grown on sucrose agar, prepared by adding filter-sterilised sucrose and serum to sterile BMA to give a final concentration of 0.5% (w/v) of both. Levan producing colonies appeared as large and mucoid after incubation for 4 d.

Production of dextran

The method of Cowan & Steel (1974) was used. 500ml of a filter-sterilised 10% (w/v) sucrose solution was added to 500ml of double strength BMB. Samples were inoculated in 15 x 150mm test tubes and incubated. 0.5ml amounts of each sample were added to equal volumes of ethanol. This flocculated any dextran to produce a fluffy white precipitate, which was considered a positive reaction.

Digestion of casein

The method of Cowan & Steel (1974) was used. 500ml of Skim milk medium (Difco) was sterilised at 115°C for 10 min. This was added to 500ml of double strength BMA. This was inoculated and incubated for 3 d. Casein digestion appeared as a clear zone around the colonies. As clearing may be due to acid or alkaline end products instead of true digestion, acid mercuric chloride solution was flooded onto all positive plates. Only clearing which remained after this was scored as positive.

Acid meruric chloride solution consisted of;

Mercuric chloride	12g
37% (w/v) HCl	16ml
Distilled water	80ml

Gelatin liquefaction

The method 2 of Cowan & Steel (1974) was used. Nutrient gelatin was inoculated in 15 x 150mm tubes with a straight wire and incubated. After 2, 5, 10 and 14 d the tubes were cooled for 1 hr at 4°C before readings were taken. Gelatin liquefaction was shown by liquefaction of the media.

Nutrient gelatin consisted of:

Gelatin (Oxoid)	120g
Beef extract (Difco)	3g
Bacto peptone (Difco)	5g
Distilled water to	1000ml

Indole production

Method 2 of Cowan & Steel (1974) was used. 6ml of BMB in 10 x 80 mm tubes was inoculated and incubated for 2 d. The presence of indole was shown as a red colouration 1 min after the addition of Kovác's reagent. Kovác's reagent consisted of:

p-dimethylaminobenzaldehyde	10g
Amyl-alcohol	150ml
37% (w/v) HCl	50ml

Ethanol oxidation

The method of Skerman (1969) was used. 9ml of BMB containing 5% (v/v) filtered absolute ethanol in 15 x 150mm tubes was used. This was inoculated and incubated for 2 d. After this a few drops of a 1.6% (w/v) solution of bromocresol purple was added. This indicated any acid production from the oxidation of ethanol. A duplicate set of tubes containing only BMB were also set up to act as a control in case of acid production from the media.

Methyl red and Voges Proskauer test

15ml of BMB was used containing an added 0.5% (w/v) glucose and 0.5% (w/v) K_2HPO_4. This was inoculated in universal bottles and incubated for 7 d. 1ml samples were taken after 3 and 7 d and placed in a divided replidish. To these was added 0.5ml of methyl red solution. A positive reaction was indicated by the solution remaining

red. To the remaining samples after 7 d, 1ml of 40% (w/v) NaOH and 1ml of 5% (w/v) α-napthol alcohol in ethanol were added. These were well shaken and laid at a slope for 1-2 h. A deep red colour indicated the production of acetoin and was considered a positive reaction.

Methyl red solution consisted of:

Methyl red		0.04g
Ethanol		40ml
Distilled water	to	1000ml

DNase

The method of Smith et al. (1969) was used. DNase agar (Difco) was used with the addition of 0.1% (w/v) yeast extract (Difco). 100 x 100mm square petri dishes were inoculated and incubated for 1, 3 and 4 d. A positive reaction was seen as a clear area around the inoculum due to the bound methyl green being liberated and the pH raised.

Urease

A modification of Christensen's medium (Cowan & Steel, 1974) was used. Two sets of media were made up. To one was added a filter-sterilised urea solution. Both were inoculated and incubated as slopes in bijoux bottles containing 2ml for 5 d. A positive reaction due to the splitting of urea to ammonia was apparent as a red colouration. The second set of media acted as a control in case of ammonia production from the basal medium.

Urease medium consisted of:

Sodium chloride	5g
di-Potassium hydrogen phosphate	2g
Yeast extract (Difco)	5g
Bacto agar (Difco)	20g
D (+) glucose	1g
Phenol red (0.2% (w/v))	0.5ml
Distilled water to	1000ml

Urea solution consisted of:

Urea	20g
Distilled water to	100ml

Phosphatase

The method of Cowan & Steel (1974) was used. 10ml of a 1% (w/v) filtered solution of phenolphthalein phosphate was added to 1l of BMA. Plates were inoculated a incubated for 24 h. Filter paper disks soaked in ammonia solution (sp.gr.0.880) were then placed in the lids of the plates which were inverted. Free phenolphthalein liberated by phosphatase turned red in 3–10 min.

Decarboxylation reactions

The method of Falkow (1958) was used. Decarboxylation medium was made up in four batches. To one was added 5g l^{-1} l-arginine hydrochloride, to another 5g l^{-1} ornithine hydrochloride and to a third 5g l^{-1} lysine hydrochloride. The remaining batch was used as a control. After sterilisation of 6ml in 10 x 80 mm tubes they were all layered with sterile liquid paraffin, inoculated and incubated at 35°C for 4 d. Initial acid production turned all of the tubes yellow. Decarboxylation subsequently resulted in a

rise in the pH and the tubes returned to a purple colour. Control tubes showed only the initial acid production.

Decarboxylation medium consisted of:

Yeast extract (Difco)		3g
Bacto peptone (Difco)		5g
D (+) glucose		1g
Bromocresol purple (1.6% (w/v))		1ml
Distilled water	to	1000ml

CAMP test

This is the test of Christie, Atkins and Munch Peterson (1944). BMA was used containing 5% (v/v) washed sheep red blood cells (Gibco). Staphylococcus aureus NCTC 7428 was applied to these plates as a single streak across them. Streaks of the test organisms were made upto these streaks, but not touching them. A positive reaction was seen as the enhancement of haemolysis around the junction of the two streaks. This appeared as a large arrow or hammer head area of β-haemolysis.

2.8.1 Sugar fermentation reactions; API methods (API system S A)

The API 50E was used for the sugar fermentation reactions. This is based on the work of Buissière (1972) and consists of a plastic gallery containing 5 rows of 10 cupules. 38 of these contain carbohydrates and phenol red as an indicator. 11 contain media for other tests and 1 is left empty as a control. Organisms were grown overnight on BMA containing 7½% (v/v) horse blood. Colonies

were then suspended in the API suspending media until they reached a density of tube No. 2 on the MacFarland scale. With the streptococci, plate counts showed this density to be approximately equivalent to 9×10^7 organisms ml^{-1}.

API suspending medium consisted of:

NH_4SO_4		2g
Yeast extract		0.5g
Mineral base solution		10ml
Distilled water	to	1000ml

The cupules in the gallery were inoculated with a Pasteur pipette so that only the tubes were filled. However, for the methyl red and DNase tests, the tube and the open cupules were filled. Then all tubes, with the exception of these two and the last four for sole energy source tests, were layered with sterile liquid paraffin. This allowed anaerobiosis for the fermentative metabolism tests.

The galleries were incubated in moist trays and readings were taken after 3, 6, 24 and 48 h. The results were read as a gradation of change in the indicator. This was scored on a scale of 0–5 against a standard colour chart.

The tests performed were:

1. Acid from glycerol
2. " " erythritol
3. " " D (–) arabinose
4. " " L (+) arabinose

5. Acid from ribose
6. " " D (+) xylose
7. " " L (-) xylose
8. " " adonitol
9. " " methyl xyloside
10. " " galactose
11. " " D (+) glucose
12. " " D (-) levulose
13. " " D (+) mannose
14. " " D (-) sorbose
15. " " rhamnose
16. " " dulcitol
17. " " mesoinositol
18. " " mannitol
19. " " sorbitol
20. " " methyl-D-mannoside
21. " " methyl-D-glucoside
22. " " N-acetyl glucosamine
23. " " amygdalin
24. " " arbutin
25. Hydrolysis of aesculin
26. Acid from salicin
27. " " D (+) cellobiose
28. " " maltose
29. " " lactose
30. " " D (+) melibiose
31. " " sucrose
32. " " D (-) trehalose

33. Acid from inulin
34. " " D (+) melezitose
35. " " D (+) raffinose
36. " " dextrin
37. " " amylose
38. " " starch
39. " " glycogen
40. Methyl red (with methyl red indicator)
41. DNase (with methyl green indicator)
42. Mucate (with phenol red indicator)
43. Gluconate (with phenol red indicator)
44. Lipase (with phenol red indicator)
45. Tetrathionate reduction (with bromocresol purple indicator)
46. Pectate (with bromothymol blue indicator)
47. Christensen citrate (with bromothymol blue indicator)
48. Malonate(with bromothymol blue indicator)
49. Acetate (with naptholphthalein indicator)

2.8.2 Sugar fermentation reactions; plate methods

A plate method of fermentation reactions for starch, glycogen and dextrin was used based on the method of Whittenbury (1963). Fermentation was observed as acid production in replidishes of sloppy agar. Readings were taken after 1,3, 5 and 14 d. A yellow colour indicating a positive reaction.

Sloppy agar medium consisted of:

Bacto peptone (Difco)	10g
Sodium chloride	5g
Yeast extract (Difco)	1g
Bacto agar (Difco)	15g
Bromocresol purple (0.2% (w/v) aqueous)	15ml
Dist illed water to	1000ml

The carbohydrates were made up in 10% (w/v) aqueous solutions which were then filter-sterilised. 50ml was then added to 1l of the cooling agar.

2.9 Enzyme methods; API methods (API system S A)

The APIzym method was used to detect the presence of certain enzymes in the organisms. APIzym consists of a gallery composed of 20 cupules, the base of which forms a support to contain an enzymatic substrate and buffer. This allows the contact between the enzyme and the generally insoluble substrate. One tube is left empty as a control, and so 19 different enzymatic reactions may be observed. The reactions involve a colour change and the intensity of this colour can be taken as an indication of the concentration of the enzyme. The colour is scored on a scale of 0-5 against a supplied colour chart, 0 being a negative reaction and 5 corresponding to 40 or more nanomoles of substrate hydrolysed. Table 2.9.a shows the enzymes assayed for and the substrates used.

The strains used were grown overnight on BMA with 7½% (v/v) horse blood, and then emulsified in 2ml of sterile distilled water to produce a suspension equivalent to tube No.5 on the MacFarland scale. Each cupule was inoculated with 65 l of sample and the strip was incubated for 4 h in the plastic incubation box

provided. This contained 5ml of distilled water to prevent drying.

After incubation one drop of each of reagents A and B was added to each tube. After 5 min the gallery was placed under a 1000 watt bulb for 20 s to eliminate any yellow colouration due to excess reagent B.

Four other media were used with the APIzym strip for one strain in an attempt to observe any variations due to the media. These might be due to inducible rather than constitutive enzymes, or enzymes in the blood. The media used were; Blood Agar Base (Oxoid) with 5% horse blood; Blood Agar Base (Oxoid) with 5% sheep blood; Blood Agar Base No. 2 (Difco) and Todd Hewitt Broth (Oxoid). The strain used was PB 2, the type strain of S. faecalis. As a test that the enzymes detected were associated with the bacteria rather than the blood in the medium, sterile horse blood and sheep serum were also used as inocula for two APIzym strips.

2.10 Numerical methods

2.10.1 Coding of results

The results of all of the "classical" tests used in the taxonomy were coded as 0 for a negative result or 1 for a positive result. Where weak but definite positive reactions had been recorded, these were coded as 1 on the basis that there had been some reaction. The results from the API methods were coded

Table 2.9.a APIzym gallery.

Enzyme assayed for	Substrate used
Alkaline phosphatase	2-naphthyl-phosphate
Esterase (C4)	2-naphthyl-butyrate
Esterase or lipase (C8)	2-naphthyl-caprylate
Lipase (C14)	L-leucyl-2-naphthylamide
Cysteine arylamidase	L-cystyl-2-naphthylamide
Valine arylamidase	L-valyl-2-naphthylamide
Trypsin	N-benzyl-DL-arginine-2-naphthylamine
Chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamine
Acid phosphatase	2-naphthyl-phosphate
Phosphoamidase	Naphthol AS-BL-phosphodiamide
α-galactosidase	6-Br-2-naphthyl-α-D-galactopyranoside
β-galactosidase	2-naphthyl-βD-galactopyranoside
β-glucuronodase	Naphthol AS-B-L-BD-glucoronic acid
α-glucosidase	2-naphthyl-αD-glucopyranoside
β-glucosidase	6-B-2-naphthyl-βD-glucopyranoside

Table 2.9.a continued

Enzyme assayed for	Substrate used
N-acetyl-β-glucosaminidase	1-naphthyl-N-acetyl-βD-glucosaminide
α-mannoside	6-Br-2-naphthyl-αD-mannopyranoside
α-fucosidase	2-naphthyl-αL-fucopyranoside

Reagent A		
Tris (hydroxymethyl) aminomethane		250g
37% (w/v) HCl		110ml
Sodium laurylsulphate		100g
Distilled water	to	1000ml

Reagent B		
Sigma fast blue BB		3.5g
2-methoxyethanol	to	1000ml

as recommended by the manufacturers on a scale of 0 for a negative reaction, to 5 for a strong positive reaction. Antibiotic sensitivity tests were coded so that 1 corresponded with sensitivity and 0 with resistance or partial resistance.

For the calculation of the Simple Matching coefficient (S_{SM}) and the Pattern and vigour data, the API results were coded differently to give binary characters. The API 50E results were coded as 0 for reactions 0, 1 and 2, and as 1 for reactions of 3, 4 and 5. the APIzym results were coded as 0 for reactions of 0 and 1, and as 1 for reactions of 2, 3, 4 and 5.

The characters that were scored for the taxonomy and their initial coding states are listed in Table 2.10.a.

2.10.2 Computation

The computation of all taxonomy results was performed on the University of Nottingham's ICL 1900 computer, and the dendrograms were drawn on the University of Leicester's CDC Cyber 73 computer. The results were obtained using numerical taxonomy programs which were written by M.J.Sackin and associates of the Microbiology Department, University of Leicester. Further computations on the statistical significance of results were performed on the CDC Cyber 73.

The raw data was programmed using two programs which together make up the TAXPAK procedure. The first one used was ITBNTOMT, this stands for "integer t by n clustering", and calculates

the similarities for all possible pairs of OTUs, clusters them and produces a similarity matrix and a dendrogram in store. A Cophenetic Correlation coefficient is calculated between the matrix and the dendrogram.

2.10.3 Statistical methods

The similarity between different OTUs was calculated using the Simple Matching coefficient (S_{SM}), Gower's coefficient (S_G) and the Pattern difference (D_P).

The Simple Matching coefficient considers matching negative results as well as matching positives. S_{SM} is calculated as the number of characters similar between two OTUs, divided by the total number of characters used (ignoring any "no comparisons").

a = No. of characters positive in OTUs A and B.

b = No. of characters positive in OTU A only.

c = No. of characters positive in OTU B only.

d = No. of characters negative in OTUs A and B.

i.e.

		OTU B: 1	OTU B: 0
OTU A	1	a	b
	0	c	d

So, for OTUs A and B, S_{SM} may be represented as;

$$(a+d)/(a+b+c+d)$$

Table 2.10.a. Coding states of tests.

Character.		State.
1.	Gram-positive	0/1
2.	Colony edge entire	0/1
3.	Colony mucoid	0/1
4.	Colony convex	0/1
5.	Colony grey/white	0/1
6.	Colony yellow	0/1
7.	Oxidase	0/1
8.	Catalase	0/1
9.	α-haemolysis	0/1
10.	β-haemolysis	0/1
11.	No haemolysis	0/1
12.	Final pH below 4.25	0/1
13.	Final pH between 4.25 and 4.75	0/1
14.	Final pH between 4.75	0/1
15.	Growth at $4^{\circ}C$	0/1
16.	Growth at $10^{\circ}C$	0/1
17.	Growth at $25^{\circ}C$	0/1
18.	Growth at $45^{\circ}C$	0/1
19.	Growth with 3% NaCl	0/1
20.	Growth with 4% NaCl	0/1
21.	Growth with 6.5% NaCl	0/1
22.	Growth with sodium azide	0/1
23.	Growth with thallous acetate	0/1
24.	Growth with 0.0002% crystal violet	0/1
25.	Growth with 0.0004% crystal violet	0/1

Table 2.10.a. continued

Character.		State.
26.	Bile solubility	0/1
27.	Growth at pH 9.6	0/1
28.	Reduction of nitrate	0/1
29.	Reduction of nitrite	0/1
30.	Reduction of methylene blue milk	0/1
31.	Reduction of janus green milk	0/1
32.	Reduction of tetrazolium	0/1
33.	Reduction of tellurite	0/1
34.	Reduction of selenite	0/1
35.	Reduction of litmus milk	0/1
36.	Production of clot in litmus milk	0/1
37.	Methyl red	0/1
38.	Voges-Proskauer	0/1
39.	Gas from nitrate	0/1
40.	Gas from nitrite	0/1
41.	Indole	0/1
42.	Hippurate hydrolysis	0/1
43.	Arginine hydrolysis	0/1
44.	Aesculin hydrolysis	0/1
45.	Ethanol oxidation	0/1
46.	Ammonia from serine	0/1
47.	Growth on MacConkey agar	0/1
48.	Red colonies on MacConkey agar	0/1
49.	Growth on TCBS agar	0/1
50.	Yellow colonies on TCBS agar	0/1

Table 2.10.a. continued

Character.		State.
51.	Gelatin liquefaction	0/1
52.	Urease	0/1
53.	ONPG	0/1
54.	Phosphatase	0/1
55.	Production of H_2O_2	0/1
56.	Production of H_2S	0/1
57.	Production of dextran	0/1
58.	Production of levan	0/1
59.	Arginine decarboxylation	0/1
60.	Ornithine decarboxylation	0/1
61.	Lysine decarboxylation	0/1
62.	DNase	0/1
63.	Starch hydrolysis	0/1
64.	Acid from erythritol	0–5
65.	Acid from D (–) arabinose	0–5
66.	Acid from L (+) arabinose	0–5
67.	Acid from D (+) xylose	0–5
68.	Acid from L (–) xylose	0–5
69.	Acid from adonitol	0–5
70.	Acid from methyl xyloside	0–5
71.	Acid from galactose	0–5
72.	Acid from D (+) glucose	0–5
73.	Acid from D (–) levulose	0–5
74.	Acid from D (+) mannose	0–5
75.	Acid from L (–) sorbose	0–5

Table 2.10.a. continued

Character.	State.
76. Acid from rhamnose	0–5
77. Acid from dulcitol	0–5
78. Acid from meso–inositol	0–5
79. Acid from mannitol	0–5
80. Acid from sorbitol	0–5
81. Acid from methyl–D–glucoside	0–5
82. Acid from N–acetyl glucosamine	0–5
83. Acid from arbutin	0–5
84. Hydrolysis of aesculin (API)	0–5
85. Acid from salicin	0–5
86. Acid from D (+) cellobiose	0–5
87. Acid from maltose	0–5
88. Acid from lactose	0–5
89. Acid from D (+) melibiose	0–5
90. Acid from sucrose	0–5
91. Acid from D (–) trehalose	0–5
92. Acid from inulin	0–5
93. Acid from D (+) raffinose	0–5
94. Acid from dextrin	0–5
95. Acid from amylose	0–5
96. Acid from starch	0–5
97. Acid from glycogen	0–5
98. Methyl red (API)	0–5
99. DNase (API)	0–5
100. Mucate (fermentation of released glucose)	0–5
101. Pectate (hydrolysis of pectin)	0–5

Table 2.10.a. continued

Character.	State.
102. Citrate (utilisation as a carbon source)	0–5
103. Malonate (utilisation as sole carbon source)	0–5
104. Acetate (utilisation as sole carbon source)	0–5
105. Survival of 60°C for 1 min	0/1
106. Survival of 60°C for 15 min	0/1
107. Survival of 60°C for 1 h	0/1
108. Survival of 60°C for 2 h	0/1
109. Sensitive to penicillin G	0/1
110. Sensitive to sulphafuroxale	0/1
111. Sensitive to ampicillin	0/1
112. Sensitive to erythromycin	0/1
113. Sensitive to novobiocin	0/1
114. Sensitive to oleandomycin	0/1
115. Sensitive to furazolidone	0/1
116. Sensitive to carbenicillin	0/1
117. Sensitive to nalidixic acid	0/1
118. Sensitive to nitrofurantone	0/1
119. Sensitive to tetracycline	0/1
120. Sensitive to chloramphenicol	0/1
121. Sensitive to chlortetracycline	0/1
122. Sensitive to oxytetracycline	0/1
123. Colony diameter less than 0.2 mm	0/1
124. Colony diameter between 0.2 and 0.4 mm	0/1
125. Colony diameter greater than 0.4 mm	0/1
126. Acid from glycerol	0–5
127. Acid from ribose	0–5

Table 2.10.a. continued

Character.	State.
128. Acid from methyl-D-mannoside	0–5
129. Acid from amygdalin	0–5
130. Acid from D (+) melezitose	0–5
131. Gluconate (fermentation of released galactose)	0–5
132. Lipase (butyric acid from tributyrine)	0–5
133. Tetrathionate reduction	0–5
134. Growth with 10% bile	0/1
135. Casein hydrolysis	0/1
136. Growth on acetic acid-acetate agar	0/1
137. CAMP test	0/1
138. Possession of alkaline phosphatase	0–5
139. Possession of esterase (C4)	0–5
140. Possession of esterase or lipase (C8)	0–5
141. Possession of lipase (C12)	0–5
142. Possession of leucine arylamidase	0–5
143. Possession of valine arylamidase	0–5
144. Possession of cysteine arylamidase	0–5
145. Possession of trypsin	0–5
146. Possession of chymotrypsin	0–5
147. Possession of acid phosphatase	0–5
148. Possession of phosphoamidase	0–5
149. Possession of α-galactosidase	0–5
150. Possession of β-galactosidase	0–5
151. Possession of β-glucuronidase	0–5

Table 2.10.a. continued

Character.	State.
152. Possession of α-glucosidase	0–5
153. Possession of β-glucosidase	0–5
154. Possession of N-acetyl-β-glucosaminidase	0–5
155. Possession of α-mannosidase	0–5
156. Possession of α-fucosidase	0–5
157. Growth with 40% bile	0/1

For quantitative or multi-state characters, the similarity depends upon the range of possible results as well as upon their difference. This is allowed for by Gower's coefficient. The similarity in this case can be represented for each character as one minus the (difference divided by the range).

		Characters							
OTU	A	0	1	1	0	2	1	0	1
OTU	B	0	0	1	1	0	2	0	1
Range		1	1	1	1	5	5	1	1
$\underline{S}_{\underline{AB}}$		1	0	1	0	3/5	4/5	1	1

$\underline{S}_{\underline{G}} = \Sigma \underline{s}_{\underline{AB}}/\underline{n}$

$\underline{S}_{\underline{G}} = 5.4/8$

Differences between OTUs may be due not only to their fundamental differences but also to their abilities to metabolise different substrates under certain conditions. In order to take account of this both the difference in vigour ($\underline{D}_{\underline{V}}$) between strains and their Pattern difference were calculated.

Vigour was calculated as the percentage of positive reactions. The differences between these were used to calculate the Pattern difference.

$\underline{D}_{\underline{T}} = (b+c)/(a+b+c+d)$

$\underline{D}_{\underline{V}} = (b-c)/(a+b+c+d)$

$\underline{D}_{\underline{P}}^2 = \underline{D}_{\underline{T}}^2 - \underline{D}_{\underline{V}}^2$

This expression can be simplified to give;

$$\underline{D}_{\underline{P}} = (2\sqrt{bc})/(a+b+c+d)$$

The Pattern difference is an anlogue of shape difference. It considers only differences in the pattern of reactions, not the number of positive reactions, and corrects for differences due solely to vigour. Examples of the use of this are where a damaged culture yields a disproportionately high number of negative results, or where a medium induces a set of enzymes in an organism.

	Characters							
OTU A	1	0	1	1	1	1	0	1
OTU B	0	0	1	1	1	0	0	0

In the above case the Pattern difference between the two OTUs is zero. This is because the differences shown are all in the same direction, resulting in OTU A showing a greater vigour than OTU B.

The similarities between all possible pairs of OTUs were calculated by the programs mentioned. These produced two similarity matrices and dendrograms in each case. One matrix and dendrogram was based on single linkage clustering and the other on average linkage clustering. These were both clustered by using unweighted average pair group methods (UPGMA). Clustering was represented in the form of dendrograms and by similarity matrices in which the OTUs were rearranged to give the same order. The Cophenetic Correlation coefficient was determined in order to give a measure of fidelity with which the dendrograms represent the similarity matrices. Details of this coefficient and the methods entailed are given by Sneath & Sokal (1973).

2.10.4 Integer groups

The program IGROUPs was used to provide information on pre-determined groups selected from the $\underline{S_G}$ average linkage clustering. This program considers only the groups of OTUs selected and the test results for them. This enables a table to be constructed of characters that are positive or negative for each group. The program

also considers the orientation of these groups in relation to one another, calculating the Euclidean distances between centroids of groups, the mean inter- and intra- group similarities, and their variances and standard deviations. Also, the inter-centroid distances and their standard deviations along these axis are ordered into a format for use with the program OVCLUST (Sneath, 1979a) to determine cluster overlap statistics.

2.10.5 Distinctness of clusters

The distinctness of clusters was determined using the program OVCLUST (Sneath, 1979a). This program considers the Euclidean distances and related statistics generated by the integer group program. These are accepted by the program as data and from this an index of disjunction ($\underline{W}_{LM}$) was calculated. This index of disjunction may vary from zero for complete overlap to infinity for complete separation; and so corresponds to an index of overlap ($\underline{V}_G$). $\underline{V}_G$ is twice the standardised Gaussian integral for $\underline{W}$ and so varies from 1.0 for complete overlap to zero for complete disjunction. The significance of the $\underline{W}$ value is tested by means of a non-central $\underline{t}$ - test.

The program provides an option to test whether the observed overlap is significantly less than some chosen value, this is entered as a critical $\underline{V}_G$ value and then converted to the corresponding $\underline{W}_{crit.}$ value.

The observed value of $\underline{d}_{LM}$, the Euclidean distance between clusters L and M, is not unbiased if the OTUs are samples of larger populations. A corrected estimate may be obtained by subtracting a correction factor. This is the sum of the standard errors squared of the position of the centroids. This may however lead to overcorrection and so care must be given in the interpretation of these results.

The calculations were performed twice, once for a critical $\underline{V}_G$ of 0.025 and once for a rectangular distribution.

The calculation of the inter-centroid distances for use in the overlap calculations depends upon the similarities of the groups and the number of strains in each. The calculation of the index of disjunction and other calculations depends upon the number of characters. If the mean similarity between a pair of groups was 50% from fifteen characters and then eighty-five further characters were added which were all positive, then the final similarity would be 92.5%. In this case fifteen could be taken as the effective number of characters. The differences in calculations using the total number of characters ($\underline{n}$) and the effective number of characters ($\underline{n}'$), may affect the results of the overlap calculations. If this effect is large no overlap may be seen using $\underline{n}$, although there may be some degree of overlap if $\underline{n}'$ is used. The differences that this may give were investigated. The effective number of characters may be given by this formula:

$$\underline{n}' = 2\underline{n} \ (1-\underline{\bar{S}}_{SM})$$

The program INTGROV (file PHS8B06) was used to calculate inter-centroid distances and the relevant overlap statistics from the S_{SM} coefficient. Six taxon pairs were selected and computer runs using this program were performed for both the total and effective numbers of characters. This enabled the effect on the final overlap results to be observed. In each case a critical V_G value of 0.025 was used as in the OVCLUST calculations.

A further measure of dispersion of clusters was considered. This was the average Euclidean distance from the centroid for each group. This was calculated using the program PDBAED2, listed in Appendix I.

2.10.6 Identification matrices

Two identification matrices were constructed. One of these was based on previously published results from a variety of sources. This matrix is described in Section 3.5. The identification matrices were constructed for use on the University of Leicester's CDC Cyber 73 computer. The second matrix consisted of the sixty most diagnostically useful tests for the separation of the integer groups. The program CHARSEP (Sneath, 1979c) was used to determine these tests. The total test results represented as a percentage positive for each integer group were used as data and the program calculated separation indices for these. The separation indices calculated were; Gyllenberg's Sum of C, Gyllenberg's R, Niemela's index, the VSP index and the CSP index.

The VSP and CSP indices are independant of the cut-off level required by the other indices and VSP was chosen to rank characters by merit in the running program. The sixty characters with the highest VSP values were used to construct the matrix.

The identification matrices were tested by the use of two programs, MOSTTYP (Sneath, 1980a) which found the best identification score obtainable and OVERMAT (Sneath, 1980b) which measured the observed overlap between taxa.

MOSTTYP calculates the best identification scores that an entirely typical example of each group could achieve. The program lists the Willcox probability, the taxonomic distance, the standard error score and the Gaussian integral of the standard error of a hypothetical median organism for each group.

OVERMAT calculates values of observed overlap between groups. Values for $\underline{W}_{\underline{LM}}$ are given and these may be compared against a chosen critical value as in the program OVCLUST.

2.11 Test reproducibility

The test reproducibility was calculated by using split cultures to give duplicates. Twenty cultures covering a range of species were split at the start of the numerical taxonomy and tested independantly at the same time as the other strains. The difference between these duplicates was calculated by dividing the number of differences between the pairs of strains by the total number of characters observed. Test error was taken as being half the pair difference.

Another method used was to repeat a number of the tests on all of the strains. A total of fifty-seven classical tests were used in the taxonomy. These gave rise to eighty characters as more than one character was obtained from some tests (i.e. growth on blood agar plates gave five morphological characters; and reduction of nitrite and gas from nitrite were performed together). As near to 20% as was possible of these were repeated. This involved repeating fourteen tests, these gave rise to nineteen characters. The tests to be repeated were selected to give as broad a spectrum of types as possible. From these results the percentage difference was calculated. Sneath & Johnson (1972) recommended between 10 and 15% difference as being the upper limit of reproducibility. As a result of this tests showing greater than 13% difference were not used for the computations of similarity.

With the API galleries and antibiotic discs, it was not practicable to repeat individual tests. As a result the reproducibility of these methods was estimated by repeating the entire range of tests on twenty duplicated cultures. The differences found by this method were taken as being estimates of the reproducibility of individual reactions. However, this method is dependant to a certain extent on the reproducibility found in individual strains.

The reproducibility of the sugar reactions on the API 50E gallery was compared with that of more classical methods. Three different sugar fermentations were performed using the semi-

solid agar technique given in Section 2.8.2. The score on the API gallery was considered equivalent to a positive reaction by the plate method if it was a value of 3 or greater. Similarly an API reaction was considered negative if it gave a score of less than 3.

2.12 DNA methods

2.12.1 DNA extraction and purification

Bacterial strains were grown to their logarithmic stage in a yeast glucose phosphate broth. This contained glycine to encourage the production of weak cell walls. Yeast glucose phosphate broth was based on the method of Garvie (1978) and consisted of:

Peptone (Oxoid, code L 37)	10g
Lablemco (Oxoid)	8g
Glycine	8g*
NaCl	5g
Yeast extract (Oxoid)	3g
KH_2PO_4	1.5g
$MgSO_4 \cdot 7H_2O$	0.2g
$MnSO_4 \cdot 4H_2O$	0.05g
Distilled water to	1l

This was adjusted to pH 6.8 before sterilising at 115°C for 20 min.

* Some strains were found to be inhibited by glycine, particularly the pyogenic organisms. The glycine concentration was reduced for strains which did not show good growth in the medium, 3g being used.

The logarithmic phase of cell growth was found from growth curves measured by optical density at 550 nm in the yeast glucose phosphate broth over a total of 24 h.

All strains were incubated in static culture at 35°C except for strains of Leuconostoc sp. which were incubated at 25°C.

Cells were harvested by centrifugation at 15,000 x G for 10 min using an MSE 18 high speed centrifuge. The cell pellet was then stored frozen at -20°C. The DNA was extracted using the method of Garvie (1976) with a few adaptations

DNA preparation reagents

EDTA solution. 0.1 M EDTA in distilled water.

EDTA / acetate. 3.0 M Na acetate (anhydrous), 0.001 M EDTA, pH 7.0.

Phenol water. 1 Kg phenol dissolved in 100 ml distilled water. This was brought to pH 6.8 by adding 20 ml of 10 M phosphate buffer and 400 ml SSC. This was separated in a separating funnel at 4°C overnight and the lower layer was collected.

CSC. 1.5 M NaCl, 0.15 M trisodium citrate, pH 7.0.

SSC. 1 : 9 dilution of CSC.

DSC. 1 : 9 dilution of SSC.

Tri-iso-propyl-napthalene sulphonic acid. 6.25% in distilled water.

Chloroform-iso amyl alcohol. 24 : 1 (v/v).

Phosphate buffer. 50 ml of (A) added to 42 ml of (B).

(A) K_2HPO_4 8.709 g 50 ml^{-1}.

(B) KH_2PO_4 6.804 g 50 ml^{-1}.

Deoxycholate solution.	2% (w/v) of sodium deoxycholate (Koch Light) made up in SSC.
4-amino salicylic acid.	12% (w/v) of sodium salt made up in SSC.
Pronase buffer.	0.1 M tris-HCl, 0.05 M NaCl, 0.01 M EDTA, 0.5% (w/v) sodium lauryl sulphate.

For ease of manipulation, the extraction and purification procedure was spread over eight days.

Cell lysis (Day 1)

Cells were suspended in 10 ml of distilled water for each gram of cells. Usually 3-4g was found to give a satisfactory yield. 10 mg of lysozyme (Analytical Supplies Ltd., Derby) dissolved in EDTA solution was added for each 2.3 g of cells and incubated at 35°C for 1 h. Pronase (Analytical Supplies Ltd.) at 0.4 mg for each ml of cell suspension was dissolved in pronase buffer and pre-incubated at 35°C for 1 h. This was then added to the cell suspension after the lysozyme treatment. After incubation for 1 h at 35°C, one ninth of the total volume of concentrated saline citrate buffer (CSC) was added to give a concentration of buffer equivalent to standard saline citrate (SSC). A 12% solution of 4-amino salicylic acid was added as 0.366 of the total volume. This suspension was then incubated overnight at 35°C.

Phenol treatment (Day 2)

Cell suspensions were chilled in ice for 10 min. Tri-iso-propyl-napthalene sulphonic acid was added in the proportion of total volume/12.2. This mixture was then heated to 60°C for 10 min in a water bath before returning to ice for 10 min. Phenol water

was added as an equal volume and this was then shaken at room temperature for 15 min using a Gallenkamp orbital shaker. This mixture was then centrifuged in an MSE 18 for 10 min at 15,000 x G. The aqueous upper layer was removed with a wide pipette and shaken at room temperature for 15 min with an equal volume of chloroform-iso amyl alcohol. After a further centrifugation at 15,000 x G as before, the top layer was removed with a wide pipette. To this fraction was added a double volume of ethanol so as to form two layers. The DNA was precipitated and collected by stirring with a sealed pasteur pipette. The DNA on the pipette was then transferred to a drum vial and covered with between 2 and 5 ml of dilute saline citrate (DSC), depending upon the yield. This was then stored overnight at 4°C.

First deoxycholate treatment (Day 3)

The DNA was gently dissolved in DSC at 35°C. This was then adjusted with CSC to give SSC. For each 100 ml of DNA solution, 6 mg of ribonuclease (Boehringer Corp.) was added dissolved in 0.15 M NaCl. This solution was preheated at 80°C for 10 min before use. This was then incubated for 1 h at 35°C. 0.4 mg pronase for each ml of solution was added after first being dissolved in pronase buffer and pre-incubated as before. This was followed, after incubation, by an equal volume of 4 M NaCl / citrate. 2% deoxycholate solution was added as one ninth of the total volume. The resulting mixture was incubated at 35°C for 1 h. Any gel that formed was dispersed after incubation and the preparation was then stored at 4°C overnight.

Second ethanol precipitation (Day 4)

Any gel present in the samples was dispersed. The samples were centrifuged at 15,000 x G and the liquid was poured off. To this liquid was added a double volume of ethanol and the DNA was precipitated and collected as before. The resulting DNA sample was stored under DSC in a drum vial overnight at 4°C.

Second deoxycholate treatment (Day 5)

The procedure as described for Day 3 was repeated with the exception that the pronase step was omitted.

Third ethanol precipitation (Day 6)

The procedure as described for Day 4 was repeated.

Iso propyl alcohol precipitation (Day 7)

The DNA was dissolved gently in DSC. To this was added EDTA / acetate solution to 10%. An equal volume of iso popyl alcohol was used. Half of this was added to the DNA solution immediately. The remainder was then added dropwise using a fine buerette tilted at 30°. While this was added the DNA was collected on a sealed pipette by stirring. The purified DNA was then washed in ethanol water mixes of 85, 90 and 95% ethanol before being stored overnight at 4°C in 0.5 ml DSC.

Dialysis (Day 8)

The DNA was dissolved gently in DSC and then adjusted to SSC. This was then dialysed in a dialysis bag at 4°C against SSC for 16 h. The final pure DNA was stored in a screw topped universal bottle containing a drop of chloroform. This was stored at 4°C for upto 1 month.

2.12.2 Estimation of DNA

The purified DNA was estimated by its absorbance ratio at 260 and 280 nm using the nomogram shown in Figure 2.12.1. This was achieved by plotting a straight line between the two absorbance readings. This line was then extended in each direction to give an estimate of the concentrations of protein and nucleic acid. The concentration of DNA was also estimated by chemical analysis.

Chemical analysis

1 ml of diphenylalanine reagent was added to a few drops of DNA solution. This was heated in a boiling water bath for 15 min. A blue colour on cooling indicated the presence of DNA. The intensity of the colour produced was dependant on the concentration of the DNA present. A calibration curve was plotted for this using known DNA solutions and this was used to estimate the DNA content of the sample.

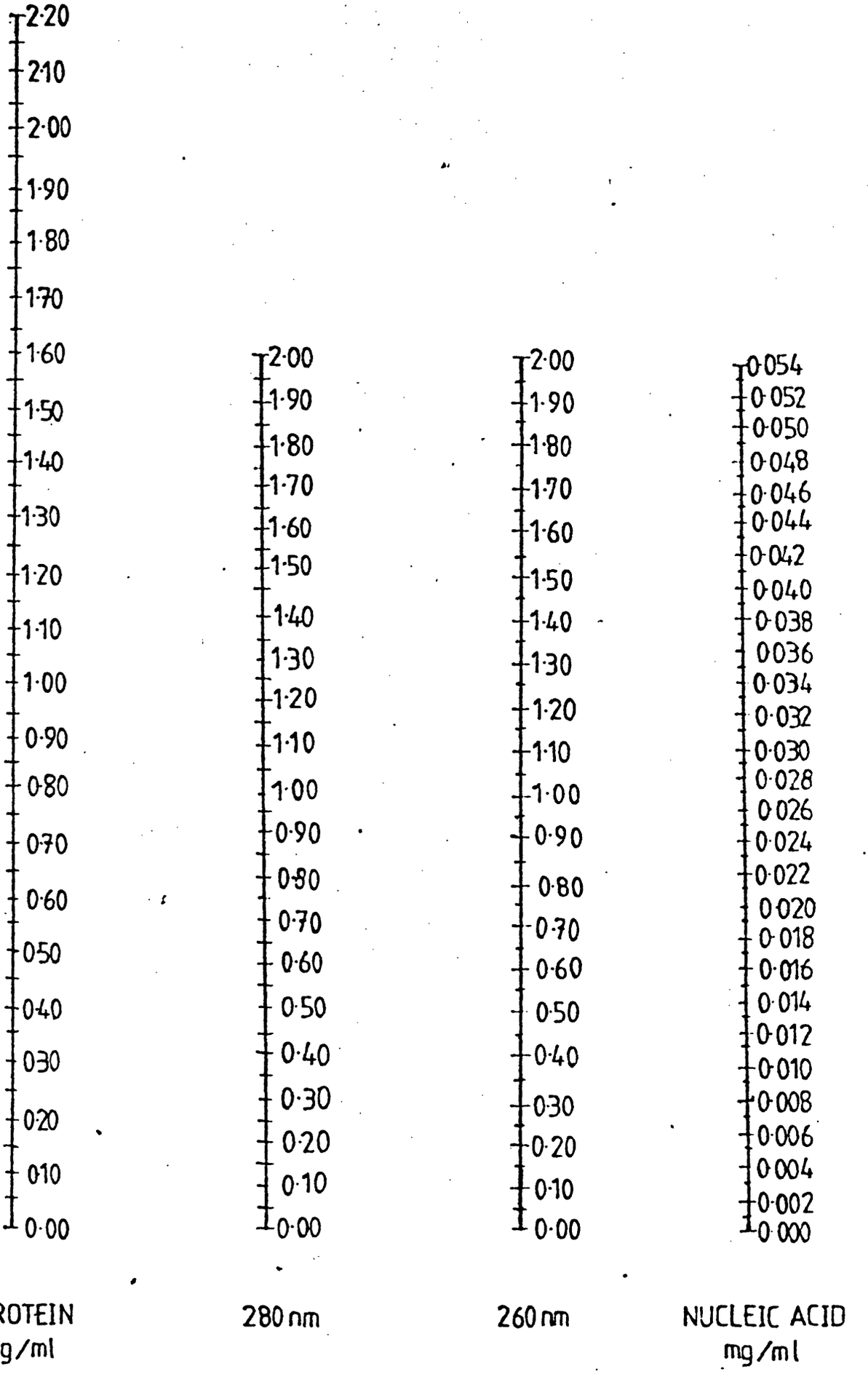
Diphenylalanine reagent consisted of:

0.5 g diphenylalanine dissolved in 50 ml glacial acetic acid and 2 ml 10 M H_2SO_4.

2.12.3 Melting curves

The melting of the DNA was carried out in SSC. The curves were followed using a Beckman Model 35 spectrophotometer, with a Stanton Redcroft Linear temperature Variable rate Programmer and an Edale thermistor thermometer with a Grant instruments temperature probe. The temperature probe was fitted through a small hole drilled in the centre of the cuvette stopper.

Figure 2.12.1 Nomogram for protein and nucleic acid determination.



The hypochromism of individual samples was calculated using the following formula:

Hypochromism (h) = $A_d/A_o - 1$ (Owen & Lapage, 1978)

Where A_d is the absorbance at 260 nm of the disordered (denatured) sample.

A_o is the absorbance at 260 nm of the ordered (unheated) sample.

The unheated sample was the sample at room temperature before melting and the denatured sample was the sample after melting, when absorbance was constant.

The following formula was used to calculate the Mol % G+C ratio of the samples from their melting points;

Mol % G+C = $(T_m - 69.4)\ 2.44$ (De Ley, 1970)

The melting point was taken as being the steepest point on the curve. All of these calculations were performed by means of a simple BASIC program, PDBGC2, written for the University of Leicester CDC Cyber 73 computer. A listing of this program is given in Appendix I.

2.13 Protein extraction

Cultures were grown in yeast glucose phosphate broth as described in Section 2.12. Each strain was grown up using 1l of medium in a 2l flask, incubated without aeration for 16 h at 35°C. The cells were harvested by centrifugation in an MSE 18 centrifuge at 15,000 x G for 10 min. The resulting pellet was washed in sterile distilled water and resuspended as a thick suspension at 1 g ml^{-1} in sterile distilled water.

The bacterial suspensions were disrupted by use of a French Pressure Cell (Aminco), each suspension was passed through the cell three times. This process gave between 65 and 85% disruption as observed by phase contrast microscopy. Strains showing only 65 to 75% disruption were passed through the cell a fourth time. This appeared to only marginally increase disruption. The disrupted cell isolates were clarified by centrifugation in an MSE 18 centrifuge at 30,000 x G for 2 h.

The amount of whole cell soluble protein in each extract was determined by the method of Lowry et al. (1951). Bovine serum albumin (BDH) was used as a standard for this method, five different concentrations being used to give each calibration curve. A new calibration curve was used for each batch of protein preparations. A typical curve is shown in Figure 2.13.1.

The extracts were stored in 250 µl aliquots at -20°C until required in an attempt to minimise freezing-thawing effects.

2.14.1 Electrophoresis of proteins

The electrophoresis of the protein samples was carried out in a polyacrylamide gel gradient. This gradient was established by mixing solutions of 5.25 and 21% (w/v) acrylamide in a gradient mixer. The top centimetre of the gel was made up of a "stacker" gel with an acrylamide concentration of less than 5.25% (w/v).

A 4 mm glass plate, 125 x 260 mm, was clamped vertically to a plastic slot former of the same dimensions. The sides of the mould and the bottom edge was sealed with a rubber gasket. This gasket was inserted between the plates about a third of the

distance up, to give a final mould of 125 x 155 mm. The slot former consisted of a plastic plate with 9 small blocks fitted in a line 1 cm from the top of the plate. These blocks produced slots of approximately 55 µl capacity.

Stock solutions

Solution A. 28% (w/v) acrylamide (BDH) and 0.4% (w/v) n, n-methylene bis acrylamide (Eastman) in distilled water.

Solution B. 0.5% (w/v) tris (hydroxymethyl) aminomethane (Sigma) in distilled water adjusted to pH 6.7 with 37% (w/v) HCl.

Solution C. A freshly prepared solution of 0.16 g ammonium persulphate (BDH) in 25 ml distilled water.

The gels were catalysed by the addition of 11 µl n,n,n,n-tetramethylethylenediamine (TMED) to each.

The gels consisted of:

21% (w/v) gel 12 ml A, 2 ml B, 2 ml C.

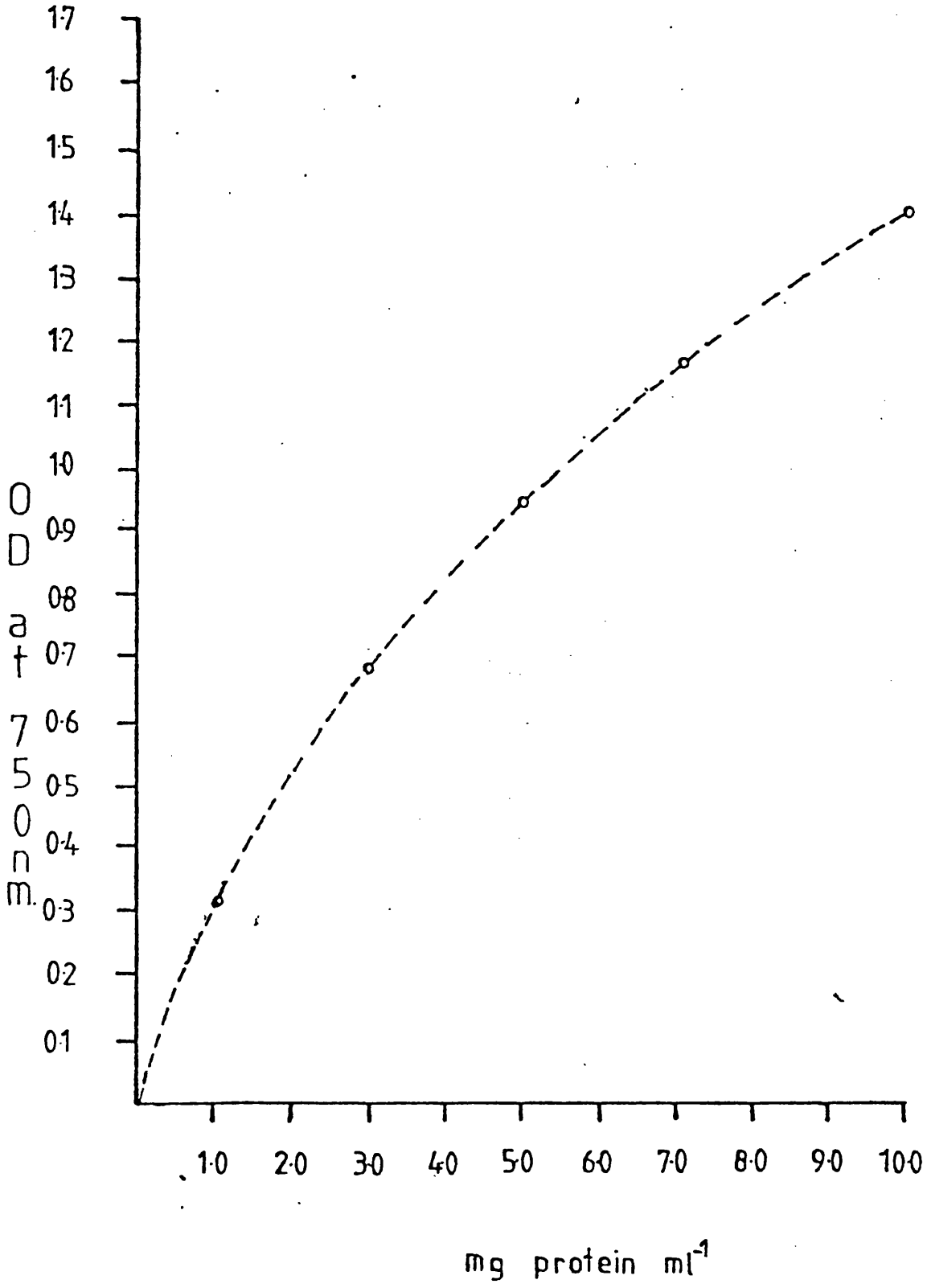
5.25% (w/v) gel 3 ml A, 2 ml B, 2 ml C, 9 ml water.

"Stacker" gel 2 ml A, 2 ml B, 2 ml C, 10 ml water.

The running buffer was a tris-glycine system made up in distilled water and consisted of:

Tris (hydroxymethyl) aminomethane		3 g	
Glycine		14.4 g	
Distilled water	to	1 l	pH 8.3

Figure 2.13.1 A typical calibration curve for the estimation of protein concentration.



The gels were run for 16 h at a constant voltage using a 4 mA current. An LKB electrophoresis tank was used with a platform cooled by running tap water. Filter paper wicks (Whatman grade 1) of the same width as the gels were used. The system was driven by a Shandon Southern power pack.

Gels were loaded with 50 μl of a 4 mg ml^{-1} protein solution. This concentration was selected as giving the optimum definition without excessive smearing. 5 μl of a 0.1% (w/v) aqueous bromophenol blue solution was added to each well to act as a marker. The two wells on either side of the gel were loaded with a 4 mg ml^{-1} solution of ovalbumin (BDH) to provide a reference standard of a known molecular weight.

The gels were removed after the bromophenol blue had travelled 12 cm and then stained at room temperature for between 3 and 8 h. The protein bands were then developed by successive destaining for up to 24 h in a methanol-acetic acid solution.

The stain consisted of:

Page blue	2.5 g
Methanol	450 ml
Glacial acetic acid	100 ml
Distilled water	450 ml

The destain consisted of:

Methanol		100 ml
Glacial acetic acid		70 ml
Distilled water	to	1 l

The destained gels were photographed with Ilford Pan F 35 mm film and printed as 100 x 125 mm positive transparencies. The transparancies were scanned using a Beckman model R-112 scanning micro-densitometer at 550 nm. The resulting traces were digitised by hand and computed as described in Section 2.14.3.

Three samples were extracted and developed in duplicate in order to obtain some estimate of the reproducibility of the method. Similarly, three further tracks were scanned directly from the gel as well as from the transparancies.

2.14.2 Electrophoresis of esterases

Soluble protein samples were prepared as for the protein analysis in the previous section. Electrophoresis was carried out in a 9.5% (w/v) poly-acrylamide gel made up from the following stock solutions.

Solution A. 22.5% (w/v) acrylamide (BDH), 0.6% (w/v) n,n-methylene bis acrylamide (Eastman) in distilled water.

Solution B. 1.37% (w/v) tris (hydroxymethyl) aminomethane, 0.0525% (w/v) citric acid in distilled water.

Solution C. 3.55% (w/v) ammonium persulphate in distilled water.

The working gel consisted of:

A	19.8 ml
B	15 ml
C	2.25 ml
Water	7.8 ml
TMED	30 µl

The running buffer was the tris-glycine system described in Section 2.14.1. The flat gels were made up and run as in Section 2.14.2. The gels were run at a constant voltage with an initial current of 15 mA, rising to 20 mA. The gels were run for 5–7 h until the bromophenol blue marker had travelled 12 cm.

The gels were stained using the method of Lawrence et al. (1960). The gels were flooded with a freshly prepared solution consisting of:

1% (w/v) α-naphthyl acetate in 50% (v/v) acetone		2 ml
Fast blue BB (Sigma)		50 mg
Tris-maleate buffer (0.1 M, pH 6.4)*		50 ml

*Tris-maleate buffer consisted of:

Tris (hydroxymethyl) aminomethane		12.1 g
Maleic anhydride		9.8 g
Distilled water	to	1000 ml

Esterase activity was shown by the appearance of dark brown bands in the gel which developed in 30 min to 2 h at room temperature.

Gels were run at two different protein concentrations in order to ascertain whether there were any appreciable differences in the patterns of bands obtained.

2.14.3 Numerical analysis of protein patterns

The densitometric traces were standardised by marking on each the positions of the origin, the bromophenol blue marker and ovalbumin. The distance between the origin and the furthest marker, bromophenol blue was taken as being 100 units.

The distance from the origin to the ovalbumin marker was compared to this and taken as being 80 units. The height of the trace was measured on a scale of 100 units, and the height of the trace at each of the 80 points along its length was recorded. Two methods of comparison of these results were used; both were based on the method of Feltham & Sneath (1979) and involved the use of the taxonomic distance and the cosine θ coefficient. All resulting similarity matrices were clustered by unweighted average pair groups methods.

(a) The first method involved calculating the two similarity coefficients between each pair of traces. Each trace was considered as a single OTU of 80 characters, each between 0 and 100.

The distance coefficient estimates an average function of the vertical distances between the two traces. The coefficient is sensitive to different protein concentrations, as these give different peak heights. The distance coefficient was used such that the distance ($\underline{d}_{jk}$) between two traces j and k is:

$$\underline{d}_{jk} \qquad 1/\underline{n} \left[\sum_{i=0}^{i=n} (\underline{Y}_{ij} - \underline{Y}_{ik})^2 \right]^{\frac{1}{2}}$$

Where $\underline{n}$ is the number of points on the trace and $\underline{Y}_{ij}$ is the height of trace j at point i. This gives a measure of the Euclidean distance between the traces. If the distance between any two traces is small, then the positions in $\underline{n}$-dimensional space are close and so the traces are similar. The greater the distance, the lower the similarity.

The cosine θ coefficient is a measure of the similarity between shapes. If two traces j and k are represented in $\underline{n}$-dimensional space where $\underline{n}$ is the number of characters, then $\underline{\theta}_{jk}$ is the angle between the vectors linking j and k to the origin, where the value of all $\underline{n}$ characters is zero. The cosine of a small angle tends to 1 and the cosine of an angle of 90° is zero. So the higher the cosine value the small the angle between the two traces. This is a shape coefficient and as such it is not so dependant upon concentration as the overall shape of the trace is considered rather than the heights of individual peaks. For the two traces j and k, the cosine θ coefficient, $\cos \underline{\theta}_{jk}$ may be represented as:

$$\text{Cos } \underline{\theta}_{jk} = \frac{\sum_{i=0}^{i=n} \underline{Y}_{ij} \cdot \underline{Y}_{ik}}{\left[\left(\sum_{i=0}^{i=n} \underline{Y}_{ij}^{2}\right)\left(\sum_{i=0}^{i=n} \underline{Y}_{ik}^{2}\right)\right]^{\frac{1}{2}}}$$

(b) The second method involved considering any distortions on the X-axis. These may affect considerably the similarity coefficients and may be due to discrepancies in gel composition, uneven running times, voltage variations or a stretching of the gel during handling. Trend surface analysis was used for this where the lateral displacement (shift) was considered as the horizontal axis of the surface and the stretch-factor was considered as the vertical axis. Amended values $\underline{X}'_{ij}$

used for this method are given by the equation:

$$\underline{X'_{ij}} = a + b\,\underline{X_{ij}}$$

where a represents the shift value and b the stretching value. These calculations and the clustering for both methods were performed by using modifications of existing computer programs developed in the Department of Microbiology at the University of Leicester (see Appendix I).

2.15 Serological methods

Strains to be tested for the possession of the different Lancefield group antigens were grown up in 30 ml of Nutrient Broth No. 2 (Oxoid), for 48 h. These were then harvested at 10,000 x G for 10 min in an MSE 18 centrifuge. The resulting pellet was re-suspended in HCl-saline (0.85% (w/v) NaCl made up in 0.5 M HCl) and placed in a boiling water bath for between 10 and 12 min. After this the samples were cooled in ice and one drop of 0.002% (w/v) phenol red was added to each. The samples were then neutralised with 0.5 M NaOH which was added drop wise until the indicator was decolourised.

Microcapillaries drawn from pasteur pipettes were used to suck up small amounts of grouping serum (Wellcome Laboratories) and this was sealed inside the capillaries at one end. A few drops of the neutralised samples were added on top of this using a micro-pipette. A white line appearing at the junction of the two liquids within 30 min was taken as a positive reaction. Type species were used as positive controls.

RESULTS

3.1 Test reproducibility

In order to ascertain the reproducibility of the results given in this section, twenty strains used in the taxonomy were repeated as described in Section 2.11. The results of these are given in Table 3.1.a. The similarity between duplicates averaged 95.04%. This corresponds to an error of less than 5%, which is within the upper limit suggested by Sneath & Johnson (1972). The mean overall value of test error is 2.48%.

The difference recorded between tests was calculated as described in Section 2.11. Certain tests may be suspected as being less reproducible than others, (Lapage et al., 1970; Sneath & Collins, 1974) particularly those that rely on subjective judgements. The results of the test reproducibility for the individual tests are shown in Appendix II. The twelve tests showing a difference of more than 15% were not used for the numerical taxonomy. As the API methods consists of galleries containing a number of individual tests, the reproducibility of the galleries was also calculated. The API 50E gallery gave an overall 11% difference and the APIzym gallery gave 1.6%. Although some individual tests in these galleries gave differences over 15%, these were included in the taxonomy. This was because it was considered that one gallery constituted one fixed group of tests and as a result it would be inappropriate to consider individual tests in isolation. The greatest differences were found in the antibiotic sensitivity tests. Some of these, namely cloxacillin, methicillin, colistin

Table 3.1.a. Error shown between duplicated strains.

Strain pair	Error (%)
PB 1 PB 1A	3.18
PB 14 PB 14A	0.96
PB 35 PB 35A	3.82
PB 40 PB 40A	3.18
PB 50 PB 50A	2.54
PB 100 PB 100A	0.96
PB 132 PB 132A	1.91
PB 144 PB 144A	2.54
PB 150 PB 150A	2.54
PB 192 PB 192A	2.23
PB 193 PB 193A	2.86
PB 194 PB 194A	2.54
PB 195 PB 195A	3.82
PB 196 PB 196A	3.18
PB 197 PB 197A	4.46

Table 3.1.a. Error shown between duplicated strains continued.

Strain pair	Error (%)
PB 198 PB 198A	1.59
PB 199 PB 199A	1.91
PB 200 PB 200A	0.96
PB 201 PB 201A	2.54
PB 202 PB 202A	1.91

sulphate and streptomycin and others showed 50% or more difference. Possible explanations of this are given in the discussion.

The overall difference between all the tests retained in the taxonomy was estimated from these tables as 5.8%. The tests that were not used in the taxonomy were cell arrangement, catalase from haemin and resistance to ten antibiotics. These are listed in Appendix II. A further study on APIzym reproducibility was carried out as described in Section 2.11. This is described more fully in the discussion. The results obtained for S. faecalis, PB 2, (NCTC 775) on five different growth media are shown in Table 3.1.b. These results showed that different growth media may give rise to different APIzym results. The sheep serum was included to see if any results were due directly to a carry over of the media. This seems unlikely from these results. They do however highlight the need to use the same media throughout a study of this kind.

Two tests in particular on the API 50E gallery appeared to be very irreproducible: these were ribose and tetrathionate reductase. These are discussed further in the discussion section, but were thought to show batch variation.

The correlation between a selection of the API 50E sugars and more classical sugar fermentation methods was estimated. This was done by growing all of the strains in a sloppy agar medium containing glycogen, starch or dextrin. The results of this are summarised below.

Table 3.1.b. Different APIzym results for strain PB 2 grown on different media.

		Difco BAB2 + 5% horse blood	Oxoid BAB + 5% horse blood	Oxoid BAB + 5% sheep blood	Oxoid Todd-Hewitt broth	Difco BAB2	Sheep serum inoculum
	0	0	0	0	0	0	0
	1	1	1	0	0	1	4
	2	2	1	0	1	2	0
	3	4	5	2	4	4	3
	4	0	0	0	0	0	2
T	5	5	4	2	5	3	3
E	6	0	0	0	0	0	0
S	7	2	2	0	1	2	1
T	8	0	0	0	0	0	0
	9	5	2	1	4	5	0
N	10	5	4	2	5	5	3
U	11	5	4	3	2	4	3
M	12	0	0	0	0	0	0
B	13	3	2	0	0	4	0
E	14	0	0	0	0	0	0
R	15	5	5	5	3	5	3
	16	3	4	0	0	4	0
	17	0	0	0	0	1	0
	18	0	0	0	0	0	0
	19	0	0	0	0	0	0

	Glycogen	Starch	Dextrin
No. of reactions positive on API 50E gallery	42	131	156
No. of reactions positive in sloppy agar	20	119	158
No. of reactions positive in both methods	20	113	154
No. of reactions negative in both methods	160	71	29
Percentage similarity expressed as $\underline{S_G}$	89.1	91	90.6

The correlation between the two methods is within the same range as the test reproducibility.

3.2 Numerical taxonomy results

3.2.1 Dendrograms and similarity matrices

The coded results of the 157 tests used in the study are listed in Appendix III. These were computed as described earlier in Section 2.10 to produce the dendrograms and similarity matrices shown in Figures 3.2.1 and 3.2.2. The similarity matrix is shown in Appendix IV.

Figure 3.2.1 shows the average linkage dendrogram derived from the data using Gower's coefficient. The Cophenetic Correlation coefficient for this dendrogram is 0.71. It can be seen from this dendrogram that the majority of the clustering occurs at above 85% similarity. The base lines joining the clusters, however, are close together and also of high similarity indicating that there is a large amount of similarity between different clusters. This can be seen clearly on the similarity matrix shown in Appendix IV.

Figure 3.2.1 Average linkage dendrogram using Gower's coefficient for 157 characters and 202 strains. Cophenetic correlation coefficient is 0.71085.

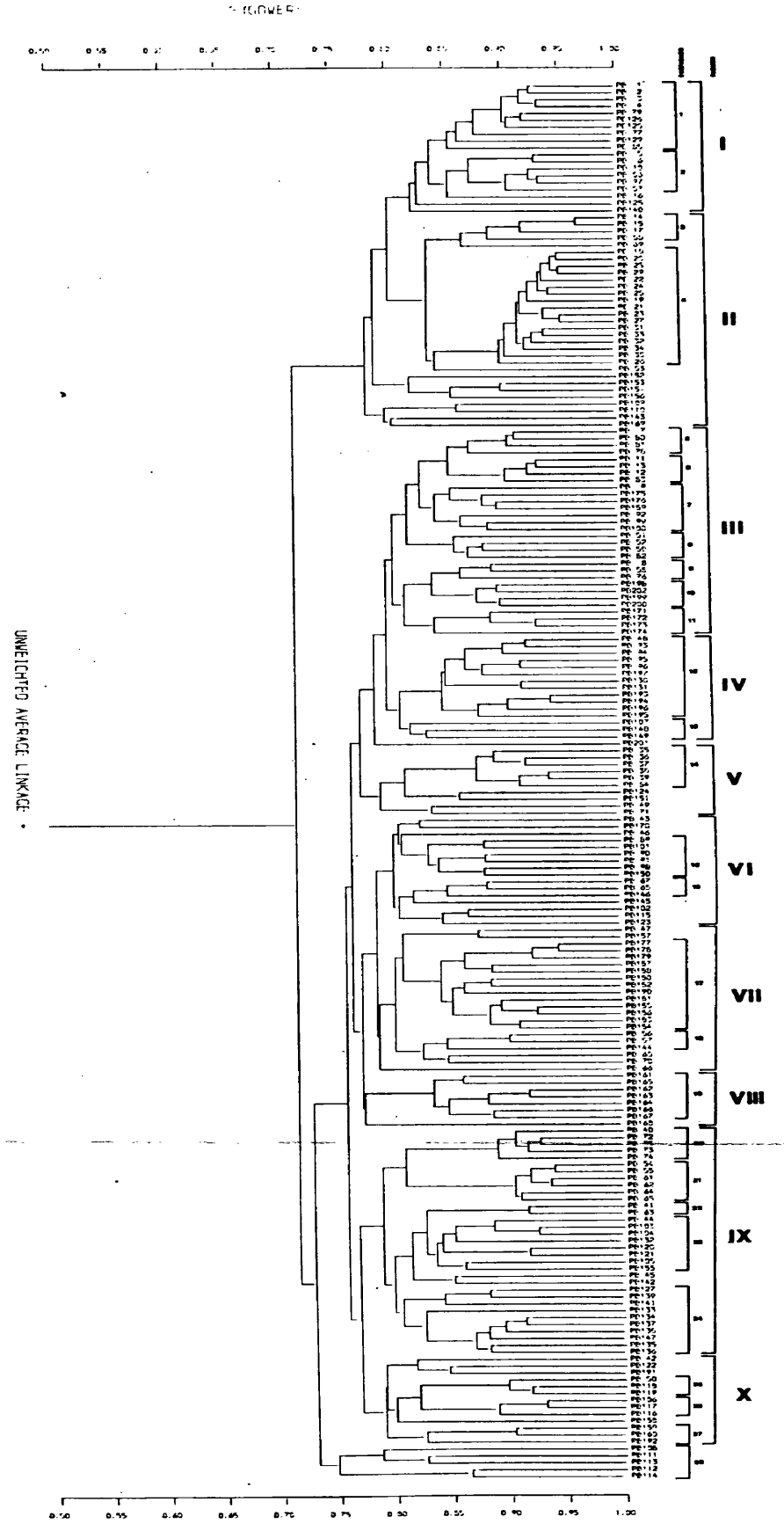
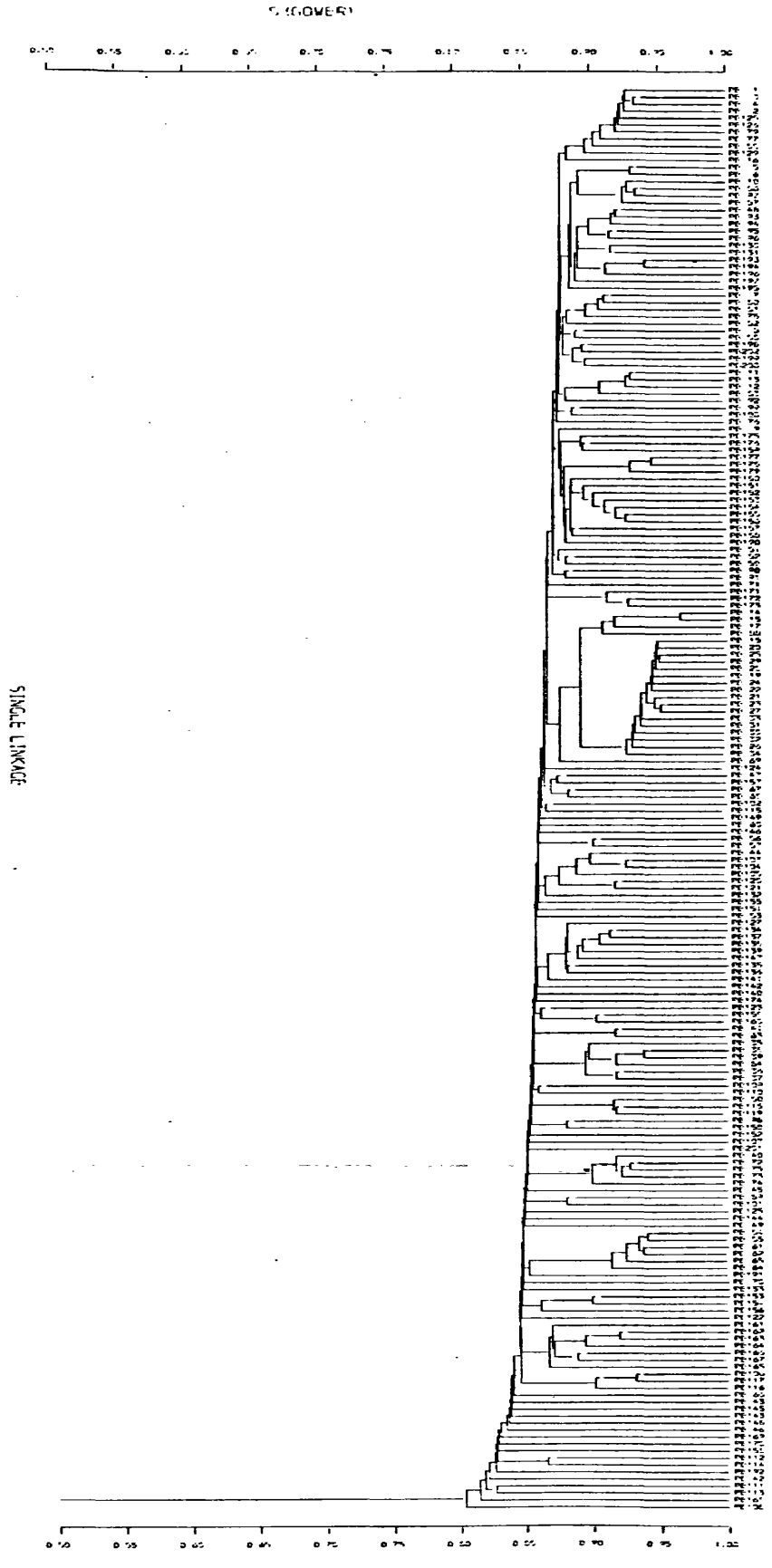


Figure 3.2.2 Single linkage dendrogram using Gower's coefficient for 157 characters and 202 strains. Cophenetic correlation coefficient is 0.3561.



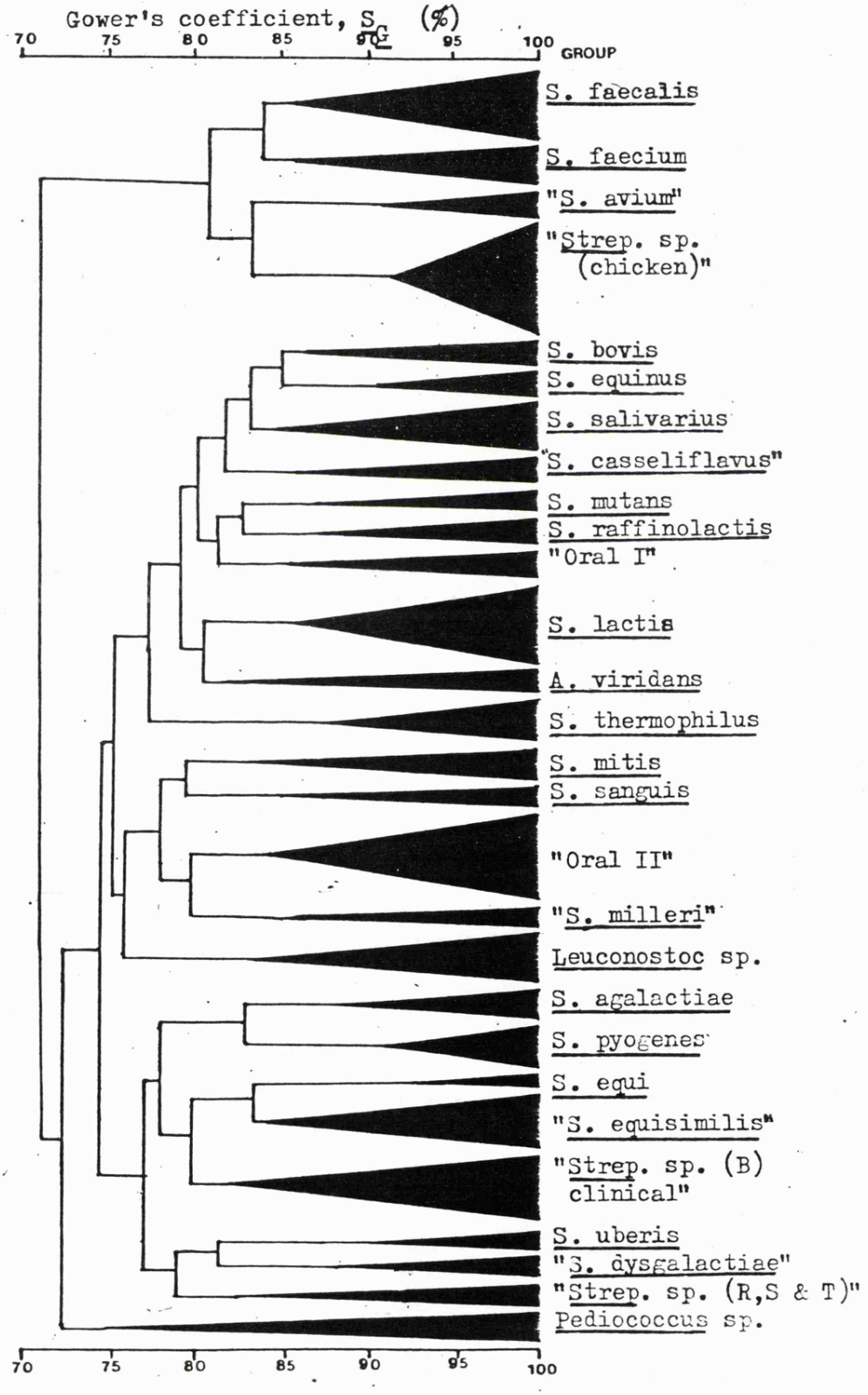
Here areas of high similarity of around 80% may be seen some distance from the clusters. Although distinct clusters have formed, it appears that only those at the top of the dendrogram are markedly different from the others. This conclusion is reinforced by the single linkage dendrogram which shows the base lines of the clusters to be even closer together. The single linkage dendrogram is dependant upon the similarity of only one OTU in a group. As a result of this, the representation does not give such a good overall representation as the average linkage. Consequently, the single linkage clustering is often less stable to strain composition. As a result of this it was decided to use the mathematically more acceptable average linkage results.

An average linkage dendrogram was also produced for the Simple Matching coefficient (S_{SM}). This is shown in Figure 3.2.4. The same overall high similarity between clusters may also be seen in this dendrogram.

Figure 3.2.3 shows a simplified version of the S_G average linkage dendrogram. Satellite and ungrouped strains have been removed to show ten major groups. This is however only a two dimensional representation of an n dimensional arrangement, where n is the number of characters used. As a result the stems shown on the clusters in the dendrogram may revolve around each other to give a different ordering in the groups. The groups themselves may also revolve around on their stems.

The ten phenons shown in the average linkage dendrogram (Fig. 3.2.1) contain twenty-seven subphenons that were considered

Figure 3.2.3 The simplified dendrogram using Gower's coefficient and average linkage clustering.



to be reasonably separate, with the addition of a loosely linked subphenon shown at the bottom of the dendrogram. The subphenons were named from the presence of type or reference strains (Sneath & Skerman, 1966: Skerman et al., 1980). As noted above, the average linkage dendrogram on S_G was thought to represent the taxonomy best, and the following discussion relates to Figure 3.2.1 and the simplified dendrogram in Figure 3.2.3.

3.2.2 Average linkage clustering

Phenon I

Phenon I contains nineteen strains, sixteen of these cluster in two subphenons. Subphenon 1 contains the type strain of S. faecalis (PB 2) and nine other strains (PB 1, PB 3, PB 4, PB 79, PB 126, PB 128, PB 77, PB 129 and PB 85). These were all received as either S. faecalis or varieties of the species S. faecalis. One strain, PB 129, was labelled S. faecium, but it is possible that this has been mislabelled. Subphenon 2 contains the type strain of S. faecium (PB 87) and five other strains (PB 5, PB 6, PB 10, PB 86 and PB 97). These were all received as representatives of either S. faecium or "S. durans".

Phenon II

Phenon II contains twenty-one strains and these are arranged into two subphenons. Subphenon 3 contains four strains of serological group Q. One of these (PB 88) is a Guthof reference strain of "S. avium" and this subphenon has been named from this.

Subphenon 4 contains seventeen strains isolated from

chickens. These have been reported as being similar to S. faecium (Barnes et al., 1978). Loosely linked to these clusters are two satellite strains, strain PB 69 (which was received as "S. faecalis subsp. malodoratus" and appears as a satellite to subphenon 3) and strain PB 53 (which was received as "S. faecium subsp. mobilis" and appears as a satellite to subphenon 4). Also represented in phenon II are eight loosely linked and ungrouped strains.

Phenon III

Phenon III contains twenty-nine strains forming seven subphenons. Subphenon 5 contains four strains (PB 7, PB 80, PB 81 and PB 78) all of which were labelled as S. bovis. Subphenon 6 contains four strains (PB 11, PB 13, PB 12 and PB 83) which were labelled as S. equinus. The type strain of this species (PB 83) appears in this subphenon. Subphenon 7 contains seven strains. Three of these, PB 175, PB 176 and PB 189, are oral isolates from the human mouth isolated by Prof. Carlsson (1968). These he considered to be similar to S. salivarius. Three strains received as S. salivarius, PB 9, PB 99 and PB 100, are also present in this subphenon. PB 9 was received as the type strain of this species. The remaining strain in this subphenon, PB 92, was received as "Streptococcus sp. (MG)". Subphenon 8 contains four strains; two of these, PB 51 and PB 52 were received as examples of "S. faecium subsp. casseliflavus". The other two strains were received as "S. sobrinus" (PB 59) and S. equinus (PB 82). Subphenon 8 was tentatively named as "S. casseliflavus". Subphenon 9 contains three strains, two of these, PB 58 and PB 76, are representatives of

S. rattus and S. mutans. However, the third strain was received as S. bovis (PB 8). The presence of the strain of S. mutans and the phenotypically similar S. rattus was used to name this cluster S. mutans. Subphenon 10 contains four strains, two of which, PB 199 and PB 200, were received as representatives of the species S. raffinolactis. The remaining two strains, PB 198 and PB 202, were received as "Streptococcus sp., possible S. cremoris variants" (NCDO catalogue, 1976). Subphenon 11 contained four strains, PB 171, PB 172, PB 173 and PB 174, all of which were received as human oral isolates from Prof. Carlsson. This subphenon appears to be equivalent with the group II found in his numerical study with the addition of one strain, PB 174, from his group III which was closely linked (Carlsson, 1968). In the absence of any named reference strains this subphenon was named "Oral I".

Phenon IV

Phenon IV consists of sixteen strains which form two subphenons. One strain, PB 201, remaining ungrouped. Subphenon 12 contains twelve strains, which were all received as representatives of serological group N. The subphenon is made up of three strains of S. lactis (PB 93, PB 94 and PB 196), three strains of S. cremoris (PB 95, PB 96 and PB 197), four strains of "S. lactis subsp. diacetylactis" (PB 130, PB 131, PB 193 and PB 195) and one strain of "S. cremoris subsp. alactosus" (PB 194). One other strain is also present (PB 48) and this was received as "Streptococcus sp. (Lancefield group N)". This subphenon equates with the lactic streptococci. However, the subphenon contains two type strains and one

reference strain. These are PB 93, the type strain of S. lactis, PB 95, the type strain of S. cremoris and the reference strain PB 193, "S. lactis subsp. diacetylactis". This subphenon was therefore considered to represent S. lactis. The last four strains in this subphenon appear on a distinct stem at 85% similarity, but this is considered here as part of the single subphenon on the basis of its compactness and its relation to its nearest neighbour. Subphenon 13 consists of three strains loosely linked at 83% similarity. One of these strains, PB 107, was received as Aerococcus viridans. The other strains, PB 147 and PB 148, were received as "Streptococcus sp.(viridans type)". This subphenon was tentatively named as A. viridans. The strain PB 201 appears as an ungrouped entity at the base of phenons III and IV. It is linked at 79% similarity and was received as Streptococcus sp.

Phenon V

Phenon V consists of ten strains forming one subphenon with four strains remaining ungrouped. Subphenon 14 consists of six strains of streptococci received as S. thermophilus (PB 35, PB 36, PB 37, PB 38, PB 39 and PB 84). One of these, PB 84, is the type strain of the species and so this cluster was named S. thermophilus. The other four strains in this phenon are; PB 124, received as "S. cremoris subsp. alactosus", PB 151 (S. mutans), PB 49 ("Streptococcus sp. serological group O") and PB 71 ("Streptococcus sp. serological group N").

Phenon VI

Phenon VI consists of sixteen strains which form two subphenons (15 and 16). Seven strains are either ungrouped or

loosely linked. Strain PB 43 was received as a representative of serological group F, PB 170 was received as Streptococcus sp. of human origin and strain PB 46 was received as a representative of serological group K. These three strains are loosely linked to subphenon 15. This subphenon consists of six strains linked in three pairs. Two of these strains, PB 90 and PB 91, were received as examples of streptococci of serological group 0, and these appear as a pair in the centre of the cluster. Two strains received as "Streptococcus sp. (viridans type)" are present on either side of this pair and they are linked with two strains received as S. mitis so as to form two mixed pairs on either side of the central pair. Due to the presence of the strains of S. mitis in this cluster it was named S. mitis. Subphenon 16 consists of three strains all of which were received as S. sanguis (PB 67, PB 68 and PB 146). The type strain PB 67 was included in this cluster which was named S. sanguis. Four further strains are loosely linked to subphenon 16. Two of these, PB 145 and PB 102, were received as human isolates. These had been named "S. mitior" and "Streptococcus sp. (viridans type)" respectively. Strain PB 145 is loosely linked to subphenon 16 alone at 81% similarity. Strain PB 102 is linked at 84% similarity to two strains received as "S. suis", PB 115 and PB 123.

Phenon VII

Phenon VII consists of twenty-one strains which form two subphenons (17 and 18), five strains remaining ungrouped. PB 47 and PB 157 appear as a loosely linked pair of strains at 81%

similarity with subphenon 17. Both of these strains were received as "Streptococcus sp. (serological group M)". They show 86.5% similarity to one another. Subphenon 17 consists of thirteen strains. These are human oral streptococci donated by Prof. Carlsson and they group in a similar way to that seen in his study. The two strains PB 178 and PB 179 were both received as being representatives of his group IB. These are found grouped together in subphenon 17 at 92% similarity. Strains PB 187 and PB 188 are representatives of his group VB and these link at 88% similarity. Strains PB 180, PB 182 and PB 181 are all members of Carlsson's group IA and clustering with these is strain PB 190 which is a representative of his group IV. The remaining strains in this subphenon are all members of his group VA. As no type strains are present in this subphenon it was decided to name it as "Oral II". Subphenon 18 contains three strains all received as "S. milleri", so that name was applied to this subphenon. Two strains are loosely linked to this subphenon. These are strains PB 60 and PB 70, these were received as "S. sobrinus" and S. pneumoniae. One strain, PB 66, is linked to both subphenon 17 and subphenon 18 at 78% similarity. This was received as an atypical strain of S. sanguis (Cole & Kolstad, 1974).

Phenon VIII

Phenon VIII consists of eight strains. Seven of these form subphenon 19, one strain remaining loosely linked to it. Subphenon 19 consists of strain PB 161, received as Gemella haemolysans and six other strains received as examples of the genus Leuconostoc

(PB 165, PB 162, PB 163, PB 164, PB 166 and PB 167). This subphenon separates from the previous phenons VI and VII at 77%. Separated at 77.5% similarity from subphenon 19 is the ungrouped strain PB 168. This was received as a strain of Pediococcus halophilus.

Phenon IX

Phenon IX consists of thirty-three strains forming five subphenons (20-24), with two strains remaining ungrouped. Subphenon 20 consists of five strains. These were all received as strains of S. agalactiae. Strain PB 40 was received as the type strain of this species and as a result this name was given to the subphenon. Subphenon 21 consists of six strains that were all received as strains of S. pyogenes, as strain PB 54 was the type strain this name was allotted to this subphenon. Subphenon 22 consists of only two strains. These are the only strains of S. equi included in the study (strain PB 41 was the type strain for this species). Subphenon 23 consists of eight strains. Four of these were received as "S. equisimilis" (strains PB 103, PB 104, PB 132 and PB 105). Also present were two strains of "S. zooepidemicus" (strains PB 120 and PB 121). Included with these Lancefield group C organisms in this cluster were two representatives of other serological groups. These are PB 44, received as an example of "Streptococcus sp. serological group G" and PB 155 received as "Streptococcus sp. serological group L". This group was named "S. equisimilis". Two strains were linked to subphenons 22 and 23 at 82% similarity. These are PB 45, received as "Streptococcus sp. serological group H" and PB 142, received as "Streptococcus sp. serological group G".

Subphenon 24 consists of ten strains, all of which were received as "Streptococcus sp. serological group B". These were all human clinical isolates. These may be further subdivided into two groups within the subphenon. The first consisting of PB 127, PB 139 and PB 141, linking at 84% similarity and the remaining strains, PB 133, PB 134, PB 137, PB 138, PB 147, PB 135 and PB 136, linking at 82.5%. This subphenon was tentatively labelled as "Streptococcus sp. (B) clinical".

Phenon X

Phenon X consists of thirteen strains which form three subphenons (25–27). Loosely linked to subphenon 25 and subphenon 26 are three strains, PB 42, PB 122 and PB 191. The first two were received as strains of "Streptococcus sp. serological group E" and the other strain was received as "Streptococcus sp. serological group M". The two serological group E strains cluster together at 82% similarity with the serological group M strain. These three are in turn linked to the two subphenons at 79% similarity. Subphenon 25 consists of three strains received as S. uberis. Strain PB 118, which appears in the centre of the cluster, was received as the type strain and so this name was assigned to the subphenon. Subphenon 26 consists of three strains all received as "S. dysgalactiae". These showed over 88% similarity to each other and linked to subphenon 25 at 82% similarity. Subphenon 27 consists of three strains, PB 159, PB 160 and PB 192, these were received as strains of the Lancefield groups R, S and T respectively. Linked between this subphenon and subphenon 26 was a single strain, PB 158. This was received as a member of serological group F,

and it linked to subphenon 27 at 79% similarity and to subphenon 26 at 80% similarity.

Subphenon 28

Subphenon 28 appears at the bottom of the dendrogram and is a separate cluster. It links only loosely to the other phenons at 73% similarity, but does not appear tight enough to be considered as a phenon in its own right, resembling more a collection of loosely linked strains. It consists of five strains, four of which were received as representatives of the genus Pediococcus (PB 108, PB 111, PB 113 and PB 114) and one strain, PB 112, which was received as a strain of Aerococcus viridans.

3.2.3 Single linkage clustering

The single linkage dendrogram obtained from the numerical data using the S_G coefficient is shown in Figure 3.2.2. All of the strains are more than 80% similar to each other; the vast majority have a similarity of above 83% to each other. The positions of the previously mentioned subphenons are altered, some having moved and some having split into two or more smaller groups. Group numbers follow the average linkage S_G convention where they may be equated.

Subphenon 1 appears in much the same position and form as previously, although the strain order is different. Subphenon 2 is however identical in both dendrograms. Subphenons 3 and 4 have moved considerably in position while still maintaining their original composition. Their position has been taken by subphenon 12, which has also maintained its composition. Subphenon 5 is the

same as before. This is also true of subphenon 6, although it has moved to appear further down the dendrogram. Subphenon 7 is still linked to subphenon 6 although it has split into two branches. The strains received as S. salivarius are arranged on one branch and those received from Prof. Carlsson as being human oral isolates similar to S. salivarius are on the other. Subphenon 8 has split up. The single strain of S. equinus, PB 82, has become linked to subphenon 5 (S. bovis), while the remaining three strains have stayed together and are linked in the same order as they were. Subphenon 9 has also split up. Two strains, PB 8, received as S. bovis and PB 58, received as S. rattus are loosely linked between subphenons 5 and 10. The third strain, PB 76, received as S. mutans, has become linked between the two branches of subphenon 7.

Subphenon 10 is the same as before, as is subphenon 11, although the latter has moved. Subphenon 13 (A. viridans) has in effect exploded, all three strains being scattered about the dendrogram. Subphenon 14 has been conserved although its position has changed. Subphenon 15 has split up into three pairs of strains seen originally in the average linkage dendrogram as one cluster. Subphenon 16 has been altered by the acquisition of some previously ungrouped strains and one strain from subphenon 13. Subphenon 17 has remained intact but has moved considerably up the dendrogram to link between subphenons 7 and 8. As a result of this the majority of the Carlsson strains are now found together. Subphenon 18, "S. milleri" has split up. The two culture collection strains, PB 56 and 57, are found together, the other strain received as a clinical isolate, PB 144, has become separated.

Subphenon 19 has remained intact although it has lost the satellite strain of PB 168. Subphenons 21 and 22 have retained their form and composition, although their positioning has altered, unlike subphenon 20 which has remained unchanged. Subphenon 23 has likewise retained its original composition. Subphenon 24 is now linked closest to one part of subphenon 27, but is otherwise undisturbed. Subphenons 25 and 26 have become separated, subphenon 25 linking to subphenon 14 and subphenon 26 linking to subphenon 19. Subphenon 27 has split up, strain PB 192 occupying its original position, while strains PB 159 and PB 160 have moved up the dendrogram to link with subphenon 24. Subphenon 28, the loosely linked strains of mainly Pediococcus sp., has changed, acquiring strain PB 168 (the satellite strain from subphenon 19). This strain was received as Pediococcus halophilus. Strains PB 112 and PB 114, both of which were seen in this cluster in the average linkage dendrogram, have become slightly more separated.

3.2.4 Simple Matching coefficient

A dendrogram was constructed using the Simple Matching coefficient, S_{SM} and average linkage clustering. This coefficient only considers test results in binary states. As a result a less distinct version of the S_G dendrogram would be expected due to the reduced amount of information employed. The average linkage dendrogram is shown in Figure 3.2.4, a simplified version being shown in Figure 3.2.5.

Figure 3.2.4 Average linkage dendrogram using the Simple Matching coefficient for 157 characters and 202 strains. Cophenetic correlation coefficient is 0.6958.

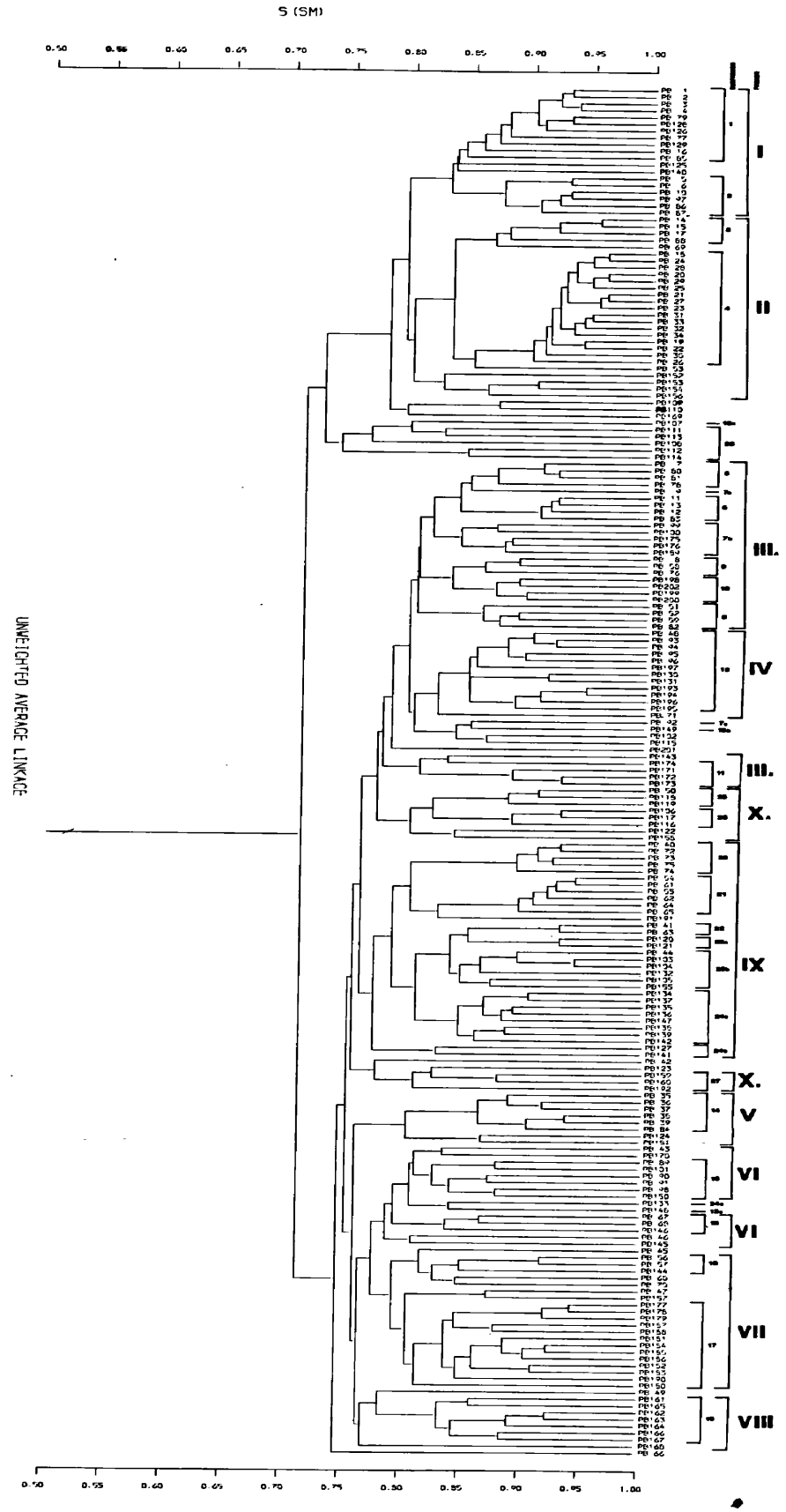
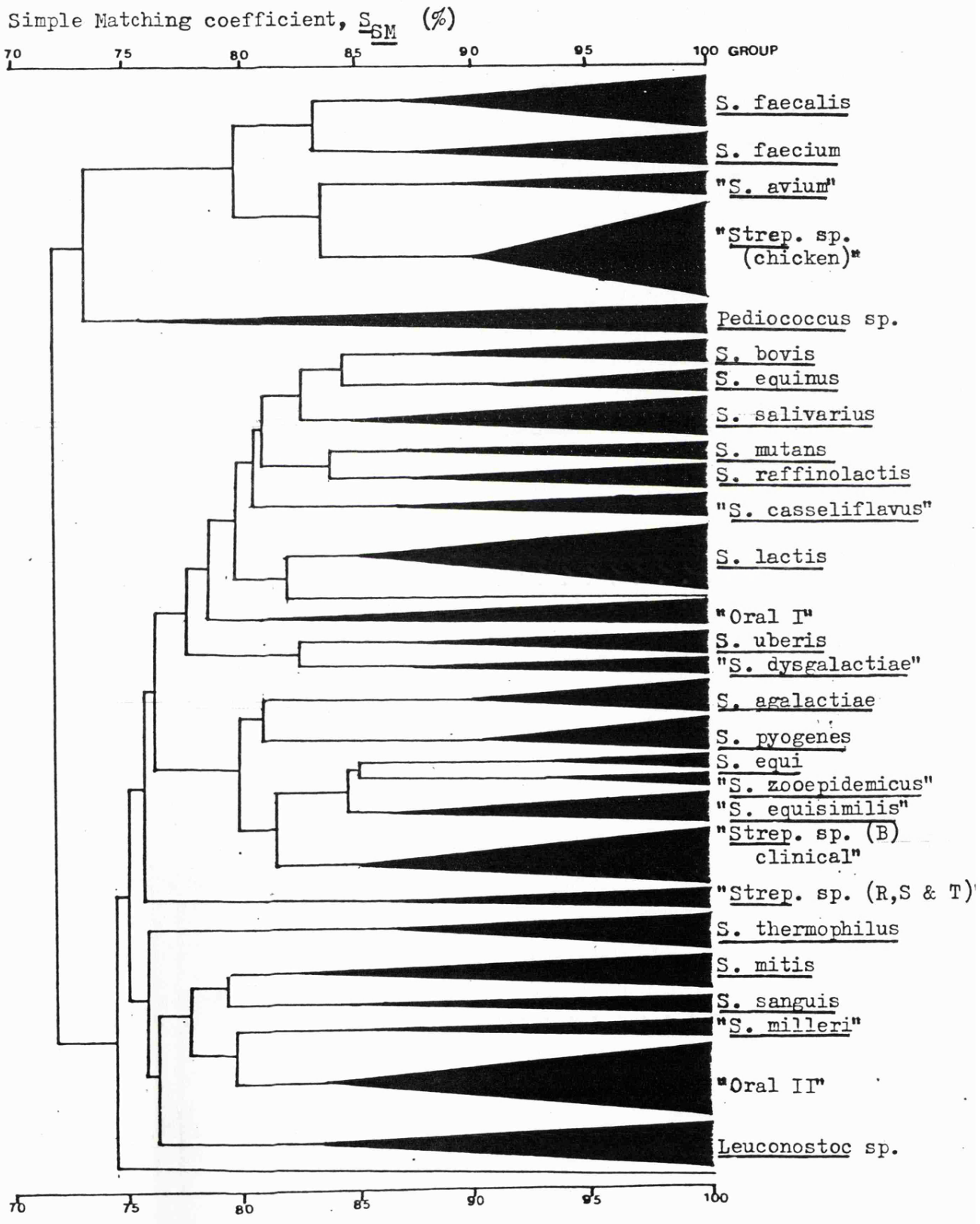


Figure 3.2.5 Simplified dendrogram using the Simple Matching coefficient and average linkage clustering.



Phenons I and II are the same as in the Gower dendrogram with the exception that some loosely linked strains, mainly of serological group D, from the base of subphenon 2, S. faecium, now link with subphenon 1, S. faecalis. There has however been some change in phenon III. Subphenons 5, 6, 8, 9 and 10 are the same as before, although they are now arranged in a different order. Subphenon 7, S. salivarius, has lost one strain, PB 9. This strain has moved to link between subphenons 5 and 6, S. bovis and S. equinus. Subphenon 11, "Oral I", has moved out of this cluster and is now found further down the dendrogram. Subphenon 28, the group of loosely linked strains, has moved from the base of the dendrogram to link between phenons II and III.

Phenon IV remains much as before, subphenon 12, S. lactis, remaining the same. However, two members of subphenon 13, A. viridans, have moved from this cluster, leaving only one strain, PB 149, as a representative.

Phenon V has moved and lost two of its previously loosely linked strains, PB 49 and PB 71. It however remains linked to phenon IV. Phenon IV has similarly lost some of the loosely linked strains but remains linked to phenon V and phenon VII, having acquired strain PB 148 from subphenon 13.

Phenon VII is the same as before with the exception that it is in a different position and has acquired strain PB 45 as a satellite. This strain was previously loosely linked between subphenons 23 and 24.

Phenon VIII is as before save for the acquisition of a satellite strain, PB 49, which was previously loosely linked to the base of phenon V. These last four phenons have in effect swung round on their common stem to change places with phenons IX and X.

Phenon IX remains as it was with the exception that two strains, PB 120 and PB 121, from subphenon 23 now show greater affinity with subphenon 22. Subphenon 22 consists of the two strains received as S. equi; subphenon 23 consists of some other Lancefield group C organisms. The two strains PB 120 and PB 121 are the only strains received as "S. zooepidemicus"; as these have broken away from subphenon 23, this now contains mainly strains received as "S. equisimilis".

Phenon X has split up into two arms. These were visible in the Gower dendrogram but were considered as one phenon on the basis of being over 78% similar. The lower of these two arms retains its original position from the Gower dendrogram at the base of subphenon 24. This arm comprises of subphenon 27, "Streptococcus sp. (groups R, S and T)" as well as two satelitte strains from the original phenon, strains PB 42 and PB 123. The other arm of the phenon has linked to the base of phenon IV and contains the subphenons 25 and 26 as well as two loosely linked strains, PB 122 and PB 158.

3.2.5 Pattern coefficient.

The Pattern coefficient ($\underline{D_P}$) is described in Section 2.10.3. It is a measure of difference between OTUs and is also related to their vigour values. As a result, strains showing a large vigour difference may appear more similar when their Pattern difference is considered than with the more conventional $\underline{S_G}$ or $\underline{S_{SM}}$. A table of vigour values for the 202 OTUs, derived from the method given in Section 2.10.3, is shown in Appendix V.

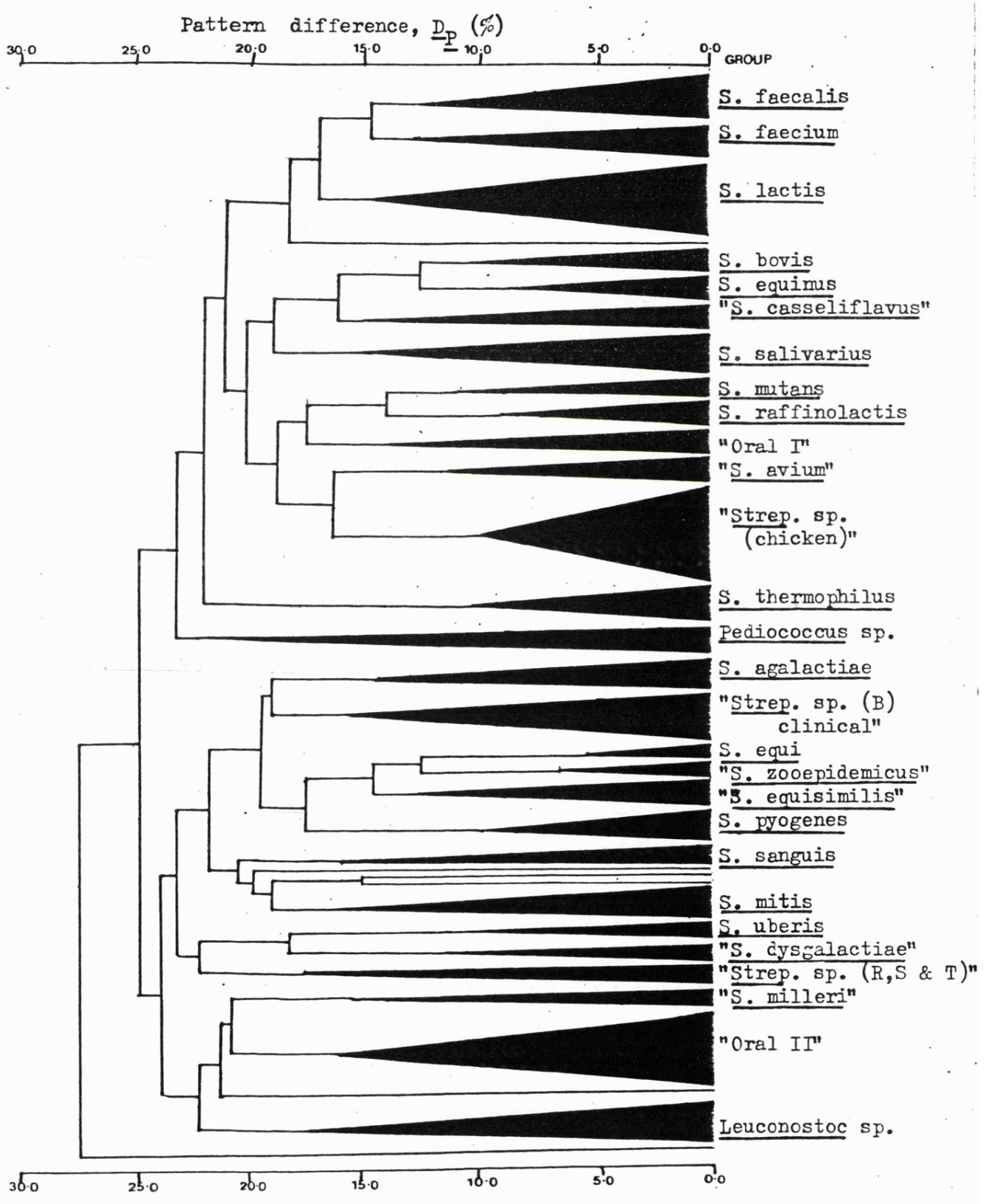
Figure 3.2.6 shows the dendrogram representing the difference between OTUs as described by the Pattern coefficient and average linkage clustering. Figure 3.2.7 shows a simplied version of this dendrogram (which has had all the loosely linked and ungrouped strains removed). The Pattern coefficient is calculated on binary characters although recent work (Sackin, 1981) has enabled its use with quantitiative characters.

The ten major phenons seen in the Gower dendrogram are all represented in the Pattern dendrogram and these contain all of the previously mentioned subphenons. The only exception to this is seen in phenon IV, where subphenon 13 has become scattered; the same occurred in the $\underline{S_{SM}}$ dendrogram. The numbers from the $\underline{S_G}$ average linkage dendrogram are used.

Phenon I is as described for the $\underline{S_G}$ dendrogram with three exceptions. Two strains, PB 77 and PB 85, from subphenon 1 have been lost. The three loosely linked strains shown in the $\underline{S_G}$

Figure 3.2.6 Average linkage dendrogram using Pattern difference for 157 characters and 202 strains. Cophenetic correlation coefficient is 0.6308.

Figure 3.2.7 Simplified dendrogram using the Pattern difference and average linkage clustering.



dendrogram, PB 16, PB 125 and PB 140, have also been lost and appear further down the dendrogram. Strain PB 92, received as "Streptococcus sp. (MG)", has moved up the dendrogram from subphenon 7 (S. salivarius) and now appears loosely linked to subphenon 1. Subphenon 2 (S. faecium) remains unchanged.

Phenon II has moved to a position further down the dendrogram. However, this is the only change as subphenons 3 and 4 remain as before.

Phenon III occupies the same position in this dendrogram as in the Gower representation. Subphenons 7 and 8 have changed positions. Subphenon 9 (S. mutans) now consists of only two strains, but one strain, PB 76, has been lost. Subphenons 10 and 11 remain unchanged.

Phenon I has moved up the dendrogram and now occupies the position previously held by phenon II. The strains previously assigned to subphenon 12(S. lactis)are still present although one strain, PB 77, previously a member of subphenon 1 (S. faecalis) and received as S. faecalis, has joined at the centre of this cluster. At the base of this subphenon there are five loosely linked strains. Two of these PB 125 and PB 140, were previously loosely linked to the base of subphenon 2. Strain PB 107, which is also present constituted part of subphenon 13. It is now the only representative of the subphenon linked to subphenon 12. This strain is also loosely linked to two other strains PB 124 and PB 151. These strains had been previously loosely linked to the base of subphenon 14 (S. thermophilus).

Phenon V contains subphenon 14. This appears as before in the Gower dendrogram although the previously loosely linked strains in this phenon have been lost.

Phenon VI has moved further down the dendrogram. Subphenon 15 appears with five loosely linked strains attached to it. Two of these, PB 89 and PB 101, were previously assigned to this subphenon, (S. mitis) whereas one strain, PB 148 has been acquired from the scattered subphenon 13. One strain appeared as a loosely linked strain, PB 170, in this position in the S_G dendrogram. The fifth strain, PB 133, had previously been considered as a central strain in subphenon 24, "Streptococcus sp. (B) clinical". Subphenon 16, S. sanguis is as seen before. Also appearing with subphenon 16 however, are three strains that were previously loosely linked to it. Attached to these in turn is one strain, PB 149, which was previously considered a member of subphenon 13.

Phenon VII appears as before although now it has moved down the dendrogram. Subphenons 17 and 18 are the same as before. One strain, PB 43, appears loosely linked to subphenon 18, "S. milleri". This strain had previously been loosely linked to subphenon 15. Four strains appear loosely linked to subphenon 17, "Oral II". Three of these, PB 60, PB 66 and PB 70, had previously been loosely attached to subphenon 18, while one strain, PB 76 had previously been assigned to subphenon 9 (S. mutans).

Phenon VIII has moved to the bottom of the dendrogram but links as previously with subphenon VII. Phenon VIII consists wholly of subphenon 19 (Leuconostoc sp.) and this remained unchanged.

Phenon IX consists of five subphenons, 20-24. The phenon appears higher up the dendrogram than before but remains complete. Subphenon 20 (S. agalactiae) is as before, as is subphenon 21 (S. pyogenes). However, subphenon 21 has moved away from subphenon 20 to which it was previously linked. The position adjacent to subphenon 20 has been taken by subphenon 24, "Streptococcus sp. (B) clinical". One strain previously assigned to this group, PB 133, has been lost and now appears as a loosely linked strain at the base of subphenon 16. The arrangement of subphenons 22 and 23, S. equi and "S. equisimilis" are as seen in the S_{SM} dendrogram, with the two strains of "S. zooepidemicus" linking to subphenon 22 instead of subphenon 23 as in the S_G dendrogram.

Phenon X is as described in the S_G dendrogram with the exception of one strain, PB 123. This strain appears as a loosely linked strain at the base of the phenon. This strain was received as "S. suis". On the S_G dendrogram it was loosely linked at the base of phenon VI.

Subphenon 28 has moved to the centre of the D_P dendrogram, joining at the base of the cluster containing phenons I, II, III, IV and V. One strain, PB 108, remains in its original position at the base of phenon X, as seen in the S_G dendrogram. Three strains, PB 109, PB 110 and PB 169 have joined subphenon 28. These were all previously loosely linked in this position on the S_G dendrogram.

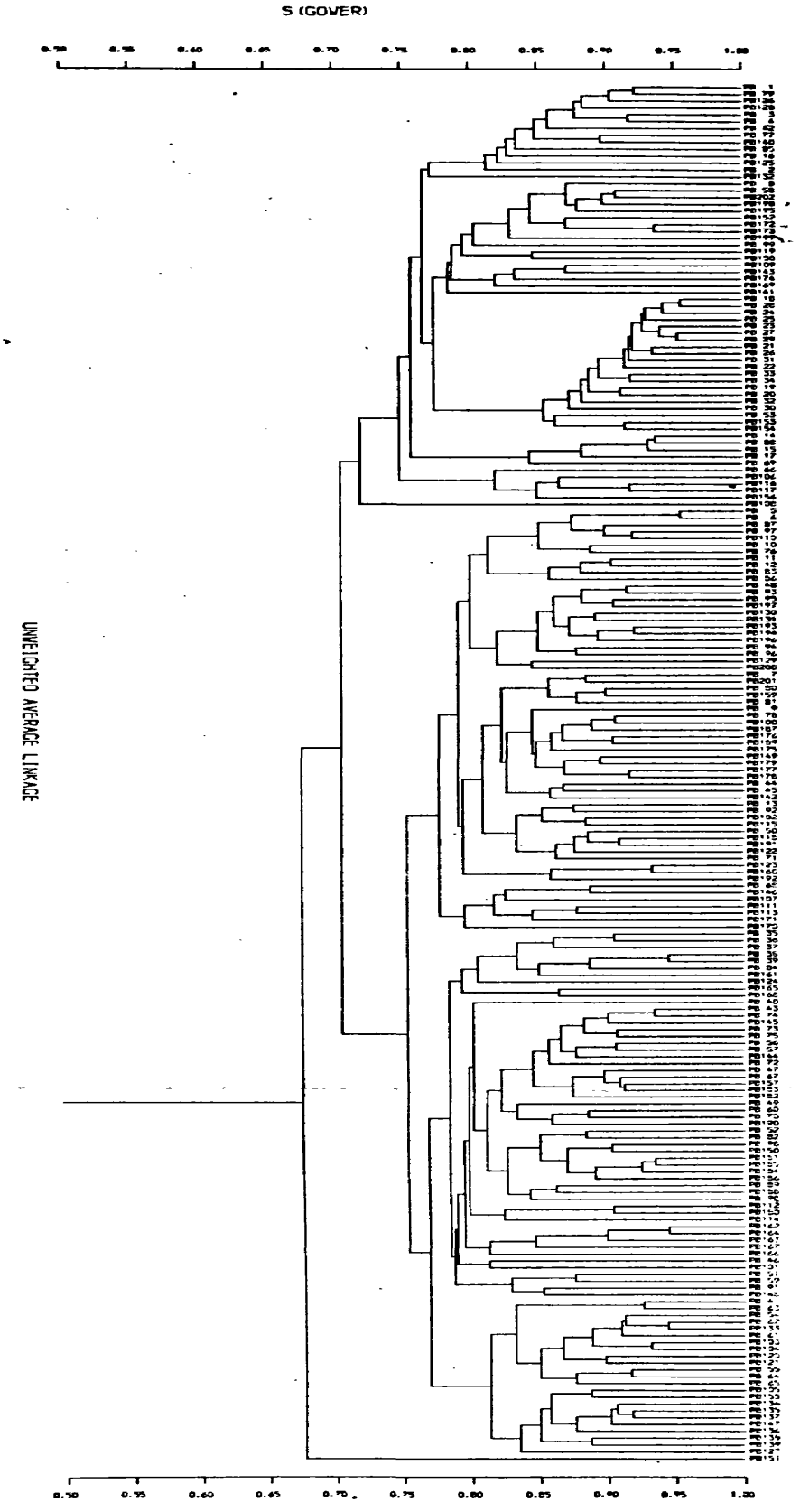
3.2.6 $\underline{S_G}$ dendrogram from test kit results

Test results for all characters other than those contained on the two API galleries were deleted. This enabled the construction of a similarity matrix and dendrogram using the $\underline{S_G}$ coefficient and average linkage clustering based solely on the fourty-nine API 50E tests and the nineteen APIzym tests. This dendrogram is shown in Figure 3.2.8. The Cophenetic Correlation coefficient of this dendrogram was 0.645. The upper arm of the dendrogram is separated at 70% from the rest of the dendrogram, the most dissimilar strain being separated at 67.5%. The upper arm of the dendrogram consists of three clusters and eleven other strains. As before clusters are numbered as in the full $\underline{S_G}$ dendrogram.

The first cluster on the dendrogram corresponds with subphenon 1 (S. faecalis) as shown on the full $\underline{S_G}$ dendrogram, with the addition of five strains and the loss of one. Three of these strains, PB 140, PB 16 and PB 125, were previously loosely linked within phenon I, one other strain, PB 42, was previously loosely linked within phenon X. The remaining strain, PB 132, has been acquired from subphenon 23, "S. equisimilis". The strain lost from subphenon 1 is PB 129.

The second cluster contains representatives from subphenon 9, S. mutans, (PB 8 and PB 58), and subphenon 10, S. raffinolactis, (PB 202 and PB 198). All three strains assigned to subphenon 11, "Oral I", are present in this cluster, although one strain, PB 174, is separated from the other two, PB 172 and PB 173. Nine other strains are also present. Four of them,

Figure 3.2.8 Average linkage dendrogram using Gower's coefficient for the 68 characters contained in the API 50E and APIzym galleries and 202 strains. Cophenetic correlation coefficient is 0.6454.



PB 152, PB 109, PB 143 and PB 169, were previously loosely linked in phenon II. One other strain, PB 158, was previously loosely linked in phenon X to subphenons 25 and 26, S. uberis and "S. dysgalactiae". The other four strains, PB 195, PB 99, PB 119 and PB 141, were all previously assigned to a diverse collection of subphenons. These were 12, S. lactis, 7, S. salivarius, 25, S. uberis and 24, "Streptococcus sp. (B) clinical".

The third cluster is similar to the previously described subphenon 4, although arranged in a different order. The last three strains, PB 53, PB 153 and PB 154 were all previously linked loosely within phenon II.

The eleven strains at the base of this arm form two branches, one strain, PB 108, remaining separate. The upper branch of this cluster corresponds to subphenon 3, "S. avium", and includes the strain, PB 69, which previously appeared as a satellite of this group. The lower branch consists of three strains, PB 106, PB 116 and PB 117. These previously formed subphenon 26, "S. dysgalactiae". Two other strains present on this arm are PB 66 and PB 156. Strain PB 66 was previously loosely linked in phenon VII and strain PB 156 was previously loosely linked in phenon II. The single strain, PB 108, seen on this arm was previously seen in the loosely linked subphenon 28.

The next arm of the dendrogram contains six main clusters, all linked at about 78% similarity. The first cluster contains eleven strains. Seven of these, PB 5, PB 6, PB 87, PB 97, PB 10,

PB 76 and PB 86 were all received as either S. faecium or "S. durans" and were previously seen in subphenon 2 (S. faecium). Three strains, PB 11, PB 12 and PB 83, were previously seen in subphenon 6 (S. equinus). One strain, PB 110, was previously loosely linked to the base of subphenon 4, "Streptococcus sp. (chicken)". The second cluster consists of thirteen strains. The first eleven of these, from PB 48 to PB 96, were all previously assigned to subphenon 12 (S. lactis). Of the remaining two strains, one, PB 129, was previously seen in subphenon 1 (S. faecalis), and the other, PB 200, was previously assigned to subphenon 10 (S. raffinolactis).

The third cluster contains five smaller subclusters of at least 80% similarity. The first of these subclusters contains five strains. Three of these PB 7, PB 80 and PB 81 were previously assigned to subphenon 5 (S. bovis). Of the other two strains, PB 201 was previously loosely linked to phenons III and IV, and PB 159 was previously assigned to subphenon 27 "Streptococcus sp. (groups R, S and T)". The second subcluster contains eleven strains. Five of these, strains PB 9, PB 100, PB 176, PB 189 and PB 175, were previously assigned to subphenon 7 (S. salivarius). Also in this cluster are four strains, PB 187, PB 179, PB 177 and PB 178. These were previously assigned to subphenon 17, "Oral II". Also in this cluster are two strains, PB 78 and PB 149. These were previously assigned to subphenon 5 (S. bovis) and subphenon 13 (A. viridans) respectively. The third subcluster contains three strains. One of these, PB 44 was

previously placed in subphenon 23, "S. equisimilis". The other two strains, PB 45 and PB 142, were both previously observed as loosely linked to this subphenon. The fourth subcluster contains nine strains. The three central strains, PB 115, PB 50 and PB 118 were previously assigned to subphenon 25 (S. uberis). The two strains, PB 191 and PB 122 were previously loosely linked to this group. Of the four remaining strains, one, PB 13 was previously assigned to subphenon 6, one, PB 92, to subphenon 7. The remaining two strains were both previously loosely linked; PB 102 to subphenon 16 and the other, PB 71, to subphenon 14. The fifth subcluster contains three strains, two of which, PB 160 and PB 192, were previously assigned to subphenon 27, "Streptococcus sp. (groups R, S and T)". The third strain, PB 123, had been previously loosely linked to subphenon 16 (S. sanguis).

The next cluster consists of seven strains. Two of these, PB 68 and PB 146 were previously found in subphenon 16 and one strain, PB 107 was previously assigned to the loosely linked subphenon 28. Strain PB 171 had previously been assigned to subphenon 11, "Oral I", while strain PB 170, which is loosely linked to this cluster had previously been loosely linked to subphenon 15 (S. mitis).

The next arm of the dendrogram contains a cluster of seven strains with three other strains linked to it. Strains PB 35, PB 36, PB 37, PB 38, PB 39 and PB 84 are arranged in this cluster with the strain PB 161. The former were all

previously assigned to subphenon 14 (S. thermophilus) the latter having been placed in subphenon 19 (Leuconostoc sp.). Strain PB 161 was received as Gemella haemolysans. The remaining three strains in this cluster were loosely grouped. Strains PB 165 and PB 168 formed a pair at the base of the cluster. PB 165 was received as Leuconostoc lactis and was also previously assigned to subphenon 19. Strain PB 168 was received as Pediococcus halophilus and was previously linked as a satellite to subphenon 19. The other strain in this cluster, PB 124, was previously loosely linked to subphenon 14.

The next strain to appear in the dendrogram, PB 40, is loosely linked to the next two clusters but appears on its own. It was previously assigned to subphenon 20 (S. agalactiae).

The next cluster is a large one of eighteen strains. Four of these, PB 74, PB 73, PB 75 and PB 72, were previously assigned to subphenon 20 along with the strain PB 40. Although in this case they all appear separated. Three strains, PB 56, PB 57 and PB 144 appear together. These strains previously constituted subphenon 18, "S. milleri". Strains PB 60 and PB 70 which appear near the base of this cluster were previously loosely linked to subphenon 18. Three strains, PB 183, PB 182 and PB 190 appear separated in this cluster. These are "oral" strains which were previously assigned to subphenon 17, "Oral II". Strains PB 47 and PB 157 were previously loosely linked to this subphenon. One strain PB 67 was previously assigned to subphenon 16 (S. sanguis). The three remaining strains PB 43, PB 145

and PB 49, were all previously loosely linked strains, being linked to subphenons 15, 16 and 14 respectively.

The next cluster contains eleven strains. Five of these, PB 181, PB 185, PB 184, PB 186, and PB 188, were all previously assigned to subphenon 17, "Oral II". Four strains, PB 98, PB 150, PB 89 and PB 90 were previously assigned to subphenon 15 (S. mitis). The two remaining strains, PB 52 and PB 82 were previously assigned to subphenon 8, "S. casseliflavus".

The next arm consists of three loosely linked strains. Two of these, PB 112 and PB 114 were previously assigned to the loosely linked subphenon 28. The third strain, PB 180 had been seen in the full S_G dendrogram in subphenon 17, "Oral II".

The next section of the dendrogram consists of three groups which show 79% similarity and are very closely linked. The first of these contains the five strains PB 162, PB 164, PB 163, PB 167 and PB 166. These are all representatives of the genus Leuconsotoc and were named as such in subphenon 19. The second group consists of two loosely linked strains, PB 46 and PB 101. These were previously associated with subphenon 15 (S. mitis), PB 101 being assigned to that group and PB 46 being loosely linked to it. The third group consists of four strains linked as two pairs. One pair, PB 51 and PB 59 were previously assigned to subphenon 8, "S. casseliflavus". The other pair consists of PB 91 and PB 148; these were previously assigned to subphenons 15 and 13 respectively.

The next cluster on the dendrogram consists of thirteen strains. Six of these, PB 54, PB 62, PB 61, PB 55, PB 64 and PB 65 represent the previously designated subphenon 21 (S. pyogenes), although they are now no longer as distinct as seen in the full S_G dendrogram. Four of the remaining strains, PB 103, PB 104, PB 120 and PB 121 were previously assigned to subphenon 23, "S. equisimilis". Of the remaining strains, PB 41 and PB 63 represent the previously designated subphenon 22 (S. equi). The strain PB 133 appears near the centre of the cluster. This is a representative of subphenon 24, "Streptococcus sp. (B) clinical". The next cluster consists of ten strains. The first two of these, PB 105 and PB 155 were previously assigned to subphenon 23. The remaining eight strains, PB 134, PB 135, PB 137, PB 147, PB 136, PB 138, PB 139 and PB 127, were all previously assigned to subphenon 24 with the strain PB 133.

The final strain shown in the dendrogram, PB 151, was previously loosely linked to subphenon 14 (S. thermophilus). However, in this case it appears as a single strain linked at 67.5% similarity to the rest of the strains used in the study.

3.3 Integer groups

The twenty-eight subphenons seen in the full S_G dendrogram were considered as separate groups. These contained one hundred and sixty-five OTUs, thirty-seven strains remaining ungrouped. The OTUs assigned to each group are listed in Table 3.3.a. These strains were used in these groups as data for the I-GROUPS program. This program used all of the characters considered in

Table 3.3.a.

The OTUs assigned to the twenty-eight subphenons.

Subphenon 1. S. faecalis 10 OTUs.
PB 1, PB 2, PB 3, PB 4, PB 79, PB 126, PB 128, PB 77, PB 129, PB 85.
Subphenon 2. S. faecium 6 OTUs.
PB 5, PB 6, PB 10, PB 86, PB 97, PB 87.
Subphenon 3. "S. avium" 4 OTUs.
PB 14, PB 15, PB 17, PB 88.
Subphenon 4. "Streptococcus sp. (chicken)" 17 OTUs.
PB 18, PB 20, PB 25, PB 29, PB 22, PB 24, PB 28, PB 19, PB 21,
PB 23, PB 27, PB 31, PB 33, PB 32, PB 34, PB 30, PB 26.
Subphenon 5. S. bovis 4 OTUs.
PB 7, PB 80, PB 81, PB 78.
Subphenon 6. S. equinus 4 OTUs.
PB 11, PB 13, PB 12, PB 83.
Subphenon 7. S. salivarius 7 OTUs.
PB 9, PB 175, PB 176, PB 189, PB 92, PB 99, PB 100.
Subphenon 8. "S. casseliflavus" 4 OTUs.
PB 51, PB 52, PB 59, PB 82.
Subphenon 9. S. mutans 3 OTUs.
PB 8, PB 58, PB 76.
Subphenon 10. S. raffinolactis 4 OTUs.
PB 198, PB 202, PB 199, PB 200.
Subphenon 11. "Oral I" 4 OTUs.
PB 171, PB 172, PB 173, PB 174.
Subphenon 12. S. lactis 12 OTUs.
PB 48, PB 93, PB 94, PB 95, PB 96, PB 197, PB 130, PB 131,
PB 193, PB 194, PB 196, PB 195.
Subphenon 13. A. viridans 3 OTUs.
PB 107, PB 148, PB 149.
Subphenon 14. S. thermophilus 6 OTUs.
PB 35, PB 36, PB 37, PB 38, PB 39, PB 84.

Table 3.3.a. continued

Subphenon 15. S. mitis 6 OTUs.
PB 89, PB 101, PB 90, PB 91, PB 98, PB 150.
Subphenon 16. S. sanguis 3 OTUs.
PB 67, PB 68, PB 146.
Subphenon 17. "Oral II" 13 OTUs.
PB 177, PB 178, PB 179, PB 187, PB 188, PB 180, PB 182, PB 190,
PB 181, PB 185, PB 186, PB 183, PB 184.
Subphenon 18. "S. milleri" 3 OTUs.
PB 56, PB 57, PB 144.
Subphenon 19. Leuconostoc sp. 7 OTUs.
PB 161, PB 165, PB 162, PB 163, PB 164, PB 166, PB 167.
Subphenon 20. S. agalactiae 5 OTUs,
PB 40, PB 72, PB 75, PB 73, PB 74.
Subphenon 21. S. pyogenes 6 OTUs.
PB 54, PB 55, PB 61, PB 62, PB 64, PB 65.
Subphenon 22. S. equi 2 OTUs.
PB 41, PB 63.
Subphenon 23. "S. equisimilis" 8 OTUs.
PB 44, PB 103, PB 104, PB 132, PB 120, PB 121, PB 105, PB 155.
Subphenon 24. "Streptococcus sp. (B) clinical" 10 OTUs.
PB 127, PB 139, PB 141, PB 133, PB 134, PB 137, PB 138, PB 147,
PB 135, PB 136.
Subphenon 25. S. uberis 3 OTUs.
PB 50, PB 118, PB 119.

Table 3.3.a. continued

Subphenon 26. "S. dysgalactiae" 3 OTUs.

PB 106, PB 117, PB 116.

Subphenon 27. "Streptococcus sp. (R, S and T)" 3 OTUs.

PB 159, PB 160, PB 192.

Subphenon 28. Pediococcus sp. 5 OTUs.

PB 108, PB 111, PB 113, PB 112, PB 114.

the taxonomy. The percentage positive results for each character are listed in Appendix VI. The information contained in Appendix VI was used to derive tables that differentiated between the different phenons and subphenons as described in Section 2.10.4. Table 3.3.b lists tests that may be used to differentiate between the ten phenons and also the loosely linked subphenon 28. If a positive result is taken as being a level of 80% or above, then fifty-three of the fifty-five possible pairs of phenons may be separated. There is however no single character that will differentiate between phenons VI and IX or phenons VII and IX. Tables 3.3.c-3.3.g list tests that may aid in the differentiation of subphenons within each phenon. Table 3.3.h lists tests that will aid in the differentiation of subphenons of phenons VI, VII and IX. The API test results were considered as positive if a reaction of 3 or above was seen for the API 50E and 2 or above for the APIzym galleries.

Six further tables, 3.3.j-3.3.p list tests that may be used in the differentiation of some further subphenons. These were chosen to demonstrate both similarities and differences outside the phenon groupings.

The I-GROUPS program also calculates the inter- and intra-group similarities. These were used to produce a combined matrix constructed from the mean variance and standard deviation of these. This matrix is shown in Figure 3.3.1. The Euclidean distances between individual strains and the centroids of all of the groups were also calculated. The average Euclidean distances between groups were used to provide data for the OVCLUST program

Table 3.3.b Test results that may aid in the differentiation of the main phenons.

	Phenons										
Character	I	II	III	IV	V	VI	VII	VIII	IX	X	28
11. No haemolysis	44	0	57	33	33	0	6	0	3	33	69
13. Final pH between 4.25 and 4.75	0	0	3	7	0	56	6	0	45	89	40
16. Growth at 10°C	100	100	17	93	0	22	13	43	10	55	60
18. Growth at 45°C	100	100	70	20	100	100	25	0	26	11	80
20. Growth with 4% NaCl	100	100	67	60	17	22	0	67	68	67	80
21. Growth with 6.5% NaCl	100	100	3	7	0	0	0	0	0	0	60
24. Growth with 0.0002% crystal violet	19	52	53	33	100	22	100	57	39	78	100
25. Growth with 0.0004% crystal violet	6	14	20	7	0	0	88	100	35	78	80
27. Growth at pH 9.6	100	100	7	7	0	0	0	0	0	0	20
31. Reduction of janus green B milk	88	100	40	80	100	0	0	14	52	78	80
36. Production of clot in litmus milk	50	19	57	60	100	67	13	0	39	0	80
38. V.P.	94	100	60	53	17	11	38	14	10	33	80
42. Hydrolysis of hippurate	56	10	3	60	0	33	6	14	55	89	40
43. Hydrolysis of arginine	100	5	17	73	17	89	38	0	100	100	80
44. Hydrolysis of aesculin	100	100	100	73	33	0	13	43	19	100	80

Table 3.3.b continued

Character	Phenons I	II	III	IV	V	VI	VII	VIII	IX	X	28
47. Growth on MacConkey agar	100	100	3	13	0	0	0	0	39	0	100
48. Red colonies on MacConkey agar	100	62	3	13	0	0	0	0	32	0	100
50. Yellow colonies on TCBS agar	50	90	0	13	0	0	0	0	0	0	60
54. Phosphatase (plate)	56	100	0	13	33	11	19	14	74	22	40
59. Arginine decarboxylation	88	10	23	93	0	44	88	43	77	78	100
66. Acid from L (+) arabinaose	44	100	10	20	0	11	0	71	10	11	60
67. Acid from D (+) xylose	25	100	23	47	17	0	0	14	10	0	0
76. Acid from rhamnose	38	29	20	0	0	11	6	0	3	22	60
79. Acid from mannitol	94	100	68	20	17	11	25	57	16	78	20
80. Acid from sorbitol	94	100	53	13	17	11	19	0	19	89	0
81. Acid from methyl-D-glucoside	13	100	40	47	0	44	25	71	61	78	40
82. Acid from N-acetyl glucosamine	100	100	100	100	0	100	100	86	100	100	100
84. Hydrolysis of aesculin (API)	100	100	87	87	0	22	13	14	19	78	60
98. Methyl red (API)	88	95	73	100	83	44	25	0	71	67	60
129. Acid from amygdalin	100	100	67	67	0	22	13	14	13	67	60

Table 3.3.b continued

Character	Phenons										
	I	II	III	IV	V	VI	VII	VIII	IX	X	28
134. Growth with 10% bile	100	100	70	100	100	67	13	100	68	56	20
142. Possession of leucine arylamidase	100	100	80	80	0	89	56	0	65	100	60
147. Possession of acid phosphatase	56	86	63	93	0	56	50	14	10	67	40
157. Growth with 40% bile	100	100	50	53	0	44	0	43	32	22	0

Table 3.3.c Test results which may aid in the differentiation of subphenons of phenons I and II.

	Subphenons			
Character	1	2	3	4
15. Growth at 4°C	10	100	0	6
30. Reduction of methylene blue milk	100	67	25	0
42. Hydrolysis of hippurate	90	0	0	100
43. Hydrolysis of arginine	100	100	25	0
65. Acid from D (−) arabinose	40	0	100	6
67. Acid from D (+) xylose	40	0	100	100
146. Possession of chymotrypsin	90	17	0	100
147. Possession of acid phosphatase	90	0	25	100
150. Possession of β-galactosidase	90	50	75	100

Table 3.3.d Test results that may aid in the differentiation of subphenons of phenon III.

Character	Subphenons 5	6	7	8	9	10	11
18. Growth at 45°C	100	100	83	100	100	0	25
24. Growth with 0.0002% crystal violet	0	0	67	25	100	75	100
29. Reduction of nitrite	100	100	50	100	100	0	100
32. Reduction of tetrazolium	100	0	83	0	100	25	50
34. Reduction of selenite	0	0	100	25	0	100	50
36. Production of clot in litmus milk	100	25	100	50	70	50	0
38. V.P.	100	0	67	100	70	0	100
80. Acid from sorbitol	0	0	17	100	100	100	75
81. Acid from methyl-D-glucoside	25	0	17	75	100	100	0
88. Acid from lactose	100	0	0	100	100	100	75
89. Acid from D (+) melibiose	100	0	50	50	100	75	75
92. Acid from inulin	50	25	50	0	100	75	50
96. Acid from starch	100	0	33	100	70	75	50
97. Acid from glycogen	75	0	0	0	30	0	0
123. Colony diameter below 0.2 mm	25	0	0	0	100	50	75

Table 3.3.d continued

Character	Subphenons 5	6	7	8	9	10	11
124. Colony diameter between 0.2 and 0.4 mm	50	100	100	100	0	50	25
126. Acid from glycerol	25	0	0	25	30	50	100
128. Acid from methyl-D-mannoside	0	0	0	100	70	25	0
130. Acid from D (+) melezitose	0	50	33	100	30	75	75
132. Lipase	50	100	67	100	70	0	50
133. Tetrathionate reductase	100	100	67	100	70	50	0
134. Growth with 10% bile	100	100	33	100	100	100	0
147. Possession of acid phosphatase	100	0	83	25	70	100	50
153. Possession of β-glucosidase	50	100	50	0	100	100	25
157. Growth with 40% bile	100	100	17	100	70	0	0

Table 3.3.e Test results which may aid in the differentiation of subphenons of phenons IV and V.

Character	Subphenons 12	13	14
16. Growth at 10°C	92	100	0
18. Growth at 45°C	0	100	100
30. Reduction of methylene blue milk	75	100	0
42. Hydrolysis of hippurate	50	100	0
59. Decarboxylation of arginine	92	100	0
63. Hydrolysis of starch	8	100	83
82. Acid from N-acetyl glucosamine	100	100	0
86. Acid from D (+) cellobiose	92	100	0
147. Possession of acid phosphatase	92	100	0
148. Possession of phosphoamidase	92	33	0
153. Possession of β-glucosidase	92	0	0
157. Growth with 40% bile	42	0	100

Table 3.3.f Test results which may aid in the differentiation of subphenons of phenons VII and VIII.

Character	Subphenons 17	18	19
30. Reduction of methylene blue milk	8	67	100
43. Hydrolysis of arginine	31	100	0
51. Gelatin liquefaction	0	0	100
55. Production of H_2O_2	100	67	0
134. Growth with 10% bile	15	0	100
135. Hydrolysis of casein	0	100	0
136. Growth on acetic acid-acetate agar	0	0	100
142. Possession of leucine arylamidase	61	100	0
148. Possession of phosphoamidase	54	100	0

Table 3.3.g Test results which may aid in the differentiation of subphenons of phenon X and subphenon 28.

Character	Subphenons 25	26	27	28
9. ∠-haemolysis	100	100	0	40
16. Growth at 10°C	100	0	67	60
18. Growth at 45°C	0	0	33	80
21. Growth with 6.5% NaCl	0	0	0	60
34. Reduction of selenite	0	100	0	40
38. V.P.	100	0	0	80
47. Growth on MacConkey agar	0	0	0	100
48. Red colonies on MacConkey agar	0	0	0	100
80. Acid from sorbitol	100	100	67	0
92. Acid from inulin	100	0	100	0
123. Colony diameter less than 0.2 mm	0	100	0	20
126. Acid from glycerol	100	100	0	100
134. Growth with 10% bile	100	0	67	20
138. Possession of alkaline phosphatase	100	100	0	40
147. Possession of acid phosphatase	100	100	0	40
148. Possession of phosphoamidase	100	33	0	20

Table 3.3.h Test results that may aid in the differentiation of phenons VI, VII and IX.

	Subphenons								
Character	15	16	17	18	20	21	22	23	24
9. α-haemolysis	83	100	100	33	20	0	0	12	60
10. β-haemolysis	17	0	0	33	80	100	100	88	30
12. Final pH below 4.25	33	67	92	100	100	0	50	50	70
18. Growth at 45°C	100	100	38	33	20	0	0	0	70
19. Growth with 3% NaCl	100	100	0	67	100	100	100	88	100
20. Growth with 4% NaCl	33	0	0	67	100	0	0	62	100
22. Growth with sodium azide	33	0	54	100	100	100	100	75	40
24. Growth with 0.0002% crystal violet	17	33	100	100	100	100	0	0	10
25. Growth with 0.0004% crystal violet	0	0	100	33	100	100	0	0	0
28. Reduction of nitrate	17	0	8	67	0	0	100	25	0
29. Reduction of nitrite	0	67	92	67	0	83	100	75	50
30. Reduction of methylene blue milk	100	0	8	67	0	0	50	12	70
31. Reduction of janus green B milk	0	0	0	0	0	0	0	100	80
32. Reduction of tetrazolium	67	0	100	67	100	100	0	100	80
33. Reduction of tellurite	0	0	0	0	0	0	100	88	60

Table 3.3.h continued

Character	Subphenons								
	15	16	17	18	20	21	22	23	24
34. Reduction of selenite	67	33	69	33	80	100	0	75	100
38. V.P.	0	33	38	100	20	0	0	12	10
42. Hydrolysis of hippurate	50	0	8	0	100	17	0	12	100
45. Oxidation of ethanol	17	67	54	0	100	33	0	25	0
47. Growth on MacConkey agar	0	0	0	0	100	50	0	0	40
49. Growth on TCBS agar	0	0	0	0	20	0	100	0	0
54. Phosphatase (plate)	17	0	23	0	100	0	0	100	100
62. DNase	0	0	0	0	40	100	0	25	50
81. Acid from methyl-D-glucoside	50	33	38	0	0	50	100	88	70
84. Hydrolysis of aesculin (API)	0	67	15	0	0	33	100	12	10
88. Acid from lactose	67	100	100	100	100	100	0	62	60
91. Acid from D (-) trehalose	17	100	77	100	100	100	0	75	100
92. Acid from inulin	17	100	31	0	0	0	0	12	10
94. Acid from dextrin	100	67	77	0	40	100	0	100	100
96. Acid from starch	50	33	31	0	20	83	100	100	80

Table 3.3.h continued

Character	Subphenons 15	16	17	18	20	21	22	23	24
97. Acid from glycogen	0	33	23	0	0	50	100	88	10
98. Methyl red (API)	50	33	38	0	40	33	100	88	90
106. Survival of 60°C for 15 min	87	67	15	33	100	0	0	85	100
123. Colony diameter less than 0.2 mm	17	33	38	100	0	0	0	0	10
126. Acid from glycerol	0	100	54	0	20	17	50	88	30
127. Acid from ribose	50	67	92	100	100	0	100	100	90
133. Tetrathionate reductase	17	33	92	100	80	100	100	100	0
134. Growth with 10% bile	83	67	15	0	100	0	50	75	70
135. Hydrolysis of casein	0	0	0	100	0	33	0	0	10
144. Possession of cysteine arylamidase	0	0	0	67	67	67	0	62	90
147. Possession of acid phosphatase	0	33	46	100	100	100	100	100	100
148. Possession of phosphoamidase	67	33	54	100	0	100	100	100	100
151. Possession of β-glucuronidase	0	0	0	0	17	17	100	88	90
152. Possession of α-glucosidase	0	33	38	100	0	100	50	100	100
157. Growth with 40% bile	33	67	0	0	0	0	0	0	50

Table 3.3.j Test results that may aid in the differentiation of subphenon 1 (*S. faecalis*) and subphenon 21 (*S. pyogenes*).

Character	Subphenons 1	21
10. β-haemolysis	0	100
12. Final pH below 4.25	100	0
13. Final pH between 4.25 and 4.75	0	100
16. Growth at 10°C	100	0
18. Growth at 45°C	100	0
20. Growth with 4% NaCl	100	0
21. Growth with 6.5% NaCl	100	0
25. Growth with 0.0004% crystal violet	10	100
27. Growth at pH 9.6	100	0
30. Reduction of methylene blue milk	100	0
31. Reduction of janus green B milk	100	0
33. Reduction of tellurite	100	0
35. Reduction of litmus milk	90	17
38. V.P.	100	0
42. Hydrolysis of hippurate	90	17
49. Growth on TCBS agar	100	0
50. Yellow colonies on TCBS agar	50	0
54. Phosphatase (plate)	90	0
62. DNase	0	100
76. Acid from rhamnose	50	0
78. Acid from meso-inositol	70	0

Table 3.3.j continued

Character	Subphenons 1	21
102. Citrate	50	0
106. Survival of 60°C for 15 min	100	0
107. Survival of 60°C for 1 h	100	0
125. Colony diameter above 0.4 mm	70	0
126. Acid from glycerol	100	17
127. Acid from ribose	100	0
129. Acid from amygdalin	100	0
131. Gluconate	70	0
134. Growth with 10% bile	100	0
146. Possession of chymotrypsin	90	0
150. Possession of β-galactosidase	90	0
153. Possession of β-glucosidase	100	0
157. Growth with 40% bile	100	0

Table 3.3.k Test results that may aid in the differentiation of subphenon 1 (*S. faecalis*) and subphenon 19 (*Leuconostoc* sp.)

Character	Subphenons 1	19
12. Final pH below 4.25	100	14
14. Final pH above 4.75	0	86
18. Growth at 45°C	100	0
19. Growth with 3% NaCl	100	29
20. Growth with 4% NaCl	100	0
21. Growth with 6.5% NaCl	100	0
22. Growth with sodium azide	100	14
25. Growth with 0.0004% crystal violet	10	100
27. Growth at pH 9.6	100	0
31. Reduction of janus green B milk	100	14
33. Reduction of tellurite	100	0
38. V.P.	100	14
42. Hydrolysis of hippurate	90	14
43. Hydrolysis of arginine	100	0
47. Growth on MacConkey agar	100	0
48. Red colonies on MacConkey agar	100	0
49. Growth on TCBS agar	100	0
50. Yellow colonies on TCBS agar	50	0
54. Phosphatase (plate)	90	14
76. Acid from rhamnose	50	0
78. Acid from meso-inositol	70	0

Table 3.3.k continued

Character	Subphenons 1	19
80. Acid from sorbitol	100	0
84. Hydrolysis of aesculin (API)	100	14
85. Acid from salicin	100	14
86. Acid from D (+) cellobiose	100	14
98. Methyl red (API)	80	0
126. Acid from glycerol	100	0
129. Acid from amygdalin	100	14
130. Acid from D (+) melezitose	80	14
136. Growth on acetic acid-acetate agar	0	100
142. Possession of leucine arylamidase	100	0
146. Possession of chymotrypsin	90	0
147. Possession of acid phosphatase	90	14
148. Possession of phosphoamidase	100	0
150. Possession of β-galactosidase	90	14
153. Possession of β-glucosidase	100	0

Table 3.3.1 Test results that may aid in the differentiation of streptococci of the Lancefield group C.

Character	Subphenons 22	23	26
10. β-haemolysis	100	88	0
24. Growth with 0.0002% crystal violet	0	0	100
28. Reduction of nitrate	100	25	0
31. Reduction of janus green B milk	0	100	100
32. Reduction of tetrazolium	0	100	67
33. Reduction of tellurite	100	85	0
34. Reduction of selenite	0	75	100
42. Hydrolysis of hippurate	0	12	100
49. Growth on TCBS agar	100	0	0
54. Phosphatase (plate)	0	100	0
79. Acid from mannitol	0	0	100
80. Acid from sorbitol	0	38	100
88. Acid from lactose	0	62	100
89. Acid from D (+) melibiose	0	12	100
91. Acid from D (-) trehalose	0	75	100
94. Acid from dextrin	0	100	100
106. Survival of 60°C for 15 min	0	88	100
123. Colony diameter less than 0.2 mm	0	0	100
132. Lipase	0	25	100

Table 3.3.m Test results that may aid in the differentiation of subphenons 13, 19 and 28 (*A. viridans*, *Leuconostoc* sp. and *Pediococcus* sp.).

	Subphenons		
Character	13	19	28
18. Growth at 45°C	100	0	80
43. Hydrolysis of arginine	100	0	80
47. Growth on MacConkey agar	0	0	80
48. Red colonies on MacConkey agar	0	0	100
51. Liquefaction of gelatin	0	100	0
86. Acid from D (+) cellobiose	100	14	80
136. Growth on acetic acid-acetate agar	0	100	80

Table 3.3.n Test results that may aid in the differentiation of subphenon 12 (*S. lactis*) from subphenon 1 (*S. faecalis*) and subphenon 2 (*S. faecium*).

Character	Subphenons 1	2	12
18. Growth at 45°C	100	100	0
21. Growth with 6.5% NaCl	100	100	0
27. Growth at pH 9.6	100	100	0
29. Reduction of nitrite	100	100	25
50. Yellow colonies on TCBS agar	50	50	0
79. Acid from mannitol	100	83	17
126. Acid from glycerol	100	100	0

Table 3.3.p Test results that may aid in the differentiation of subphenons 2 (*S. faecium*) and 8, "*S. casseliflavus*".

Character	Subphenons 2	8
15. Growth at 4°C	100	0
21. Growth with 6.5% NaCl	100	0
30. Reduction of methylene blue milk	67	0
31. Reduction of janus green B milk	67	0
49. Growth on TCBS agar	83	0
66. Acid from L (+) arabinose	67	0
81. Acid from methyl-D-glucoside	0	75
128. Acid from methyl-D-mannoside	0	100
130. Acid from D (+) melezitose	0	100
153. Possession of β-glucosidase	100	0

Figure 3.3.1 Combined matrix showing the mean inter- and intra-group similarities (based on the $\underline{D}_P$ coefficient). Below this is printed the variance of the mean inter- and intra- group similarities, and below these is the standard deviation of the mean inter- and intra-group similarities.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	0.101																											
	0.001																											
	0.034																											
2	0.147	0.098																										
	0.001	0.001																										
	0.023	0.029																										
3	0.194	0.171	0.092																									
	0.000	0.000	0.001																									
	0.020	0.019	0.034																									
4	0.202	0.186	0.163	0.076																								
	0.001	0.000	0.001	0.000																								
	0.023	0.021	0.035	0.020																								
5	0.210	0.198	0.221	0.193	0.091																							
	0.001	0.000	0.000	0.000	0.001																							
	0.026	0.021	0.021	0.021	0.033																							
6	0.195	0.179	0.195	0.209	0.135	0.086																						
	0.001	0.001	0.001	0.002	0.001	0.000																						
	0.027	0.026	0.036	0.038	0.031	0.006																						
7	0.219	0.218	0.203	0.200	0.160	0.182	0.153																					
	0.002	0.001	0.001	0.001	0.000	0.001	0.001																					
	0.044	0.033	0.027	0.028	0.020	0.024	0.025																					
8	0.198	0.177	0.184	0.189	0.174	0.180	0.201	0.128																				
	0.001	0.000	0.001	0.001	0.001	0.000	0.001	0.000																				
	0.031	0.022	0.026	0.028	0.027	0.021	0.025	0.015																				
9	0.228	0.237	0.230	0.211	0.160	0.190	0.182	0.185	0.126																			
	0.000	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.000																			
	0.018	0.025	0.018	0.025	0.029	0.013	0.022	0.018	0.015																			
10	0.195	0.222	0.198	0.181	0.190	0.202	0.176	0.193	0.153	0.102																		
	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.000	0.001	0.000																		
	0.030	0.029	0.027	0.025	0.024	0.019	0.024	0.021	0.024	0.010																		
11	0.238	0.247	0.221	0.202	0.199	0.223	0.188	0.224	0.169	0.189	0.115																	
	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.001																	
	0.025	0.023	0.026	0.030	0.018	0.023	0.036	0.017	0.030	0.026	0.035																	
12	0.183	0.182	0.203	0.210	0.179	0.195	0.183	0.205	0.201	0.170	0.232	0.121																
	0.001	0.002	0.001	0.001	0.000	0.000	0.001	0.001	0.001	0.001	0.001	0.001																
	0.033	0.034	0.030	0.028	0.020	0.018	0.030	0.024	0.024	0.027	0.028	0.035																
13	0.215	0.225	0.256	0.244	0.205	0.243	0.202	0.216	0.239	0.215	0.214	0.191	0.182															
	0.001	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.000	0.000															
	0.027	0.031	0.033	0.022	0.023	0.026	0.024	0.023	0.019	0.023	0.026	0.019	0.007															
14	0.221	0.204	0.173	0.204	0.189	0.209	0.204	0.198	0.238	0.246	0.251	0.235	0.245	0.109														
	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.002	0.001														
	0.038	0.030	0.034	0.031	0.032	0.032	0.029	0.027	0.023	0.029	0.013	0.037	0.039	0.025														
15	0.243	0.236	0.239	0.256	0.195	0.218	0.203	0.201	0.226	0.222	0.257	0.205	0.205	0.215	0.155													
	0.002	0.002	0.003	0.002	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001													
	0.040	0.039	0.043	0.045	0.029	0.023	0.028	0.045	0.029	0.029	0.026	0.032	0.028	0.036	0.028													
16	0.238	0.217	0.237	0.245	0.198	0.215	0.216	0.208	0.222	0.232	0.232	0.217	0.211	0.229	0.203	0.151												
	0.001	0.001	0.000	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.001												
	0.042	0.030	0.021	0.030	0.016	0.026	0.030	0.020	0.017	0.016	0.027	0.027	0.024	0.033	0.014	0.028												
17	0.285	0.270	0.259	0.251	0.224	0.257	0.212	0.233	0.229	0.245	0.203	0.251	0.242	0.230	0.225	0.205	0.146											
	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001											
	0.032	0.029	0.026	0.037	0.031	0.032	0.037	0.028	0.028	0.026	0.035	0.034	0.027	0.023	0.031	0.040	0.035											
18	0.219	0.245	0.265	0.266	0.209	0.230	0.199	0.228	0.201	0.220	0.238	0.216	0.241	0.238	0.226	0.215	0.198	0.127										
	0.000	0.000	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.001										
	0.017	0.016	0.029	0.028	0.016	0.027	0.026	0.022	0.028	0.027	0.020	0.022	0.021	0.025	0.020	0.019	0.028	0.033										
19	0.284	0.266	0.251	0.244	0.246	0.264	0.237	0.248	0.261	0.254	0.238	0.241	0.251	0.236	0.226	0.238	0.217	0.242	0.141									
	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002									
	0.023	0.028	0.025	0.034	0.019	0.027	0.024	0.031	0.027	0.030	0.029	0.025	0.029	0.028	0.030	0.019	0.025	0.025	0.039									
20	0.234	0.255	0.265	0.284	0.241	0.268	0.237	0.251	0.271	0.246	0.298	0.224	0.258	0.230	0.227	0.232	0.238	0.203	0.259	0.092								
	0.001	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000								
	0.026	0.015	0.021	0.025	0.024	0.023	0.029	0.021	0.014	0.022	0.012	0.030	0.025	0.028	0.027	0.031	0.026	0.024	0.023	0.017								
21	0.271	0.281	0.303	0.314	0.245	0.284	0.222	0.236	0.259	0.222	0.270	0.224	0.249	0.270	0.226	0.231	0.222	0.199	0.261	0.188	0.088							
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000							
	0.021	0.016	0.020	0.022	0.019	0.019	0.019	0.019	0.019	0.022	0.018	0.022	0.019	0.035	0.030	0.031	0.031	0.021	0.019	0.015	0.018							
22	0.253	0.247	0.290	0.271	0.201	0.229	0.237	0.226	0.229	0.233	0.275	0.206	0.247	0.267	0.219	0.221	0.251	0.216	0.272	0.226	0.174	0.070						
	0.001	0.001	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000						
	0.037	0.025	0.022	0.023	0.010	0.013	0.029	0.014	0.014	0.017	0.018	0.025	0.025	0.025	0.022	0.016	0.028	0.022	0.019	0.014	0.017	0.000						
23	0.227	0.263	0.272	0.252	0.214	0.253	0.233	0.236	0.249	0.219	0.274	0.196	0.244	0.248	0.215	0.227	0.244	0.217	0.266	0.208	0.186	0.152	0.136					
	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001					
	0.027	0.030	0.019	0.024	0.034	0.024	0.030	0.025	0.020	0.023	0.027	0.030	0.030	0.029	0.033	0.027	0.027	0.021	0.024	0.025	0.026	0.025	0.037					
24	0.227	0.265	0.275	0.280	0.228	0.242	0.232	0.235	0.269	0.237	0.274	0.201	0.224	0.235	0.199	0.235	0.243	0.228	0.261	0.197	0.224	0.219	0.180	0.162				
	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.000	0.000	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002				
	0.032	0.029	0.024	0.023	0.031	0.032	0.039	0.026	0.020	0.021	0.029	0.031	0.035	0.043	0.035	0.028	0.035	0.030	0.029	0.031	0.028	0.024	0.032	0.046				
25	0.236	0.245	0.241	0.221	0.199	0.218	0.199	0.208	0.220	0.202	0.208	0.194	0.207	0.249	0.223	0.206	0.228	0.230	0.250	0.230	0.233	0.248	0.216	0.239	0.103			
	0.001	0.000	0.001	0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.000			
	0.023	0.012	0.025	0.019	0.015	0.026	0.023	0.022	0.024	0.016	0.022	0.021	0.018	0.027	0.036	0.013	0.030	0.023	0.015	0.019	0.014	0.012	0.022	0.024	0.019			
26	0.271	0.273	0.273	0.247	0.202	0.241	0.219	0.208	0.222	0.191	0.190	0.215	0.231	0.242	0.210	0.217	0.196	0.209	0.241	0.239	0.199	0.205	0.190	0.212	0.176	0.094		
	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.000	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.001	0.001	0.000	0.000		
	0.021	0.017	0.020	0.017	0.014	0.022	0.029	0.023	0.018	0.024	0.021	0.025	0.028	0.015	0.022	0.013	0.025	0.016	0.025	0.016	0.022	0.013	0.023	0.025	0.008	0.021		
27	0.283	0.283	0.307	0.267	0.212	0.228	0.221	0.240	0.216	0.229	0.232	0.234	0.243	0.275	0.222	0.233	0.220	0.248	0.244	0.291	0.223	0.228	0.235	0.283	0.205	0.213	0.155	
	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.002	0.002	0.001	0.000	0.001	0.000	0.002	0.000	0.000	0.001	0.001	0.001	0.001	0.001	0.001	
	0.028	0.033	0.041	0.030	0.028	0.025	0.035	0.039	0.025	0.024	0.036	0.031	0.041	0.039	0.035	0.019	0.031	0.021	0.041	0.019	0.017	0.024	0.027	0.038	0.025	0.032	0.028	
28	0.237	0.236	0.236	0.257	0.251	0.265	0.255	0.272	0.290	0.279	0.257	0.261	0.285	0.283	0.260	0.258	0.269	0.265	0.273	0.259	0.285	0.292	0.292	0.282	0.255	0.266	0.297	0.229
	0.002	0.002	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.002	0.001	0.001	0.002	0.003
	0.041	0.040	0.036	0.026	0.019	0.023	0.028	0.021	0.033	0.022	0.026	0.034	0.034	0.036	0.028	0.048	0.029	0.025	0.025	0.031	0.022	0.020	0.031	0.041	0.036	0.026	0.045	0.052

(Sneath, 1979a).

The number of results that gave a clear cut positive or negative result was investigated. It would be expected that maximum separation of strains would depend more on these clear cut results than on others where a difference was recorded only in terms of different degrees of variability (Sneath, 1974). As a result of this the Figure 3.3.2 was produced. This shows the distribution of % positive test results within the twenty-eight groups that were considered for the I-groups computations. It can be seen from this figure that the majority of the test results are in the ranges 0-9% and 90-99% positive. This indicates that the results previously shown for the different phenons and subphenons are based primarily on clear cut positive or negative reactions.

The average Euclidean distances of each subphenon member from its centroid was investigated. The average distance was calculated as the root mean square of the squared distances. This method gives an idea of the relative compactness of some groups. However, in this case it was found that the majority of groups were of comparable sizes. The calculations were performed on the Leicester University Cyber 73 computer using the program PDBAED2. This program is listed in Appendix I. The results obtained by this method are shown in Table 3.3.q. These are based on the Euclidean distance measurements provided by the I-GROUPS program with binary characters. The division of the RMS by the square root of the number of characters gives a standardised value.

Figure 3.3.2 Distribution of positive test results for twenty-eight I-groups.

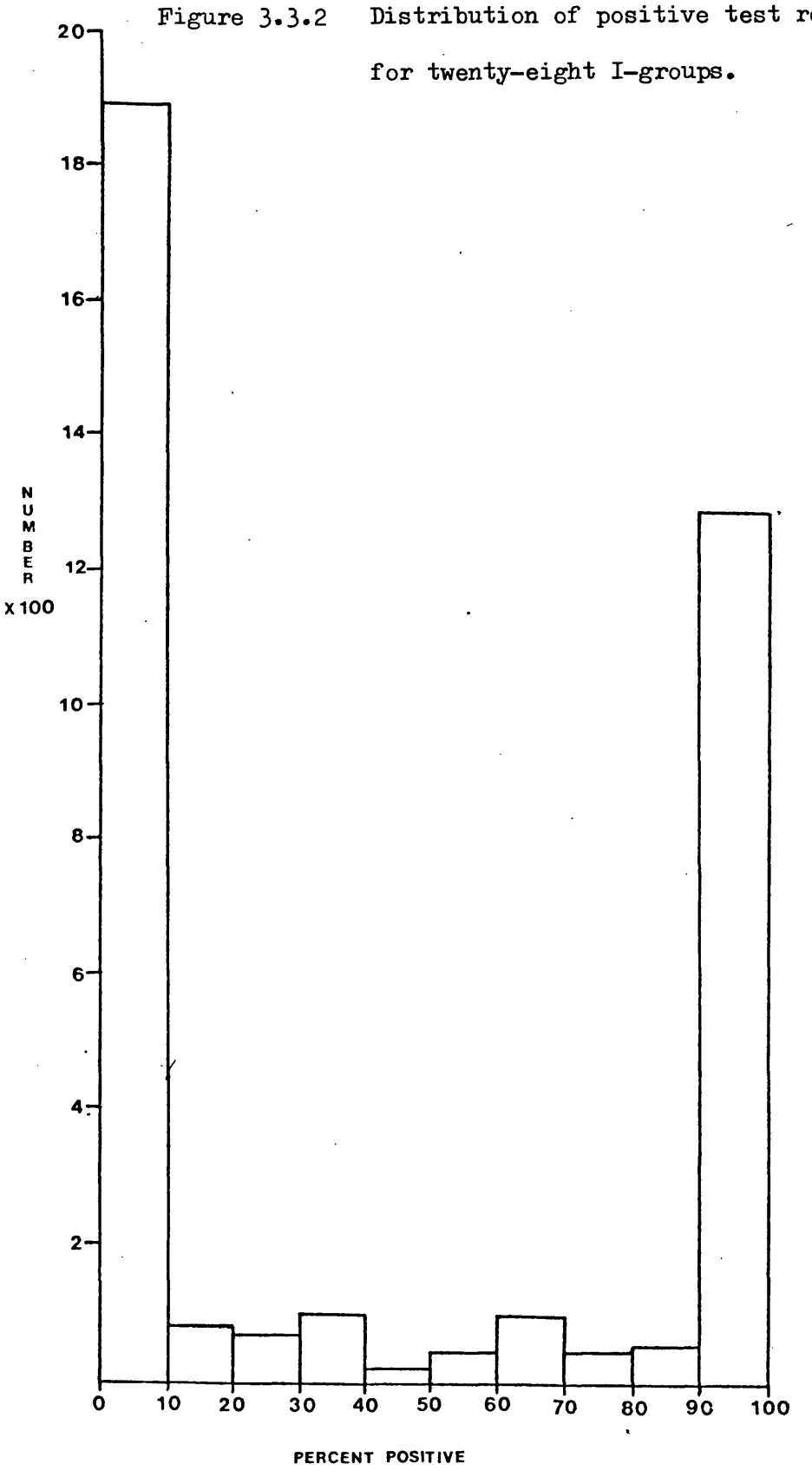


Table 3.3.q Root mean square Euclidean distances from centroids.

$$RMS=_2 \sqrt{\frac{\text{Sum of the squared distances}}{(\text{No. of group members} - 1)}}$$

Subphenon	RMS
1. *S. faecalis*	3.009
2. *S. faecium*	2.886
3. "*S. avium*"	2.723
4. "*Streptococcus* sp. (chicken)"	2.482
5. *S. bovis*	2.887
6. *S. equinus*	2.598
7. *S. salivarius*	3.517
8. "*S. casseliflavus*"	3.176
9. *S. mutans*	3.162
10. *S. raffinolactis*	3.069
11. "Oral I"	3.240
12. *S. lactis*	3.265
13. *A. viridans*	3.828
14. *S. thermophilus*	3.055
15. *S. mitis*	3.540
16. *S. sanguis*	3.464
17. "Oral II"	3.458
18. "*S. milleri*"	3.162
19. *Leuconostoc* sp.	3.430
20. *S. agalactiae*	2.702

Table 3.3.q continued

Subphenon	RMS
21. *S. pyogenes*	2.646
22. *S. equi*	2.345
23. "*S. equisimilis*"	3.325
24. "*Streptococcus* sp. (B) clinical"	3.642
25. *S. uberis*	2.887
26. "*S. dysgalactiae*"	2.769
27. "*Streptococcus* sp. (groups R, S and T)"	3.559
28. *Pediococcus* sp.	4.324

3.4 Integer group overlap calculations

The cluster overlap statistics between the groups used were calculated using data provided by the I-GROUPS program. The overlap was calculated by the program OVCLUST (Sneath 1979a). This is described in Section 2.10.5. The program was used to calculate parameters of the q distributions using a critical overlap value ($V_{(0)}$). This allows for the program to calculate whether the observed overlap is significantly less than this chosen critical value. This is achieved by a non-central t-test. The critical overlap value chosen for these calculations was 0.025. This corresponds to an index of disjunction measure ($W_{(0)}$) of 2.24138. This resulted in an overlap of that order or less being ignored. An estimated correction of error due to the number of strains and characters was used in these calculations, resulting in two results being given for the t-test for each pair of groups. Due to statistical inaccuracies inherent in comparing groups of small numbers of OTUs, only those groups containing more than three OTUs were considered.

The large number of subphenons considered in the study made it impractical to consider the apparent overlap between all possible pairs of groups. Overlap between groups that were adjacent in the phenograms was first considered and the results of this are given in Appendix VI. In cases where the adjacent group contained three or less members, the next nearest neighbour with more than three OTUs has been considered. The one exception to this is subphenon 9 (S. mutans). This was

considered in the calculations as although it contained only three OTUs, its nearest neighbours only contained four OTUs. As a result of this it was thought that the statistical inaccuracies may be less than in cases where a small group is compared to a large one. However, care should be taken with the results for S. mutans as they may not be representative. A further table in Appendix VI shows the recorded overlap between pairs of groups where some overlap might have been expected on the basis of previously published information.

Table 3.4.a shows a simplified form of these two tables.

An interactive version of the program OVCLUST was also used to give some measure of the amount of overlap between groups. This version (MJS8B19) did not require the entering of data from a terminal. Instead the data was taken directly from the I-GROUPS computations. This enabled all possible pairs to be studied.

In this case the critical overlap was set to that expected for a rectangular distribution. A rectangular distribution may contain between 5 and 8% overlap, depending upon the sample size (Sneath, 1979a). The observed overlap ($\underline{V}_G$) was considered for each pair of groups. No pair gave an uncorrected $\underline{V}_G$ value of greater than 1×10^{-3}.

Fourty-five pairs showed a $\underline{V}_G$ value of between 1×10^{-3} and 1×10^{-6}. Of these, sixteen were calculated on less than four degrees of freedom and so are not statistically valid. Of the remaining twenty-nine pairs, sixteen show an uncorrected

Table 3.4.a Recorded overlap between two groups (L and M). Critical overlap was 0.025. The t-test result means that there is that percentage probability that the observed overlap is less than the critical value. The groups shown here are; (1) those that appeared adjacent; and (2) those that may be expected to show some overlap.

$N_{(L)}$ = Number in group L

$N_{(M)}$ = Number in group M

W = Disjunction

$V_{(G)}$ = Overlap value

W (EST) = Disjunction after correction

$V_{(G,EST)}$ = Overlap after correction

Subphenons 1 and 2.

S. faecalis and S. faecium

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W (EST)	$V_{(G,EST)}$
10	6	4.0158	5.92522E-5	2.51293	1.9734E-2

t-test Above 90%

t-test (corrected) Below 90%

Subphenons 2 and 3.

S. faecium and "S. avium"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W (EST)	$V_{(G,EST)}$
6	4	9.60732	2.09359E-18	8.75217	2.09359E-18

t-test Above 99%

t-test (corrected) Above 99%

Table 3.4.a continued

Subphenons 3 and 4.

"S. avium" and "Streptococcus sp. (chicken)"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
4	17	2.74686	6.01690E-3	0.262824	0.792686

t-test: Below 90%

t-test (corrected): Below 90%

Subphenons 4 and 5.

"Streptococcus sp. (chicken)" and S. bovis

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
17	4	5.44288	5.25498E-8	4.70624	2.52617E-6

t-test: Above 90%

t-test (corrected): Above 90%

Subphenons 5 and 6.

S. bovis and S. equinus

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
4	4	8.57078	1.02826E-17	7.33712	2.81360E-13

t-test: Above 99%

t-test (corrected): Above 99%

Subphenons 6 and 7.

S. equinus and S. salivarius

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
4	6	4.29228	1.76970E-5	1.65037	9.88682E-2

t-test: Above 95%

t-test (corrected): Below 90%

Subphenons 7 and 8.

S. salivarius and "S. casseliflavus"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
6	4	4.34948	1.36563E-5	1.79388	7.28321E-2

t-test: Above 95%

t-test (corrected): Below 90%

Table 3.4.a continued

Subphenons 8 and 9.

"S. casseliflavus" and S. mutans

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W (EST)	$V_{(G,EST)}$
4	3	13.1197	2.53863E-39	12.2351	2.01773E-34

t-test: Above 99%

t-test (corrected): Above 99%

Subphenons 9 and 10.

S. mutans and S. raffinolactis

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W (EST)	$V_{(G,EST)}$
3	4	5.11857	3.08413E-7	1.94194	5.21438E-2

t-test: Above 95%

t-test (corrected): Below 90%

Subphenons 10 and 11.

S. raffinolactis and "Oral I"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W (EST)	$V_{(G,EST)}$
4	4	6.13702	8.41429E-10	4.24712	2.16676E-5

t-test: Above 95%

t-test (corrected): Above 90%

Subphenons 11 and 12.

"Oral I" and S. lactis

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W (EST)	$V_{(G,EST)}$
4	12	3.60225	3.15551E-4	1.77868	7.52928E-2

t-test: Below 90%

t-test (corrected): Below 90%

Subphenons 14 and 15

S. thermophilus and S. mitis

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W (EST)	$V_{(G,EST)}$
6	6	4.53454	5.77843E-6	2.73473	6.24332E-3

t-test: Above 95%

t-test (corrected): Below 90%

Table 3.4.a continued

Subphenons 17 and 19.

"Oral II" and Leuconostoc sp.

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
13	7	4.99041	6.03464E-7	4.43518	9.20731E-6

t-test: Above 99%

t-test (corrected): Above 99%

Subphenons 19 and 20.

Leuconostoc sp. and S. agalactiae

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
7	5	7.39029	1.46590E-10	6.44461	1.15976E-10

t-test: Above 99%

t-test (corrected): Above 99%

Subphenons 20 and 21.

S. agalactiae and S. pyogenes

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
5	6	9.0241	1.81246E-19	8.19522	2.50246E-16

t-test: Above 99%

t-test (corrected): Above 99%

Subphenons 23 and 24.

"Streptococcus sp. (B) clinical" and "S. equisimilis"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
8	10	3.52929	4.16759E-4	1.93227	5.33259E-2

t-test: Above 95%

t-test (corrected): Below 90%

Subphenons 10 and 12.

S. raffinolactis and S. lactis

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
4	12	3.88218	1.03141E-4	2.2949	2.17389E-2

t-test: Above 95%

t-test (corrected): Below 90%

Table 3.4.a continued

Subphenons 7 and 11.

S. salivarius and "Oral I"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
6	4	2.60951	9.06730E-3	Correction gives negative	

t-test Negative value after correction

Below 90% indicates that observed overlap greater than critical overlap.

Subphenons 2 and 8.

S. faecium and "S. casseliflavus"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
6	4	7.20821	5.67246E-13	6.02148	1.72955E-9

t-test t-test (corrected)

Above 99% Above 95%

Subphenons 1 and 12.

S. faecalis and S. lactis

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
10	12	4.08031	4.9991E-5	3.08425	2.04082E-3

t-test t-test (corrected)

Above 99% Above 90%

Subphenons 11 and 17.

"Oral I" and"Oral II"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
4	13	3.95942	7.51639E-5	2.53806	0.011147

t-test t-test (corrected)

Above 95% Below 90%

Subphenons 15 and 17.

S. mitis and "Oral II"

$N_{(L)}$	$N_{(M)}$	W	$V_{(G)}$	W(EST)	$V_{(G,EST)}$
6	13	5.44279	5.25761E-8	4.62177	3.80876E-6

t-test t-test (corrected)

Above 99% Above 99%

t-test of greater than 95% confidence and so may be distinct. As a result of different numbers of strains in different groups it is possible that the corrected values may give an overcorrection. This leaves thirteen pairs of groups where there may be some degree of overlap. The results for these pairs are given in Table 3.4.b. The $V_{(O)}$ values givein in both this table and the previous one are proportional. As such a $V_{(O)}$ value of 0.0307 indicates 3.07% overlap. In Table 3.4.b. this value ranges from 2.56% to 5.63%.

The effect of the effective number of characters (n') was investigated as described in Section 2.10.5. The program INTGROV (Sneath, unpublished) was used and a critical $V_{(O)}$ of 0.025 was used. The following taxon pairs were investigated; S. mutans/S. raffinolactis; S. faecalis/S. faecium; "S. avium/ "Streptococcus sp. (chicken)"; S. bovis/S. equinus; S. agalactiae/ S. pyogenes; S. faecium/"S.avium". The effective number of characters for each pair is given in Table 3.4.c. In each case it was found that no instances of overlap were given, print-out from the program being suppressed if the $V_{(O)}$ value is less than the critical value.

3.5 Identification matrices

Two identification matrices were constructed for use with the computer identification programs MJSSKR and MATIDEN (Sneath, 1979b) available on the Leicester University CDC Cyber 73 machine.

Table 3.4.b Rectangular OVCLUST.

```
D(L,M) IS THE DISTANCE BETWEEN CENTROIDS L AND M;
W IS THE DISJUNCTION D(L,M)/SQRT((N(L) +
N(M))*(S(L)*S(L)/N(L) + S(M)*S(M)/N(M))),
WHERE S(L), S(M) ARE THE STANDARD DEVIATIONS
ON THE INTERCENTROID (Q) AXIS. NOTE THAT M
IS USED HERE AS A TAXON LABEL AS WELL AS THE
NUMBER OF CHARACTERS, N, INSIDE THE PROGRAM.
V(G) IS THE OVERLAP ITSELF AS A PROPORTION,
AND IS 2 GAU(W).

N O T E   3 WARNING INDICATIONS.   IN CASES (1) AND (2) THE THREE
VALUES T(0) MAY BE INACCURATE,   E S P E C I A L L Y   CASE (2)
   (1) X  IN  CARE  COL. INDICATES EXTREME INEQUALITY IN SAMPLE
       SIZES OR VARIANCES
   (2) F  IN  CARE  COL. INDICATES F<4.0
   (3) R  IN  CARE  COL. SERVES AS A REMINDER THAT OVERLAP IS
       BEING TESTED AGAINST RECTANGULAR DISTRIBUTION,
       I.E.  V0 < 0  IN INPUT DATA

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
  5 11  4.359   .510   .533    4    4   157  .0307 2.161  4.1783 2.939E-005

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 11.8179   5.9884  CORRECTION GIVES D(L,M)<0    10.4426  12.2061  16.8586   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
  7 11  3.569   .710   .642    6    4   157  .0389 2.065  2.6095 9.067E-003

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
  8.2528   7.8675  CORRECTION GIVES D(L,M)<0    10.5906  12.1838  16.2705   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
  7 12  3.110   .662   .270    6   12   157  .0563 1.909  2.6061 9.158E-003

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 11.0568   5.8479  CORRECTION GIVES D(L,M)<0    13.7328  16.0307  22.1186   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
  8 12  3.932   .431   .326    4   12   157  .0534 1.932  4.1803 2.913E-005

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 16.7211   4.2116  2.7681 5.639E-003  11.0722  14.8798  18.0286  26.5643   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
  8 15  3.957   .659   .545    4    6   157  .0389 2.065  3.1473 1.648E-003

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
  9.9526   5.6542  CORRECTION GIVES D(L,M)<0    11.3202  13.2940  18.5483   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
  8 27  4.681   .565   .649    4    3   157  .0256 2.232  3.7703 1.631E-004

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
  9.9752   4.0479  CORRECTION GIVES D(L,M)<0    11.7193  14.3309  21.4635   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
 11 12  4.544   .582   .421    4   12   157  .0534 1.932  3.6022 3.156E-004

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 14.4090   4.1037  1.7787 7.529E-002   7.1147  15.0540  18.2999  27.0847   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
 11 24  5.068   .676   .489    4   10   157  .0497 1.963  3.6441 2.684E-004

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 13.6349   4.3241  1.4370 1.507E-001   5.3768  14.0060  16.9250  24.8545   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
 12 28  4.170   .368   .588   12    5   157  .0549 1.920  3.5661 3.624E-004

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 14.7033   5.3603  1.8659 6.205E-002   7.6934  13.8386  16.3018  22.9006   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
 15 28  4.266   .350   .652    6    5   157  .0421 2.032  3.9612 7.461E-005

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 13.1378   5.8805  1.1910 2.337E-001   3.9500  11.5122  13.4609  18.6180   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
 16 28  4.489   .317   .842    3    5   157  .0307 2.161  3.7232 1.966E-004

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 10.5308   5.4993  CORRECTION GIVES D(L,M)<0    10.7344  12.6546  17.7880   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
 24 28  4.269   .456   .708   10    5   157  .0516 1.946  3.1681 1.534E-003

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 12.2702   5.7220  CORRECTION GIVES D(L,M)<0    12.9168  15.1227  20.9841   R

  L  M D(L,M) S(Q,L) S(Q,M)  N(L) N(M)   N    V(0)  W(0)     W         V(G)
 27 28  4.885   .468   .784    3    5   157  .0307 2.161  3.9017 9.554E-005

   T(W)       F      W(EST)  V(G,EST)  T(W,EST) T(0,.90) T(0,.95) T(0,.99) CARE
 11.8358   5.9586  CORRECTION GIVES D(L,M)<0    13.4588  12.2309  16.9100   R
```

Table 3.4.c. Taxon pairs used in the INTGROV calculations and their effective number of characters.

Taxon pair	Effective number.
S. mutans/S. raffinolactis	53
S. faecalis/S. faecium	53
"S. avium"/"Streptococcus sp. (chicken)"	51
S. bovis/S. equinus	50
S. agalactiae/S. pyogenes	61
S. faecium/"S. avium"	57

The first matrix, PDBSTP2, was constructed from the results obtained from the integer group program. The program CHARSEP (Sneath, 1979c) was used to designate the sixty most diagnostic tests used for the twenty-eight subphenons. These tests were then used to construct the matrix. The tests selected and their separation indices in the form of VSP values are listed in Table 3.5.a. VSP stands for four times variance strain potential and is based upon the number of taxa giving positive or negative results and the total number of taxa. The matrix itself is shown in Table 3.5.b. This matrix was tested by use of the program MOSTTYP, described in Section 2.10.6. This program showed that the best score obtainable for every group in the matrix was 1.0 as a Willcox probability (Willcox et al., 1973). The results of this program are listed in Appendix VII. The identification matrix are also tested in a further way. The program MOSTTYP considers the score obtainable by a wholly typical member of each taxon. It was considered that the type or centroid strains from each cluster were practically the most typical available and so these were used in the identification program MATIDEN (Sneath, 1980a). The identification scores obtained for each strain as Willcox probabilities and taxonomic distances are shown in Table 3.5.c.

The second matrix, PDBSTP, was based upon information obtained from this study and from other sources already published. Seven other sets of information were considered. These were the studies of Diebel & Seeley (1974), Cowan & Steel (1974), Jones (1978), Carlsson (1968), Facklam (1972; 1977), Feltham

Table 3.5.a Separation indices of the sixty most diagnostically useful tests used in the numerical taxonomy.

Character	VSP index %	Number of taxa +ve	Number of taxa -ve
Growth at 45°C	72.4165	12	8
Growth with 40% bile	68.5147	8	12
V.P.	68.4351	9	8
Acid from sorbitol	67.1716	8	9
Reduction of janus green B milk	65.8147	8	9
Arginine hydrolysis	65.3657	13	6
Acid from glycerol	63.3772	9	7
Aesculin hydrolysis (API)	61.6266	13	5
Growth at 10°C	61.4795	7	12
Aesculin hydrolysis	59.2146	14	5
Starch hydrolysis	58.8886	7	7
Possession of acid phosphatase	58.6333	13	5
Possession of phosphoamidase	58.2677	11	5
Acid from mannitol	58.0232	9	5
Growth with 0.0002% crystal violet	57.7252	10	5
Growth with 0.0004% crystal violet	57.4956	6	14
Possession of α-glucosidase	56.9394	8	6
Growth with 4% NaCl	56.9192	10	6
Possession of β-glucosidase	56.3603	7	12
Reduction of selenite	55.6362	10	6
Arginine decarboxylation	55.0354	9	5
Acid from amygdalin	54.5126	7	5
Acid from methyl-D-glucoside	54.0635	7	6
Production of clot in litmus milk	52.0642	5	9

Table 3.5.a continued

Character	VSP index %	Number of taxa +ve	Number of taxa -ve
∝-haemolysis	51.8177	7	5
Hippurate hydrolysis	51.1715	5	13
Reduction of methylene blue milk	50.0533	4	12
Phosphatase (plate)	49.7423	5	12
Growth on MacConkey agar	49.133	6	18
Tolerance of 60°C for 1 hour	48.6171	4	10
Acid from arbutin	48.5481	9	4
Possession of alkaline phosphatase	48.4823	6	5
Growth with 10% bile	46.7045	15	4
Acid from D (+) raffinose	46.0874	4	7
Starch hydrolysis	44.0904	10	2
Acid from glycogen	42.9429	4	17
Lipase	41.6995	5	5
Methyl red (API)	41.5511	10	3
Acid from inulin	41.4577	4	10
Reduction of nitrite	41.3017	12	4
Colony diameter between 0.2 and 0.4 mm	39.8052	8	3
Acid from D (+) melibiose	39.5024	4	4
Final pH below 4.25	38.5276	16	3
Reduction of tetrazolium	38.5257	13	4
No haemolysis	37.6676	3	13
Tetrathionate reductase	37.6401	12	3
Growth on TCBS agar	37.5902	4	19
Tolerance of 60°C for 15 min	37.1328	13	2
Red colonies on MacConkey agar	35.4748	3	18
Acid from dextrin	35.1525	13	2
Production of H_2O_2	34.9304	1	12

Table 3.5.a continued

Character	VSP index %	Number of taxa +ve	Number of taxa -ve
Final pH between 4.25 and 4.75	32.6139	3	17
Possession of β-glucuronidase	31.8663	2	19
Acid from gluconate	31.3964	1	11
Acid from D (+) cellobiose	30.8513	16	1
Growth with 6.5% NaCl	30.3091	4	21
Possession of β-galactosidase	29.4669	1	14
Growth at pH 9.6	28.9978	4	21
Colony diameter below 0.2 mm	28.3637	3	16

Table 3.5.b Listing of identification matrix PDBSTP2.

TAXA:

10 S.FAECALIS
20 S.FAECIUM
30 S.AVIUM
40 S.SP.(CHICKEN)
50 S.BOVIS
60 S.EQUINUS
70 S.SALIVARIUS
80 S.CASSELIFLAVUS
90 S.MUTANS
100 S.RAFFINOLACTIS
110 ORAL 1
120 S.LACTIS
130 A.VIRIDANS
140 S.THERMOPHILUS
150 STREP.MITIS
160 S.SANGUIS
170 ORAL 2
180 S.MILLERI
190 LEUCONOSTOC
200 S.AGALACTIAE
210 S.PYOGENES
220 S.EQUI
230 S.EQUISIMI
240 STREP.SP.B
250 S.UBERIS
260 S.DYSGALACTIAE
270 STREP.SP.R,S,T
280 PEDIOCOCCUS

TESTS:

GROWTH AT 45 C
40% BILE
V.P
SORBITOL
REDN. OF JANUS G
ARGININE HYDROL.
GLYCEROL
AESCULIN (API)
GROWTH AT 10 C
AESCULIN HYDROL.
STARCH HYDROL.
ALCO PHOSPHATASE
PHOSPHORAMIDASE
MANNITOL
0.0002% CRYST.VIOL.
0.0004% CRYST. VIOL.
A GLUCOSIDASE
TOLERANCE OF 4%NACL
B GLUCOSIDASE
REDN. OF SELENITE
ARGININE DECARBOX.
AMYGDALIN
METHYL-D-GLUCOSIDE
CLOT IN LITMUS
ALPHA HAEMOL.
HIPPURATE HYDROL.
REDN. OF METH.BLUE
PHOSPHATASE
GROWTH ON MAC.AGAR.
60 DEGREES FOR 1 HR
ARBUTIN
ALK.PHOSPHATASE
10% BILE
RAFFINOSE
STARCH
GLYCOGEN
LIPASE
METHYL RED(API)
INULIN
REDN.OF NITRITE
COL.DIAM.0.2-0.4MM.
MELIBIOSE
FINAL PH<4.25
REDN.OF TETRAZ.
NO HAEMOL.
TETRATHIONATE
GROWTH ON TCBS AGAR
60 C FOR 15 MINS
GROWTH WITH NA AZIDE
ACID ON MAC. C AGAR
DEXTRIN
H2O2 PRODN.
FINAL PH4.25-4.75
B GLUCORONIDASE
GLUCONATE
D(+) CELLOBIOSE
TOLERANCE OF 6.5%NACL
B GALACTOSIDASE
GROWTH AT PH 9.6
COL.DIAM.<0.2 MM.

TAXA	GROWTH AT 45 C	40% BILE	V.P	SORBITOL	REDN. OF JANUS G	ARGININE HYDROL.	GLYCEROL	AESCULIN (API)	GROWTH AT 10 C	AESCULIN HYDROL.	STARCH HYDROL.	ACID PHOSPHATASE	PHOSPHOAMIDASE	MANNITOL	0.0002% CRYST.VIOL.	0.0004% CRYST. VIOL.	A GLUCOSIDASE	TOLERANCE OF 4%NACL	B GLUCOSIDASE	REDN. OF SELENITE	ARGININE DECARBOX.	AMYGDALIN	METHYL-D-GLUCOSIDE	CLOTTING LITMUS	ALPHA HAEMOL.	HIPPURATE HYDROL.	REDN. OF METH.BLUE	PHOSPHATASE	GROWTH ON MAC.AGAR.	60 DEGREES FOR 1 HR
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1 S.FAECALIS	99	99	99	99	99	99	99	99	99	99	1	90	99	99	20	10	99	99	99	99	80	99	20	40	40	90	99	90	99	99
2 S.FAECIUM	99	99	83	83	67	99	99	99	99	99	1	1	67	83	17	1	1	99	99	99	99	99	1	67	83	1	67	1	99	99
3 S.AVIUM	99	99	99	99	99	25	99	99	99	99	25	25	99	99	75	25	25	99	50	99	1	99	99	99	99	1	25	99	99	25
4 S.SP.(CHICKEN)	99	99	99	99	99	1	99	99	99	99	77	99	99	99	53	12	1	99	99	99	10	99	99	1	99	12	1	99	99	1
5 S.BOVIS	99	99	99	1	75	1	25	75	1	99	99	99	25	50	1	1	99	99	50	1	75	75	25	99	50	1	1	1	1	75
6 S.EQUINUS	99	99	1	1	1	1	1	99	1	99	99	1	25	50	1	1	99	99	99	1	25	99	1	25	25	1	1	1	1	75
7 S.SALIVARIUS	83	17	67	17	67	17	1	99	17	99	83	83	83	33	67	50	67	50	50	99	1	67	17	99	67	1	33	1	1	17
8 S.CASSELIFLAVUS	99	99	99	99	1	75	25	25	25	99	25	25	75	99	25	1	25	99	1	25	50	75	75	50	75	1	1	1	25	50
9 S.MUTANS	99	70	70	99	30	33	30	99	1	99	33	70	99	99	99	1	70	67	99	1	1	99	99	70	1	1	70	1	1	1
10 S.RAFFINOLACTIS	1	1	1	99	50	1	50	99	75	99	50	99	99	99	75	1	99	75	99	99	1	50	99	50	1	25	1	1	1	1
11 ORAL 1	25	1	99	75	50	1	99	99	1	99	99	50	25	99	99	75	25	1	25	50	25	75	1	1	25	1	25	1	1	1
12 S.LACTIS	1	42	42	17	99	67	1	92	92	75	8	92	92	17	25	1	83	75	92	92	92	92	67	50	75	67	50	75	8	17	33
13 A.VIRIDANS	99	1	99	1	33	99	33	67	99	99	99	99	33	33	67	33	33	33	1	67	99	33	33	33	67	99	99	33	1	99
14 S.THERMOPHILUS	99	99	17	17	99	17	33	1	1	33	83	1	1	17	99	1	1	17	1	83	1	1	1	99	67	1	1	33	1	83
15 S.MITIS	99	33	1	17	1	83	1	1	33	1	83	1	67	17	17	1	1	33	17	67	17	1	50	99	83	50	99	17	1	50
16 S.SANGUIS	99	67	33	1	1	99	99	67	1	1	1	33	33	1	33	1	33	1	1	33	99	67	33	1	99	1	1	1	1	33
17 ORAL 2	38	1	38	23	1	31	54	15	15	8	46	46	54	38	99	99	38	1	1	69	99	15	38	15	99	8	8	23	1	1
18 S.MILLERI	33	1	99	1	1	99	1	1	1	33	1	99	99	1	99	33	99	67	33	33	33	1	1	1	33	1	67	1	1	33
19 LEUCONOSTOC	1	43	14	1	14	1	1	14	43	43	14	14	1	57	57	99	29	1	1	86	43	14	71	1	99	14	99	14	1	1
20 S.AGALACTIAE	20	99	20	1	1	99	20	1	1	1	1	99	99	1	99	99	60	99	1	80	80	20	1	40	20	99	1	99	99	1
21 S.PYOGENES	1	1	1	33	1	99	17	33	1	50	1	99	99	33	99	99	99	1	1	99	99	1	50	17	1	17	1	1	50	1
22 S.EQUI	1	1	1	1	1	99	50	99	1	1	50	99	99	1	1	1	50	1	1	1	99	1	99	50	1	1	50	1	1	1
23 S.EQUISIMI	1	1	12	38	99	99	88	12	38	12	62	99	99	1	1	1	99	62	12	75	62	12	88	38	12	12	12	99	1	25
24 STREP.SP.B	70	50	10	20	30	99	30	10	1	20	80	99	99	30	10	1	99	99	10	99	70	20	70	60	60	99	70	99	40	90
25 S.UBERIS	1	67	99	99	99	99	99	99	99	99	99	99	99	99	99	99	67	99	1	1	57	99	33	1	99	99	1	67	1	33
26 S.DYSGALACTIAE	1	1	1	99	99	99	99	33	1	33	99	99	33	99	99	33	99	67	1	99	99	67	99	1	99	99	1	1	1	1
27 STREP.SP.R,S,T	33	1	1	67	33	99	1	99	67	99	99	1	1	33	33	99	67	33	33	1	57	33	99	1	1	67	67	1	1	1
28 PEDIOCOCCUS	80	1	80	1	80	80	99	60	60	80	80	40	20	20	99	80	20	80	20	40	99	60	40	80	40	40	40	40	99	80

	ARBUTIN	ALK.PHOSPHATASE	10% BILE	RAFFINOSE	STARCH	GLYCOGEN	LIPASE	METHYL RED(API)	INULIN	REDN.OF NITRITE	COL.DIAM.0.2-0.4MM.	MELIBIOSE	FINAL PH<4.25	REDN.OF TETRAZ.	NO HAEMOL.	TETRATHIONATE	GROWTH ON TCBS AGAR	60 C FOR 15 MINS	GROWTH WITH NA AZIDE	ACID ON MAC.C AGAR	DEXTRIN	H2O2 PRODN.	FINAL PH4.25-4.75	B GLUCURONIDASE	GLUCONATE	D(+) CELLOBIOSE	TOLERANCE OF 6.5%NACL	B GALACTOSIDASE	GROWTH AT PH 9.6	COL.DIAM.<0.2 MM.
TAXA:	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
1 S.FAECALIS	10	60	99	1	99	10	40	80	10	99	30	1	99	90	60	70	99	99	99	99	90	10	1	1	70	99	99	90	99	1
2 S.FAECIUM	99	17	99	1	67	1	67	99	17	99	83	50	99	50	17	99	83	99	99	99	50	33	1	1	67	99	99	50	99	1
3 S.AVIUM	99	1	99	25	25	1	99	99	25	75	75	25	99	99	1	99	99	99	99	75	75	75	1	1	75	99	99	75	99	1
4 S.SP.(CHICKEN)	99	29	99	99	99	6	88	94	77	99	77	99	99	99	1	82	94	77	99	65	99	82	1	88	99	99	99	99	99	1
5 S.BOVIS	75	25	99	99	99	75	50	99	50	99	50	99	99	99	50	99	1	75	75	1	75	25	1	1	1	99	1	25	1	25
6 S.EQUINUS	99	1	99	25	1	1	99	75	25	99	99	1	99	1	75	99	1	99	99	1	25	50	1	1	25	99	1	1	1	1
7 S.SALIVARIUS	99	33	33	83	33	1	67	50	50	50	99	50	99	83	33	67	1	83	83	1	67	17	1	1	17	99	1	67	1	1
8 S.CASSELIFLAVUS	75	25	99	50	99	1	99	99	1	99	99	50	75	1	25	99	1	75	99	25	99	25	25	1	50	75	1	1	50	1
9 S.MUTANS	99	30	99	99	70	30	70	99	99	99	1	99	99	99	99	70	1	30	99	1	99	1	1	1	1	99	1	30	1	99
10 S.RAFFINOLACTIS	75	50	99	75	75	1	1	75	75	1	50	75	99	25	99	50	1	99	99	1	50	1	1	1	25	99	25	1	1	50
11 ORAL 1	99	25	1	75	50	1	50	99	50	99	25	75	99	50	75	1	1	25	1	1	99	1	1	1	75	99	1	1	1	75
12 S.LACTIS	92	33	99	8	99	8	67	99	17	25	83	17	92	93	33	42	17	92	83	17	99	1	8	1	50	92	1	8	1	8
13 A.VIRIDANS	67	67	33	67	99	1	1	99	33	33	99	33	99	67	33	1	50	99	1	1	99	67	1	1	33	99	33	1	33	1
14 S.THERMOPHILUS	33	1	99	17	17	1	67	83	1	99	83	33	99	99	33	67	1	99	83	1	17	1	1	1	1	1	1	33	1	17
15 S.MITIS	1	17	83	67	50	1	33	50	17	1	67	67	33	67	1	17	1	83	33	1	99	33	67	1	17	33	1	50	1	17
16 S.SANGUIS	33	1	67	1	33	33	1	33	99	67	67	1	67	1	1	33	1	67	1	1	67	67	33	1	1	67	1	1	1	33
17 ORAL 2	38	31	15	61	31	23	61	38	31	92	61	77	92	99	1	92	1	18	54	1	77	99	8	1	15	31	1	15	1	38
18 S.MILLERI	33	67	1	1	1	1	67	1	1	67	1	33	99	67	33	99	1	33	99	1	1	67	1	1	1	33	1	67	1	99
19 LEUCONOSTOC	14	29	99	43	29	1	57	1	14	99	71	57	14	99	1	57	1	71	14	1	57	1	1	1	71	14	1	14	1	29
20 S.AGALACTIAE	20	99	99	20	20	1	20	40	1	1	20	20	99	99	1	80	20	99	99	80	40	1	1	80	1	20	1	1	1	1
21 S.PYOGENES	1	99	1	17	83	50	1	33	1	83	99	17	1	99	1	99	1	1	99	50	99	1	99	17	1	83	1	1	1	1
22 S.EQUI	1	99	50	50	99	99	1	99	1	99	99	1	50	1	1	99	99	1	99	1	1	1	50	99	1	99	1	1	1	1
23 S.EQUISIMI	25	88	75	1	99	88	25	88	12	75	75	12	50	99	1	99	1	88	75	1	99	12	50	88	1	62	1	25	1	1
24 STREP.SP.B	60	99	70	1	80	10	60	90	10	50	60	30	70	80	10	1	1	99	40	30	99	1	30	90	60	30	1	1	1	10
25 S.UBERIS	99	99	99	33	33	33	99	67	99	1	99	33	1	99	1	67	1	99	67	1	99	67	99	33	33	99	1	1	1	1
26 S.DYSGALACTIAE	67	99	1	67	99	99	99	99	1	67	1	99	33	67	1	99	1	99	99	1	99	67	67	99	67	99	1	1	1	99
27 STREP.SP.R,S,T	67	1	67	99	99	99	67	33	99	67	99	67	1	99	99	99	1	67	67	1	99	67	99	33	1	99	1	67	1	1
28 PEDIOCOCCUS	40	40	20	40	40	1	60	20	1	60	20	40	60	99	60	40	60	99	60	99	20	40	40	1	60	80	60	1	20	20

Table 3.5.c Identification scores of typical (PB 2 -PB 113) and loosely linked (PB 16 -PB 158) strains.

Number.	Received as.	Identified as.	Willcox	Distance
PB 2	S. faecalis	S. faecalis	1	0.281179
PB 87	S. faecium	S. faecium	1	0.253114
PB 15	"S. avium"	"S. avium"	1	0.179884
PB 18	"Streptococcus sp. (chicken)"	"Streptococcus sp. (chicken)"	1	0.148694
PB 81	S. bovis	S. bovis	1	0.2671
PB 83	S. equinus	S. equinus	1	0.248065
PB 9	S. salivarius	S. salivarius	1	0.366801
PB 52	"S. faecium subsp. casseliflavus"	"S. casseliflavus"	1	0.294123
PB 58	S. rattus	S. mutans	1	0.216829
PB 199	S. raffinolactis	S. raffinolactis	1	0.28332
PB 172	Streptococcus sp.	"Oral I"	1	0.32605
PB 93	S. lactis	S. lactis	1	0.27419
PB 107	A. viridans	A. viridans	1	0.342595
PB 98	S. mitis	S. mitis	1	0.321114
PB 68	S. sanguis	S. sanguis	1	0.274918
PB 179	Streptococcus sp.	"Oral II"	1	0.388739
PB 57	"S. milleri"	"S. milleri"	1	0.246671

Table 3.5.c continued

Number	Received as.	Identified as.	Willcox	Distance
PB 40	*S. agalactiae*	*S. agalactiae*	1	0.298993
PB 54	*S. pyogenes*	*S. pyogenes*	1	0.255926
PB 41	*S. equi*	*S. equi*	1	0.193869
PB 103	"*S. equisimilis*"	"*S. equisimilis*"	1	0.226381
PB 137	"*Streptococcus* sp. (B) clinical"	"*Streptococcus* sp. (B) clinical"	1	0.284371
PB 118	*S. uberis*	*S. uberis*	1	0.21474
PB 117	"*S. dysgalactiae*"	"*S. dysgalactiae*"	1	0.219439
PB 160	"*Streptococcus* sp. (R,S and T)"	"*Streptococcus* sp. (R, S and T)"	1	0.288594
PB 39	*S. thermophilus*	*S. thermophilus*	1	0.218556
PB 163	*Leuconostoc oenos*	*Leuconostoc* sp.	1	0.292783
PB 113	*Pediococcus halophilus*	*Pediococcus* sp.	1	0.432074
PB 16	"*S. avium*"	*S. faecalis*	0.69233	0.39715
PB 125	*S. faecalis*	*S. faecalis*	0.99988	0.429412
PB 140	"*Streptococcus* sp. (D)"	*S. faecalis*	0.999998	0.31368
PB 152	"*Streptococcus* sp. (D)"	*S. lactis*	0.16795	0.521069
PB 153	"*Streptococcus* sp. (D)"	*S. lactis*	0.999895	0.461714
PB 154	"*Streptococcus* sp. (K)"	*S. lactis*	0.999999	0.471006

Table 3.5.c continued

Number.	Received as.	Identified as.	Willcox	Distance
PB 156	"Streptococcus sp. (L)"	S. lactis	0.994952	0.48564
PB 109	A. catalyticus	Pediococcus sp.	1	0.51545
PB 110	A. catalyticus	Pediococcus sp.	0.999987	0.461543
PB 143	S. salivarius	"Streptococcus sp. (B) clinical"	0.900627	0.496185
PB 169	Pediococcus acidilacti	Pediococcus sp.	1	0.482032
PB 201	Streptococcus sp.	S. salivarius	0.999978	0.451237
PB 71	"Streptococcus sp. (N)"	S. salivarius	0.546155	0.464344
PB 43	"Streptococcus sp. (F)"	"Oral II"	0.996346	0.483513
PB 170	Streptococcus sp.	S. mitis	0.999997	0.450892
PB 149	"Streptococcus sp. (O)"	"S. casseliflavus"	0.980903	0.471533
PB 46	"Streptococcus sp. (K)"	S. mitis	0.703517	0.486453
PB 115	"S. suis"	A. viridans	0.708902	0.462643
PB 123	"S. suis"	S. mitis	0.882513	0.48611
PB 102	Streptococcus sp.	S. lactis	5.321E-2	0.496501
PB 124	"S. cremoris subsp. alactosus"	S. lactis	0.999998	0.451125
PB 47	"Streptococcus sp. (M)"	"Oral II"	0.958434	0.422041
PB 157	"Streptococcus sp. (M)"	Leuconostoc sp.	0.994798	0.420779

Table 3.5.c continued

Number	Received as.	Identified as.	Willcox	Distance
PB 60	"S. sobrinus"	"S. milleri"	2.672E-4	0.491779
PB 70	S. pneumoniae	"Oral II"	0.910377	0.493408
PB 66	S. sanguis	"Oral II"	0.984522	0.494083
PB 45	"Streptococcus sp. (H)"	"Oral II"	0.686796	0.505422
PB 142	"Streptoococcus sp. (G)"	"Streptococcus sp. (B) clinical"	0.999999	0.428797
PB 42	"Streptococcus sp. (E)"	"S. equisimilis"	0.178213	0.537198
PB 122	"Streptococcus sp. (E)"	"S. equisimilis"	0.739857	0.537508
PB 191	"Streptococcus sp. (M)"	"S. equisimilis"	0.27788	0.504891
PB 158	"Streptococcus sp. (P)"	S. uberis	5.956E-4	0.520045

(1979) and unpublished data) and Crowley, Bradley & Darrel (1969). The results from these sources and this study are tabulated in Appendix VII, with the studies shown by the initials of the authors' names. The identification matrix derived from this information is listed in Table 3.5.d. The program MOSTTYP was also used to determine the best score obtainable for each group. These ranged from 0.995472 to 1.0 as Willcox probabilities. The results of this are shown in Appendix VII.

3.6 Identification matrix overlap

The apparent overlap between groups in the identification matrices was determined with the program OVERMAT (as described in Section 2.10.6). A critical overlap was used for the t-test. This was a V_0 of 0.025, corresponding to a W_0 of 2.24138. These are the values used earlier in the OVCLUST program.

The matrix PDBSTP used results from both this work and other sources. As a result the number of strains on which each group is based is unknown. In order to use the program a value was required, and so an arbitary figure of fifteen was used. As a result the matrix overlap was not corrected for sample size. This may not give an exact representation but should enable the overall pattern to be seen.

The matrix PDBSTP2 was also tested for overlap; in this case the numbers in each group were known. However, for ease of comparison between the two matrices this was not corrected for sample size either. The variances and the standard deviations were found for such subphenon and these are listed in Appendix VII.

Table 3.5.d Listing of identification matrix PDBSTP.

	TAXA:	GROWTH AT 10 DEGREES 1	GROWTH AT 45 DEGREES 2	60 DEGREES FOR 30 MI 3	TOLERANCE OF 10% 3TL 4	TOLERANCE OF 40% BIL 5	TOLERANCE OF TELLURI 6	TOLERANCE OF TETRAZO 7	GROWTH AT PH 9.6 8	TOLERANCE OF 4% NACL 9	TOLERANCE OF 6.5% NA 10	AESCULIN HYDROL. 11	ARGININE HYDROL. 12	HIPPURATE HYDROL. 13	STARCH HYDROL. 14	GELATIN HYDROL 15	ACID FROM ARABINOSE 16	ACID FROM MALTOSE 17	ACID FROM TREHALOSE 18	ACID FROM RAFFINOSE 19	ACID FROM SALICIN 20	ACID FROM GLYCEROL 21	ACID FROM MANNITOL 22	ACID FROM SORBITOL 23	ACID FROM LACTOSE 24	ACID FROM INULIN 25	ACID FROM SUCROSE 26	ACID FROM MELIBIOSE 27	ACID FROM MELEZITOSE 28	ACID FROM GLUCOSE 29	ACID FROM XYLOSE 30
1	STREP. FAECALIS	99	99	99	99	99	99	99	99	99	99	99	99	99	1	50	25	99	99	1	99	99	99	99	99	5	90	1	85	99	20
2	STREP. FAECIUM	99	99	99	99	99	25	25	99	99	99	99	99	25	1	1	50	99	65	10	99	99	80	50	99	12	55	60	1	99	10
3	STREP. AVIUM	75	99	99	99	99	33	50	99	99	99	99	12	1	16	1	99	99	99	12	99	99	99	99	99	25	75	25	99	99	75
4	STREP. SP.(CHICKEN)	99	99	99	99	99	18	99	99	99	99	99	1	12	77	1	30	99	99	99	99	99	99	99	99	78	99	99	55	99	55
5	STREP. BOVIS	1	99	50	99	99	1	75	10	99	1	99	1	1	99	1	20	99	40	99	99	12	50	1	99	50	99	99	1	99	1
6	STREP. EQUINUS	1	99	50	99	99	1	1	1	75	1	99	1	1	75	1	1	99	30	20	99	1	20	1	1	20	70	1	25	99	1
7	STREP. SALIVARIUS	8	50	8	50	12	10	50	1	20	1	99	4	0	30	1	1	99	85	90	99	1	6	2	80	85	99	18	15	99	1
8	STREP. CASSELIFLAVU	75	80	50	99	99	45	33	75	99	99	99	75	25	8	1	70	99	99	75	50	35	99	50	99	33	99	75	50	99	1
9	STREP. MUTANS	1	75	1	65	45	1	65	1	55	8	75	11	1	11	1	1	99	99	80	99	8	99	95	99	90	99	60	15	99	18
10	STREP. RAFFINOLACTI	75	1	1	99	1	1	25	1	75	25	99	1	25	50	1	6	99	99	99	99	25	50	50	55	45	99	45	45	99	18
11	ORAL STREPTOCOCCI 1	1	20	1	1	1	1	50	1	32	5	99	5	1	99	1	12	99	99	85	99	25	95	85	85	70	99	82	50	99	12
12	STREP. LACTIS	99	1	40	99	75	67	83	1	62	1	50	50	50	1	1	40	90	70	25	70	1	20	8	85	1	85	11	1	99	40
13	AEROCOCCUS VIRIDANS	99	50	99	33	1	1	67	50	33	50	75	50	99	99	1	40	99	85	55	55	65	65	20	80	16	80	16	25	99	16
14	STREP. THERMOPHILUS	1	99	99	99	99	1	99	1	17	1	8	8	1	30	1	1	70	35	4	1	13	4	4	99	1	99	16	34	99	25
15	STREP. MITIS	16	50	18	70	9	1	34	1	50	1	1	50	10	75	1	1	99	45	50	50	1	2	2	85	3	85	50	25	99	1
16	STREP. SANGUIS	1	60	45	80	80	1	1	1	1	1	68	99	1	1	1	4	99	90	40	80	15	2	2	95	80	99	24	10	99	17
17	ORAL STREPTOCOCCI 2	15	26	1	15	1	30	99	1	14	1	22	40	4	46	1	2	99	68	58	59	26	19	13	99	34	99	61	35	99	1
18	STREP. MILLERI	1	16	15	1	1	25	67	1	58	1	75	99	1	1	1	1	99	95	2	85	1	2	1	85	2	99	27	1	99	1
19	LEUCONOSTOC/GEMELLA	71	1	1	99	43	1	99	1	1	1	46	1	14	14	99	34	99	71	43	14	1	57	1	43	14	99	57	14	99	14
20	STREP. AGALACTIAE	1	7	1	99	99	1	99	1	99	1	1	99	99	1	1	3	99	98	5	65	65	1	1	80	1	99	13	1	99	1
21	STREP. PYOGENES	1	1	1	1	1	1	99	1	1	1	48	99	4	1	1	1	99	99	4	99	7	14	14	99	1	99	6	5	99	4
22	STREP. EQUI	1	1	1	50	1	99	1	1	1	1	12	99	1	50	1	1	99	1	10	99	24	1	1	1	1	99	1	1	99	1
23	STREP. EQUISIMI	19	1	8	67	1	85	99	1	62	1	38	99	3	56	1	1	95	54	1	85	60	2	50	58	3	99	7	19	99	21
24	STREP. SP B	1	70	99	70	50	60	80	1	99	1	10	99	99	80	1	32	99	99	1	97	50	1	1	40	6	99	30	27	99	10
25	STREP. UBERIS	99	50	70	99	37	1	75	1	99	17	99	99	99	45	1	1	99	99	10	99	63	99	99	99	99	99	11	17	99	4
26	STREP. DYSGALACTIAE	1	1	1	1	1	1	67	1	67	1	23	99	70	99	1	4	99	90	14	75	23	22	60	80	1	99	52	34	99	1
27	STREP. SP R,S,T	67	33	1	67	1	33	99	1	33	1	99	99	1	99	1	1	99	99	75	99	1	16	34	99	99	99	67	33	99	1

CHARACTERS

Table 3.5.d continued.

	TAXA:	REDN. OF METHYLENE B	ACID IN LITMUS MILK	CLOT IN LITMUS MILK	V.P	PRODUCTION OF H_2O_2	ALPHA HAEMOLYSIS	BETA HAEMOLYSIS
		31	32	33	34	35	36	37
1	STREP. FAECALIS	99	99	99	99	10	1	66
2	STREP. FAECIUM	99	85	65	83	33	99	33
3	STREP. AVIUM	12	99	50	99	75	99	1
4	STREP. SP.(CHICKEN)	1	99	1	99	82	99	1
5	STREP. BOVIS	20	99	70	99	25	99	1
6	STREP. EQUINUS	1	50	12	1	50	99	1
7	STREP. SALIVARIUS	10	99	99	65	6	30	1
8	STREP. CASSELIFLAVU	50	50	50	99	25	99	1
9	STREP. MUTANS	35	99	70	85	25	1	1
10	STREP. RAFFINOLACTI	1	75	1	1	1	50	1
11	ORAL STREPTOCOCCI 1	25	99	1	99	1	25	25
12	STREP. LACTIS	35	99	85	45	1	60	1
13	AEROCOCCUS VIRIDANS	99	99	16	99	67	99	1
14	STREP. THERMOPHILUS	1	99	99	17	1	50	1
15	STREP. MITIS	33	99	99	25	66	99	1
16	STREP. SANGUIS	1	99	50	16	50	60	1
17	ORAL STREPTOCOCCI 2	1	99	1	33	58	33	25
18	STREP. MILLERI	8	99	15	70	50	30	30
19	LEUCONOSTOC/GEMELLA	67	67	1	99	67	50	1
20	STREP. AGALACTIAE	1	70	70	20	1	50	50
21	STREP. PYOGENES	1	60	8	1	1	1	99
22	STREP. EQUI	25	75	25	1	1	1	99
23	STREP. EQUISIMI	6	87	44	12	12	1	99
24	STREP. SP B	70	99	60	10	1	50	50
25	STREP. UBERIS	1	99	50	99	67	90	1
26	STREP. DYSGALACTIAE	1	99	25	1	67	90	1
27	STREP. SP R,S,T	67	67	1	1	67	1	1

Using the matrix PDBSTP, fourteen pairs of groups were found to give a *t*-test result of less than 95%. This indicates that there was less than 95% confidence that the true overlap was less than 0.025. Twenty pairs were found using this criterion in the matrix PDBSTP2. These pairs are listed in Table 3.6.a. The V_G was observed for these pairs and note was taken if it was above 9×10^{-3}. Four of these instances were found in the matrix PDBSTP and none were found in the matrix PDBSTP2. These are listed in Table 3.6.b.

3.7 Dendrogram derived from the identification matrix PDBSTP2.

The strains assigned to the twenty-eight subphenons and the sixty tests used in the matrix PDBSTP2 were used as data for a further S_G dendrogram. This is shown in Figure 3.7.1 and a simplified version is shown in Figure 3.7.2. The Cophenetic Correlation coefficient of this dendrogram was found to be 0.736 and clustering was by average linkage. As loosely linked and satellite strains have been removed from the data, the majority of the dendrogram consists of separated clusters.

This dendrogram is very similar to that seen as the S_G average linkage one for all characters. The first four clusters are separated from the rest of the dendrogram. With one exception these four clusters correspond to the subphenons of *S. faecalis*, *S. faecium*, "*S. avium*" and "*Streptococcus* sp. (chicken)" respectively. The one exception is the strain PB 88. This appears adjacent to but not within the bottom cluster, subphenon 4,

Table 3.6.a Pairs of taxa where the t-test shows less than 95% confidence.

PDBSTP

S. faecium/"S. casseliflavus"

S. salivarius/S. mutans

S. salivarius/S. sanguis

S. salivarius/"Oral II"

S. mutans/"Oral I"

S. mitis/S. sanguis

S. mitis/"Oral II"

S. mitis/"S. dysgalactiae"

"Oral II"/"S. milleri"

"Oral II"/"S. equisimilis"

"Oral II"/"S. dysgalactiae"

"Oral II"/"Streptococcus sp. (R, S and T)"

"S. milleri"/S. pyogenes

S. equi/"S. equisimilis"

PDBSTP2

S. faecalis/Pediococcus sp.

"Streptococcus sp. (chicken)"/Pediococcus sp.

S. bovis/S. salivarius

S. bovis/"S. casseliflavus"

S. bovis/S. lactis

S. salivarius/S. mutans

S. salivarius/S. lactis

S. salivarius/"Oral II"

S. salivarius/"S. milleri"

S. salivarius/Pediococcus sp.

S. salivarius/Pediococcus sp.

"S. casseliflavus"/S. lactis

S. raffinolactis/S. lactis

"Oral I"/"Oral II"

S. lactis/Pediococcus sp.

A. viridans/Pediococcus sp.

S. mitis/S. sanguis

S. sanguis/Pediococcus sp.

"Oral II"/Leuconostoc sp.

"Oral II"/Pediococcus sp.

"Streptococcus sp. (B) clinical"/Pediococcus sp.

Table 3.6.b Pairs of taxa where the $V_{(G)}$ value is above 9×10^{-3}.

PDBSTP	$V_{(G)}$
S. faecium/"S. casseliflavus"	1.39118E-2
S. mitis/"Oral II"	1.23285E-2
"Oral II"/"S. milleri"	1.99308E-2
"Oral II"/"S. dysgalactiae"	1.09549E-2

Figure 3.7.1 Average linkage dendrogram using Gower's coefficient for the 60 characters used in the identification matrix PDBSTP2 and 165 strains. Cophenetic correlation coefficient is 0.7364.

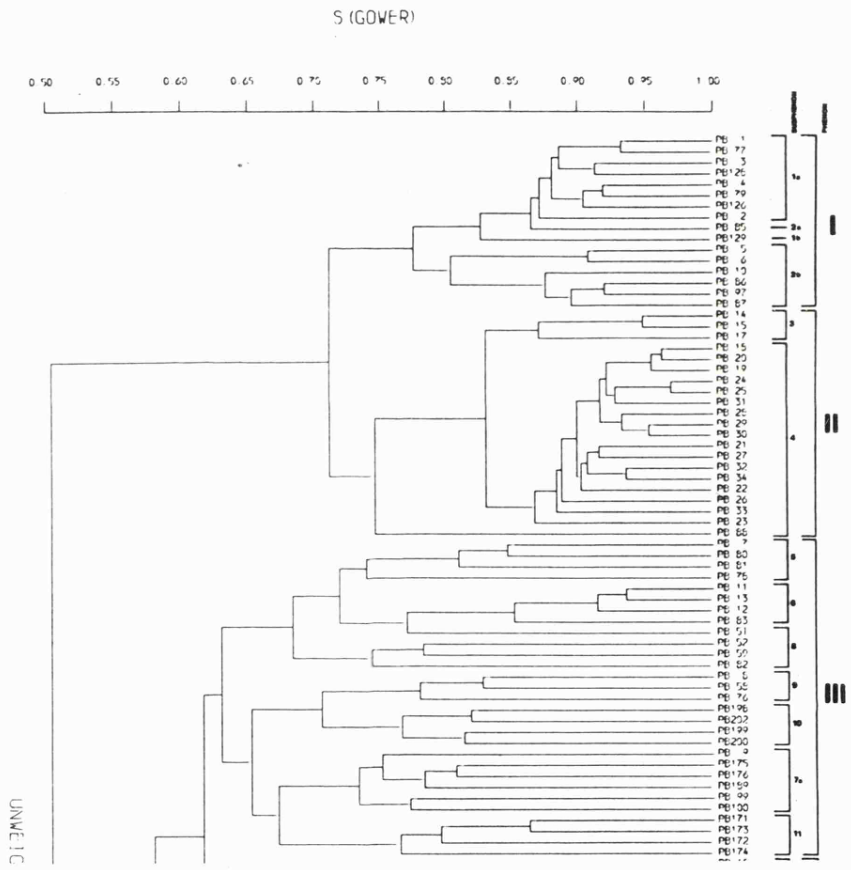
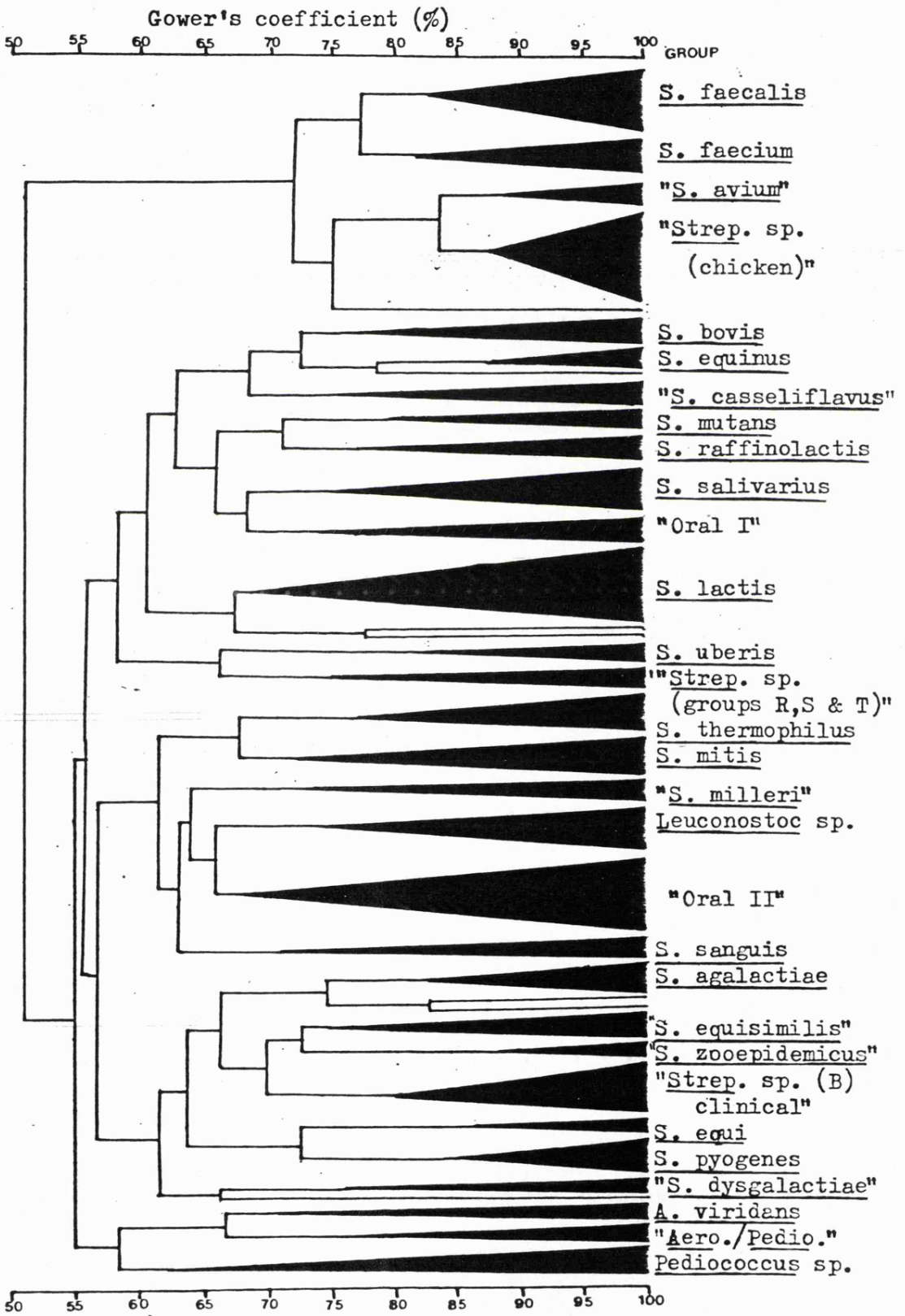


Figure 3.7.2 Simplified version of the dendrogram obtained from the identification matrix PDBSTP2. This representation uses Gower's coefficient and average linkage clustering.



"Streptococcus sp. (chicken)". It was previously assigned to subphenon 3, "S. avium".

The next group of clusters constitutes a separate arm of the dendrogram. The clusters are linked together at 63% similarity. These clusters correspond to subphenons 5, 6, 8, 9, 10, 7, 11 and 12 respectively which made up phenon III with the inclusion of S. lactis. Linked to the base of S. lactis at 67% similarity are two strains, PB 92 and PB 149. PB 92 was previously assigned to S. salivarius and PB 149 was previously a member of subphenon 13, A. viridans. Linked to this arm at 58% similarity are two clusters. The first of these corresponds to subphenon 25 (S. uberis) and the other to subphenon 27, "Streptococcus sp. (groups R, S and T)".

The next arm of the dendrogram consists of six clusters linked at 63% similarity. The top cluster corresponds to subphenon 14 (S. thermophilus). The second cluster corresponds to subphenon 15 (S. mitis). The third cluster appears below these two clusters as part of a separate subgroup. This cluster corresponds to subphenon 18, "S. milleri". The next cluster contains the strains of the genus Leuconostoc and corresponds to subphenon 19. The next cluster and one satellite strain corresponds to subphenon 17, "Oral II". The final cluster at the base of this arm corresponds to subphenon 16 (S. sanguis). This group of clusters represents phenons V, VI, VII and VIII.

The next arm of the dendrogram consists of six clusters corresponding with phenon IX, the pyogenic organisms. The

first cluster corresponds to subphenon 20 (S. agalactiae). Included in this cluster however are two extra strains, PB 127 and PB 139. These are strains received as "Streptococcus sp. (B) clinical" and they were previously assigned to subphenon 24. The members of subphenon 24 previously linked to S. agalactiae with the D_p coefficient. Below this the next cluster corresponds to part of subphenon 23, "S. equisimilis". The first cluster contains six strains. PB 44 appears as a satellite to a tighter cluster of four strains, PB 103, PB 104, PB 105 and PB 132. PB 44 was received as an example of streptococci of serological group G. The four strains in the tight group were all received as examples of "S. equisimilis". Linked to these four strains is the single strain PB 155, received as an example of serological group L. There are two further strains at the base of this cluster which are shown as a separate group on the simplifed dendrogram. These were both received as examples of "S. zooepidemicus", PB 120 and PB 121. These are linked to the main cluster at 72% similarity.

The next cluster consists of the majority of subphenon 24. One strain, PB 133 appears as a satellite to this group, although it had previously been assigned to it. The two clusters below this correspond to subphenons 22 and 21 (S. equi and S. pyogenes). The final cluster on this arm of the dendrogram corresponds to subphenon 26, "S. dysgalactiae". One strain, PB 141 is linked to the base of this cluster at 64% similarity. This had previously been assigned to subphenon 24.

The bottom arm of the dendrogram consists of two clusters. The first cluster consists of four strains. Two of these strains, PB 107 and PB 148, were previously assigned with one other strain to subphenon 13 (A. viridans). The remaining two strains, PB 112 and PB 114 were previously assigned to the loosely linked subphenon 28 (Pediococcus sp.). The lower cluster contains the rest of the strains from this loosely linked group.

3.8 DNA results

The mole percent G+C content of DNA was determined by thermal denaturation for thirty-two strains. These represented twenty-five of the twenty-eight groups. One additional strain, PB 70, was also included as it was received as the type strain of S. pneumoniae. The type strain of Eschericia coli (NCTC 9001) was also included as a control. The strain PB 80 was initially chosen as it was close to the centroid of subphenon 5, S. bovis. However this particular strain was found to be very difficult to lyse and gave poor yields of DNA. Therefore PB 81 was used as a representative of this cluster. Strains PB 58, PB 179 and PB 183 also gave poor samples of DNA. It was seen for these particular strains that although the DNA yield appeared high from absorbance readings, the melting process was very slow and the degree of denaturation was poor. This was improved considerably by performing a further two iso-propyl alcohol precipitations before dialysis (some possible reasons for this are given in Section 4).

The results obtained are presented in Table 3.8.a, showing the average mol %G+C. This was obtained from three or more determinations for each strain. Those strains that required more than three determinations to give reproducible results were PB 15, PB 81, PB 58, PB 179 and PB 183. As a measure of the range of the results obtained, the standard deviation for each set of determinations is given. However, this may not be statistically significant as it is based on a relatively small number of figures. As a result, care should be taken when considering them.

The melting point of the DNA was determined from the point on the denaturation curve where the greatest increase in absorbance was seen. A representative curve from strain PB 137 is shown in Figure 3.8.1. The average hypochromism for each sample was also calculated as described in Section 2.10. These results are listed in Table 3.8.a.

3.9 Esterase results

The thirty-four strains of streptococci used in the DNA work were tested for the presence of active esterase enzymes in polyacrylamide gels. Whole cell preparations were used as described in Section 2.14.2. 50μl of a 3 mg ml^{-1} sample was used for each, giving a "loading" in each track of 150μg. Each gel was able to take nine samples. However, the outer tracks on either side were used for duplicates to avoid edge effects.

Active esterases were seen in the polyacrylamide gels and these varied in number from a single faint band up to four intense bands. Active esterases were seen in twenty of the

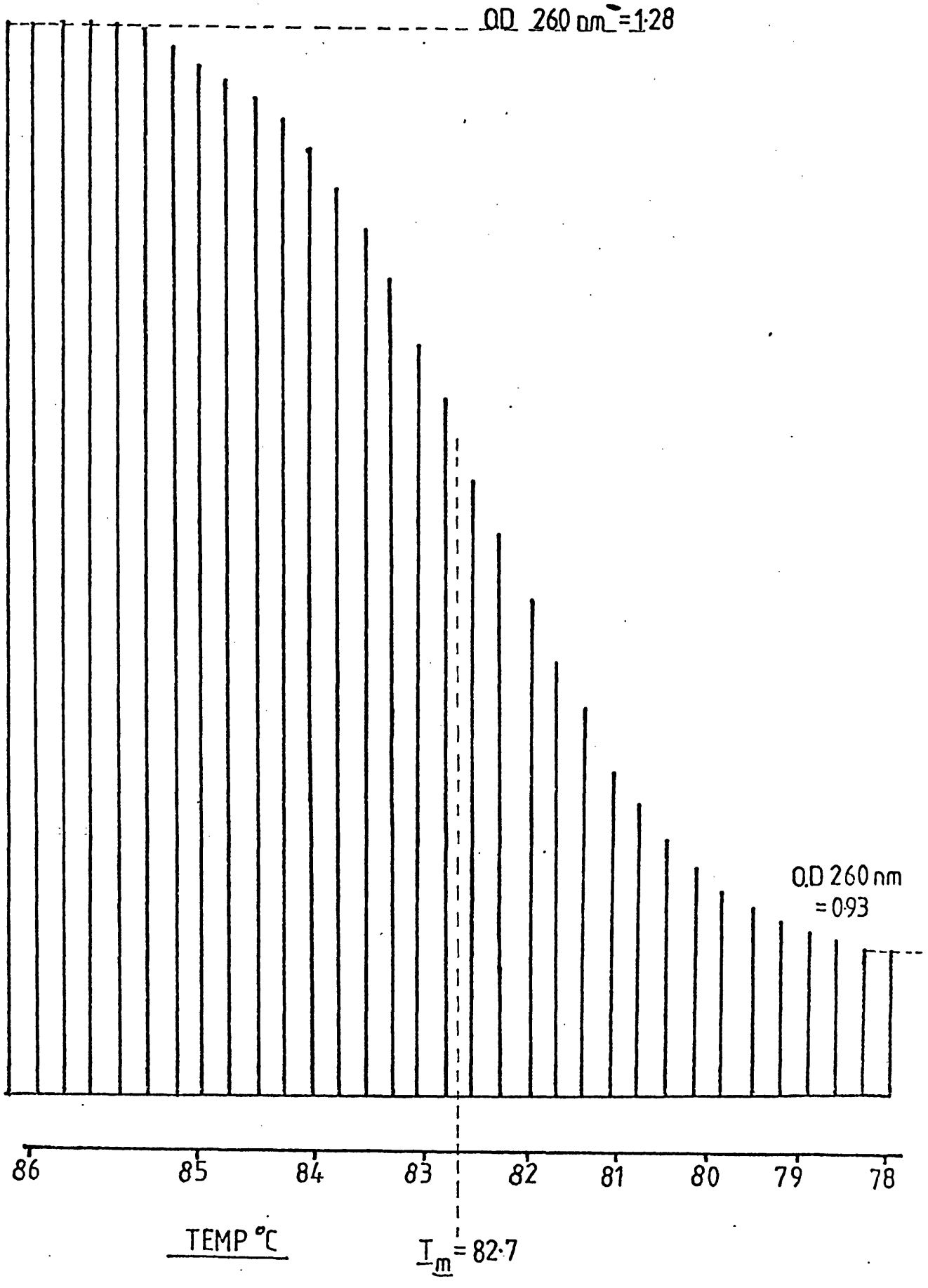
Table 3.8.a DNA results.

Sub-phenon	Strain	Received as.	Mol %G+C	S.D.	Hypo-chromism.
1	PB 2	S. faecalis	38.40	0.43	30.5%
2	PB 86	"S. durans"	36.98	0.62	27.8%
2	PB 87	S. faecium	38.95	0.35	34.1%
3	PB 15	"S. avium"	39.89	0.61	36.7%
4	PB 18	"Strep. sp. (chicken)"	35.62	0.25	31.6%
4	PB 21	"Strep. sp. (chicken)"	39.15	0.65	36.2%
5	PB 81	S. bovis	39.30	0.04	36.4%
6	PB 83	S. equinus	35.60	0.00	38.3%
7	PB 9	S. salivarius	38.93	0.49	28.2%
8	PB 52	"S. casseliflavus"	39.90	0.10	34.8%
9	PB 58	S. rattus	38.77	0.27	33.7%
10	PB 202	Streptococcus sp.	40.40	0.43	40.3%
10	PB 199	S. raffinolactis	39.77	0.25	35.4%
11	PB 172	Streptococcus sp.	38.12	0.12	30.5%
12	PB 93	S. lactis	35.50	0.10	33.4%
12	PB 193	"S. lactis subsp. diacetylactis"	34.75	1.35	32.0%
12	PB 95	S. cremoris	34.05	0.15	33.8%
13	PB 107	A. viridans	39.90	0.10	31.1%
13	PB 148	Streptococcus sp.	40.20	0.20	28.2%
15	PB 98	S. mitis	38.85	0.09	34.5%
16	PB 68	S. sanguis	39.29	0.25	34.5%
17	PB 179	Streptococcus sp.	39.89	0.13	40.5%
17	PB 183	Streptococcus sp.	40.01	0.49	34.6%

Table 3.8.a continued

Sub-phenon	Strain	Received as.	Mol %G+C	S.D.	Hypo-chromism.
18	PB 57	"S. milleri"	34.05	0.35	22.3%
20	PB 40	S. agalactiae	36.60	0.20	30.1%
21	PB 54	S. pyogenes	35.75	0.15	30.2%
22	PB 41	S. equi	39.28	0.00	32.9%
23	PB 103	"S. equisimilis"	37.33	0.25	40.8%
23	PB 155	"Streptococcus sp. L"	38.06	0.15	30.2%
24	PB 137	"Streptococcus sp. B"	33.18	0.73	34.6%
25	PB 118	S. uberis	36.40	0.20	41.6%
26	PB 117	"S. dysgalactiae"	37.21	0.12	38.1%
27	PB 160	"Streptococcus sp. S"	40.15	0.15	32.5%
—	PB 70	S. pneumoniae	35.14	0.00	35.4%
—	NCTC 9001	E. coli	51.48	0.40	35.9%

Figure 3.8.1 A typical melting curve for strain PB 137.



cell extracts. Five gels were used for the thirty-four samples and tracings of these are shown in Figures 3.9.1-3.9.5. The strain PB 40 was included in gel V as well as in gel IV in order to make up the same total loading.

The positions occupied by the esterase bands in the gels were measured from normalised traces. In the absence of a known marker esterase and the presence of apparent bands close to the bromophenol blue marker, bromophenol blue was used to represent the maximum distance travelled. The distance was considered as being 100 units from the origin. The position of each band could then be measured on a scale of 0-100 and compared between gels. Using this method the bands were classified into thirteen major groups. In the majority of cases it was decided that these groups should be wide enough to allow for experimental errors which may be present as a result of inconsistencies in the gels or irregularly running fronts. It was found that there were four apparent bands between 60 and 64 units. These were grouped into three subgroups on the ground that strain PB 2 showed two of them. A list of the different groups of bands and the distances assigned to them is given in Table 3.9.a.

Groups 12 and 13 contained bands that had travelled between 92 and 100 units. Bromophenol blue, which travelled 100 units on this scale, has a molecular weight of 670 daltons. It would therefore be expected that the components of these bands were either of low molecular weight or very highly charged. It is unlikely that they were of low molecular weight as they showed esterase activity.

Figure 3.9.1 Esterase gel I.

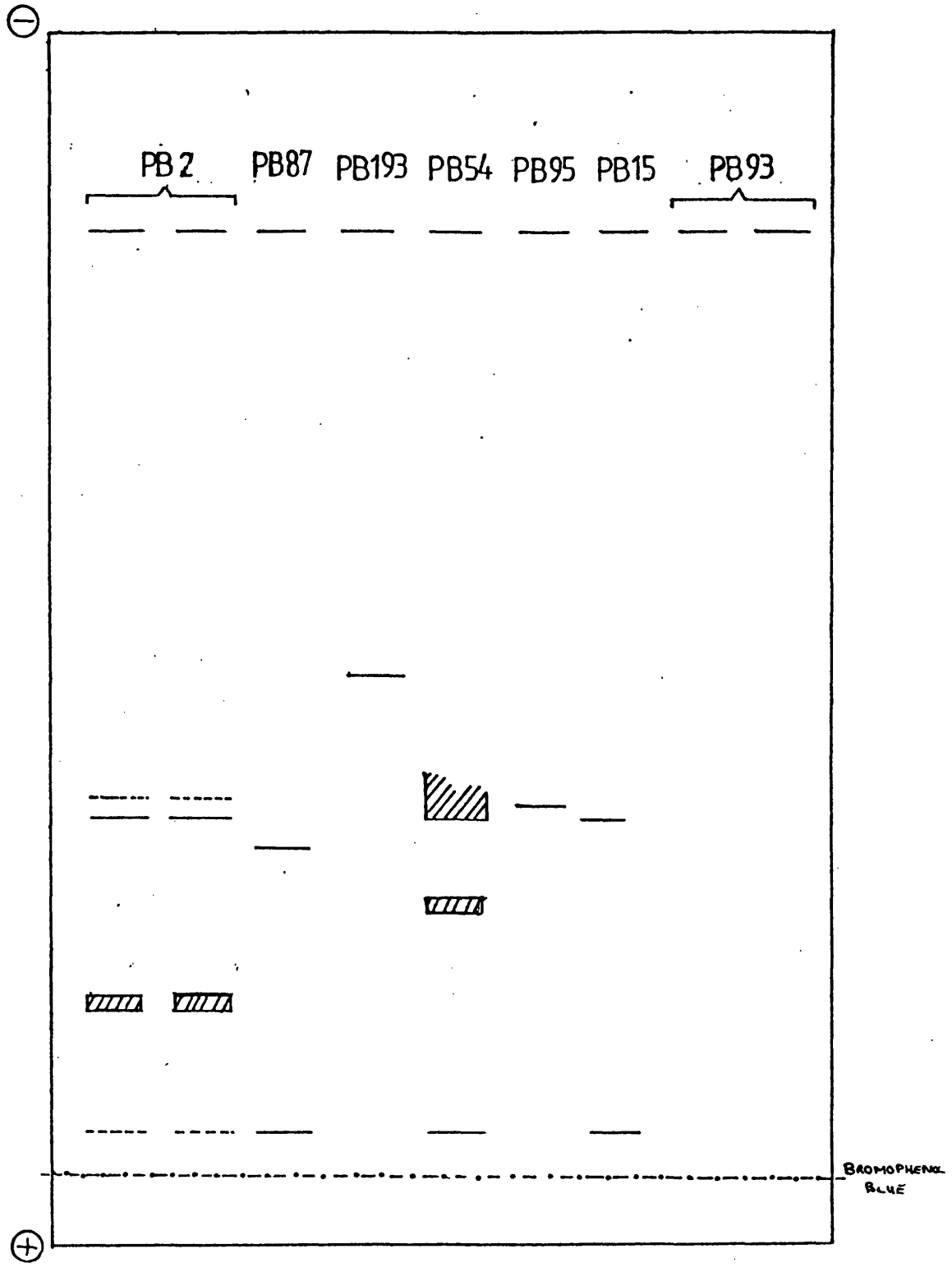


Figure 3.9.2 Esterase gel II.

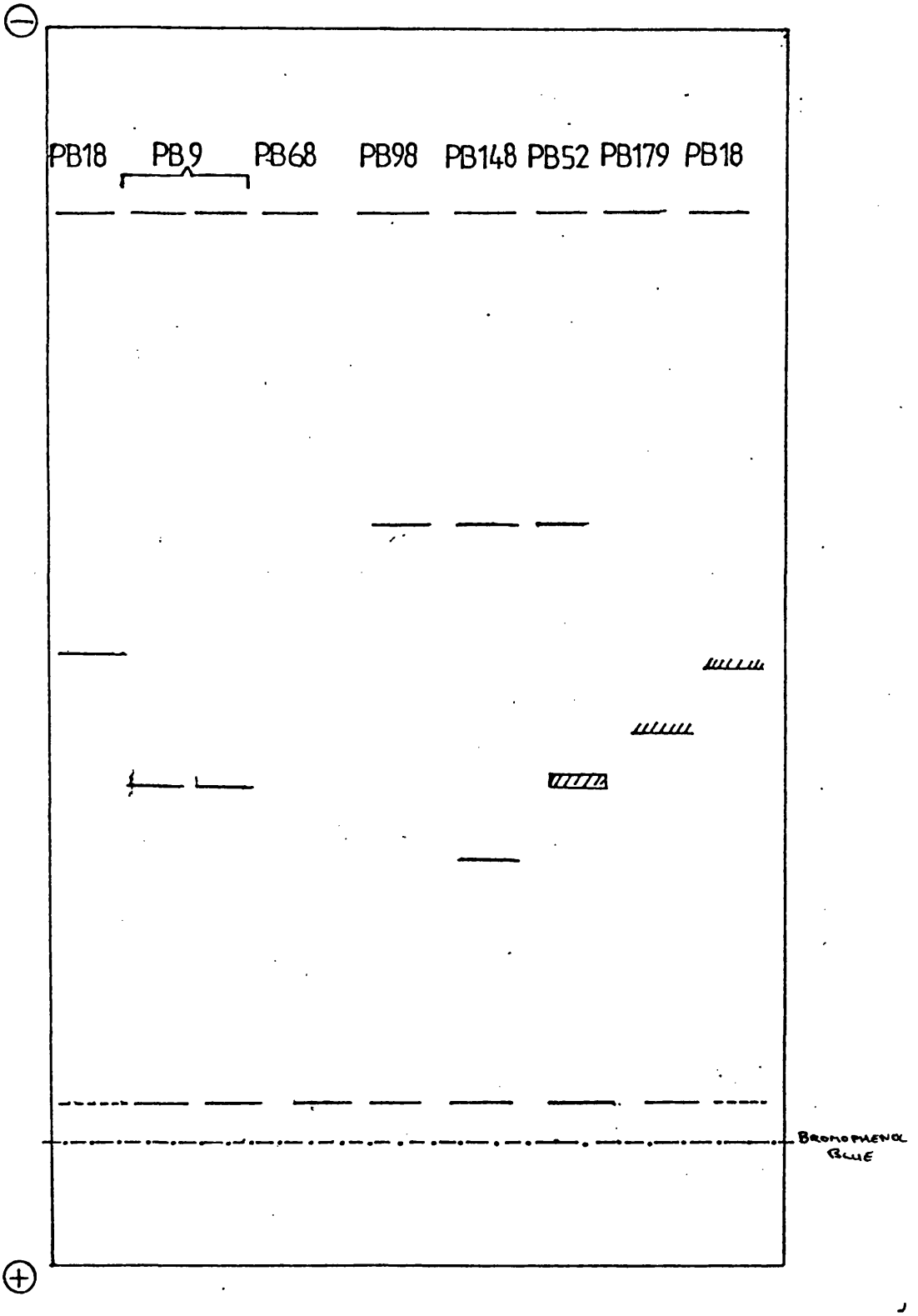


Figure 3.9.3 Esterase gel III.

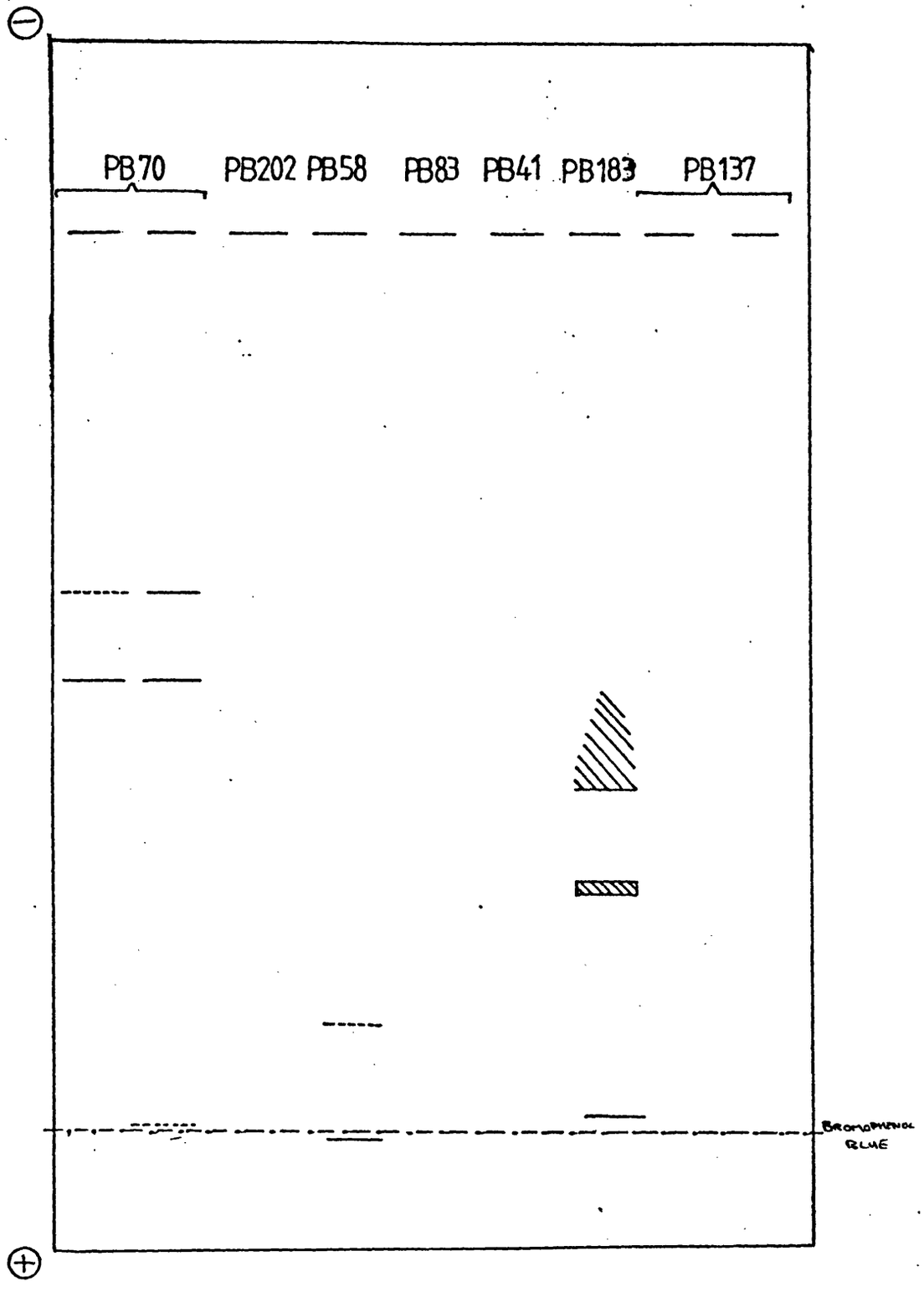


Figure 3.9.4 Esterase gel IV.

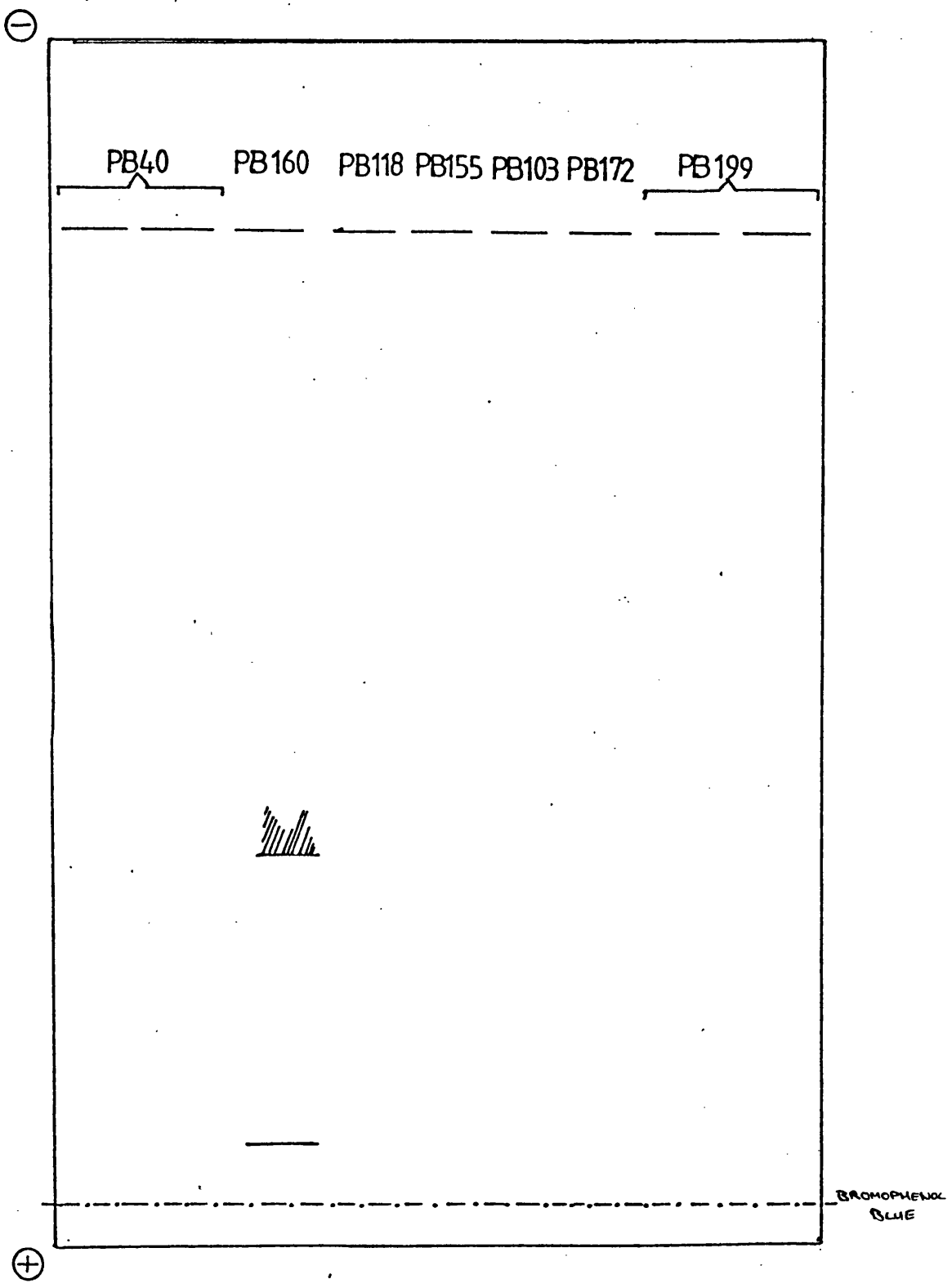


Figure 3.9.5 Esterase gel V.

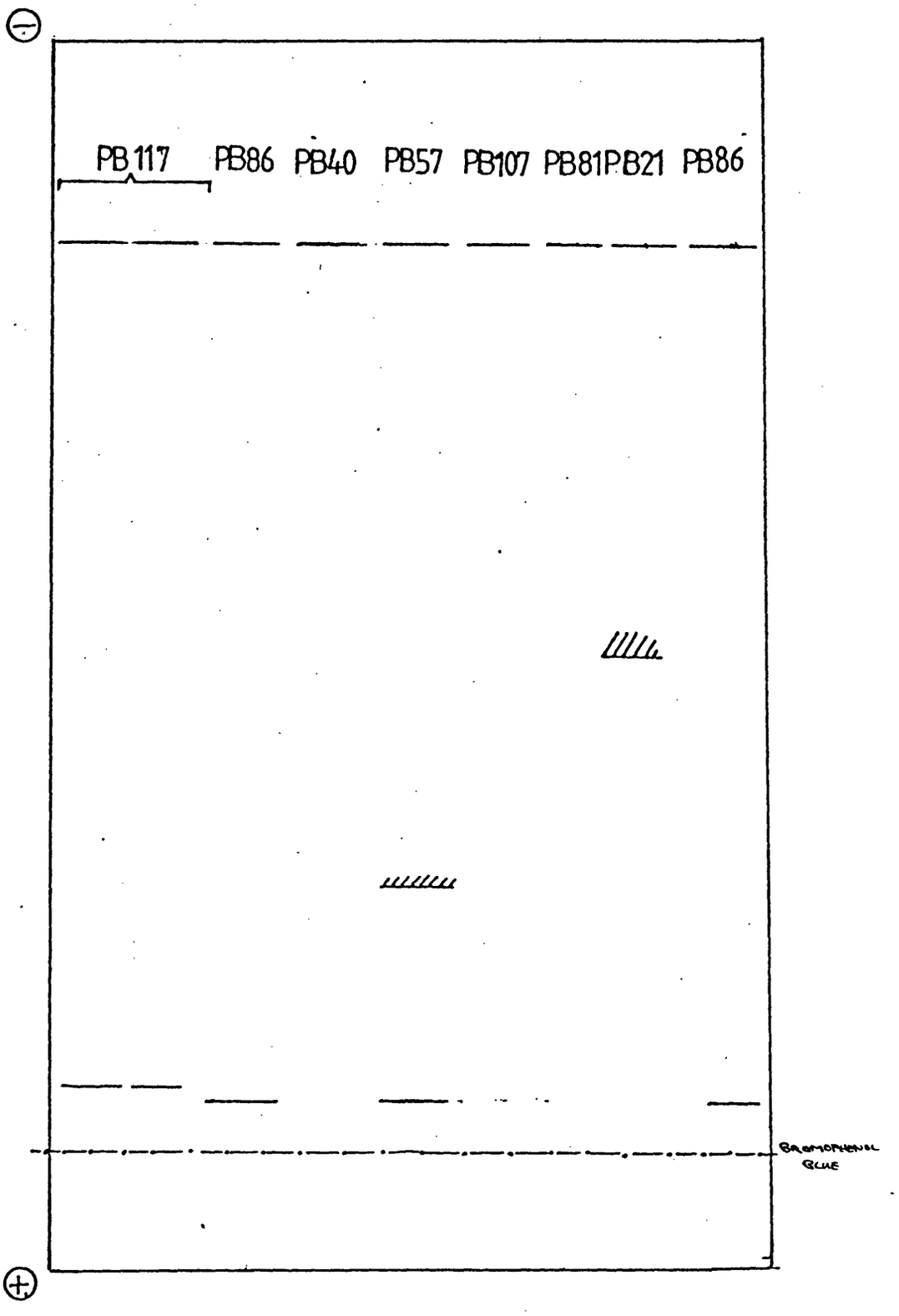


Table 3.9.a The distances migrated by the major esterase bands expressed as a percentage of the total distance.

Group.	Distance migrated.
1	33
2	39
3	42-43
4	47.3-49
5	52-55
6a	60
6b	61
6c	62-62.5
7	65-66
8	69
9	71-73
10	82
11	88
12	92-96
13	97-100

The results of these gels can be ordered to show the occurrence of esterases within the different phenons found in the numerical taxonomy. The results of this are shown in Tables 3.9.b and 3.9.c.

Two further gels were also used. One consisted of the samples used in gel I, but taken from a 6 mg ml^{-1} solution, giving a loading of 300 μg in each track. The resulting gel is shown in Figure 3.9.6. All of the bands previously seen are present. However, apart from the increased density and smearing only one extra band was seen. This was in strain PB 93 and it migrated 79 units, falling between groups 9 and 10. The heavy smearing at this concentration made reading difficult and further gels were not thought worthwhile.

The second gel consisted of seven strains from the original thirty-four. These were grown and harvested as before and proteins were extracted as before. This was done to give an idea of the reproducibility of the methods used. Three of the samples, PB 117, PB 81 and PB 83 failed to demonstrate any esterase activity. This was the same result as seen previously. Strain PB 86 showed a single band at a distance of 93 units and strain PB 18 showed a single band at 48 units. Both of these were seen before. Strain PB 21 had previously shown a band at 42.5 units. Strain PB 98, which previously showed a band at 33 units and one at 94 units, showed two smears, one between 27 and 34 units and one between 94 and 96 units.

Table 3.9.b Distribution of esterase bands within phenons I - IV.

Phenons I and II.

	Bands.														
Strain.	1	2	3	4	5	6a	6b	6c	7	8	9	10	11	12	13
PB 2						+		+				+		+	
PB 87									+					+	
PB 86														+	
PB 15								+						+	
PB 18				+										+	
PB 21			+												

Phenon III.

	Bands.														
Strain.	1	2	3	4	5	6a	6b	6c	7	8	9	10	11	12	13
PB 81															
PB 83															
PB 9								+						+	
PB 52	+							+							
PB 58													+		+
PB 199															
PB 202															
PB 172															

Table 3.9.b continued

Phenon IV

	Bands.													
Strain.	1	2	3	4	5	6a	6b	6c	7	8	9	10	11	12	13
PB 93															
PB 95							+								
PB 193				+											
PB 107															
PB 148	+									+				+	

Table 3.9.c Distribution of esterase bands within phenons VI, VII, XI and X.

Phenons VI and VII.

	Bands.														
Strains.	1	2	3	4	5	6a	6b	6c	7	8	9	10	11	12	13
PB 98	+													+	
PB 68															
PB 179					+									+	
PB 183							+				+				+
PB 57											+			+	

Phenon IX

	Bands.														
Strains.	1	2	3	4	5	6a	6b	6c	7	8	9	10	11	12	13
PB 40															
PB 54								+			+			+	
PB 41															
PB 103															
PB 155															
PB 137															

Table 3.9.c continued

Phenon X

	Bands.														
Strains.	1	2	3	4	5	6a	6b	6c	7	8	9	10	11	12	13
PB 118															
PB 117														+	
PB 160									+					+	

Figure 3.9.6 Concentrated esterase gel I.

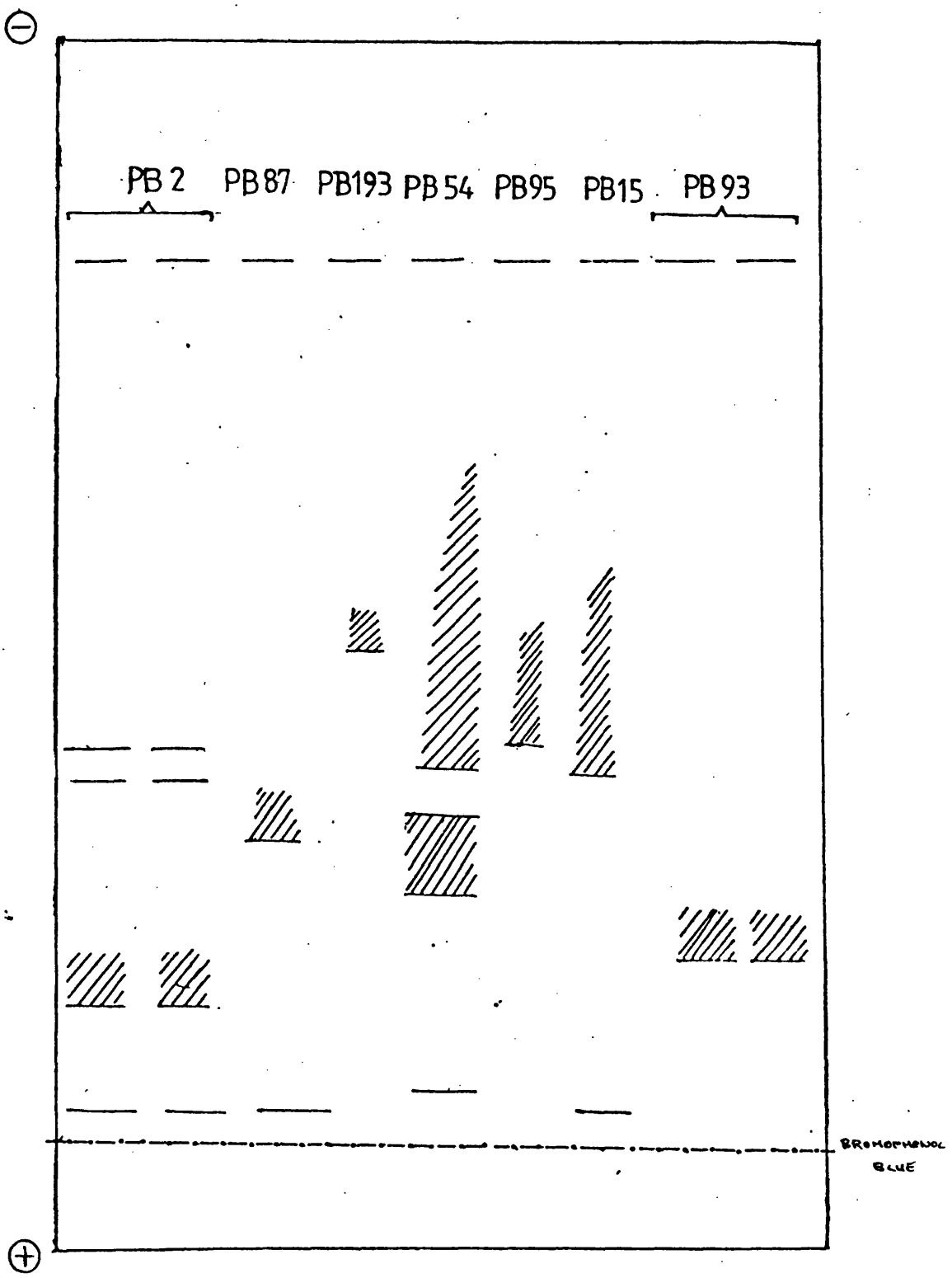
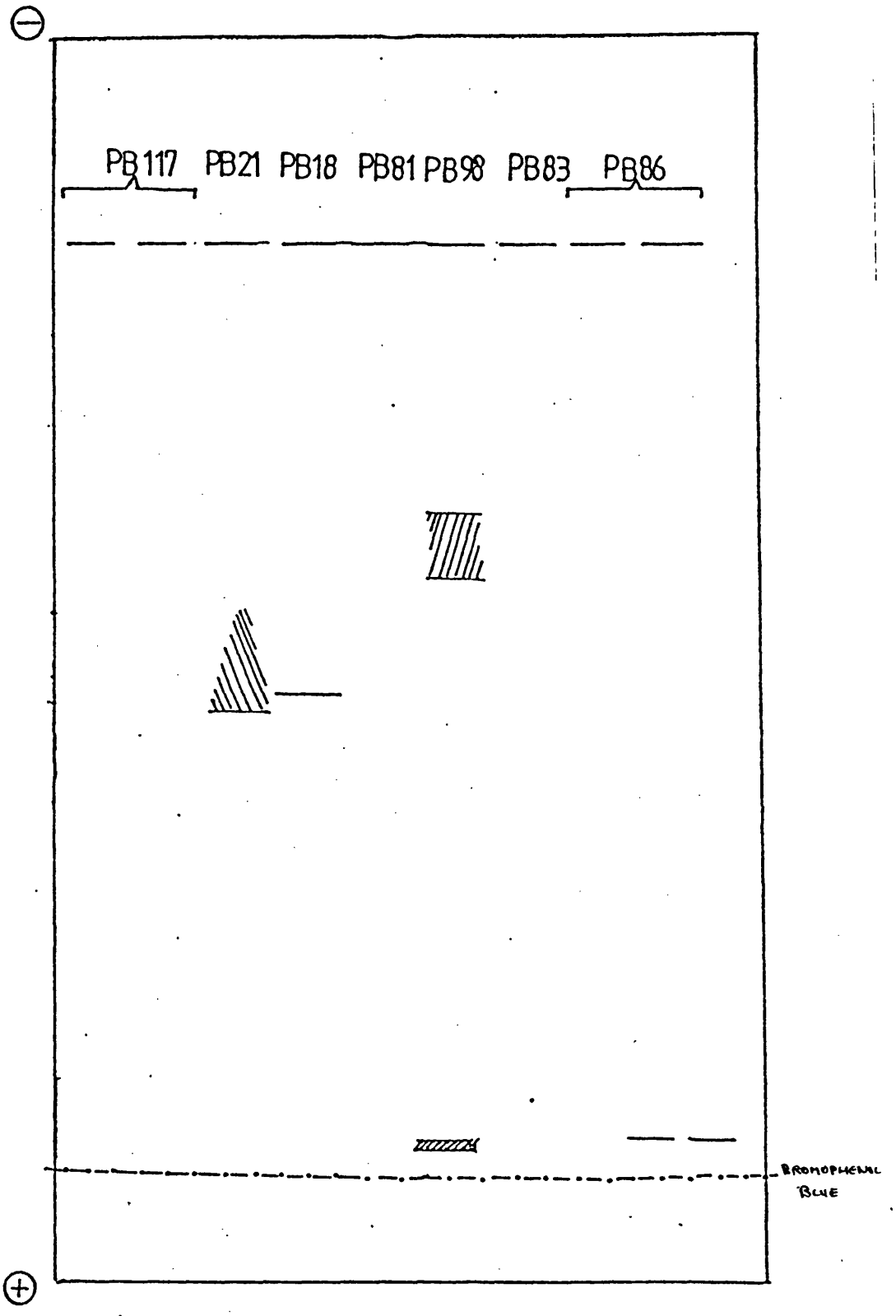


Figure 3.9.7 Repeated esterase gel.



This gel is shown in Figure 3.9.7

3.10 Numerical analysis of protein patterns

The method previously described (Section 2.15) was used to produce densitometric traces of all of the protein patterns obtained. These were digitised as described. Figure 3.10.1 shows a representative protein trace obtained from strain PB 54. Figure 3.10.2 shows the trace obtained from ovalbumin. It was decided that any peaks seen below the level of ovalbumin would consist largely of broken protein. As a result only the first eighty positions were considered for the numerical analysis.

The numerical data was used to calculate the taxonomic distance and the cosine θ coefficient as described earlier. The resulting dendrograms are shown in Figure 3.10.3 and 3.10.4. The names of the strains are the same as given for the DNA work (Section 3.8).

Figure 3.10.3 shows the two dendrograms obtained from the taxonomic distance for the two different methods. The distance between duplicates of the same strains is less than 1.2 units. Although the two dendrograms were produced by two methods they show several areas of similarity. The most evident of these is the group of strains that appear to form a tight group. These are strains PB 81, PB 9, PB 68, PB 172, PB 52, PB 83 and PB 137. Also present in both dendrograms is the looser group of strains consisting of PB 21, PB 193, PB 18 and PB 98. The first four strains in the dendrogram PB 15, PB 15a, PB 40 and PB 41 are the same in both instances. With these exceptions the two dendrograms show little structure, the rest of the strains being loosely linked. However, in both cases the strains PB 179 and

Figure 3.10.1. A typical protein trace.

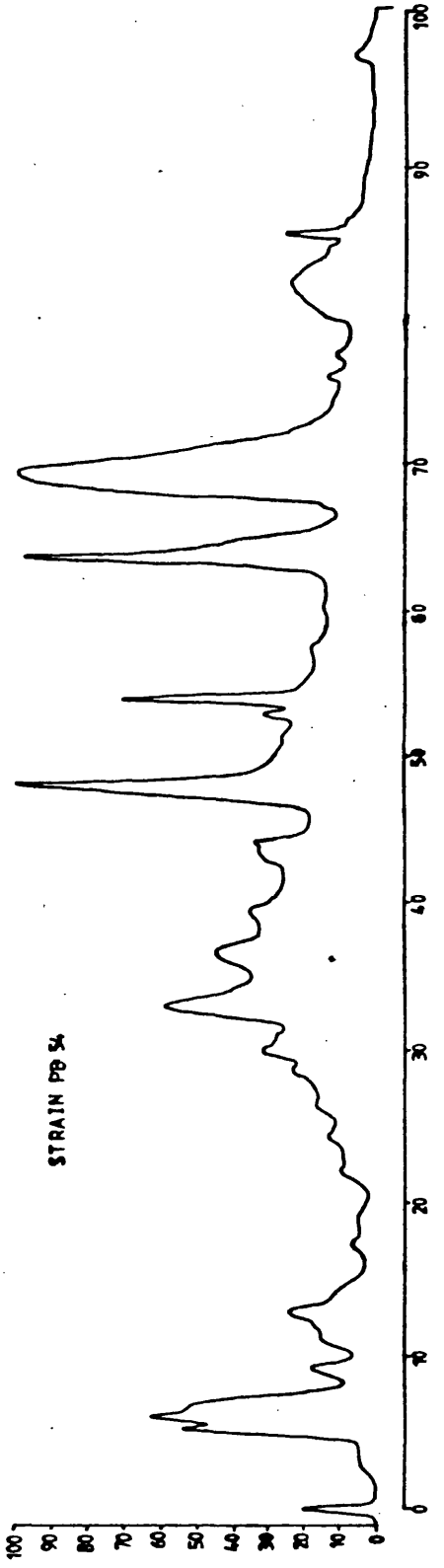


Figure 3.10. 2 Densitometric trace of ovalbumin.

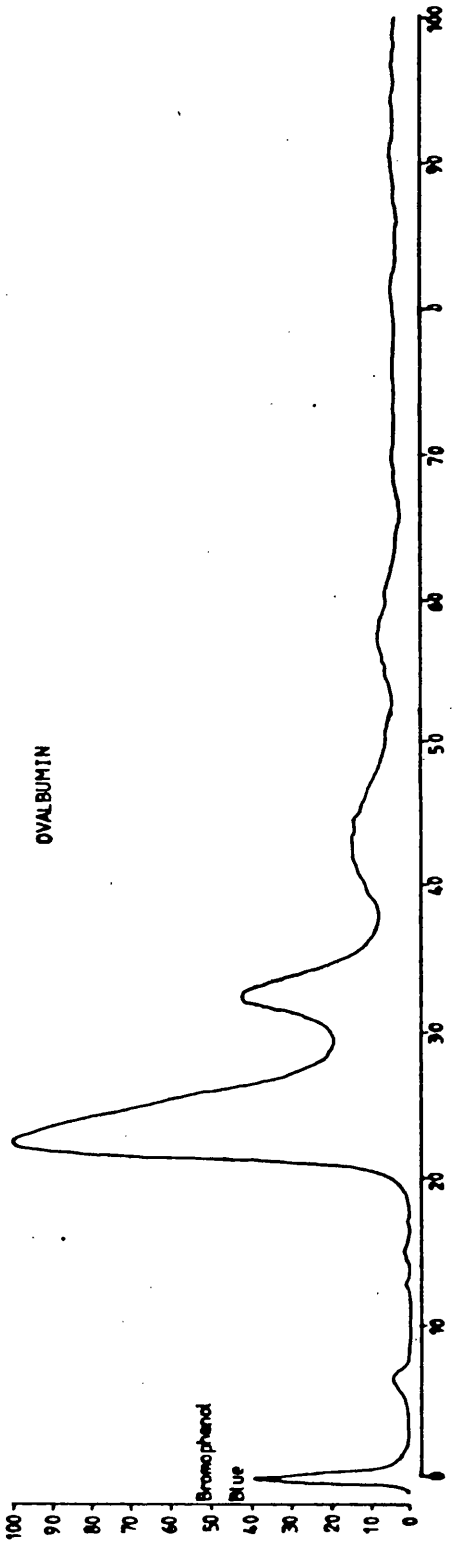


Figure 3.10. 3 Dendrograms obtained from the protein traces using taxonomic distance and average linkage. (a) uncorrected; (b) corrected.

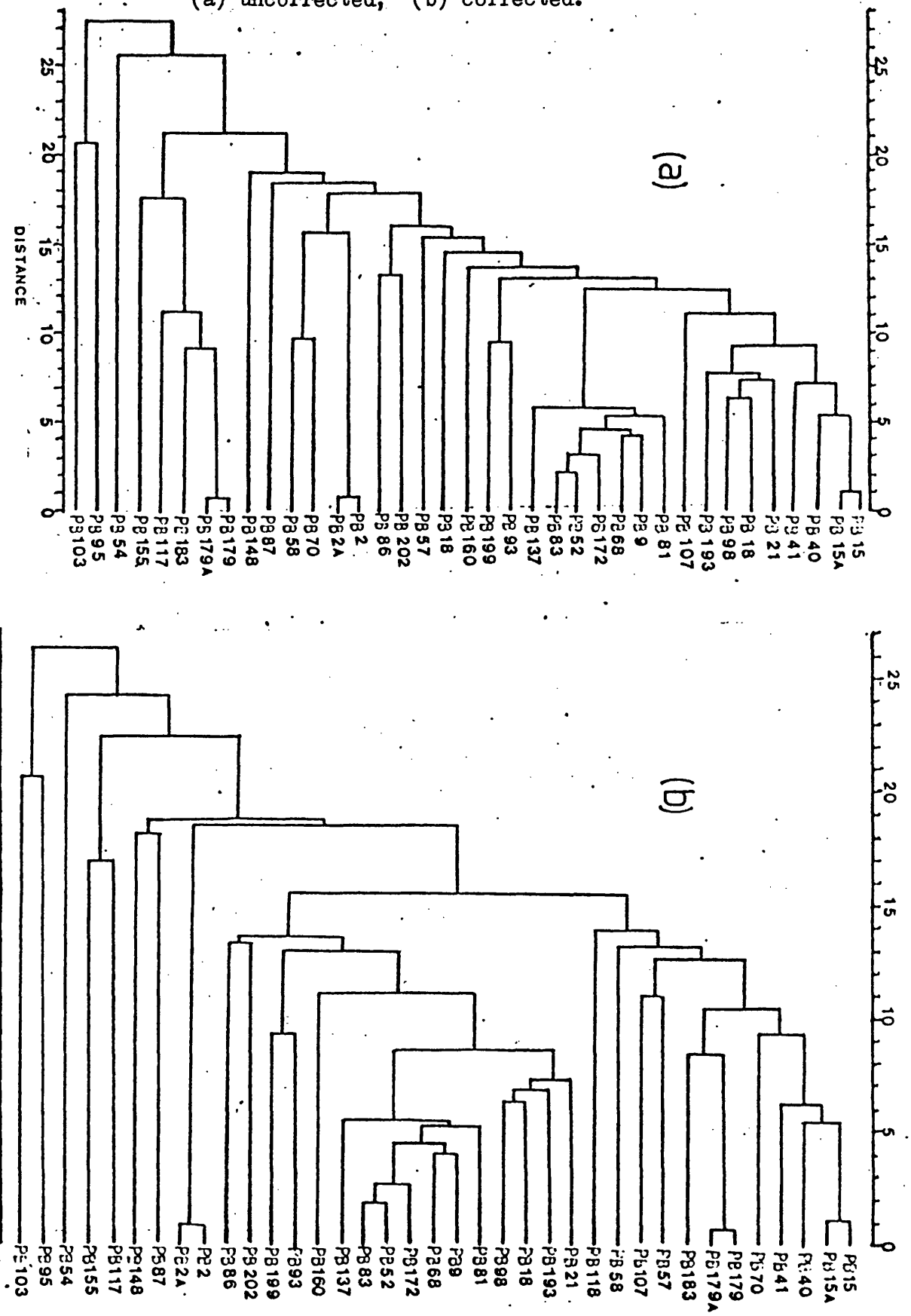
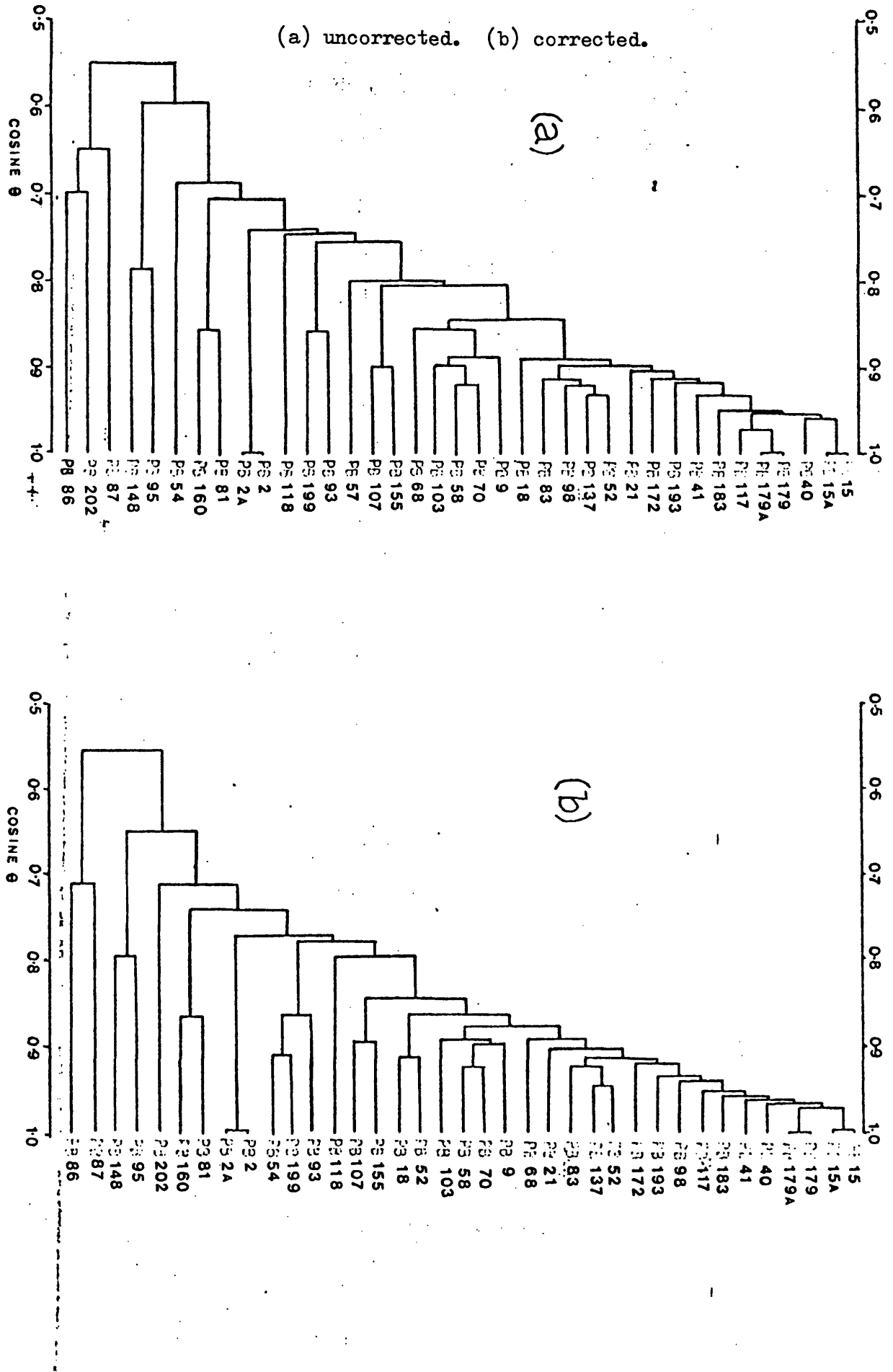


Figure 3.10. 4 Dendrograms from protein traces based on the cosine θ coefficient.

(a) uncorrected. (b) corrected.



PB 183, both members of the "Oral II" group, appear together. The pairs of PB 18 and PB 21, and PB 86 and PB 87, also represent groups where two members were studied and these do not link together. There appears to be little agreement between the groups found in the numerical taxonomy and these representatives.

The program OVCLUST was used to determine whether the first four strains and the two apparent groups were statistically distinct. In each case this showed a $\underline{t}$-test of below 90% confidence for these groups and a $\underline{V_G}$ value of greater than 1×10^{-3}. This shows a low level of disjunction and so it is unlikely that they are significantly different from the rest of the strains. The taxonomic distance for these methods ranges from less than 1 for some duplicates to just over 27 for the most dissimilar.

Figure 3.10.4 shows the two cosine θ dendrograms obtained from the above methods. These both show a large number of strains linking at above 0.8, with the lower strains on the dendrograms linking loosely to them. In both cases these looser linked strains are the same. These are PB 81, PB 160, PB 202, PB 95, PB 148, PB 87 and PB 86. Overall, the order of the strains is very similar in each dendrogram but the apparent groups seen in the distance dendrograms are not present. The duplicate strains all link at above 0.99 in both dendrograms.

The high similarity shown between replicates, that is seen with both coefficients, indicates that each trace is distinct from the others and the methods employed appear to be reproducible.

It appears that different strains may therefore be differentiated. However, quite a range of differences may be seen between organisms from the same cluster. Strains PB 18 and PB 21 are both members of subphenon 4, "Streptococcus sp. (chicken)". They appear together when taxonomic distance is used without corrections and close when the corrections are applied. However, they do not appear together when the cosine θ coefficient is used.

Alternatively, strains PB 86 and PB 87, which are both members of subphenon 2, S. faecium, appear closer with the cosine θ coefficient in both cases than they do with the taxonomic distance. As a result of differences such as these and the high levels of similarity shown, it is not possible to attempt to group these representative strains into any arrangement other than that each strain appears different.

3.11 Serology results

Thirteen strains that were received as being assigned to particular Lancefield groups were serogrouped again. The method described in Section 2.15 was used. The strains used and the results obtained are given in Table 3.11.a. The strains had all been received as possessing the Lancefield group B or D antigens. All were found to give a positive reaction to the expected antiserum. However, two strains, PB 53 and PB 69 gave only weak positive results, a very faint line of precipitation being seen.

Table 3.11.a Serological results

Strain.	Received as.	Group B		Group D
PB 51	"*S. faecium* subsp. *casseliflavus*"	/		+
PB 52	"*S. faecium* subsp. *casseliflavus*"	/		+
PB 53	"*S. faecium* subsp. *mobilis*"	/	Weak	+
PB 69	"*S. faecalis* subsp. *malodoratus*"	/	Weak	+
PB 127	"*Streptococcus* sp. (group B)"	+		/
PB 134	"*Streptococcus* sp. (group B)"	+		/
PB 135	"*Streptococcus* sp. (group B)"	+		/
PB 137	"*Streptococcus* sp. (group B)"	+		/
PB 138	"*Streptococcus* sp. (group B)"	+		/
PB 139	"*Streptococcus* sp. (group B)"	+		/
PB 141	"*Streptococcus* sp. (group B)"	+		/
PB 147	"*Streptococcus* sp. (group B)"	+		/

/ = not tested

DISCUSSION

4.1 Test reproducibility discussion

The percentage error between duplicate strains was shown in Table 3.1.a to have an overall value of 2.48% which corresponds to a similarity of 95.04%. This would indicate that the strains tested were stable under the conditions used. These figures also helped in evaluating the clustering methods. It would be expected that different strains would cluster at below this level. Only two pairs of strains in the S_G average linkage dendrogram (Figure 3.2.1), link close to this at 95% similarity. These strains, PB 23 and PB 27, and PB 25 and PB 29, are all isolates of serological group D organisms which were isolated from chickens (Barnes et al., 1978). Two strains in this dendrogram link at above 95% similarity. These are strains PB 14 and PB 15, which were received as different isolates of "S. avium".

The differences between tests were recorded in Appendix II. Sneath & Johnson (1972) suggested that when test error is over about 10%, the error of similarity values becomes unacceptably large. It was hoped by removing "bad" tests, the similarity values found would be an accurate representation of the classification.

Morphological tests were found to give, in general, irreproducible results, particularly cellular aggregation. This test relies on observations based on the majority of the cells seen in a number of microscope fields. Discrepancies may enter this test in the form of differences in the concentration of the suspension, the age and condition of the cells, the spreading of the slides, the treatment of cultures during preparation, and strain variation. Most of these may be standardised in one way or another, and attempts

were made to do this in the numerical taxonomy (see Section 2); the same amount of inoculum, the same media and the same incubation times and temperatures were used. However, the high difference figure seen indicates that either the methods were not sufficiently standardised or that cellular aggregation is very variable and may not remain constant for one strain. The use of cellular aggregation for some organisms has been found by other workers to give some of the less reproducible results (Sneath & Johnson, 1972).

The antibiotic sensitivity tests proved to be very irreproducible. Of the twenty-four tests carried out, fourteen were considered "good" enough to be scored in the taxonomy. However, of these, six were found to give all positive or all negative results. The reasons for the irreproducibility of the "bad" tests are not immediately clear. The method involved spread plates, and so serious contamination would probably have been noticed. The multodisks used were all either near, or at the end of their recommended usage period and possibly this may account for some of the discrepancies. Similarly, different batches were used and batch variation may also be a contributory factor. Antibiotic resistance in streptococci may be plasmid mediated (Dunney & Clewell, 1975; Harwood, 1980). However, if this were to account for any of the variations found, the expression of the plasmid would have to be affected by the conditions used.

The production of a catalase from haemin was found to be just outside the chosen reproducibility limits. Again in this case there appeared to be some form of batch variation, the majority of

discrepancies appearing in one batch of plates. This may be due to variation in the composition of the media. However, when the method was repeated, variation was again seen in one batch of plates. This may be due to the haemin being unevenly distributed throughout the medium.

There were some "bad" tests on the API 50E gallery. Eight of the forty-nine tests gave results which were above the upper limit of 15% difference. Two of these, variation in ribose and tetrathionate reduction, appeared to be due to batch variation. Two batches of the API galleries were used in the numerical taxonomy. In one batch ribose always gave a positive result, often reaching 3 on a scale of 1-5 on inoculation. In this batch tetrathionate reduction was usually negative. In the other batch ribose gave varied results and tetrathionate reduction was usually positive. Acid from ribose could be expected to give varied results for the streptococci and tetrathionate reduction could be expected to be usually negative (Feltham, 1979), and so both tests are affected. Two of the eight "bad" tests, gluconate and lipase gave both positive and negative results. However, it was found that an average score of 2 was obtained on inoculation. As a result a small change that would otherwise be considered as negative or only weakly positive, will appear as a strong reaction. No immediate reason was seen for the variations in the other four "bad" tests. The concept of batch variation must be taken into account when considering test variation between galleries. The API gallery is intended primarily for use as part of a characterisation scheme, and as such may be considered

as a single test method. The tests on the gallery were intended for use with the Enterobacteriaceae, although other workers (Feltham, 1979; Logan & Berkeley, 1981) have used this system for gram-positive organisms.

The results of the comparison study between the API sugar methods and the more conventional sloppy agar medium were given in Section 3.1. The error of 5.4% seen for this is within the chosen upper limit of 15% for repeated tests. In general a greater number of positive test results were seen with the API methods than the sloppy agar methods. It is possible that for the API tests scoring above 2 as positive may be too low for comparison with conventional methods. The work of Dolezil & Kirsop (1977) and Power (1978) indicated that the API system may be used with good effect in place of other methods, and compares well with them.

The test reproducibility of the APIzym gallery indicates that these are "good" tests. However, there are some discrepancies between different studies. Humble et al., (1977) and Waitkins et al., (1980)both used the APIzym system with streptococci. Both of these studies involved α-haemolytic and non-haemolytic organisms. These organisms gave differing results and these results in turn differed from those found in this study. A comparison of all these results is shown in Table 4.1.a, showing the organisms, the study, the number of strains used and the number of strong and weak positive reactions. In each case only 0 was considered as a negative reaction, a weak reaction was taken as 1 or 2, and a strong reaction was 3, 4 or 5. Some tests, such as leucine arylamidase (5), α-glucosidase (15) and β-glucosidase(16)

Table 4.1.a Comparison of APIzym results from different sources; number of strains giving strong and weak reactions.

Organism	Study	No.	Level	Enzyme number*																	
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
S. faecalis	Humble	7	strong	0	2	3	/	0	/	/	/	/	1	3	0	0	/	6	6	/	/	/
			weak	0	5	4	/	0	/	/	/	/	6	3	0	0	/	1	0	/	/	/
	Waitkins	21	s	0	19	21	0	21	0	19	0	0	20	21	0	0	0	21	0	0	0	0
			w	1	2	0	0	0	0	2	1	1	0	0	0	0	0	0	0	1	0	0
	Bridge	11	s	2	4	10	0	9	0	1	0	8	9	9	0	8	0	9	10	2	0	0
			w	9	7	1	0	2	0	8	0	1	2	2	0	2	0	0	0	3	0	0
S. bovis	H	4	s	0	0	0	/	0	/	/	/	/	0	0	3	0	/	2	3	/	/	/
			w	0	1	1	/	4	/	/	/	/	4	1	1	0	/	2	1	/	/	/
	W	18	s	18	18	0	0	18	0	17	0	0	0	0	0	0	0	17	0	0	0	0
			w	0	0	0	0	0	2	1	0	4	0	0	3	1	0	0	0	1	0	0
	B	5	s	1	1	2	0	3	0	0	0	0	3	1	0	1	0	2	1	0	0	0
			w	4	4	3	0	2	0	0	0	0	2	3	0	0	0	1	1	0	0	0
"S. milleri"	H	10	s	10	0	0	/	5	/	/	/	/	10	0	0	0	/	0	0	/	/	/
			w	0	9	0	/	5	/	/	/	/	0	1	2	2	/	7	2	/	/	/
	W	17	s	17	16	0	0	17	17	17	0	0	16	0	0	0	0	8	0	0	0	0
			w	0	1	0	0	0	0	0	0	0	1	1	3	0	0	1	0	0	0	0
	B	3	s	2	1	2	0	3	0	1	0	0	3	3	0	2	0	3	3	0	0	0
			w	1	2	1	0	0	0	2	0	2	0	0	0	1	0	0	0	0	0	0
S. mutans	H	3	s	0	0	0	/	0	/	/	/	/	0	0	0	0	/	1	0	/	/	/
			w	0	2	0	/	1	/	/	/	/	0	0	0	0	/	2	0	/	/	/
	W	9	s	8	6	8	1	7	1	0	0	3	7	0	0	1	0	0	0	1	0	0
			w	0	3	0	0	0	2	0	0	2	0	2	0	1	0	5	0	0	0	0
	B	5	s	0	3	4	0	3	0	1	0	1	1	1	0	1	0	2	2	0	0	0
			w	5	2	1	0	1	0	1	0	0	1	4	0	0	0	0	1	0	0	0
"S. mitior"	H	7	s	0	1	0	/	5	/	/	/	/	1	0	4	1	/	1	0	/	/	/
			w	1	5	1	/	2	/	/	/	/	1	0	2	1	/	4	0	/	/	/
	W.	14	s	6	9	1	2	13	14	14	0	2	14	11	1	2	4	4	0	6	0	0
			w	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	3	s	1	0	2	0	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0
			w	2	3	1	0	2	1	0	0	1	1	3	0	2	0	2	0	1	0	0
S. sanguis	H	8	s	2	0	0	/	4	/	/	/	/	4	0	1	1	/	2	0	/	/	/
			w	2	6	0	/	4	/	/	/	/	2	0	3	1	/	2	0	/	/	/
	W	17	s	16	14	4	0	16	13	15	0	6	16	10	2	1	2	12	0	1	2	0
			w	0	0	2	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0
	B	4	s	1	0	1	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0
			w	3	4	3	0	1	0	1	0	1	3	4	0	1	0	3	1	0	0	0

/ = no information.

* Enzymes listed in Table 2.9.a.

give in general comparable results. However, others such as esterase/lipase (3) and ∝-galacto-sidase (12) show considerably less similarity. An explanation for this may be the different media and preparations used by the different workers. These differences in media, inocula and methods are shown in Table 4.1.b. Odds et al. (1978) drew attention to variations in hydrolytic enzyme activity on APIzym strips of Candida albicans grown on different peptones. The results (Table 3.1.b) obtained from the same strain of S. faecalis on various media suggest that the differences may be due to media differences. The APIzym system detects the enzymes present in the sample at the time of testing. As a result, inducible enzymes, induced by a particular medium will also be present and may be detected. Thus, different media may be expected to give different results. The differences between the column that records the enzymes in sheep serum and those for the cells of S. faecalis indicate that there is little carry over from the growth media. Enzymes such as alkaline phosphatase (1) and chymotrypsin (9) show differences in both directions, and no enzyme gives a positive result for all strains; therefore it is unlikely that any results are due totally to the media. Thus although the APIzym test strip is easy and quick to use, and gives "good" reproducible results, it is very media dependant. For future studies with this method it will be necessary to fix on a standard growth medium for the organisms, otherwise comparisons may not be valid.

Table 4.1.b. Comparison of APIzym methods from different sources.

Study.	Medium.	Inoculum.	Suspending medium.	Reagent amount.
Humble	Blood agar	40µl	Peptone water	20µl
Waitkins	Todd Hewitt	40µl	Saline	40µl
Bridge	Blood agar	65µl	Distilled water	35µl

4.2 Similarity coefficients and dendrograms.

4.2.1 S_G average linkage dendrogram.

The twenty-eight subphenons seen in the average linkage dendrograms (Figures 3.2.1 and 3.2.3) in general correspond to species groupings. This certainly is so for groups such as S. thermophilus and S. pyogenes, but it is less clear for some of the other groups such as Leuconostoc sp. (subphenon 19) and Pediococcus sp. (subphenon 28).

The enterococcus region

The top arm of the dendrogram, phenons I and II, corresponds to the enterococcus group. The term enterococcus is used here as described by Sherman (1937), although he only described S. faecalis and its varieties and "S. durans". Other species which also possess the Lancefield group D antigen, such as S. bovis and S. equinus are not considered in this group in this study. The group of strains constituting subphenon 4 were considered by Barnes et al. (1978) to be related to S. faecalis/S. faecium and are included in this group on the basis of test results rather than their possession of the group D antigen. In this dendrogram (Figure 3.2.1) the enterococcus region shows a relatively low level of similarity to the other organisms in the study. This may justify it being considered as a natural group. It shows less similarity to the other streptococci than the genera of Leuconostoc, Gemella, Pediococcus and Aerococcus and so gives some support to the contention of Kalina (1970) that these species may form a separate genus.

The region of pyogenic streptococci

Near the base of the dendrogram, phenon IX and possibly phenon X, correspond to a pyogenic group. Phenon IX contains representatives of most of the classical pyogenic organisms. Although "S. equisimilis" is shown here as a single species consisting of "S. equisimilis" and "S. zooepidemicus", it appeared from further work that these two species could be better considered separate. This is discussed further in Section 4.2.3. The species named here as "Streptococcus sp. (B) clinical" does not correspond to any named species. There is some evidence for considering the human and animal strains of Lancefield group B to be distinct (Jones, 1978; Feltham, 1979). This is discussed further in Section 4.2.3.

It is difficult to decide upon the status of phenon X due to the presence of subphenons 25 and 27. Subphenon 25 contains strains of S. uberis. This was not considered to be a pyogenic organism by Wilson & Miles (1975) or Jones (1978). Subphenon 27 contained the strains received as "Streptococcus sp. (groups R, S and T)". These organisms were tentatively placed in the pyogenic group by Colman (1968) and Jones (1978). Strains of serological group S have been reported as having identical characteristics to "S. suis" (Elliot, 1966). This was not found in this study; the two strains received as "S. suis" appeared loosely linked some distance away from this group. On the basis of this they are considered separate in this study.

The region of lactic streptococci and certain allied organisms.

The lactic organisms are represented by subphenon 12. The species S. lactis and S. cremoris were not differentiated as separate species in this dendrogram and this supports the view that they may constitute only one species (Jones, 1978; Collins & Jones, 1979). S. cremoris has not been isolated outside the dairy industry (Jones, 1978), and therefore this subphenon was named S. lactis. This subphenon, however, is linked to the looser group of Aerococcus viridans which has previously been considered to be physiologically similar to the enterococci (Whittenbury, 1965b). The presence of only one named strain of A. viridans in this cluster raises some doubt to its positioning here. This is discussed further in Section 4.2.3.

The region of oral streptococci

Phenons III and VII correspond in the main to the oral streptococci with the addition of S. bovis, S. equinus, "S. casseliflavus" and S. raffinolactis. The unsatisfactoriness of grouping S. bovis and S. equinus with the enterococci was discussed in Section 1.4.3, as were the differences between the enterococci and "S. casseliflavus". It is interesting that the strain received as "S. faecium subsp. mobilis", PB 53, appeared as a satellite strain to subphenon 4 in the enterococci. This was reported by Collins & Jones (1979) as probably being closely related to the organisms described as "S. faecium subsp. casseliflavus". This observation, coupled with the presence of a further motile strain of serological group D (PB 153) in phenon II gives some doubt to the validity of the group "S. casseliflavus". Further work may be needed to clarify these relationships.

S. raffinolactis was listed in Section 1.4.4 as a lactic organism. It was originally described as one by Orla-Jensen & Hansen (1932). Although it belongs to the serological group N, it was considered by Garvie (1978) and Collins & Jones (1979) to be not closely related to either S. lactis or S. cremoris.

The oral isolates received from Carlsson (1968), grouped in these phenons in three clusters. The first (subphenon 7), contained organisms that he considered similar to S. salivarius and it is therefore satisfactory to see that strains received under this name grouped in this subphenon. The subphenon named "Oral I" (subphenon 11) contained strains he considered similar to S. mutans. No other strains were placed in this subphenon and although S. mutans is grouped nearby, it is difficult to completely endorse his view. This situation is further complicated by the separation of some of the strains of S. mutans in this study. Subphenon 17 ("Oral II"), contained the strains that Carlsson considered similar to S. mitis and S. sanguis. The positioning of "Oral II" close to these groups may support this view. This is discussed further in Sections 4.2.3 and 4.3.

Other organisms

Subphenon 15 (S. thermophilus) was loosely linked at the base of phenons III and IV. This organism was considered by Wilson & Miles (1975) to be in the group "other streptococci". Its relatedness to other groups is discussed more fully in Sections 4.2.3 and 4.3.

The genus of Leuconostoc is present in this study as a subphenon (subphenon 19). This is not surprising as members

of a separate genus could be expected to show a much higher level of similarity to each other than to another genus. What does seem a little surprising is the position of this subphenon with the oral organisms of phenons VI and VII. Garvie (1974) reported that members of the genus may be separated into two groups, although no evidence of this was seen in this study. The presence of the only strain of the genus Gemella in this subphenon would indicate that it is more similar to Leuconostoc than to Streptococcus. Further studies with a larger number of strains would be required to confirm this.

The only strain of S. pneumoniae used in the study, (PB 70) was loosely linked at the base of phenon VII. The cell wall studies on this species by Colman & Williams (1965) indicated that it had a unique cell wall structure. The work of London & Kline (1973) showed that the FDP aldolases of this species were similar to those of S. bovis and S. equinus. As a result of these findings and some physiological characters, such as haemolysis and bile solubility, it appears that S. pneumoniae is separate from the pyogenic organisms. This study endorses this view but the level of similarity does not definitely indicate a close relationship with the oral organisms.

The organisms known as "S. milleri" have been considered to be in both the pyogenic and oral regions (see Section 1.4). Its position in this study in the oral region is therefore not surprising although this is discussed further in Section 4.2.3.

The final group in the dendrogram is the loosely linked subphenon 28. This contains a mixture of strains received as

as either Aerococcus sp. or Pediococcus sp. Other information (Sections 4.2.3 and 4.2.4) indicates that these genera may be related to each other and more closely to the enterococci than is apparent here.

4.2.2 S_G single linkage dendrogram

In the single linkage dendrogram (Figure 3.2.2) two subphenons (5 and 8) are slightly altered and five subphenons (7, 13, 15, 16 and 18) appear to have split up to some degree. In general, the loosely linked strains attached to groups in the average linkage dendrogram are not in these positions in the single linkage dendrogram, but are instead in the lower half of the dendrogram.

The most obvious difference between the two dendrograms can be seen at the top of the figures (Figures 3.2.1 and 3.2.3). In the average linkage case four subphenons constituting the enterococci are separated on a single arm from the rest of the organisms. This separate arm is not seen in the single linkage dendrogram, but instead although subphenons 1 and 2 (S. faecalis and S. faecium) are present, subphenon 12 (S. lactis) has exchanged positions with subphenons 3 and 4 ("S. avium" and "Streptococcus sp. (chicken)". There is some historical precedence for considering the lactic organisms as close to the enterococci because they share a substantial number of properties. Sherman (1937) commented on the difficulties experienced by early workers in separating S. lactis and S. faecalis. Recently Mundt (1975) has isolated some streptococci from plants that appear to have properties similar to both S. faecalis and S. lactis.

The high degree of similarity seen between S. bovis and S. equinus has long been known (See Section 1). Subphenon 6 (S. equinus) however appears some distance from S. bovis in the single linkage dendrogram. Linked to S. bovis is a single strain (PB 82). This was received as S. equinus although it clustered in subphenon 8 ("S. casseliflavus") in the average linkage dendrogram. This may support the high level of similarity between the species of S. bovis and S. equinus, although it then throws some doubt on its positioning in the average linkage case. This may be accounted for by the high level of similarity seen in phenon III, as part of subphenon 7 (S. salivarius) is seen in this dendrogram linked to S. equinus. This does however seem to be only thin evidence.

However, further evidence of the existance of an oral region as described in the average linkage dendrogram is given by the remainder of S. salivarius which has linked with subphenon 17, "Oral II". This is further supported by the presence of S. mitis and S. sanguis in this region. Both of these subphenons, however, appear split in the single linkage case and this adds to the confusion. Subphenon 18 ("S. milleri") is also associated with these species but is split. These last three species are seen in the single linkage dendrogram associated with both oral and pyogenic organisms. This could have been expected in the case of "S. milleri" (see Section 1.4). It may be explained by the very close base lines in this figure. The similarity between the two oral regions in the average linkage case was a little above

75%. The similarity between all of these organisms and the pyogenic organisms was a little below 75%. In the single linkage dendrogram all of the clustering is compressed in an area between 82 and 87% similarity. As the Cophenetic Correlation coefficient is only 0.356, these groupings may not be significant. This may be suspected by the high level of similarity between apparently unrelated strains.

4.2.3 Comparison of S_G with S_{SM} and D_P dendrograms.

In addition to the average linkage dendrograms using Gower's coefficient (Figures 3.2.1 and 3.2.3) further average linkage dendrograms were constructed using the Simple Matching coefficient and the Pattern difference. The full dendrograms were shown in Figures 3.2.4 and 3.2.6. Simplified dendrograms were shown in Figures 3.2.5 and 3.2.7. Brief comments are given below on how the phenons of Figure 3.2.1 are treated in these additional average linkage dendrograms.

Phenon I.

Phenon I (*S. faecalis* and *S. faecium*) is unchanged between the three different coefficients, although some extra loosely linked strains are present with the S_{SM} coefficient. This strengthens the case for considering these species to be closely related and distinct from other streptococci.

Phenon II.

Phenon II ("*S. avium*", "*Streptococcus* sp. (chicken)", "*S. faecalis* subsp. *malodoratus*" and "*S. faecium* subsp. *mobilis*") is distinct in each representation, and "*S. avium*" and

"Streptococcus sp. (chicken)" may be distinct species. In the S_G average linkage dendrogram and in the S_{SM} dendrogram phenon II links with phenon I to form the eneterococcus group. The D_P dendrogram shows a similar arrangement to that in the S_G single linkage dendrogram, with the lactic organisms of phenon IV linking to phenon I. A possible reason for this may be the high vigour values shown by phenon II. Phenon II shows an average vigour of 0.578, compared to an average vigour of 0.429 for phenon IV. As was described in Section 2.10.3 the Pattern coefficient corrects for the vigour of the strains. The results of this movement between phenons II and IV, is that the previously well separated enterococcus group is not seen in the D_P dendrogram. The strains grouped in phenon II are from a variety of sources, the majority, i.e. those in subphenons 3 and 4, were isolated from chickens (Nowlan & Diebel, 1967a; Barnes et al., 1978). However, PB 69, "S. faecalis subsp. malodoratus" was received as an isolate from cheese and plant material (Langston et al., 1960).

Phenon III.

Phenon III contains the species S. bovis, S. equinus, S. salivarius, S. mutans, S. raffinolactis, "S. casseliflavus" and "Oral I". This phenon corresponds to a mixed "oral and other organisms" group. Contained in this group in the S_G average linkage dendrogram was the strain PB 92, received as "Streptococcus sp. (MG)". This was found in the enterococci in the D_P dendrogram and loosely linked at the base of phenon IV in the S_{SM} dendrogram. This organism has been considered similar to "S. milleri" (Wilson & Miles, 1975; Jones, 1978). The reasons for its appearance in these positions are not clear.

S. bovis and S. equinus appear as closely similar but distinct species. Their relationship to the other organisms in phenon III is unclear. S. bovis and S. equinus have been grouped with the enterococci on some occasions (e.g. Wilson & Miles, 1975), although this may be a bad suggestion (see Section 1.4). As was seen in Table 3.3 there are very few clear cut test results that separate these two species.

S. salivarius is unchanged in the three dendrograms (although it also contains strain PB 92 in the S_G dendrogram). Its position in phenon III is altered in the D_P dendrogram. This is due to "S. casseliflavus" appearing closer to S. bovis and S. equinus (discussed later). The constancy of S. salivarius strengthens the case for the strains received as S. salivarius from Carlsson as they appear in this cluster with the type strain of the species.

"S. casseliflavus" contains the strains received as "S. faecium subsp. casseliflavus". These strains may not be an accurate representation of that subspecies. The characteristic yellow colour seen in that species was not convincingly present in these strains and the majority of the differences between this cluster and S. faecium were found to be in the same direction, usually negative in "S. casseliflavus". This can be seen from the vigour results for the two groups; S. faecium shows an average vigour value of 0.503 compared to an average of only 0.428 for "S. casseliflavus". The single strain of "S. faecium subsp. mobilis" (PB 53) gives a vigour value of 0.548. It is possible that the strains in the cluster "S. casseliflavus" are clustered

in phenon III in all three representations partly because of a lower metabolic activity. This is partly supported by the movement of this group up the dendrogram in the D_P representation. However, the movement is not large and other factors may also be involved, such as contamination of the original strains.

S. mutans appears in a similar position in both the S_G and S_{SM} average linkage dendrograms. It is split up in the D_P dendrogram. This may be due to the range of vigour values for these strains, because these range from 0.446 to 0.471. It is possible that this is not a very good group. A further strain, similar to S. mutans, "S. sobrinus" (PB 59) is present in the adjacent subphenon 8 ("S. casseliflavus") A further strain of "S. sobrinus" (PB 60) and one strain received as S. mutans (PB 151) were found loosely linked in all three representations. It may be that S. mutans consists of two groups, one made up of "S. sobrinus" and one made up of S. mutans and S. rattus. A similar situation was found by Feltham (1979) but this does not explain the presence of PB 59 in subphenon 8.

"Oral I" (subphenon 11) contains the strains considered by Carlsson (1968) to be similar to S. mutans. This subphenon is present in all three representations, where it is either adjacent or close to the strains of subphenon 9 (S. mutans).

S. raffinolactis appeared in phenon III in all three representations. It seems to be a distinct species.

Phenon IV.

Phenon IV consists of two groups, S. lactis and A. viridans in the S_G average linkage dendrogram. S. lactis appears on its own, near phenon III, in the other representations. The movement of S. lactis in the D_P dendrogram has already been discussed. It is not possible to differentiate S. lactis and S. cremoris, or indeed any of their subspecies, in any of the dendrograms. This strengthens the case for considering these organisms as one species (see Section 1.4).

A. viridans proved to be very unstable in its position. The strains assigned to it in the S_G average linkage dendrogram are scattered about in the other representations. Of the three dendrograms, only the S_G representation uses multi-state characters. Possibly the A. viridans cluster is only apparent when degrees of positive reactions are considered. This may be due to numerous intermediate results which are made either positive or negative by binary coding.

Phenon V.

Phenon V (S. thermophilus) varies in its position considerably, although the structure within the phenon is conserved in each dendrogram. In the S_G dendrogram it was seen loosely linked at the base of phenons III and IV. In the S_{SM} dendrogram it appeared loosely linked to phenons VI, VII and VIII. The D_P figure shows it loosely linked to phenons I, II, III and IV. The group appears as a satellite in all three representations and as such it is unlikely that much significance can be placed on any of these positions. S. thermophilus gave a very low overall vigour value of only 0.339. This was possibly due to the difficulties encountered in culturing these organisms.

Phenon VI.

Phenon VI (S. mitis and S. sanguis) appears in each dendrogram although S. mitis appears split in the D_P dendrogram. It is interesting that in each dendrogram the S. mitis group contains strains received as "Streptococcus sp. (0)" as well as those received as S. mitis or "Streptococcus sp. (viridans)". S. mitis has been reported as reacting with the Lancefield group 0 antisera and similarities between these organisms have also been noted (Wilson & Miles, 1975; Jones, 1978). The only strain received as serological group 0 that did not cluster with S. mitis was PB 49. This showed properties distinct from the other organisms in growing on TCBS agar, giving a positive ONPG reaction, and growing with 0.0002% crystal violet, but its serological group was not checked in this study.

One strain of S. sanguis (PB 66) is separated from phenon VI, appearing loosely linked at the base of phenon VII. This was received as an atypical strain (Cole & Kolstad, 1974) and these results show that it is quite different from the other strains.

Phenon VII.

Phenon VII ("S. milleri" and "Oral II") is linked to phenon VI in the S_G and S_{SM} dendrograms and so the two phenons may be closely related. However, in the D_P dendrogram these two phenons are separated by phenon X, a mixed group of organisms. "S. milleri" is not well studied taxonomically and it is not included in the Approved Lists of Bacterial Names (Skerman et al., 1980).

It seems likely from the position of "S. milleri" in the three dendrograms that it should be classified with the oral organisms and not with the pyogenic organisms as has been suggested (see Section 1.4).

"Oral II" is very similar to "S. milleri" and they may be closely related.

Phenon VIII.

Phenon VIII (Leuconostoc sp.) was seen at the base of the dendrogram in the S_{SM} and D_P dendrograms (Figures 3.2.4 and 3.2.6). In each case the composition was as seen in the S_G average linkage dendrogram. The inclusion of the strain of Gemella in phenon VIII in each case probably indicates that this organism is more closely related to Leuconostoc than Streptococcus. The relationship of phenon VIII to the streptococci is unclear. In every dendrogram it was loosely linked to "S. milleri" and "Oral II". It was not expected that Leuconostoc would form a group that in every dendrogram showed higher levels of similarity to some oral streptococci than some of the other streptococcal species (i.e. S. faecalis and S. mutans), and the reason is not clear.

Phenon IX.

Phenon IX (the pyogenic region, S. agalactiae, S. pyogenes, S. equi, "S. equisimilis" and"Streptococcus sp. (B) clinical") is present in each dendrogram. S. agalactiae and S. pyogenes are both distinct tight species. The clinical (human) isolates of serological group B however, are found separate from the bovine S. agalactiae in both the S_G and S_{SM} dendrograms. They are adjacent in the D_P dendrogram. The possibility that these groups are

separate species was raised in Section 4.2.1. However, in this study the bovine strains were all culture collection strains and they showed a lower vigour value than the clinical (human) isolates (0.406 as opposed to 0.467). This may account for their separation in the S_G and S_{SM} dendrograms and they may be more closely related than at first thought.

The group "S. equisimilis" from the S_G average linkage dendrogram contained strains received as both "S. equisimilis" and "S. zooepidemicus". These species separate in both the S_{SM} and D_P dendrograms. As with A. viridans, the different representations of "S. equisimilis" correspond to the different coding states. It is possible that the differences are due to levels of positive reactions rather than clear cut differences in the properties of the strains. "S. zooepidemicus" has an average vigour of 0.424 and "S. equisimilis" has an average vigour of 0.43 and so it is unlikely that this could account for the separation of the two species.

Phenon X.

Phenon X (S. uberis, "S. dysgalactiae" and "Streptococcus sp. (groups R, S and T)") is split up in the S_{SM} dendrogram, although it appears as a single phenon in the S_G and D_P representations. "Streptococcus sp. (groups R, S and T)" appears as a satellite group to phenon IX in the S_{SM} dendrogram. In each dendrogram this subphenon appears separated from the strains received as "S. suis". It has previously been considered that these may be very similar organisms (Diebel & Seeley, 1974; Jones, 1978). This does not appear to be so for the strains used in this study.

The unusual grouping of "S. dysgalactiae" with S. uberis is maintained in each of the dendrograms. This may indicate that these organisms are less similar to the other Lancefield group C species of S. equi, "S. equisimilis" and "S. zooepidemicus", and closer to each other than previously thought. "S. dysgalactiae" and S. uberis are both isolated from similar sources and this may have some effect on their relationship. The group "S. dysgalactiae" is discussed further in Section 4.4.

Subphenon 28.

Subphenon 28 (Pediococcus sp.) is seen in a different position in each of the three representations. It undergoes a relatively large amount of internal alteration between each dendrogram. It would appear from this that this subphenon consists of strains that are only slightly similar to each other and to the rest of the streptococci. It is interesting to note that some of the strains received as "Aerococcus catalyticus" are found in this subphenon, and that others are present as loosely linked strains at the base of phenon II. In both the D_P and the S_{SM} dendrograms subphenon 28 is found loosely linked with these strains at the base of phenon II. It has previously been thought that both Pediococcus and Aerococcus may be similar to the enterococci, and this may be a more realistic position for this group than that seen in the S_G dendrogram.

4.2.4 Test kit dendrogram

The dendrogram shown in Figure 3.2.8 was obtained using Gower's coefficient and average linkage clustering with the sixty-eight test results obtained from the two API galleries.

The majority of the clusters seen in the S_G dendrogram earlier (Figure 3.2.1) are present although their positions are often changed.

The enterococcus region

The enterococcus region in this dendrogram is not as distinct as in the other dendrograms. S. faecium has been lost to further down the dendrogram. The four subphenons, S. mutans, S. raffinolactis, "Oral I" and "S. dysgalactiae" have been added to this cluster. The separation of S. faecium was not expected, as this organism is often difficult to distinguish from S. faecalis (Whittenbury, 1965a).

The region of pyogenic streptococci

The pyogenic streptococci are recognisable in this dendrogram as a loose group of clusters (PB 41-PB 127). It is interesting that S. agalactiae is missing from this group and is found further up the dendrogram. It was suggested earlier that S. agalactiae and the subphenon "Streptococcus sp. (B) clinical" may only be separated due to vigour differences. However, S. agalactiae is not separated from the rest of the pyogenic organisms on the basis of vigour, and so this does not explain this difference in position.

Phenon X is well separated from the other pyogenic organisms and this may be further evidence that it is not closely related. The separation of "S. dysgalactiae" from the other serological group C organisms is more pronounced than seen earlier. This may indicate that the major difference between this species and the other pyogenic organisms is the production of acid from

particular carbohydrates. The same may also be true for the "S. equisimilis"/"S. zooepidemicus" area, which appears as two species in this dendrogram.

The region of oral streptococci

The mixed group phenon III has altered considerably. S. salivarius has remained almost entire, though it has acquired some strains from the "Oral II" subphenon (PB 187, PB 179, PB 177 and PB 178). It has lost strain PB 99 to the "Oral I" subphenon. These gains and losses are perhaps not surprising because these strains are all from the same habitat and as such could be expected to metabolise many of the same sugars. If this was a full explanation, one would expect most of the oral groups to cluster together in this dendrogram. This is however not so. S. mutans and "Oral I" have moved up the dendrogram to cluster with the enterococci. The other oral groups, phenons VI and VII, are not seen in this representation and this gives further doubt to the premise that these organisms would metabolise similar sugars. From the test results shown in Appendix III it can be seen that the subphenons of phenon III, S. salivarius, S. mutans and "Oral I" give similar sugar reactions and the subphenons of phenon VI give similar reactions (S. mitis and S. sanguis). "Oral II" gives carbohydrate reactions that are similar to both phenons. Beighton et al. (1979) has shown that the composition of diet may affect the oral population. The results from this dendrogram support this, with different oral species metabolising different sets of carbohydrates.

S. bovis and S. equinus were previously seen with some oral organisms in phenon III. In this dendrogram they have clustered with S. faecium and S. lactis. The similarities between these two organisms have already been mentioned and it would appear that the similarities are highest in the area of carbohydrate metabolism. This can be seen from the test results in Appendix III. This was not expected. S. bovis, S. equinus and S. faecium are all faecal organisms and would be expected to metabolise different sugars from S. lactis. This seems so for S. bovis and S. equinus, and S. faecium and S. lactis show similar carbohydrate profiles.

"S. casseliflavus", which from previous observations may have been expected to cluster near this area is not present as a subphenon in this dendrogram. The individual strains are found down the dendrogram and this reinforces the earlier view that the strains used in this study may not adequately represent these organisms.

The region of lactic streptococci and certain allied organisms

The positions of S. lactis and S. raffinolactis in this dendrogram have already been mentioned. S. raffinolactis appears closely linked to S. mutans as in the previous dendrograms, although in this case they are found in the enterococcus region. There are some similarities between S. lactis and S. raffinolactis in their carbohydrate metabolism, and this may be seen in the clustering of each species with members of the enterococci. However, they appear distinct and are probably not closely related.

The subphenon named as A. viridans in the S_G average linkage dendrogram is not present in this representation, all three strains appearing independantly. This reinforced the view

that this is not a true group.

Other organisms

S. thermophilus is present as a distinct group and is linked as a satellite to a much larger group containing mainly oral organisms. The carbohydrate reactions of S. thermophilus were distinctive in consisting of more negative reactions than the other streptococci. This gives no further information as to its true position in the genus.

Leuconostoc sp. is present as a loosely linked group with two strains previously assigned to this subphenon (PB 161 and PB 165) being loosely linked to S. thermophilus.

Representatives of Pediococcus are found throughout the dendrogram. This may indicate that carbohydrate reactions are of use in differentiating the species of Pediococcus from each other.

The grouping of the streptococci on the basis of only carbohydrate reactions has previously led to some confusion. (Bergey et al., 1926; Sherman, 1937) although the number of carbohydrates then used was small. It is encouraging to find that most of the species may be differentiated to some extent by this method when a large number of carbohydrates are used. This does not apply so well to the larger areas such as the oral organisms. These larger areas are only partly defined, the enterococci perhaps being an exception. It is possible that the species-level groupings are the only true ones within the genus. However, in this case the low Cophenetic Correlation coefficient, 0.645, casts some doubt on the validity of the dendrogram.

Several of the tests on the APIzym strip were for enzymes related to carbohydrate metabolism. For example, β-galactosidase is involved with lactose fermentation in the Enterobacteriaceae (Cowan & Steel, 1974). However, in the gram-positive organisms the position is less clear. The enzyme β-phosphogalactosidase is thought to be involved in the fermentation of lactose in the streptococci (Farrow, 1980). The possession of these enzymes and the rate of lactose metabolism have been found to differ in different strains of starter streptococci (Farrow, 1980). It is possible that a similar system is found for other carbohydrates where more than one enzyme is involved either directly or indirectly in fermentation. Most of the test reactions given as variable by Cowan & Steel (1974) and Diebel & Seeley (1974) are carbohydrate reactions. This may indicate that carbohydrate fermentations have a higher level of strain specificity than of species specificity. An example of this is strains PB 9, PB 99 and PB 100 in S. salivarius and strains PB 67 and PB 68 in S. sanguis. These strains all produce acid from raffinose, yet this property is listed as variable for both groups in Cowan & Steel (1974). This specificity may in turn be linked to the habitat of the organism or may be due merely to the strains selected for the present study.

4.3 Overlap discussion

The method described in Section 2.10.5 was used to determine the observed overlap between groups and the results of this are given in Tables 3,4.a, 3.4.b, and Appendix VI.

First, overlap greater than that corresponding to a $V_{(0)}$ of 0.025 was investigated. The only pairs of subphenons in Table 3.4.a that show less than 95% confidence that the true overlap is less than 0.025 are S. salivarius with "Oral I" and S. lactis with "Oral I". However, many pairs showed less than 95% after correction for the inter-group distance. As already stated, the correction is applied to take account of the fact that the OTUs are small samples from larger groups, and the number of test results are also considered by this statistic. It is known that comparisons between a large and a small group may give erroneous results, as may the comparison of two very small groups.

Not all the characters in the taxonomy show differences between a given pair of subphenons. Some, such as the gram-stain, gave all positive results, while others, such as indol production, gave all negative results. Similarly (as was seen in Section 4.2.3), some of the enzyme tests may detect enzymes that are also detected in other tests. It may therefore be erroneous to use all the 157 tests for the determination of inter- and intra-centroid distances and then to correct at a later stage. Possibly only those characters that showed different results between a pair of subphenons should be considered. When, however, some representative pairs of groups were treated in this manner in Section 3.4, it was found that there was no substantial difference in the statistical significance in terms of the critical $V_{(0)}$.

Care should be taken when interpreting the overlap results, especially when using the correction factor. It was decided in this study to consider the results using all 157 characters, but to take into account the above comments when interpreting them.

In Table 3.4.a an apparent example of overlap may be seen between subphenons 3 and 4, "S. avium" and "Streptococcus sp. (chicken)". However, this shows less than four degrees of freedom. Any result showing less than four degrees of freedom may not be statistically valid and therefore no conclusions can be drawn from it. The other extreme is seen in Appendix VI where the comparison of subphenons 17 and 19, "Oral II" and Leuconostoc sp., gives more than twenty-seven degrees of freedom: in such cases conclusions are very reliable. In general, the apparent overlap between groups is low. Ten pairs show a t-test of below 95% after correction. However, one of these (subphenon 3 and subphenon 4) involves a comparison between groups of four and seventeen members and so may be inaccurate. Of the nine remaining pairs, two give over 90% confidence after correction and so may still be considered distinct. The other seven pairs are all adjacent in the S_G dendrogram with the exception of subphenons 7 and 11.

Similarly, overlap of 1.11% may be seen after correction between subphenons 11 and 17 ("Oral I" and "Oral II"), although in this case the difference in numbers between members of the subphenons (4 and 13) may make this result unreliable. There may be a little overlap of about 1.9% between S. faecalis and S. faecium. There appears to be a little overlap between subphenons 6 and 7, and 7 and 8. These are all positioned next to each other in phenon III and were named S. equinus, S. salivarius and "S. casseliflavus". It is interesting that there is only a low level of overlap between subphenons 8 and 9 (2.0E-32%), although higher levels are seen between subphenons 9 and 10 (5%) and 11 and

12 (7%). Overlap between subphenons 10 and 11 is again low (0.002%). This may indicate that phenon III consists of a swarm of OTUs rather than clear cut groups (Sneath, 1977). However, another explanation may be that some species, such as subphenon 7, S. salivarius, and subphenon 10, S. raffinolactis are less well defined than others, such as subphenon 5, S. bovis. Although there appears to be some overlap between S. raffinolactis and S. lactis, the inequality of sizes means that this must be treated with some care. Low levels of overlap are seen between the subphenons of S. bovis and S. equinus; and S. faecium and "S. casseliflavus". Both of these pairs had been described as being similar (Seeley & Dain, 1960; Mundt, 1975) and some degree of overlap had been expected.

There appears to be some significant overlap between S. thermophilus and S. mitis. S. thermophilus was seen to link with different phenons in different dendrograms and so some overlap with other species may not be surprising. It may not be as distinct as first thought, though it has been noted that suboptimal growth conditions may cause difficulties. The groups "S. equisimilis" and "Streptococcus sp. (B) clinical" appear to overlap after correction. However, "S. equisimilis" has been split into two arms in all but the S_G average linkage dendrogram and so it may be a rather heterogeneous cluster; the significance of this result is thus not clear. It is possible that the strains of "S. equisimilis" which clustered furthest from S. equi may show some similarity to the group B strains. Unfortunately the group S. equi, which appeared distinct, contained too few strains to be tested statistically.

Second, the level of overlap expected if a rectangular distribution in hyper-space was also examined. The results for this were shown in Table 3.4.b. Eight of the recorded instances gave negative values for $D_{(L,M)}$ after correction, although without correction none showed a V_G value of above 1×10^{-3}. This indicates that the overlap at this level is less than one in one thousand. It was noted that some subphenons appear in the table more frequently than others. Subphenons 11, 12 and 28, "Oral I", S. lactis and Pediococcus sp., occur a total of thirteen times, and they account for all but two of the cases of undue overlap. The two remaining occurances both involve group 8, "S. casseliflavus", which shows some overlap with S. mitis and with "Streptococcus sp. (groups R, S and T)". As already stated, "S. casseliflavus" is in some ways suspect, with low vigour values. This may account for the apparent overlap, which may be due to negative similarities rather than positives. This may also account for its positions in the dendrograms. However, the overlap may also be due to the relatively small numbers of OTUs (4) in this group.

The "Oral I" and S. lactis subphenons are also prominent in the tables for $V_{(0)}=0.025$, although Pediococcus sp. is absent from these. They account for only one case at $V_{(0)}=0.025$ where the confidence limit is less than 90%. There are only 4 OTUs in "Oral I" and 5 in Pediococcus sp.. There are twelve OTUs in S. lactis and this may account for some of the overlap.

Pediococcus sp. was seen in the dendrogram as a loosely linked group and so may be expected to show some overlap. Similarly, both Pediococcus sp. and S. lactis changed positions

between different dendrograms. This may also account for some of the possible instances of overlap.

Table 3.3.q showed the root mean squared average Euclidean distances (AED) of strains to the centroids of the subphenons, in order to give an estimate of the sizes in hyper-space of the different subphenons. If these sizes were related to the instances of overlap, then high values (i.e. groups with strains widely displaced) would be expected for the groups showing the most overlap. However, these figures do not correlate well with the overlap results. Two examples of this are: (1) Pediococcus sp., this showed the highest AED value and although there appeared to be some overlap when a rectangular distribution was used, all confidence limits were above 90% for $V_{(0)}=0.025$; (2) "Oral I" showed many occurancies where there might be overlap, but it showed an AED of 3.24 compared to an average value for all subphenons of 3.148.

In summary, twenty-five of the twenty-eight groups appear reasonably distinct at the $V_{(0)}=0.025$ level by overlap calculations. There may be some underlying overlap between subphenons in phenon III. In general, areas where overlap may be expected show only low levels. Examples of this are "Oral I" and S. mutans; and S. agalactiae and "Streptococcus sp. (B) clinical". The three separate subphenons of "Oral I", S. lactis and Pediococcus sp. show several instances of overlap and may not be as distinct as they appear in the dendrograms.

A further matter to consider in relation to overlap is the number of characters that gave nearly all positive or negative results. The graph shown in Figure 3.5.1 showed the number of results obtained in this study and their distribution

as percent positive within phenons. It can be seen from this that the majority of the tests that defined the phenons were either mostly positive or mostly negative within phenons, that is to say, few had frequencies near 50% within the phenons.

4.4 Comparison of results with those in the literature

A comparison of test results between this taxonomy and other published studies was carried out in Section 2.10.6. The results of this, on which the matrix PDBSTP was based, are shown in Appendix VII. The first three tables show the different results obtained from acid from carbohydrate reactions. However, this study and that of Feltham (1979) used the API system for these reactions, while the other studies used more conventional methods. As a result, these two studies may not be directly comparable with the others. This does however serve as a further test on the API methods which may be compared with that seen in Section 3.1. The number of strains used is not known for all of these studies. The significance of differences is therefore not known. One might by chance pick two strains and find one positive, which would give a result of 50%. This would be classed as variable. However, a larger sample might yield only one strain positive out of a sample of ten, which may be classed as negative. The sizes of the groups used in this study range from two to seventeen and it is likely that groups in the other studies had similar sizes. Only clear cut discrepancies involving positive or negative results will be considered, where negative is taken as 20% or below and positive is taken as 80% or above (where percentages are given).

No differences between positive and negative results, either for the two API studies or overall, were seen for arabinose, maltose, trehalose, raffinose, salicin, sorbitol, sucrose, glucose or xylose. The results of mannitol and glycerol for the group named "S. dysgalactiae" differed from those in all other studies. The results for this group on mannitol, glycerol and melibiose also differed between the two API studies. This may indicate that the group "S. dysgalactiae" referred to different groups of organisms in different studies. This study did not include a type or reference strain, nor did the API study of Feltham (1979). The group labelled "S. equisimilis" showed discrepancies in sugar reactions, giving a negative result for glycerol in Feltham's study but a positive or variable result in all the others. The same appears true of S. uberis, although Facklam (1977) also found this species to be glycerol negative. The group S. salivarius in this study was found to differ from the other studies by not producing lactose, although it was originally described as acidifying lactose and raffinose. S. raffinolactis was found to produce acid from lactose in this study and not in that of Feltham. The results obtained for acid from inulin for S. mutans and S. sanguis differed from those of Feltham, although they were in agreement with other studies. "S. casseliflavus" is reported to produce acid from melezitose but did not do so in this study.

No discrepancies were observed for hippurate, gelatin, tellurite, tetrazolium, growth at pH 9.6, survival of 60°C for 30 min, 4% NaCl, 6.5% NaCL and V.P. The test results for survival of 60°C for 30 min may not be comparable between this study and others. In this study resistance to heating to 60°C

was observed at 15 min and at 1 h. In order to arrive at results for 30 min, interpolation had to be made from these results. A better title for this test in this study would be "resistance to 60°C for at least 15 min". The results shown for tolerance of bile show three discrepancies. In this study strains assigned to the group A. viridans failed to grow on 10 or 40% bile. The strains assigned to the group S. thermophilus were found to grow with 40% bile. It was found difficult to get the latter organisms to grow under any circumstances on solid media and in this test only pin-head colonies were seen. It is possible that these small colonies did not constitute true growth of the organism, but were the result of either a heavy inoculum or a contaminant. They were seen on repeating the experiment and gram-stained as gram-positive cocci.

The results given for aesculin hydrolysis are all based on the plate method with the exception of those of Feltham who used the API micromethod. Discrepancies were found between the API and the conventional methods in this study and so this part of the table may not be directly comparable. The only discrepancy seen for the present study in the other aesculin results is S. sanguis, which did not split aesculin in the conventional plate method as expected. A comparison of results found in this study for the plate method for aesculin hydrolysis and an API method is given in Table 4.4.a. In general the API method gives a lower number of positive results. This indicates that the API method may be less sensitive than the plate method. The three exceptions to this are S. sanguis, S. lactis and S. equi which show more positive results. As this is against the general form of the results it may be that a different enzyme is involved in the API method.

S. sanguis and S. uberis failed to produce a clot in litmus milk media although the other studies found them to do so. This may have been a failure to grow well in the media although they both produced acid. More discrepancies were found in the milk tests than in the others. S. equinus produced acid in litmus milk and strains of S. pyogenes failed to do so. These results are both in disagreement with the results of other workers. In the methylene blue milk test both S. mitis and "S. casseliflavus" gave results that were opposite to those expected. However, in this study a relatively low concentration of methylene blue milk was used ($0.2g\ l^{-1}$). Litmus milk reactions are reported by Diebel & Seeley (1974) as of limited taxonomic value for the streptococci. Cooper & Ramadan (1955) found that Janus green was a better indicator in milk media than litmus. However, it has not been used in any major studies and so could not be considered in these tables.

The results on "S. casseliflavus" differed from those in the literature in its failure to grow at $10^{o}C$. S. uberis failed in this study to grow at $45^{o}C$, which disagrees with most of the published findings, although Jones (1978) reported that the property was variable for S. uberis. This may be due to the composition of the media used for the test. The strains of the group A. viridans were found in this study to hydrolyse arginine, but this was not recorded in other studies. Glucose has been found to have a diauxic effect on arginine metabolism in some strains of streptococci (Whittenbury, 1965a). This may have some effect on these results. Whittenbury also reported that some strains

Table 4.4.a Number positive in each group for aesculin hydrolysis by the two methods used.

Group.	Plate	API
S. faecalis	10/10	10/10
S. faecium	6/6	6/6
"S. avium"	4/4	4/4
"Streptococcus sp. (chicken)"	17/17	17/17
S. bovis	4/4	3/4
S. equinus	4/4	4/4
S. salivarius	7/7	7/7
"S. casseliflavus"	4/4	1/4
S. mutans	3/3	3/3
S. raffinolactis	4/4	4/4
"Oral I"	4/4	4/4
S. lactis	8/12	11/12
A. viridans	3/3	2/3
S. thermophilus	2/6	0/6
S. mitis	0/6	0/6
S. sanguis	0/3	2/3
"Oral II"	1/13	2/13
"S. milleri"	1/3	0/3
Leuconostoc sp.	3/7	1/7
S. agalactiae	0/5	0/5
S. pyogenes	3/6	2/6
S. equi	0/2	2/2
"S. equisimilis"	1/8	1/8
"Streptococcus sp. (B) clinical"	2/10	1/10
S. uberis	3/3	3/3
"S. dysgalactiae"	1/3	1/3
"Streptococcus sp. (groups R, S and T)"	3/3	3/3
Pediococcus sp.	4/5	3/5

of streptococci would deaminate serine but no evidence was found of this in the present study. However, a different basal medium was used which may account for this.

Most strains of S. salivarius were found to hydrolyse starch. Starch hydrolysis within this species was reported to be variable by Facklam (1977) although Diebel & Seeley (1974) considered it to be negative. The strains designated as "S. milleri" were found to produce H_2O_2, which is in disagreement with the other studies. As stated earlier, no clear cut positive or negative differences were found for those tests involving tellurite or tetrazolium. However, those strains in subphenon 2 (S. faecium) that were received as "S. durans" gave a positive result for tellurite and tetrazolium, whereas a negative result for S. faecium has been reported, and was seen with the strains received under this name. This was the only clear cut difference found between strains of S. faecium and "S. durans". The concentrations of tellurite, tetrazolium and other inhibitory compounds has not always been reported. It may be that the technique tests a mixture of reduction and tolerance of compounds.

Two discrepancies were shown in these tables for haemolysis on blood agar. Some α-haemolytic strains of S. faecalis were found in this study which is in disagreement with other studies. No β-haemolytic strains were found in "Oral II" although some had been reported by Carlsson (1968); a possible explanation is that too few strains were examined. There is also a third discrepancy that is not shown in these tables. Barnes et al. (1978)

described the organisms that are grouped as "Streptococcus sp. (chicken)" as β-haemolytic. However, under all conditions used throughout this study they appeared α-haemolytic. Haemolytic properties in the streptococci may be lost by repeated sub-culture and may also be plasmid borne. Both of these observations have been noted with "S. faecalis subsp. zymogenes" (Diebel & Seeley, 1974; Jones, 1978). One of the haemolysins involved is oxygen sensitive, and therefore discrepancies may be found between tests which are performed anaerobically and aerobically. All of the discrepancies seen in this study are in the same direction with β-haemolysis being expected and α-haemolysis being seen. The species that show discrepancies all show high vigour values and therefore partial haemolysis due to weakly active cells is unlikely. It is possible that some factor in the blood or the media inhibited haemolysis. This factor would however have had to be present in all batches used in the study.

A further discrepancy was seen for the group "Streptococcus sp. (chicken)" between this study and that of Barnes et al. (1978). This was the production of acid from starch. Barnes et al. found this property to be negative by broth methods over 10 d. However, in this study these strains were found to produce acid from starch by the API method after 48 h. It is possible that this may be due to the different methods used.

Discrepancies between results may be due to the organisms themselves, the methods employed, the basis of the tests or a combination of all of these. The largest number of discrepancies seen in these tables for any one test was acid from glycerol.

In Section 3.1 the difference between replicates of this test was seen to be suspiciously high, and it was only included in the study because it was part of the API gallery. The test when performed on an API gallery is layered with sterile liquid paraffin and so is partly anaerobic. The aerobic and anaerobic production of acid from glycerol are different tests involving two different biochemical pathways. Both have been used in previous studies on the streptococci. However, it is not always clear in the literature which test has been used, and therefore one may expect considerably more discrepancies for this test. Tests such as growth at 10°C and growth with different concentrations of inhibitory compounds rely to a large extent on the composition of the basal media and the period of incubation. In such cases insufficient information was given on these tests.

In this study, aberrant results have been found in general to occur in both directions in the same test, i.e. positive when negative was expected and negative when positive was expected. An overall deficiency in the media, the metabolic activity of the organisms or the sensitivity of the test reaction would tend to produce more negative results. However, if any cultures were contaminated their combined reactions would be seen and this would give more positive results. No test in this study appears in general to give all positive or all negative discrepancies. The only possible exception to this is haemolysis which was discussed earlier.

If we next consider the groups, of all those named at species level in this study, subphenon 14 (S. thermophilus) showed

the lowest overall vigour with an average value of 0.339. This group showed only one discrepancy in these tables and this gave a positive reaction where a negative one was expected. This is the opposite occurance to that expected if the discrepancy was due to the low level of activity. Subphenon 3 ("S. avium") showed some of the highest vigour values, its average value being 0.566. This group did not show any clear cut discrepancies within these tables. These two observations would seem to indicate that although differences in vigour may account for individual discrepancies, they do not appear in this study to have had any overall effect on either the comparisons or the reproducibility of the methods.

"S. dysgalactiae" appears to show more discrepancies than any group, particularly for reactions involving the production of acid from carbohydrates. It is possible that there is no single species that corresponds to "S. dysgalactiae" and it may be that the difficulty in deciding this point has led to the omission of the species from the Approved Lists of Bacterial Names (Skerman et al., 1980). It is encouraging to note that no other species consistently gives such pronounced discrepancies in these tables with the possible exception of "S. casseliflavus". It may be erroneous to consider the group of A. viridans as a species, because it is seen in only one dendrogram and shows several discrepancies in these tables.

A comparison of the APIzym methods was undertaken in Section 2.9. The results of this were discussed in Section 4.1.

4.5 Identification matrices

4.5.1 Identification scores

The identification matrix PDBSTP2 was used in Section 3.5 to identify typical organisms from the numerical taxonomy and some loosely linked strains. The identification scores have been shown in Table 3.5.c.

All of the typical organisms identified to a level of over 0.999999 as Willcox probabilities (Willcox *et al.*, 1973). The distance scores for these organisms ranged from 0.1487 for PB 18, "*Streptococcus* sp. (chicken)", to 0.432074 for PB 113, *Pediococcus* sp.. The taxonomic distance is the Euclidean distance from the centre of the group as defined by the sixty tests selected for identification. In the program MOSTTYP all groups identified to a Willcox probability of over 0.999999, these scores were printed as 1. The corresponding distances ranged from 0.14965 to 0.28871. It is interesting that apart from *A. viridans*, which showed dubious groupings in this study, the other taxa giving high distance scores in Table 3.5.c are *S. salivarius*, "Oral I", *S. mitis* and "Oral II". "Oral I" overlaps heavily with other groups. The results for *S. salivarius*, *S. mitis* and "Oral II" may indicate that they do not appear as distinct as earlier when defined by only sixty tests. They are all oral organisms, and all except *S. mitis* contain isolates from Carlsson. These organisms have not been well studied in the past and they may deserve more work.

Thirty-two strains that had appeared loosely linked in the S_G dendrogram were tested with the identification matrix. The scores ranged from 1 to 2.67248×10^{-2} as Willcox probabilities

and from 0.31368 to 0.537508 as distance scores. Six of these strains, PB 170, PB 46, PB 47, PB 60, PB 70 and PB 66, identified to the taxa to which they were most closely linked in the dendrogram, although not with very high Willcox probabilities. The one exception to this was strain PB 170. This was received as Streptococcus sp. and linked with the group S. mitis, but it identified to this group with a Willcox probability of 0.999997 and therefore it may well be a member of this species.

Six strains were found to identify to a subphenon within the same phenon as the subphenon they were most closely linked to. These were PB 16, PB 125, PB 140, PB 43, PB 123 and PB 158. Two of these strains, PB 125 and PB 140, identified to the subphenons they were received as being similar to.

Two strains identified to taxa that they had been received as being similar to, although they had not clustered near these taxa. PB 169, P. acidilacti, identified as Pediococcus sp. to a Willcox probability of 1, but this strain clustered at the base of the enterococci. Strain PB 124, received as "S. cremoris subsp. alactosus", identified as S. lactis to a Willcox probability of 0.999998, but clustered further down the dendrogram with S. thermophilus. This may be due to one of two things. Either the strains may appear to belong to a species on the basis of only those tests included in the identification matrix. Alternatively, they may not have linked with the named groups in the clustering because of some unexplained differences in test results. This may be due to the vigour of the strains. S. lactis showed an average vigour of 0.430, compared to an average of 0.374 for S. thermophilus and 0.338 for strain PB 124.

This idea is reinforced by the findings of the D_P dendrogram where this strain was seen loosely linked to S. lactis.

Six strains gave very high identification scores to apparently unrelated organisms. Three of these, PB 153, PB 154 and PB 156 clustered in the S_G dendrogram at the base of the enterococci. They all identify to S. lactis with Willcox scores of between 0.994952 and 0.999999. This is interesting because several different instances of an apparent relationship between the lactic organisms and the enterococci have been seen in both this study and in the literature (see Section 1.4.4). These loose strains were linked in the dendrogram close to the position occupied by S. lactis in the D_P dendrogram and the S_G single linkage dendrogram. The group S. lactis also showed a comparatively large amount of overlap with nearby groups. This is probably because S. lactis and the enterococci share a number of characters (such as growth at 10°C and tolerance of 10% bile) which are used in the matrix.

One strain, PB 109, was received as "Aerococcus catalyticus" but identified with a Willcox score of 1 to Pediococcus sp., although the taxonomic distance score was high. This strain was linked at the base of phenon II with another strain received as "A. catalyticus", PB 110, which also identified as Pediococcus sp. with a Willcox score of 0.999987. These two strains were in turn linked to PB 169 (received as P. acidilacti). The group Pediococcus sp. was considered a very loose group from the overlap calculations and dendrogram representations, but it linked at the base of phenon II in the S_{SM} dendrogram, thus

indicating a similarity with the enterococci. An apparent relationship between the members of the genera Pediococcus and Aerococcus was seen in the dendrogram drawn from the matrix information and this could account for the high similarity between these strains.

One strain, PB 201, received as Streptococcus sp., identified to S. salivarius with a score of 0.999978. This strain was loosely linked to the base of phenons III and IV, and would not be expected to show such a high score with S. salivarius. The species is however one of the more heterogenous ones.

The strain PB 142 was received as a representative of Lancefield serological group G although this was not checked in this study. It was found linked to "S. equisimilis" in the S_G average linkage dendrogram. It showed a Willcox probability of 0.999999 to the group "Streptococcus sp. (B) clinical". Some serological group G organisms have been grouped with the pyogenic organisms (Wilson & Miles, 1975). The only other strain of group G that was used in the study linked near it. However, as the serological group was not checked it may have been incorrectly serogrouped.

In general the low identification scores and the high taxonomic distance scores seen in Table 3.3.c on identification were expected because the majority of the organisms in that table were not assigned to the taxa used to construct the identification matrix. However, the majority of the high identification scores (greater than 0.99999) appear to make some sort of sense.

The groups S. lactis, S. salivarius, "Oral II" and Pediococcus sp. again show a high degree of similarity with many loosely linked strains. These four groups were involved in sixteen of the thirty-two results.

4.5.2 Practicality of the identification matrix

The tests used in the construction of the matrix were selected as being the most useful for differentiating all of the groups found in this study. They were obtained from different classes of tests (e.g. biochemical tests, morphological tests etc.) and so represented a good cross section of tests. Both the MOSTTYP results and the identification of typical strains shows that the matrix will differentiate and identify a wide range of species. However, the practicality of this set of tests is less certain. The identification scheme, if used in full, may be both costly and time-consuming. Test results from this study were compared with the identification system of Waitkins et al. (1980). Her system is based solely on APIzym tests and was discussed in Section 4.1. A comparison was also made with the ten APIstrep tests (API Laboratory products). It was found that the same identifications were given with typical strains. Both of these latter methods require 24 h or less for identification from a pure culture. Serological testing as a means of identification is relatively quick. However, there are problems for species such as S. mutans where there is no specific Lancefield antigen, and species such as S. salivarius where there are organisms with the same group antigen that appear physiologically different (Jones, 1978). The advantages that this matrix may have, however, are over other methods involving classical sugar fermentations, because the API method will give results in a much shorter time.

Some probably distinct groups were not included in the matrix because too few strains were used e.g. "S. suis" (Elliott, 1966), "S. infrequens" (DeMoor & Thal, 1968), the streptococci of serological group E (Diebel et al., 1964) and S. pneumoniae. On the other hand, several new but distinct groups from the numerical taxonomy have been included, e.g. "Oral I", "Oral II" and "Streptococcus sp. (chicken)".

It may have been better to use a small number of the most useful tests as a first identification system to separate the streptococci into primary groups. This could have been followed by a second matrix suited for the particular organisms in that group.

4.5.3 Dendrogram from the matrix PDBSTP2

The tests and organisms used in the identification matrix were represented in the form of an S_G average linkage dendrogram. This was done in order to see how the relationships described by these sixty tests compared to the full numerical study. This dendrogram was shown in Figure 3.7.2. The numbering of the phenons and subphenons from the full S_G average linkage dendrogram (Figure 3.2.1) has been retained as far as possible.

Phenons I and II

Phenons I and II contained the previously described enterococci, only one change was seen from the taxonomy. Strain PB 88 was received as "S. avium" and has been seen grouped in that subphenon in the previous dendrograms. In the PDBSTP2 dendrogram however, it appeared as a satellite strain, quite separate from that group. As both this group and its neighbour

"Streptococcus sp. (chicken)" appear to be distinct, this strain must show some atypical properties in the tests used for the matrix. Atypical results were found for the production of a clot in litmus milk, arginine hydrolysis, nitrite reduction and some of the APIzym tests.

Phenon III

Phenon III is present in very much the same form as in the dendrogram from all the tests. The group S. bovis is less distinct than before, as are the other subphenons in this phenon with the exception of S. equinus. This may indicate that the sixty tests are particularly suitable in differentiating this group.

However, one strain, PB 83, appears as almost a satellite strain. Surprisingly, this was received as the type strain of S. equinus. S. salivarius has moved within this phenon and now appears more similar to "Oral I" than was previously seen.

Phenon IV

In the S_G dendrogram from all the tests, phenon IV consisted of the species S. lactis and A. viridans. The grouping of only one strain of A. viridans in this phenon in the PDBSTP2 representation is in agreement with the previous findings in this study. It is interesting that it is still not possible to differentiate between S. cremoris and S. lactis, reinforcing the view that they may constitute one species.

Phenon V

Phenon V consisted of the species S. thermophilus. In the dendrogram from PDBSTP2 it was linked to phenons VI, VII and VIII. Its position may not be significant, as it did not appear to be particularly close to any one group of organisms.

Phenons VI, VII and VIII

Phenon VI appears as two arms about the two arms of phenon VII, which in turn are positioned around phenon VIII. The significance of this is not clear. A certain amount of the change may be accounted for by the swivelling of phenon VII on its stem. The reduced number of tests has resulted in these groups appearing less distinct and this must account for some of the change. However, it does appear that the two major groups have genuinely split. The strains of Leuconostoc (phenon VIII) were previously linked to phenons VI and VII, and so it seems in this case that the reduced amount of information has led to a loss of structure, although the overall pattern is still present to some extent.

Phenon IX

Phenon IX (the pyogenic group) consists of the same species as seen previously. The group "S. equisimilis" appears away from S. equi and is split into the two groups of "S. equisimilis" and "S. zooepidemicus". It would have been better to consider these as separate groups in the identification matrix. S. equi and S. pyogenes have moved to the base of the phenon. The significance of this is not clear, especially in the case of S. equi where only two strains were present.

Phenon X

Phenon X previously consisted of the three groups "S. dysgalactiae", S. uberis and "Streptococcus sp. (groups R, S and T)". In the PDBSTP2 dendrogram "S. dysgalactiae" is linked at the base of phenon IX and the other two subphenons are found further up the dendrogram. In the S_{SM} dendrogram from

all the tests only "Streptococcus sp. (groups R, S and T)" was found in this position. These movements away from phenon IX may indicate that the species in this phenon are intermediate between the organisms of phenon IX and phenons III and IV. However, this does not explain the separation of "S. dysgalactiae" from the other Lancefield group C organisms, seen in this dendrogram as well as in all the others in this study. It seems unlikely that the species in phenon IX are true members of the pyogenic group.

Subphenon 28

Subphenon 28 has split in the PDBSTP2 dendrogram into two arms, one consisting of only members of Pediococcus and one consisting of a mixture of pediococci and aerococci. In previous dendrograms strains of Aerococcus were often seen in association with some strains of Pediococcus.

Overall, the dendrogram constructed from tests used in the matrix shows a good representation of the numerical taxonomy from all tests. The reduced number of tests means that some of the structure has been lost. The number of subphenons that now show one or two satellites indicates that these tests are only just sufficient to define all the groups.

It may have been better to consider a smaller number of major phenons, rather than the original ten. The choice of species groups appears to have been a correct one, although "S. equisimilis" may have been better considered as two species. It is interesting that in both this and the S_G dendrogram from all the tests, that the enterococci are very distinct from the other streptococci. In both representations they are less similar to the other streptococci than the genera Pediococcus and Aerococcus.

The genus Leuconostoc shows a high level of similarity in each case with some of the oral organisms (it may however be easily differentiated from these organisms by its growth temperature).

4.5.4 Matrix overlap

Table 3.6.a showed the pairs of taxa from the matrices which showed less than 95% confidence for a critical overlap of 2.5%. The majority of these pairs are from the identification matrix PDBSTP. The subphenons listed have in many cases been considered to be similar (e.g. S. faecium and "S. casseliflavus"). As a result of these similarities, some overlap may have been expected.

The test results obtained from the literature for the group "S. equisimilis" were combined with those for "S. zooepidemicus" because strains from both these species were found in this cluster. Both of these organisms have been described as similar to S. equi (Jones, 1978), so again the overlap was expected.

The majority of the other taxon pairs are oral organisms. It is interesting that both "Oral I" and "Oral II", received as being similar to S. mutans and S. mitis, show overlap with these groups. Apart from the pairs involving two oral organisms, there are some occurancies of overlap between distantly related pairs. The overlap between "S. milleri" and S. pyogenes may not be significant as the t-test showed only 90% condience that the observed value of overlap of 0.3% was less than the 2.5% cutoff. The overlap of most other taxon pairs was not significant, including "Oral II" with "S. equisimilis" and "Oral II" with "Streptococcus sp. (groups R, S and T)". It was noted in Section 4.4 that the organisms

used in this study may not accurately represent the species "S. dysgalactiae", and this may have led to the apparent overlap shown between this group and nearby groups.

Two further factors in the construction of the matrix PDBSTP may have affected the overlap results. One is that each group was considered as containing fifteen organisms. This was used as an arbitary value, because one was required by the statistics. The second factor is the choice of tests. The tests were those that were widely reported in the literature. However, they were not chosen to show either similarity or dissimilarity between particular groups. As can be seen in Section 3.3, different tests appear more suitable for differentiating different groups. As a result, these may not be the most suitable tests overall. This may cause distantly related organisms to seem similar on the basis of only a few tests. Similarly, organisms that are closely related may not appear so if the tests used are the only ones in which they differ.

The tests that are used for the most usually considered groups in the literature will appear more often than others. This may mean that these tests are weighted towards species within particular groups, such as the enterococci. Species not commonly studied, such as "Oral I" and "Oral II", as a result may not be easily defined by them.

The matrix PDBSTP2 used test results solely from this study. It could be expected that the groups which showed the highest levels of overlap in the numerical taxonomy would also show high levels of overlap here. These groups were noted earlier to be "Oral I", S. lactis and Pediococcus sp.. "Oral I" is

distinct in this matrix, and the only instance where overlap is likely to be above 2.5% is "Oral I" with "Oral II". S. salivarius also overlapped to a moderate extent with "Oral II". The taxonomic position of Pediococcus sp. is not much clearer. The grouping of some strains from this group with some aerococci probably explains the overlap seen between these groups. The other instances where Pediococcus sp. shows a moderate level of overlap are less explainable. The most probable explanation is that many single species will share a few properties with the pediococci because that group represents an entire genus, and therefore could be expected to show greater variation than a species.

S. lactis shows five cases where overlap may be greater than 2.5%. Three of these, with S. raffinolactis, S. salivarius and "S. casseliflavus" were seen in the full numerical study. This supports the possibility that they may be closely related.

S. bovis appears less distinct than in the fully study and this may account for some of the instances of overlap seen for this species. Overlap was also seen between S. salivarius and S. mutans, both of which are members of phenon III. Phenon III and the lactic group, (phenon IV), may form one large group. This is however not supported by some of the earlier work, and may be due to the failure of sixty tests to fully differentiate this area.

The difficulty in differentiating within certain areas may also account for the observed overlap for pairs S. salivarius and "S. milleri", S. mitis and S. sanguis, and "Oral II" and Leuconostoc sp..

The taxon pairs in Table 3.6.b show the four instances of a $V_{(G)}$ value of above 9 x 10^{-3}. However, instances as large as this were only seen for the matrix PDBSTP. The highest value for PDBSTP2 was 3.03610E-3 for the pair S. sanguis and "Oral II", and this seems satisfactory.

These results show the taxa in the identification matrices are relatively well defined. Most of the occurances of overlap are between closely similar organisms. Problem groups such as Pediococcus sp. may be due to the high level of variation within that genus, or to the relatively small number of strains of each species used. In general the tests that are best for discriminating the phenons gave a good representation of the taxonomy.

4.6 DNA discussion

The mol % G+C ratios for thirty-four strains were shown in Table 3.8.a, with their standard deviations and the percentage hypochromism

As mentioned in Section 3.8, problems were encountered in both culturing and lysing some organisms. Some strains, particularly the pyogenic organisms, were more susceptible to growth inhibition by glycine than the others. The glycine was included in the media, initially at a level of 8 g l^{-1}, to promote "leaky" cell walls. These were needed to assist in lysis by detergent and lysozyme. The glycine concentration was lowered for the more susceptible strains to a point where good growth of easily lysing cells was obtained. For some strains this was as low as 3 g l^{-1}. Three strains (PB 84, S. thermophilus, PB 163, Leuconostoc oenos and PB 113, Pediococcus halophilus) were found to grow very slowly in this medium.

The yield of cells did not in these cases appear to be appreciably better without glycine. The same was found of other strains from the same clusters. As a result only very low yields of DNA were obtained. There may be quite considerable losses in the DNA purification procedure used, and it was thus found impossible to isolate purified DNA from these organisms.

The problem of obtaining a good yield of DNA from strain PB 80 (S. bovis) was mentioned in Section 3.8. A variety of methods was tried in an attempt to achieve good lysis, ranging from sonication to a French pressure cell and ultimately grinding in alumina in a mortar and pestle. The latter method appeared to improve the lysis but also fragmented the DNA. It was thought that this resistance to lysis may have been due to a capsule, although nothing in the strain history indicated this.

The strains PB 40, PB 41 and PB 137 were difficult to harvest. Growth was comparable with other strains but the cells appeared to be bound together in what appeared to be small flocs, possibly due to a poly-saccharide. Future work with these strains would require an adjustment of the media to prevent this happening.

The problems with the melting of the DNA from strains PB 58, PB 179 and PB 183 was mentioned in Section 3.8. It was found that the thermal denaturation was greatly improved by a further iso-propyl alcohol precipitation. A possible reason for this may be that there is a polysaccharide associated with the DNA in these strains. The DNA appeared to be quite pure from OD 260/280 determinations, but chemical determinations gave a much lower concentration. The most likely contaminant to have an absorption at this level

(260 nm) is a polysaccharide. The reason why one extra precipitation is enough to remove it is not clear. These three strains needed more than three determinations to give reproducible results, so it is likely that the DNA was still not as pure as the other samples.

In contrast to these difficulties, strains PB 18 and PB 21, both representatives of "Streptococcus sp. (chicken)", were found to be the easiest organisms to lyse. They also gave the best yields of DNA.

The hypochromism results range from 22.3% to 41.6%. The average of these was 33.7% and this gives an idea of the amount of dissociation that has occurred in any strain (See Section 2.12).

Garvie (1979) found that three different DNA samples from the same strain of S. bovis showed up to 2.8% difference in % G+C values when prepared from different samples. A possible reason given for this was the age of the cells. Throughout this study care was taken to ensure that all cultures were of the same age, growth curves being used to determine this (see Section 2.12.1). The standard deviations in the % G+C values were satisfactorily low (average 0.29).

The high standard deviation in replicate DNA preparations seen for strain PB 193, "S. lactis subsp. diacetylactis" may be due to the determinations being performed some time apart.

Jones & Sneath (1970) suggested a range of 2.5% for % G+C was about the average within a species. Results may be considered in relation to this. Subphenon 2 (S. faecium) is represented by two

strains, PB 86 and PB 87. These show a range of less than 2%. The two strains, PB 18 and PB 21, from the group "Streptococcus sp. (chicken)" show a range of just over 3%. This was not expected from the numerical study, where this group appeared as a tight cluster. The standard deviation of the determinations from PB 21 is large (0.65) and so this result may not be as accurate as that for strain PB 18. Subphenon 10 (S. raffinolactis) is represented by PB 202 and PB 199. These show less than 1% difference. The cluster of lactic organisms (subphenon 12) is represented by the strains PB 93, PB 95 and PB 193. Despite the high standard deviation of the determinations for strain PB 193 (1.35), these organisms show a range of results of about 1.5%. The difference in % G+C between strains PB 107 and PB 148 for subphenon 13 (A. viridans) is less than 1%, as is that for strains PB 179 and PB 183 (subphenon 17, "Oral II"). Therefore the range of % G+C values in the species are broadly what one would expect.

The average hypochromism of 33.7% in this study is about the same as the 38% found by Garvie (1979). The lowest hypochromism values were found for strains PB 86, PB 148 and PB 57. The reason for this is not clear, because no other problems were associated with these strains.

The % G+C results for individual species are, in general, in agreement with those given in the literature (Marmur & Doty, 1962; Hill, 1966; Diebel & Seeley, 1974; Roop et al., 1974; Garvie, 1978). Diebel & Seeley give % G+C values from methods other than melting point determinations as well.

These differ from the results of melting point methods. All of the strains in this study gave % G+C values within the ranges given for their species in the literature, with the exception of PB 70, S. pneumoniae. This strain gave a much lower value in this study than expected, 35.14, whereas 39 was expected. This strain was received as being the type strain of the species. The culture had been plated out from the broth during DNA extraction and did not seem to be contaminated. Melting points lower than expected may be due to fragmented DNA (Garvie, 1979) and this may be a possible explanation. This strain also showed a low vigour value of 0.344 in the numerical taxonomy and this may in some way be connected with this low ratio. Possibly the cells were not as viable as they should have been, leading to contamination with partially broken DNA from the dead cells.

No previous values have been found for strains of the previously unnamed groups, so no direct comparisons can be made for them.

Overall, the range of % G+C values found here for the whole genus Streptococcus is 33.18–40.4%. The accuracy of the method may not be very high and so differences of 0.25%. are probably not significant.

The taxonomic implications of % G+C values are restricted to marked differences: similar % G+C values do not necessarily imply taxonomic similarity. In this study all of the % G+C values were similar and therefore there is no strong evidence from this that the genus is heterogenous.

4.7 Esterase results

The patterns obtained from the electrophoresis and staining of esterases in polyacrylamide gels were shown in Figures 3.9.1-3.9.5. The positions of bands were found to be reproducible (Figure 3.9.7). However, although some bands appeared in the same positions in both the five initial gels and the repeat gel; some appeared as smears rather than distinct bands. This is possibly due to a high concentration of enzyme being present, because all of the bands seen with the more concentrated samples appeared as smears (Figure 3.9.6). The position of a band may be better considered as being between two points rather than as a single line at a rigorously defined distance. This idea, however, leads to further problems. Strain PB 2 (S. faecalis) for example, showed two quite distinct separate bands between the positions 60 and 63 units from the origin. Defining the bands over a wide range or using more concentrated samples would lead to these two bands being considered as one.

The bands that appeared in the gels near the bromophenol blue marker are interesting. They were always seen for the same extracts. It is unlikely that they were due to inaccuracies in the preparation of the gels or stock solutions. The molecular weight of bromophenol blue is approximately 670 daltons. These bands stained in the same way as other bands, at the same speed and to the same intensity of colour. They must therefore be considered to have some activity against α-naphthyl acetate. Although C3 esterases are relatively small enzymes, it is unlikely that an organic molecule of less than 1000 daltons could be a polypeptide. They may be oligopeptides, but as such they would

probably not show esterase activity. It is however possible that they are larger than 1000 daltons but highly charged. This seems the most likely explanation. Unfortunately marker esterases of different sizes were not available and so the relative sizes throughout the gels are not known.

It was hoped that the distribution of esterases within the streptococci would yield information like that of London & Kline (1973) for the aldolase enzymes. However, the distribution of esterases throughout the streptococci does not appear to be species specific. Strains PB 86 and PB 87 ("S. durans" and S. faecium) appeared in the same cluster in the numerical taxonomy but gave different esterase patterns. The same is true of strains PB 93, PB 95 and PB 193 in the lactic cluster, strains PB 107 and PB 148 in the A. viridans cluster and strains PB 179 and PB 183 from the "Oral II" cluster. The latter however, do appear to have one band in common. Strains PB 18 and PB 21, from the group "Streptococcus sp. (chicken)" show single bands at 43 and 48 units respectively. The band at 48 units for strain PB 21 appears as a smear rather than a distinct band and so it may be the same band as seen in PB 18.

As was seen with the APIzym strip, all of the subphenons in this study showed some level of C4 esterase activity with 2-naphthyl butyrate and C8 activity with 2-naphthyl caprylate. It was expected from the APIzym results that more strains would show C3 activity than the twenty found. However, the extra band seen with the more concentrated sample for PB 93 indicates that

there may be lower levels of enzyme present than those detectable in these gels. Consequently, the lack of any bands does not necessarily mean the lack of esterase activity at a low concentration. The concentration of esterases in these gels (judged from intensities of reaction) varied considerably between different strains. One major difference between the gel method and the APIzym method was the growth medium. A solid medium with blood was used for the APIzym method, compared to a broth medium without blood used for the gels. This means that the two methods are not comparable. Furthermore esterases may be inducible enzymes in some strains. If this was true of C3 esterases this would account for the discrepancies in these.

All members of the enterococci showed esterases in gels, whereas the strains of S. bovis and S. equinus did not. Esterases were also seen in the strain of "S. casseliflavus" (PB 52). Of the lactic organisms, strains PB 95 and PB 193 (S. cremoris and "S. lactis subsp. diacetylactis") showed faint single bands. Strain PB 93 (S. lactis) showed no bands at this concentration but one at the higher concentration. This indicates that esterase activity in the lactic organisms is less than that in the enterococci. Of the pyogenic organisms, PB 54 (S. pyogenes) showed the strongest activity and a weak band was seen for strain PB 117 ("S. dysgalactiae"). None of the other pyogenic isolates showed any activity at either of the concentrations used. Esterase activity varied among the oral isolates. In phenon III PB 9, S. salivarius, and PB 58, S. mutans, showed bands, although the S. mutans like organisms of "Oral I" did not. In phenons VI and VII, strains PB 68 and

PB 98 (S. sanguis and S. mitis) showed weak activity although the strains PB 179 and PB 183 from the phenotypically similar "Oral II" did not.

Of the two strains from the subphenon A. viridans, strain PB 107 failed to show any bands and strain PB 148 (received as Streptococcus sp.) showed three. However, there is some doubt about the relatedness within this cluster from the numerical work.

Esterase patterns have been observed in some of the serological group D streptococci by Lund (1965; 1967). She found that a major difference between strains of S. faecalis and non-motile strains of S. faecium and "S. durans" was that strains of S. faecalis showed darker bands. This was also found in this study. Lund found that the motile strains of S. faecium and its varieties gave different esterase patterns from the other group D streptococci. Only one motile strain was used in this study. This was PB 52, received as "S. faecium subsp. casseliflavus", which in this study showed an esterase pattern distinct from the other serological group D strains. Much the same esterase pattern was seen for both S. faecalis and S. faecium in this study and those of Lund , although with a reduced number of bands in some cases. It is interesting that in the study of Lund (1967) a band is shown near the anode. This may be comparable with the bands numbers 12 and 13 found in this study. However, she did not show the positions of any markers, so this is not clear. Lund also reported that the intensity of the bands depended upon the buffer used for the cell suspension during disruption. Samples in this study were disrupted in sterile

distilled water, and this may account for some of the lower level of activity seen. Also, Lund used a higher protein concentration. Within these constraints however these results appear to be generally similar.

Norris (1964) found that in strains of Bacillus thuringiensis esterase patterns were serotype specific. Lund (1965) found some agreement with serotypes. Other enzymes in the streptococci (such as lactate dehydrogenases) have been used for the characterisation of strains at species level (Garvie, 1978; Garvie & Bramley, 1979a; 1979b). Relatedness between species has been studied on the basis of aldolase enzymes (London & Kline, 1973). Differences between both strains and species of some lactic streptococci have been found in peptidase levels (Cliffe & Law, 1979).

In this study serological types were not investigated and therefore the correlation between these and the esterase patterns was not possible.

The enterococci appear to give relatively well defined patterns within the limits of the methods used, but there is some strain variation. In other groups, such as the lactic organisms, there appears to be more strain variation. In the "Oral II" cluster the presence of one very similar band in the two strains examined for esterases may be significant, especially as this band was not seen in any other strains.

The results of this survey indicate that although esterase patterns may aid in the characterisation of particular groups such as the enterococci, their value otherwise is unclear.

For comparable results between studies a particular concentration must be fixed upon which gives distinct accurate bands. The problems encountered with faint bands may not be soluble by using higher concentrations of samples, because the lower levels of activity observed may be no better for discrimination.

4.8 Protein trace results

Figure 3.10.1 showed a representative protein trace obtained from the methods given in Section 2.14.1. Figures 3.10.3 and 3.10.4 show the four dendrograms obtained using the two different methods with two different similarity coefficients, for the whole cell soluble protein extracts.

The two coefficients used; the taxonomic distance and the cosine θ coefficient, were chosen because they had previously been used with some success for this type of work (Feltham & Sneath, 1979). Correction factors were applied to try and compensate for any inaccuracies that may have been present both between gels, and different tracks on the same gel. The reasoning behind these corrections and the mathematics involved were considered earlier in Section 2.14.3.

The distance dendrograms for the corrected and uncorrected methods were shown in Figure 3.10.3. Overall, these are very similar. Duplicate traces on the same strain are closer than other traces, indicating that the patterns were highly reproducible. No distinct taxonomic groups were present (see Section 3.10) although the same grouping of certain strains is seen in both representations. The taxonomic distance considers the heights of peaks and as such it is very dependent upon the concentrations of the samples.

The cosine θ coefficient dendrograms were shown in Figure 3.10.4. These again appear very similar to each other. Again, no clear taxonomic groups were present. The cosine θ coefficient is a shape coefficient. This means that unlike the distance coefficient, concentrations of particular samples do not have such a pronounced effect on the similarity values. Concentration effects may therefore account for the grouping together of strains PB 81 to PB 137 in the distance dendrograms, a grouping that is not seen in the cosine θ dendrograms. It can be seen that the base lines for the cosine θ dendrograms are much closer together than those for the distance dendrograms. Similarly, the duplicate strains appear to be much closer. This may be due to two factors. One is the reduced range of values available for the cosine θ coefficient when compared to the taxonomic distance. Secondly, the removal of concentration effects may increase the similarity between strains.

Strains PB 18 and PB 21 are from the group "Streptococcus sp. (chicken)". This was a tight group in the numerical taxonomy. In the taxonomic distance dendrograms they both appeared on the same arm of four strains. In the cosine θ coefficients they both appeared to be much more separated from each other. However, the base lines on the cosine θ dendrograms are much closer and they may therefore not be as dissimilar as their positions suggest. Strains PB 87 and PB 86 are from the group S. faecium. The strains were quite different by taxonomic distance, but very similar by the cosine θ coefficient. Strains PB 199 and PB 202 (S. raffinolactis)

appeared widely separated in all representations, as did strains PB 93, PB 95 and PB 193, which were all members of the group S. lactis. The same was true for strains PB 107 and PB 148 from the group A. viridans. Strains PB 179 and PB 183 ("Oral II"), appeared relatively close in the cosine θ dendrograms, whereas they linked together closely in the distance dendrograms.

Differences can be seen between the dendrograms from the two different coefficients. However, there are also some differences between the corrected and uncorrected dendrograms. The program calculated the similarity coefficients for every combination of shift (A) and stretch (B) values within a given range. Then, assuming that inaccuracies in the gels or other methods would tend to make patterns less similar, the best value for both coefficients was selected. The range of similarity values is smaller after correction. The program also provided a list of the different coefficient values and the values of A and B in every case. A perfect fit would give a value of 0 for A and of 1 for B. The highest values were found for the coefficients when A was in the range 0.25 to -0.25 and B was between 1.25 and 0.75. Considering these values, and the small change that they made to the coefficients for the duplicates, it does not seem that this led to an overcorrection. The values indicate that the level of inaccuracy in the methods was generally low. The fact that the same values were found repeatedly for A and B may indicate that the source or sources of inaccuracy may be constant in many cases. One possible reason for this may be a constant factor in edge effects within each gel.

Although the mathematics and computing appears to be suited to this method, it nevertheless failed to differentiate these strains into any clear taxonomic groups. Kersters & De Ley (1975; 1980) have achieved good taxonomic groupings of Agrobacterium and Alcaligines by similar methods. Other workers have also reported good groupings from similar methods, although a lot of these studies involved members of the Enterobacteriaceae (Feltham, 1975; Kersters & De Ley, 1980). Most studies have been on whole cell soluble protein extracts, but smaller proteins such as those associated with the cell envelope or with ribosomes have been examined (Hamada & Mizuno, 1974; Kersters & De Ley, 1980). Whole cell soluble protein extracts for group D streptococci were studied by Lund (1965; 1967), who found differences in protein patterns at both species and strain levels. She considered the presence of flagellar protein among the motile strains to be of little significance to the general protein patterns, because flagella were usually lost during harvesting and preparation of samples. Membrane proteins in S. mutans and related organisms were studied by Hamada & Mizuno (1974). Here it was found possible to group these organisms without numerical methods by iso-electric focusing in polyacrylamide gels.

The proteins present in a cell are determined by the genetic material and also the environmental conditions of growth. The biochemical properties of a cell under these conditions are in turn determined by the proteins present. A study of these proteins should yield information about both the genetic and biochemical properties of the organisms. In this study this method failed to differentiate species. The extracts used were

crude. They may have further been improved by a specific extraction procedure for specific proteins, rather than relying on a specific dye with the crude extracts.

From these protein patterns it is only possible to differentiate individual strains. The APIzym test strip was useful in differentiating some species on the presence of particular enzymes, as were biochemical tests such as phosphatase.

The knowledge of which bands in the gels corresponded to which proteins would be very useful. The separation of strains of "S. equisimilis" and "S. zooepidemicus" in the numerical taxonomy appeared to be connected to the level at which positive reactions were scored. From the similarities shown in protein patterns it is possible that much of the difference between the streptococcal species may not be due to whether a protein is present or absent, but to the concentration. The alternative is that cellular proteins may be strain, or at least serotype specific. Beighton et al. (1981) showed that serotypes of S. mutans could be characterised by their protein patterns. Possibly cell protein patterns within the streptococci at species level are too complicated to study easily. The work of Hamada & Mizuno showed the value of membrane proteins in the characterisation of some oral organisms. It may be preferable to study proteins of a particular type in the streptococci, rather than a wide range of proteins. Cell wall and membrane studies on the streptococci have proved useful in differentiating certain groups (Colman & Williams, 1965; Collins & Jones, 1979). The structural proteins associated with the cell walls may perhaps give useful results in this sort of study.

4.9 Serology results

The results for the thirteen strains of serological groups B or D were given in Table 3.11.a. These were very much as expected. The weak group D reactions given by strains PB 53 and PB 69 may not be of much consequence because some group D strains give notoriously weak serological reactions (Sherman, 1937). The results mainly served to confirm the identity of the strains.

4.10 Conclusions on streptococcal taxonomy

Relationships between the streptococci and other bacteria are not clear. Protein sequences are not available to compare with homologous protein sequences of other bacteria. There is no ribosomal RNA/DNA pairing evidence that may help (De Ley et al., 1978; Mordarski et al., 1980). A ribosomal RNA catalogue is not available for the genus and so no comparisons may be made there (Woese et al., 1975). Numerical taxonomy similarity levels between the streptococci and the similar genera of Leuconostoc and Pediococcus are high (71% S_G).

Within the genus Streptococcus the similarity levels are very high at 76–82%. This compares to 65–75% (S_G) found by Power (1978) for Haemophilus and 65–75% (S_{SM}) found by Orchard et al. (1980) for Nocardia. DNA/DNA homologies within the genus (where available) typically show greater than 80% for similar organisms and less than 20% for dissimilar organisms (Roop et al., 1974; Garvie, 1978; Vaughn et al., 1979). There is also the negative evidence of % G+C that the streptococci do not show a wide range of results that would imply heterogeneity (Jones & Sneath, 1970).

The streptococci may be grouped into groups of species although the relationships between them are not always clear. The species-groups found in this study are listed in Table 4.10.a. These are based on the numerical taxonomy results and represent the main phenons. They are discussed below. Also discussed here are the genera of Leuconostoc, Gemella, Aerococcus and Pediococcus.

The enterococcus species-group

The subphenons of S. faecalis, S. faecium, "S. avium" and "Streptococcus sp. (chicken)" form a group corresponding to the enterococci. S. faecalis and S. faecium form a distinct phenon and the internal structure of this is shown in Figure 4.10.1. From this it can be seen that the subspecies of "S. faecalis subsp. zymogenes" and "S. faecalis subsp. liquefaciens" cannot be considered as separate groupings. "S. durans" does not cluster separately from S. faecium and so may not be considered as a separate group. The two species of S. faecalis and S. faecium appeared distinct from each other; only low overlap seen was between S. faecium and "S. casseliflavus" and S. faecalis and S. faecium. The solitary strain of "S. avium" in the S. faecium cluster (PB 16), showed properties that were atypical of "S. avium" such as the decarboxylation of arginine and failed to produce acid from xylose.

"S. avium" was distinct from the above strains and more closely related to "Streptococcus sp. (chicken)". All of these strains were isolated from birds, and their phenotypic similarity

Table 4.10.a The genera and species-groups within the family *Streptococcaceae*.

Genera. *Streptococcus*, *Pediococcus*, *Leuconostoc*/*Gemella*.

Species-groups (1) Enterococcus; containing *S. faecalis*, *S. faecium*, "*S. avium*" and "*Streptococcus* sp. (chicken)".

(2) Para-viridans; containing *S. bovis*, *S. equinus*, *S. salivarius*, *S. mutans*, *"*S. casseliflavus*", *S. raffinolactis* and * "Oral I".

(3) Lactic; containing *S. lactis*.

(4) Viridans; containing *S. mitis*, *S. sanguis*, "*S. milleri*" and "Oral II".

(5) Pyogenic; containing *S. agalactiae*, *S. pyogenes*, *S. equi*, *"*S. equisimilis*" and*"*S. zooepidemicus*".

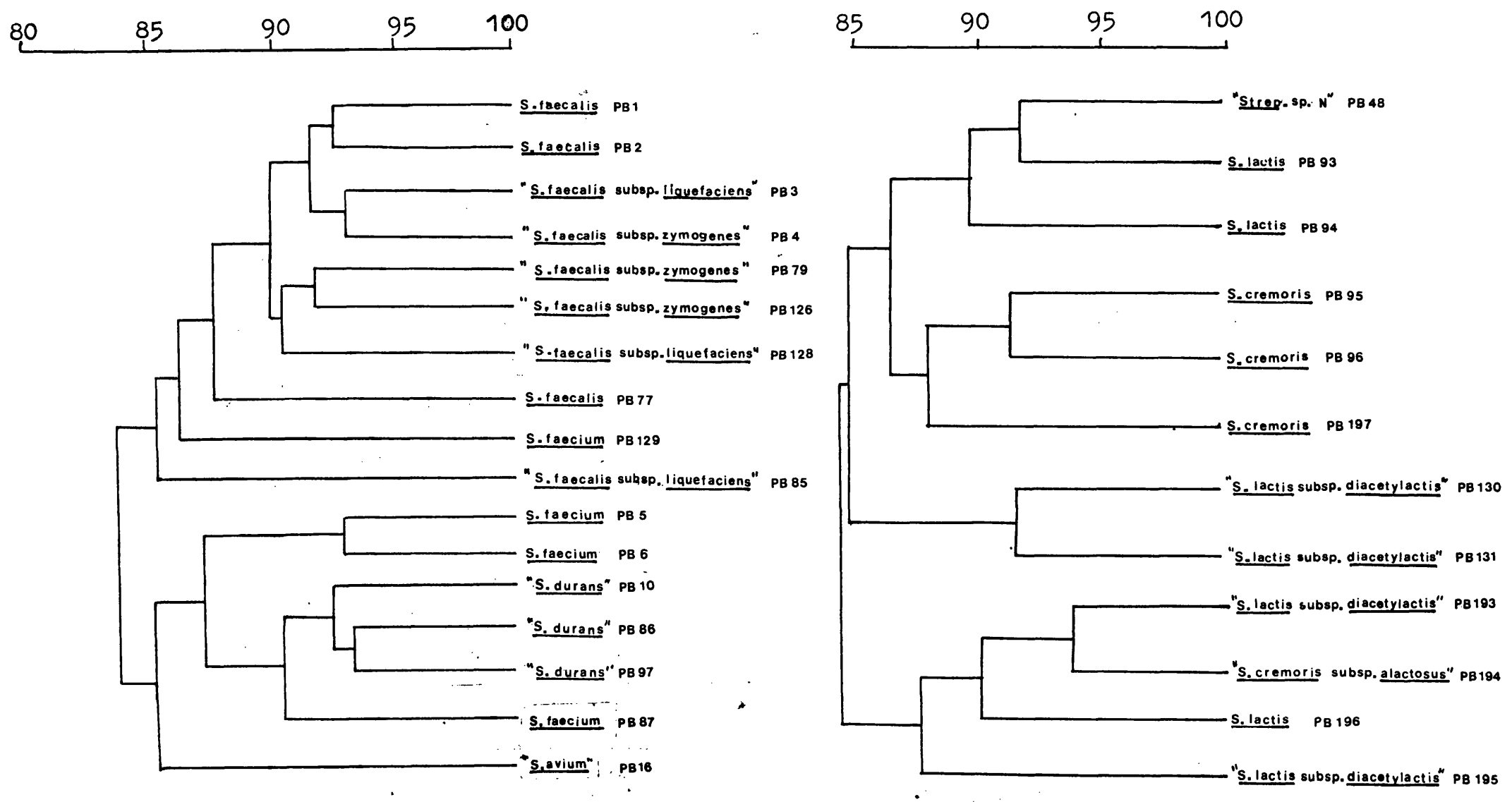
(6) Para-pyogenic; containing *S. uberis*, *"*S. dysgalactiae*" and "*Streptococcus* sp. (groups R, S and T)".

(7) *S. thermophilus*

(8) **S. pneumoniae*

* These species did not behave as expected in this study but may deserve species status.

Figure 4.10.1 Fine detail of subphenons 1, 2 and 12 from the S_G average linkage dendrogram.



may be partly due to adaption to an avian environment. Both of these subphenons showed satellite strains. PB 69, received as "S. faecalis subsp. malodoratus" was associated with "S. avium". A similar arrangement to this was reported by Feltham (1979). PB 53, received as "S. faecium subsp. mobilis", was associated with the subphenon "Streptococcus sp. (chicken)". However, this subphenon has not been reported as being motile. Both "S. avium" and "Streptococcus sp. (chicken)" are absent from the Approved Lists of Bacterial Names (Skerman et al., 1980) although from this study they appear to deserve species status. Several other strains were contained in the enterococcus species-group; the majority were received as serological group D. These may represent intermediate forms between the species, or alternatively they may be members of new species that have yet to be defined. The small number of strains present in this study makes it difficult to reach a conclusion.

It is interesting that the enterococcus species-group shows less similarity to other streptococci than strains of other genera. There may be a case for considering the enterococci to be a separate genus, as suggested by Kalina (1970). However, a lot of tests have been devised for separating the enterococci from the other streptococci and their inclusion may have weighted the results towards separation. Certainly on the basis of mol % G+C ratios, esterases and protein patterns they appear similar to the other streptococci.

The para-viridans species-group

The species S. bovis and S. equinus were seen as distinct species, separate from the enterococci and clustered in phenon III with mainly oral organisms. Although both species showed some overlap with both oral and lactic organisms they did not show large overlaps with each other or the enterococci. As was seen in Table 3.3.e, very few tests separate the species S. bovis and S. equinus. However, their low level of overlap indicates that they are separate species. One test previously used to separate them was the production of dextran. In this study dextran formation was not seen in either species. This may have been due to the conditions used (Barnes et al. (1961) warned that the growth media is important) or more likely, the difficulties encountered in reading this test (flocculation with ethanol). Although all the strains of each species did not give the same test reactions, the species were tight clusters and there was no apparent clustering within them to indicate the presence of more than one phenotype.

The strains of "S. faecium subsp. casseliflavus" clustered close to S. equinus and S. bovis. However, as already discussed, these strains showed low vigour results and therefore may not be correctly positioned in this species-group. This species may be better placed with the enterococcus group, possibly close to "S. faecium subsp. mobilis", but is left in this species-group in Table 4.10.a on the basis of the clustering.

S. salivarius, S. mutans, "Oral I" and S. raffinolactis were all placed in the para-viridans group. The overlap statistics between S. salivarius and "Oral I" indicate they may not be as distinct as they appear in the dendrograms. The possibility of S. mutans

and "S. sobrinus" forming two distinct clusters was discussed in Section 4.2.3. "Oral I" may constitute a second species that is similar to S. mutans or it may represent a group of strains that are intermediate between S. salivarius and S. mutans. Further work is necessary to clarify the positions of these organisms. The relationship of S. raffinolactis to any of these organisms is unclear. It clustered in this species-group and, on that basis, has been included here.

The lactic species-group

The lactic species-group of streptococci has previously been considered as consisting of S. lactis and S. cremoris and their subspecies. More recently S. raffinolactis has been added. S. raffinolactis was not grouped in this species-group in this study as explained earlier. S. lactis and S. cremoris clustered together in one subphenon. The internal structure of this subphenon is shown in Figure 4.10.2. As can be seen from this it is not possible to separate sharply either the named species or the subspecies. The subphenon S. lactis does not appear particularly distinct, with several instances of overlap with other subphenons having been observed. The relationships between this species group and some of the others are unclear. The overlap results and the D_P calculations both indicate that S. lactis may possibly be related to the enterococci. This relationship is further supported by some of the identification scores seen in Table 3.5.c. The lactic group appears to be a single species of which S. lactis and S. cremoris may only be phenotypes. Further work is still needed to confirm this.

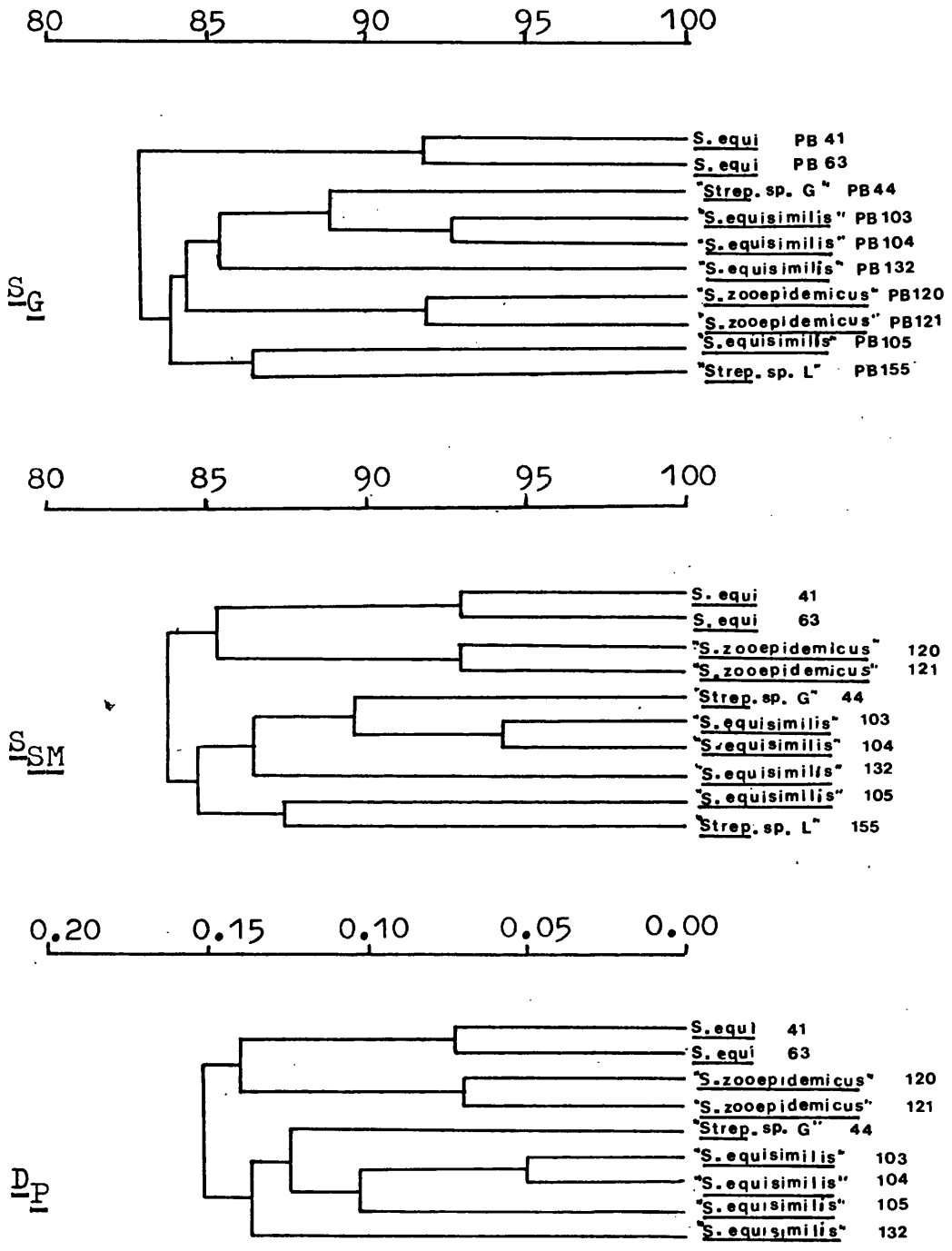
The viridans species-group

The viridans species-group consists of the subphenons S. mitis, S. sanguis, "S. milleri" and "Oral II". These all form distinct clusters and while they do show some instances of overlap, the first three deserve species status. "Oral II" contains strains that were isolated from four individuals and as such their occurance in a wider context is not known. The strains of "S. milleri" examined in this work are more similar to other oral organisms than any pyogenic ones (although a small overlap was seen between "S. milleri" and S. pyogenes). This group was named as it corresponded closely to the viridans streptococci.

The pyogenic species-group

The group previously described in the literature as the pyogenic organisms consisted of the species S. agalactiae, S. pyogenes, S. equi and "S. equisimilis". The first three of these appeared to be good distinct species. The subphenon "S. equisimilis" has upto now been considered in this study as one species, that is the species "S. zooepidemicus" has been considered as part of "S. equisimilis". The inference from the identification matrix, however, was that they were separate. The internal structure of this subphenon obtained in the three average linkage dendrograms is shown in Figure 4.10.3, and from this it appears that these two might be separate species, but closely related.

Figure 4.10.2. Fine detail of subphenons 22 and 23 from the three average linkage dendrograms.



The subphenon "Streptococcus sp. (B) clinical" appears relatively distinct and separate from S. agalactiae. A similar arrangement for human and animal group B strains was seen by Feltham (1979). This subphenon may represent a species, although it is possible that the culture collection strains, which were all of animal origin, have altered in some of their properties with repeated subculture. Further discussion of this group was given in Section 4.2.2.

The para-pyogenic species-group

The subphenons S. uberis, "S. dysgalactiae" and "Streptococcus sp. (groups R, S and T)" clustered together, close to the pyogenic organisms in the S_G dendrogram. S. uberis shares some properties with the pyogenic group of organisms, but the movement seen for this species within the different dendrograms may mean that it is not closely related to them. "Streptococcus sp. (groups R, S and T)" showed some similarity with the pyogenic species-group and the lactic and viridans groups. It may not be closely related to the pyogenic organisms although it has been described as similar to them (see Section 1). "S. dysgalactiae" was expected to cluster with the pyogenic organisms. It was however found to be distinct and clustered with S. uberis. Possible reasons for this were given in Section 4.5, and the most likely seems that these strains were not representative of the species. However, on the basis of this study it has been assigned to the parapyogenic group.

Other species-groups

S. thermophilus appeared as a distinct cluster. It showed no close similarity to any one group, appearing as a satellite group in different positions in the different dendrograms. The one strain of S. pneumoniae in the study appeared distinct from any of these groups. It is tentatively placed in a separate species-group in Table 4.10.a, although as this is only based on one strain, its validity is not certain.

Other genera

Of the other genera considered in this study, Leuconostoc and Gemella appear to be the most similar to the streptococci. They are also very similar to each other. They formed a distinct major group in the dendrograms, although whether this should be considered as a separate genus is unclear. A different genus would be expected to form a distinct cluster from the streptococci and not be embedded within Streptococcus. These genera appear very similar to the viridans species-group. The position of the only strain of Gemella used in the study in this cluster, indicates that Gemella may be more similar to Leuconostoc than Streptococcus.

Pediococcus is quite distinct from Streptococcus although it may be related in some way to the enterococci. The high levels of overlap shown by Pediococcus sp. may be due to it showing some superficial resemblance to the streptococci. However, the subphenon Pediococcus sp. showed a large proportion of variable test results which may account for a lot of the overlap. This may also have been because four species of pediococci were represented here.

The genus Aerococcus does not seem distinct from either the streptococci or the pediococci. This was best illustrated in the identification matrix. Throughout the taxonomy strains of Aerococcus have been found associated with the streptococci and the pediococci. It seems most likely that Aerococcus consists of strains that show properties intermediate between those of Pediococcus and Streptococcus.

Further work

It is possible to consider a group of organisms on the basis of their habitats. Organisms found in the same habitat and living on similar nutrients could be expected to show biochemical similarities (such as the ability to break down lactose shown by organisms found in milk). How this may relate to the taxonomy of the streptococci is not clear. Whilst the guts of horses and chickens are both enteric habitats, the nutrients available in each will be different. Certainly some relationships appear to reflect habitat, such as the organisms found in the viridans group which are all oral organisms. This does not apply in all cases, and oral organisms are found in two species-groups. Again, varied types of streptococci have been isolated from cases of bovine mastitis, particularly S. agalactiae and S. uberis, which are physiologically distinct. It is possible that further work in the areas of ecology and enzymology may help to clarify any relationships between habitats and taxonomic types. This would be particularly useful for S. bovis and S. equinus, and also for the S. salivarius/S. mutans area. The streptococci form a large group of organisms that may have diversified according to habitat and also a degree of inducible enzyme expression.

Given the high similarities within the genus, further work on ribosomal RNA and the proteins associated with it may prove helpful. Although DNA/DNA pairing techniques have been used, they have not been used for all species, and this and possibly DNA/RNA pairing may yield useful results.

APPENDICES

Appendix I

Computer program appendix

Figure A.1.1 Program listing of PDBAED2.

```
00005  REM     PROGRAM  PDBAED2
00010  REM     THIS PROGRAM CALCULATES THE AVERAGE
               EUCLIDEAN DISTANCES
00015  REM     FROM THE CENTROID FOR MEMBERS OF A GROUP USING
               DATA EX IGROUPS
00020  PRINT   "PDBAED2 CALCULATES AED (AVERAGE EUCLIDEAN DISTANCES"
00025  PRINT
00030  PRINT   "FROM THE CENTROID FOR MEMBERS WITHIN A GROUP"
00035  PRINT
00040  PRINT
00041  PRINT   "INPUT NO. OF GROUPS TO BE CALCULATED"
00042  LET  Z=0
00043  INPUT  G
00045  PRINT "INPUT NO. IN GROUP"
00050  INPUT A
00055  PRINT "INPUT DISTANCES"
00060  LET  S=0
00065  LET  X=0
00070  INPUT  D
00075  LET  X=X+1
00080  LET  S=S+D 2
00085  IF   X=A  THEN GOTO  95
00090  GOTO  70
00095  LET  B=0
00096  LET  B=A-1
00100  LET  C=S/B
00105  LET  M=SQR(C)
```

Figure A.I.1 continued.

```
00110   PRINT  "AVERAGE EUCLIDEAN DISTANCE FROM CENTROID
               IS :-  ",M
00115   PRINT
00117   LET  Z=Z+1
00118   IF  Z=G  THEN GOTO  120
00119   GOTO  045
00120   PRINT  "DO YOU REQUIRE ANOTHER CALCULATION?"
00125   PRINT
00130   PRINT  "IF YES TYPE 1, IF NO TYPE 0"
00135   INPUT Y$
00140   IF  Y$ ="1"  THEN GOTO  041
00145   IF  Y$ ="0"  THEN GOTO  150
00150   STOP
00155   END
```

Figure A.I.2 Program listing of PDBGC2.

```
00090   REM  THIS PROGRAM CALCULATES THE %G+C OF BACTERIAL
00100   REM  DNA FROM IT'S MELTING POINT IN SALINE CITRATE
00105   REM  P D BRIDGE JUNE 1980
00110   PRINT "THE G+C RATIO OF DNA MAY BE OBTAINED FROM THE"
00115   PRINT "MELTING POINT IN SSC"
00120   PRINT "1/10 SSC MAY ALSO BE USED"
00125   REM  VARIABLES USED ARE, A(I), A$ , B, B$ , C$ , D, D$ ,
             E, H, K, L, M(I)
00126   REM  N(I), P, R, S, X, Y, Z(I) AND O
00127   REM  FIRST ROUTINE TO DEFINE BUFFER AND CALCULATION
00128   REM  SECOND ROUTINE FOR SSC BUFFER. THIRD ROUTINE
00129   REM  TO DETERMINE FURTHER CALCULATIONS IF REQUIRED.
00130   PRINT  "FOR SSC TYPE S, FOR 1/10 SSC TYPE D."
00131   INPUT  D$
00132   IF  D$ ="S"  THEN GOTO  00134
00133   IF  D$ ="D"  THEN GOTO  00258
00134   PRINT "HOW MANY DETERMINATIONS ARE REQUIRED?"
00135   INPUT  X
00136   PRINT "TYPE IN EACH VALUE AFTER EACH ? SYMBOL"
00137   FOR I=1  TO X
00138   INPUT A(I)
00139   IF A(I) < 69.5 THEN GOTO 00142
00140   PRINT "MELTING POINT TOO LOW TO BE DETERMINED BY THIS METHOD"
00141   GOTO  00185
```

Figure A.I.2 continued.

```
00142  IF A(I) 100 THEN GOTO 00170
00143  PRINT "DETERMINATION SHOULD BE PERFORMED IN DSC"
00144  GOTO  00185
00170  LET  B=A(I)-69.4
00180  LET  Y=B*2.44
00184  PRINT "MOL %G+C VALUE IS "Y
00185  NEXT I
00186  PRINT "TO FIND HYPOCHROMISM TYPE H, ELSE O"
00187  INPUT  C$
00188  IF  C$ ="H" THEN GOTO 00249
00189  IF  C$ ='O' THEN GOTO 00210
00190  GOTO  00210
00191  PRINT "DO YOU REQUIRE ANOTHER CALCULATION ?
              (ENTER YES OR NO)"
00192  INPUT  A$
00193  IF A$ ="YES' THEN GOTO 00130
00194  IF A$ ="NO" THEN GOTO 00276
00200  STOP
00201  REM ROUTINE TO LIST RESULTS SEEN IN
00202  REM LITERATURE FOR STREPTOCOCCI.
00210  PRINT "TO COMPARE TO G+C RESULTS IN THE LITERATURE
              TYPE L, ELSE E"
00215  INPUT B$
00220  IF B$ ="L"  THEN GOTO 00230
00225  IF B$ ="E"  THEN GOTO 00191
00230  PRINT "S. PYOGENES, 34.5-38.5"
00231  PRINT "S. SANGUIS, 38-40 (METH NOT STATED), STRAIN CHALLIS,
                41.8 (TM)"
00232  PRINT "S. PNEUMONIAE, 39"
```

Figure A.I.2 continued.

```
00233  PRINT "S. SALIVARIUS, 37.5-39 (TM)"
00234  PRINT "S. BOVIS, 38-40 (METH NOT STATED), 36.6-39.7 (TM)
                AND 42 (TM)"
00235  PRINT "S. FAECIUM, 34-38 (METH NOT STATED) AND 40 (TM)"
00236  PRINT "S. LACTIS, 38.4-38.6 AND 36.3-36.7 (TM)"
00237  PRINT "S. CREMORIS, 38-40 (METH NOT STATED) AND 34.9
                - 35.4 (TM)"
00238  PRINT "S. RAFFINOLACTIS, 40.3-41.5"
00239  PRINT "S. MUTANS, 37-40.5"
00240  PRINT "S. UBERIS, 35.5-37"
00241  PRINT "LEUCONOSTOC, 38-42, L. LACTIS, 43-44"
00242  PRINT "PEDIOCOCCUS, 34-44 (TM)"
00243  PRINT "AEROCOCCUS, 36-44 (TM)"
00245  GOTO  00191
00246  REM  ROUTINES TO DETERMINE HYPOCHROMISM OF
00247  REM  EACH SAMPLE.  FROM LINE 00256, ROUTINE
00248  REM  TO DETERMINE MELTING POINT IN DSC.
00249  FOR  I=1  TO  X
00250  PRINT "TYPE IN INITIAL OD VALUE AT 260 NM"
00251  INPUT M(I)
00252  PRINT "TYPE IN FINAL OD VALUE AT 260 NM"
00253  INPUT N(I)
00254  LET K=N(I)/M(I))-1
00255  PRINT "HYPOCHROMISM IS "K
00256  NEXT I
00257  GOTO 00190
```

Figure A.I.2 continued.

```
00258  PRINT "HOW MANY DETERMINATIONS ARE REQUIRED ?"
00259  INPUT P
00260  PRINT "TYPE IN VALUES AFTER EACH ? SYMBOL"
00261  FOR I=1  TO  P
00262  INPUT Z(I)
00265  LET R=(Z(I)-53.9)*2.44
00266  PRINT "MOL %G+C VALUE IS "R
00267  NEXT I
00268  GOTO 00186
00276  STOP
00277  END
```

Figure A.I.3 Program specification of PDBSCX

PDBSCX (RKFSCX adapted)

Program title SCALEX

Language Fortran V

Written for interactive use on the Leicester University

CDC Cyber 73 computer.

The purpose of this program is to interpolate from a given trace and then scale the X-axis by shifting and/or stretching the values. The measure of shift is A and the measure of stretch is B. Similarity values in the form of the taxonomic distance and the cosine θ values are calculated between all pairs of traces under all elections of A and B. The highest values obtained for each pair are stored as an unclustered similarity matrix. The program allows for any number of A and B values to be calculated.

Tape 1=Input (from terminal)

Tape 2=PDBDAT (X-axis values, I4)

Tape 3=Output (to terminal)

Tape 5=PDBOUT (information previously displayed at terminal)

Tape 6=PDBIN1 (unclustered similarity matrix)

Appendix II

Reproducibility appendix

Table A.II.a Table of differences between tests

Table A.II.b List of characters not used in the taxonomy

Table A.II.a Differences between tests.

Character	Difference (%)
Cell arrangement	42.0
Catalase from haemin	15.3
Growth at 45°C	9.9
Growth with 3% NaCl	9.8
Tolerance of Na azide	8.4
Tolerance of thallous acetate	4.4
Growth with 10% bile	11.5
Growth with 0.0004% crystal violet	4.95
Reduction of selenite	15.0
Hydrolysis of casein	7.42
Hydrolysis of aesculin	7.9
Production of H_2S	0.94
DNase	3.4
Colony diameter	12.7
Acid from glycerol	35.0
Acid from erythritol	10.0
Acid from D (+) arabinose	15.0
Acid from L (+) arabinose	10.0
Acid from ribose	50.0
Acid from D (+) xylose	10.0
Acid from L (-) xylose	5.0
Acid from adonitol	5.0
Acid from methyl xyloside	0.0
Acid from D (+) glucose	0.0

Table A.II.a continued.

Character	Difference (%)
Acid from D (-) levulose	5.0
Acid from D (+) mannose	0.0
Acid from L (-) sorbose	10.0
Acid from rhamnose	10.0
Acid from dulcitol	10.0
Acid from meso-inositol	10.0
Acid from mannitol	15.0
Acid from sorbitol	15.0
Acid from methyl-D-mannoside	20.0
Acid from methyl-D-glucoside	15.0
Acid from N-acetyl glucosamine	0.0
Acid from amygdalin	20.0
Acid from arbutin	5.0
Hydrolysis of aesculin (API)	5.0
Acid from salicin	0.0
Acid from D (+) cellobiose	5.0
Acid from maltose	0.0
Acid from lactose	0.0
Acid from D (+) melibiose	0.0
Acid from sucrose	0.0
Acid from D (-) trehalose	10.0
Acid from inulin	15.0
Acid from D (+) melezitose	25.0
Acid from D (+) raffinose	10.0

Table A.II.a continued.

Character	Difference (%)
Acid from dextrin	10.0
Acid from amylose	5.0
Acid from starch	15.0
Acid from glycogen	5.0
Methyl red (API)	10.0
DNase (API)	10.0
Mucate	10.0
Gluconate	40.0
Lipase	35.0
Tetrathionate reduction	60.0
Pectate	0.0
Christensen citrate	0.0
Malonate	0.0
Acetate	0.0
Sensitive to penicillin G	5.0
Sensitive to sulphafuroxazole	15.0
Sensitive to ampicillin	0.0
Sensitive to cloxacillin	70.0
Sensitive to erythromycin	5.0
Sensitive to methicillin	60.0
Sensitive to novobiocin	0.0
Sensitive to oleandomycin	0.0
Sensitive to furazolidone	5.0
Sensitive to carbenicillin	10.0

Table A.II.a continued.

Character	Difference (%)
Sensitive to colistin sulphate	50.0
Sensitive to gentamicin	25.0
Sensitive to kanamycin	45.0
Sensitive to nalidixic avid	10.0
Sensitive to nitrofurantoin	0.0
Sensitive to polymixin B	25.0
Sensitive to tetracycline	5.0
Sensitive to cephaloridine	20.0
Sensitive to chloramphenicol	0.0
Sensitive to chlortetracycline	0.0
Sensitive to neomycin	45.0
Sensitive to oxytetracycline	0.0
Sensitive to streptomycin	50.0
Sensitive to sulphamethoxazole and trimethoprim	35.0
Alkaline phosphatase	0.0
Esterase (C4)	0.0
Esterase or lipase (C8)	0.0
Lipase (C14)	0.0
Leucine arylamidase	5.0
Valine arylamidase	0.0
Cysteine arylamidase	0.0
Trypsin	0.0
Chymotrypsin	0.0
Acid phosphatase	0.0
Phosphoamidase	0.0

Table A.II.a continued.

Character	Difference (%)
α-galactosidase	5.0
β-galactosidase	5.0
β-glucuronidase	0.0
α-glucosidase	0.0
β-glucosidase	10.0
N-acetyl-β-glucosaminidase	5.0
α-mannosidase	0.0
α-fucosidase	0.0

Table A.II.b List of characters not used in the taxonomy.

Cell arrangement

Sensitive to cloxacillin

Sensitive to methicillin

Sensitive to colistin sulphate

Sensitive to gentamicin

Sensitive to kanamycin

Sensitive to polymixin B

Sensitive to cephaloridine

Sensitive to neomycin

Sensitive to streptomycin

Sensitive to sulphamethoxazole and trimethoprim

Catalase from haemin

Appendix III

Test results appendix

Table A.III.a List of test results

Table A.III.a Test results.

Characters (See Table 2.10.a).															OTU
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 1
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 2
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 3
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 4
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 79
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB126
1	1	0	1	1	0	0	0	0	0	1	1	0	0	1	PB128
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 77
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB129
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 85
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 5
1	1	0	1	1	0	0	0	0	0	1	1	0	0	1	PB 6
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 10
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 86
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 97
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 87
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 16
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB125
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB140
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 14
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 15
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 17
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 88
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 69
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 18
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 20
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 25
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 29
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 22
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 24
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 28
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 19
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 21
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 23
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 27
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 31
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 33
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 32
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 34
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 30
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 26
1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	PB 53
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB152
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB153
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB154
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB156
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB109
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB110
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB143
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB169

Table A.III.a continued

Characters															OTU
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 7
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 80
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 81
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 78
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 11
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 13
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 12
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 83
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 9
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB175
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB176
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB189
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 92
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 90
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB100
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 51
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 52
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 59
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB 82
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 8
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 58
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 76
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB198
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB202
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB190
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB200
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB171
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB172
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB173
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB174
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 48
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 93
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 94
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 95
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 96
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB197
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB130
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB131
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB193
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB194
1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	PB196
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB195
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB107
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB148
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB149
1	1	0	1	1	0	0	0	0	0	0	1	0	0	0	PB201
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 35
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 36
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 37
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB 38

Table A.III.a continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 39
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 84
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB124
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB151
1	1	0	1	1	0	0	0	1	0	0	0	0	1	1	PB 49
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 71
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB 43
1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	PB170
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 46
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 89
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB101
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB 90
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 91
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB 98
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB150
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 67
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 68
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB146
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB145
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB102
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB115
1	1	0	1	1	0	0	0	1	0	0	0	1	0	1	PB123
1	1	0	1	1	0	0	0	1	0	0	1	0	0	1	PB 47
1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	PB157
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB177
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB178
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB179
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB187
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB188
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB180
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB182
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB190
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB181
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB185
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB186
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB183
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB184
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB 56
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 57
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB144
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 60
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB 70
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 66
1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	PB161
1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	PB165
1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	PB162
1	1	0	1	1	0	0	0	1	0	0	0	0	1	1	PB163
1	1	0	1	1	0	0	0	1	0	1	0	1	0	0	PB164
1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	PB166
1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	PB167

Table A.II.a continued

Characters															OTU
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB168
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB 40
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB 72
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB 75
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB 73
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB 74
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 54
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 55
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 61
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 62
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 64
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 65
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 41
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB 63
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 44
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB103
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB104
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB132
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB120
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB121
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB105
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB155
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB 45
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB142
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB127
1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	PB139
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB141
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB133
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB134
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB137
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB138
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB147
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB135
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB136
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB 42
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB122
1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	PB191
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB 50
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB118
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB119
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB106
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB117
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB116
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB158
1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	PB159
1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	PB160
1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	PB192
1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	PB108
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB111
1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	PB113
1	1	0	1	1	0	0	0	1	0	0	1	0	0	0	PB112
1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	PB114

Table A.III.a continued

Characters															OTU
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	PB 1
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 2
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 3
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 4
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 79
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB126
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB128
1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	PB 77
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB129
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 85
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB 5
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 6
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 10
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 86
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 97
1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	PB 87
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 16
0	1	0	1	1	1	1	1	1	1	0	1	0	1	1	PB125
1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	PB140
1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	PB 14
1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	PB 15
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 17
1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	PB 88
1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	PB 69
1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	PB 18
1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	PB 20
1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	PB 25
1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	PB 29
1	1	1	1	1	1	1	1	0	1	0	1	0	1	0	PB 22
1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	PB 24
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB 28
1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	PB 19
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 21
1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	PB 23
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 27
1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	PB 31
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB 33
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB 32
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 34
1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	PB 30
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 26
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB 53
0	1	1	1	1	0	1	1	0	0	0	0	1	0	1	PB152
1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	PB153
1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	PB154
1	1	0	0	0	0	1	1	0	0	0	0	1	0	1	PB156
0	1	0	1	1	1	1	1	1	1	0	1	0	1	1	PB109
0	1	0	1	1	1	1	1	1	1	0	1	0	0	1	PB110
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB143
0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	PB169

Table A.III.a continued

Characters															OTU
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 7
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 80
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 81
0	1	1	1	1	0	0	1	0	0	0	0	0	1	0	PB 78
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 11
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 13
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 12
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 83
0	1	1	1	0	0	1	1	0	0	0	0	0	1	0	PB 9
0	1	1	0	0	0	0	1	1	1	0	0	0	1	0	PB175
0	1	0	0	0	0	1	1	1	1	0	0	0	1	0	PB176
0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	PB189
1	1	1	1	1	0	1	1	0	0	0	0	0	0	1	PB 92
0	1	1	1	1	0	1	1	1	1	0	0	0	0	1	PB 99
0	1	1	1	1	0	1	1	1	0	0	0	0	0	0	PB100
1	1	1	1	1	0	1	1	0	0	0	1	0	1	0	PB 51
0	1	1	1	1	0	1	1	0	0	0	1	1	1	0	PB 52
0	1	1	1	1	0	1	1	1	0	0	0	1	1	0	PB 59
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 82
0	1	1	1	1	0	1	1	1	0	0	0	0	1	0	PB 8
0	1	1	0	0	0	1	1	1	0	0	0	1	1	1	PB 58
0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	PB 76
1	1	0	0	0	0	1	1	1	0	0	0	0	0	0	PB198
0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	PB202
1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	PB199
1	1	0	1	1	0	1	1	1	0	0	0	0	0	0	PB200
0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	PB171
0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	PB172
0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	PB173
0	1	1	0	0	0	0	1	1	1	0	0	0	1	0	PB174
0	1	0	1	1	0	1	1	0	0	0	0	1	1	0	PB 48
1	1	0	1	1	0	1	1	0	0	0	0	0	1	1	PB 93
1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	PB 94
1	1	0	1	0	0	1	1	0	0	0	0	0	0	1	PB 95
1	1	0	1	0	0	1	1	0	0	0	0	0	0	1	PB 96
1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	PB197
1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	PB130
1	1	0	1	1	0	1	1	1	0	0	0	0	1	1	PB131
1	1	0	1	1	0	1	1	0	0	0	0	0	0	1	PB193
1	1	0	1	1	0	1	1	0	0	0	0	0	0	1	PB194
1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	PB196
1	1	0	1	1	0	1	1	0	0	0	0	0	0	1	PB195
1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	PB107
1	1	1	1	0	0	0	1	1	0	0	0	0	0	1	PB148
1	1	1	0	0	0	0	1	0	0	0	0	0	0	1	PB149
0	1	1	0	0	0	1	1	1	1	0	0	0	0	0	PB201
0	1	1	1	0	0	1	1	1	0	0	0	0	1	0	PB 35
0	1	1	1	0	0	1	1	1	0	0	0	0	1	0	PB 36
0	1	1	1	0	0	1	1	1	0	0	0	0	1	0	PB 37
0	1	1	1	0	0	1	1	1	0	0	0	0	1	0	PB 38

Table A.III.a continued

Characters														OTU
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
0	1	1	1	0	0	0	1	1	0	0	0	0	1	0	PB 39
0	1	1	1	1	0	1	1	1	0	0	0	0	1	0	PB 84
1	1	0	1	0	0	1	1	0	0	0	0	0	0	1	PB124
1	1	1	1	1	0	1	1	1	0	0	0	1	1	1	PB151
1	1	1	1	1	0	1	1	1	0	0	0	0	1	1	PB 49
1	1	0	1	0	0	1	1	0	0	0	0	1	1	0	PB 71
1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	PB 43
0	1	1	0	0	0	0	1	0	0	0	0	0	1	1	PB170
0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	PB 46
0	1	1	1	0	0	0	1	1	0	0	0	0	0	1	PB 89
0	1	1	1	0	0	0	1	0	0	0	0	1	0	1	PB101
1	1	1	1	1	0	1	1	0	0	0	0	0	0	1	PB 90
1	1	1	1	1	0	1	1	0	0	0	0	0	0	1	PB 91
0	1	1	1	0	0	0	1	0	0	0	0	0	0	1	PB 98
0	0	1	1	0	0	0	1	0	0	0	0	0	0	1	PB150
0	1	1	1	0	0	0	1	1	0	0	0	0	1	0	PB 67
0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	PB 68
0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	PB146
0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	PB145
0	1	1	1	0	0	1	1	0	0	1	0	0	0	1	PB102
0	1	1	1	0	0	0	1	0	0	0	0	0	0	1	PB115
0	1	1	1	0	0	0	1	1	1	0	0	0	0	1	PB123
1	1	0	1	1	0	1	1	1	0	0	0	1	1	0	PB 47
1	1	0	1	1	0	1	1	1	1	0	0	0	1	1	PB157
0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	PB177
0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	PB178
0	0	0	0	0	0	1	1	1	1	0	0	1	1	0	PB179
1	1	1	0	0	0	0	1	1	1	0	0	0	1	0	PB187
1	1	1	0	0	0	0	1	1	1	0	0	0	1	0	PB188
0	0	1	0	0	0	1	1	1	1	0	0	0	1	0	PB180
0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	PB182
0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	PB190
0	0	1	0	0	0	1	1	1	1	0	0	0	1	0	PB181
0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	PB185
0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	PB186
0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	PB183
0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	PB184
0	1	1	1	1	0	1	1	1	1	0	0	1	0	1	PB 56
0	1	0	0	0	0	1	1	1	0	0	0	1	1	1	PB 57
0	1	0	1	1	0	1	1	1	0	0	0	0	1	0	PB144
0	0	1	1	0	0	1	1	1	1	0	0	0	1	1	PB 60
0	0	0	1	0	0	0	0	1	1	1	0	1	1	0	PB 70
0	1	1	1	0	0	0	1	1	1	0	0	0	1	0	PB 66
1	1	0	0	0	0	0	1	0	1	0	0	0	1	1	PB161
0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	PB165
1	1	0	1	0	0	0	1	0	1	0	0	0	1	1	PB162
1	1	0	0	0	0	0	1	1	1	0	0	0	1	1	PB163
0	1	0	1	0	0	1	1	1	1	0	0	1	1	1	PB164
0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	PB166
0	1	0	0	0	0	0	0	1	1	0	0	1	1	1	PB167

Table A.III.a continued

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
0	1	1	1	1	1	0	1	1	0	0	0	0	1	0	PB168
0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	PB 40
0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	PB 72
0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	PB 75
0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	PB 73
0	1	1	1	1	0	1	1	1	1	0	0	0	0	0	PB 74
0	1	0	1	0	0	1	1	1	1	0	0	0	1	0	PB 54
0	1	0	1	0	0	1	1	1	1	0	0	0	1	0	PB 55
0	1	0	1	0	0	1	1	1	1	0	0	0	1	0	PB 61
0	1	0	1	0	0	1	1	1	1	0	0	0	1	0	PB 62
0	1	0	1	0	0	1	1	1	1	0	0	0	0	0	PB 64
0	1	0	1	0	0	1	1	1	1	0	0	0	1	0	PB 65
0	1	0	1	0	0	1	1	0	0	0	0	1	1	0	PB 41
0	1	0	1	0	0	1	1	0	0	0	0	1	1	1	PB 63
0	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 44
1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	PB103
0	1	0	1	1	0	1	1	0	0	0	0	1	1	0	PB104
0	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB132
0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	PB120
0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	PB121
1	1	0	1	1	0	1	1	0	0	0	0	1	1	0	PB105
1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	PB155
0	1	1	1	1	0	0	1	1	0	0	0	1	1	0	PB 45
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB142
0	1	0	1	1	0	1	1	0	0	0	0	0	0	0	PB127
0	1	0	1	1	0	1	1	0	0	0	0	0	0	0	pB139
0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	pB141
0	1	1	1	1	0	0	1	0	0	0	0	0	1	1	PB133
0	1	1	1	1	0	0	1	0	0	0	0	0	1	1	PB134
0	1	1	1	1	0	1	1	0	0	0	0	0	1	1	PB137
0	1	1	1	1	0	1	1	0	0	0	0	0	0	1	PB138
0	1	1	1	1	0	0	1	0	0	0	0	0	0	1	PB147
0	1	1	1	1	0	0	1	1	0	0	0	0	1	1	PB135
0	1	1	1	1	0	0	1	0	0	0	0	0	1	1	PB136
0	0	0	0	0	0	1	1	0	0	0	0	1	1	1	PB 42
0	1	0	1	1	0	1	1	0	0	0	0	0	1	1	PB122
1	0	0	0	0	0	1	1	1	0	0	0	0	1	0	PB191
1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	PB 50
1	1	0	1	1	0	1	1	1	1	0	0	0	0	0	PB118
1	1	0	1	1	0	1	1	1	1	0	0	0	0	0	PB119
0	1	0	1	1	0	1	1	1	0	0	0	0	1	0	PB106
0	1	0	1	0	0	1	1	1	0	0	0	0	1	0	PB117
0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	PB116
1	1	1	1	1	0	1	1	1	0	0	0	0	1	1	PB158
1	1	0	0	0	0	1	1	0	1	0	0	0	1	1	PB159
1	1	0	0	0	0	0	1	1	1	0	0	0	1	1	PB160
0	0	1	1	1	0	1	1	1	1	0	0	0	0	0	PB192
1	1	0	1	0	0	0	1	1	1	0	0	0	0	0	PB108
1	1	1	1	1	0	0	1	1	1	0	0	0	1	0	PB111
0	1	1	1	1	1	1	1	1	1	0	0	0	0	1	PB113
0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	PB112
1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	PB114

: 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Table A.III.a continued

Characters														OTU
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
1	1	1	1	1	0	1	1	0	0	0	1	1	1	0	PB 1
1	1	1	1	1	0	1	1	0	0	0	1	1	1	0	PB 2
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB 3
1	1	1	1	1	0	1	1	0	0	0	1	1	1	0	PB 4
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB 79
1	1	1	1	1	0	1	1	0	0	0	1	1	1	0	PB126
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB128
1	1	1	1	1	0	1	1	0	0	0	1	1	1	0	PB 77
1	0	1	1	0	0	1	1	0	0	0	0	1	1	0	PB129
1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	PB 85
0	0	0	1	0	0	1	1	0	0	0	0	1	1	0	PB 5
0	0	0	1	1	0	1	1	0	0	0	0	1	1	0	PB 6
1	1	1	1	1	1	1	0	0	0	0	0	1	1	0	PB 10
1	1	1	1	1	1	1	1	0	0	0	0	1	1	0	PB 86
1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	PB 97
1	0	0	1	1	1	1	1	0	0	0	0	1	1	1	PB 87
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 16
0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	PB125
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB140
1	1	0	1	1	1	1	1	0	0	0	0	0	1	0	PB 14
1	1	0	1	1	1	1	1	0	0	0	0	0	1	0	PB 15
1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	PB 17
1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	PB 88
1	1	1	1	1	0	1	0	0	0	0	1	1	1	0	PB 69
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 18
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 20
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 25
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 29
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 22
1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 24
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 28
1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 19
1	1	0	1	1	0	1	1	0	0	0	1	0	1	0	PB 21
1	1	0	1	1	0	1	1	0	0	0	1	0	1	0	PB 23
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 27
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 31
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 33
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 32
1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	PB 34
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 30
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB 26
0	0	0	1	0	0	1	1	0	0	0	0	1	1	0	PB 53
0	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB152
1	1	1	1	1	0	1	1	0	0	0	0	1	1	0	PB153
1	1	0	1	1	0	1	1	0	0	0	0	1	1	0	PB154
1	1	1	1	1	0	1	1	0	0	0	1	1	0	0	PB156
1	1	0	1	1	1	1	1	0	0	0	1	0	1	0	PB109
1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	PB110
1	1	0	1	1	0	1	0	0	0	0	0	0	1	0	PB143
1	1	0	0	1	0	1	1	0	0	0	0	1	1	1	PB169

Table A.III.a continued

Characters															OTU
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
1	1	0	0	1	1	1	1	0	0	0	0	0	1	0	PB 7
0	1	0	0	1	1	1	1	0	0	0	0	0	1	0	PB 80
1	1	0	0	1	1	1	1	0	0	0	0	0	1	0	PB 81
1	1	0	0	1	1	1	1	0	0	0	0	0	1	0	PB 78
0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	PB 11
0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	PB 13
0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	PB 12
0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	PB 83
1	1	0	1	1	1	1	0	0	0	0	0	0	1	0	PB 9
0	1	0	1	1	1	1	1	0	0	0	0	0	1	0	PB175
1	1	0	1	1	1	1	1	0	0	0	0	0	1	0	PB176
0	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB189
0	0	0	1	1	1	1	1	0	0	0	0	1	1	0	PB 92
1	1	0	1	1	1	1	1	0	0	0	0	0	1	0	PB 99
1	1	0	1	1	1	1	0	0	0	0	0	0	1	0	PB100
0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	PB 51
0	0	0	0	0	1	1	1	0	0	0	0	1	1	0	PB 52
0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	PB 59
0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	PB 82
1	1	0	0	1	1	1	1	0	0	0	0	0	1	0	PB 8
0	1	1	0	1	0	1	1	0	0	0	0	0	1	0	PB 58
0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	PB 76
0	0	0	1	1	0	1	0	0	0	0	1	0	1	0	PB198
1	1	0	1	1	1	1	0	0	0	0	0	0	1	0	PB202
1	0	0	1	1	1	1	0	0	0	0	0	0	1	0	PB199
0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	PB200
0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	PB171
1	1	0	1	1	0	1	1	0	0	0	0	0	1	0	PB172
1	0	0	0	1	0	1	1	0	0	0	0	0	1	0	PB173
0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	PB174
1	1	1	1	1	0	1	0	0	0	0	0	1	1	0	PB 48
1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	PB 93
1	1	1	1	1	1	1	0	0	0	0	0	1	1	0	PB 94
1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	PB 95
1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	PB 96
1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	PB197
1	0	0	1	1	0	1	0	0	0	0	1	1	1	0	PB130
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB131
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB193
1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	PB194
1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	PB196
1	1	1	1	1	1	1	1	0	0	0	1	0	1	0	PB195
0	1	0	1	1	0	1	1	0	0	0	1	1	1	0	PB107
1	1	0	1	1	0	1	1	0	0	0	1	1	1	0	PB148
0	0	0	0	1	1	1	1	0	0	0	1	1	1	0	PB149
0	0	1	0	1	1	1	1	0	0	0	0	0	1	0	PB201
1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	PB 35
1	1	0	1	0	1	1	1	0	0	0	0	0	1	0	PB 36
1	1	0	1	0	1	1	0	0	0	0	0	0	1	0	PB 37
1	1	0	1	0	1	1	0	0	0	0	0	1	0	0	PB 38

Table A.III.a continued

Characters															OTU
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	PB 39
1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	PB 84
1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	PB124
0	1	1	1	1	1	1	0	0	0	0	0	1	1	0	PB151
0	1	0	1	1	0	1	0	0	0	0	0	1	0	0	PB 49
1	1	0	1	1	0	1	0	0	0	0	0	1	0	0	PB 71
0	1	1	0	1	0	1	1	0	0	0	0	1	0	1	PB 43
0	1	0	1	1	1	1	0	0	0	0	0	1	0	0	PB170
1	1	0	0	1	0	1	0	0	0	0	0	1	0	0	PB 46
0	1	0	1	1	1	1	0	0	0	0	1	1	0	0	PB 89
0	1	0	1	1	1	1	0	0	0	0	1	1	0	0	PB101
0	1	0	1	1	1	1	0	0	0	0	0	1	0	0	PB 90
0	1	0	1	1	1	1	0	0	0	0	0	1	0	0	PB 91
0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	PB 98
0	0	0	0	1	1	1	0	0	0	0	1	1	0	0	PB150
0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	PB 67
0	0	0	1	1	0	1	0	0	0	0	0	1	0	1	PB 68
0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	PB146
0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	PB145
0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	PB102
0	1	0	0	1	1	1	1	0	0	0	0	1	0	0	PB115
0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	PB123
0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	PB 47
0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	PB157
0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	PB177
0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	PB178
0	1	0	1	1	0	1	0	0	0	0	0	1	1	0	PB179
0	1	0	1	1	1	1	0	0	0	0	0	1	0	0	PB187
0	1	0	1	1	0	1	0	0	0	0	0	1	0	0	PB188
0	1	0	1	1	1	1	1	0	0	0	0	0	0	1	PB180
0	1	0	1	1	0	1	1	0	0	0	0	0	0	1	PB182
0	1	0	1	1	0	1	1	0	0	0	1	0	0	1	PB190
0	1	0	1	1	0	1	1	0	0	0	0	0	0	1	PB181
0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	PB185
0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	PB186
0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	PB183
0	1	0	0	1	0	1	0	0	0	0	0	1	0	1	PB184
0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	PB 56
0	1	0	1	1	0	1	1	0	0	0	0	1	1	0	PB 57
0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	PB144
0	1	1	0	1	1	1	0	0	0	0	0	1	0	0	PB 60
0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	PB 70
0	0	0	0	1	0	1	1	0	0	0	0	1	0	1	PB 66
0	1	0	1	1	0	1	1	0	0	0	0	0	0	0	PB161
0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	PB165
0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	PB162
0	1	0	1	1	0	1	0	0	0	0	1	0	0	0	PB163
1	1	0	1	1	0	1	0	0	0	0	0	0	1	0	PB164
0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	PB166
0	1	0	1	1	0	1	0	0	0	0	0	0	1	0	PB167

Table A.III.a continued

31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	OTU
0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	PB168
0	1	0	1	0	0	1	0	0	0	0	1	1	0	1	PB 40
0	1	0	1	0	0	1	0	0	0	0	1	1	0	1	PB 72
0	1	0	1	0	0	1	0	0	0	0	1	1	0	1	PB 75
0	1	0	1	1	1	1	0	0	0	0	1	1	0	1	PB 73
0	1	0	0	1	1	1	1	0	0	0	1	1	0	1	PB 74
0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	PB 54
0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	PB 55
0	1	0	1	0	0	1	0	0	0	0	0	1	1	0	PB 61
0	1	0	1	1	1	1	0	0	0	0	0	1	1	0	PB 62
0	1	0	1	0	0	1	0	0	0	0	1	1	1	1	PB 64
0	1	0	1	0	0	1	0	0	0	0	0	1	0	1	PB 65
0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	PB 41
0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	PB 63
1	1	0	1	1	0	1	0	0	0	0	0	1	0	1	PB 44
1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	PB103
1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	PB104
1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	PB132
1	1	1	0	0	0	1	1	0	0	0	0	1	0	0	PB120
1	1	1	0	1	1	1	0	0	0	0	0	1	1	0	PB121
1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	PB105
1	1	1	1	1	0	1	0	0	0	0	1	1	0	0	PB155
0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	PB 45
1	1	0	1	1	0	1	1	0	0	0	1	1	0	0	PB142
1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	PB127
1	1	1	1	1	1	1	1	0	0	0	1	1	0	0	PB139
0	1	1	1	0	1	1	0	0	0	0	1	1	0	0	PB141
0	1	0	1	1	0	1	0	0	0	0	1	1	1	0	PB133
1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	PB134
1	1	0	1	1	1	1	0	0	0	0	1	1	0	0	PB137
1	1	1	1	1	0	1	0	0	0	0	1	1	0	0	PB138
1	0	0	1	1	0	1	0	0	0	0	1	1	1	0	PB147
1	1	0	1	1	1	1	0	0	0	0	1	1	0	0	PB135
1	0	1	1	1	0	1	0	0	0	0	1	1	0	0	PB136
0	1	1	0	1	0	0	0	0	0	0	0	1	1	0	PB 42
1	1	1	0	1	0	1	1	0	0	0	1	1	1	0	PB122
1	0	0	0	1	0	1	1	0	0	0	0	1	0	1	PB191
1	1	0	0	1	0	1	1	0	0	0	1	1	1	0	PB 50
1	1	0	0	1	0	1	1	0	0	0	1	1	1	0	PB118
1	1	0	0	1	0	1	1	0	0	0	1	1	1	0	PB119
1	0	0	1	1	0	1	0	0	0	0	1	1	0	0	PB106
1	1	0	1	1	0	1	0	0	0	0	1	1	0	0	PB117
1	1	0	1	1	0	1	0	0	0	0	1	1	1	0	PB116
1	1	0	0	1	0	1	1	0	0	0	0	1	1	0	PB158
0	1	0	0	1	0	1	0	0	0	0	1	1	1	0	PB159
0	1	0	0	1	0	1	0	0	0	0	1	1	1	0	PB160
1	1	1	0	0	0	1	0	0	0	0	0	1	1	1	PB192
0	1	0	0	1	1	1	1	0	0	0	0	1	1	0	PB108
1	1	0	0	1	1	1	1	0	0	0	0	1	1	0	PB111
1	1	0	0	1	0	1	0	0	0	0	0	1	0	0	PB113
1	1	0	1	1	1	1	1	0	0	0	1	0	1	0	PB112
1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	PB114

Table A.III.a continued

Characters															OTU
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
0	1	1	1	1	0	0	1	1	0	0	0	0	1	0	PB 1
0	1	1	1	1	0	0	1	1	0	0	0	0	1	0	PB 2
0	1	1	1	1	1	0	1	1	0	0	0	0	1	0	PB 3
0	1	1	1	1	1	0	1	1	0	0	0	0	1	0	PB 4
0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	PB 79
0	1	1	1	1	0	0	1	1	0	0	0	0	1	0	PB126
0	1	1	1	1	1	0	0	1	0	0	0	0	1	0	PB128
0	1	1	1	0	0	0	1	1	0	0	0	0	1	0	PB 77
0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	PB129
0	1	1	1	1	1	1	0	1	1	0	0	0	0	1	PB 85
0	1	1	1	1	0	0	1	0	0	0	0	0	1	0	PB 5
0	1	1	1	1	0	0	1	0	1	0	0	0	1	0	PB 6
0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	PB 10
0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	PB 86
0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	PB 97
0	1	1	1	1	0	0	1	0	1	0	0	0	1	0	PB 87
0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	PB 16
0	1	1	1	1	0	0	1	0	1	0	0	0	1	0	PB125
0	1	1	1	1	0	0	1	0	0	0	0	1	1	0	PB140
0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	PB 14
0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	PB 15
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 17
0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	PB 88
0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	PB 69
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 18
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 20
0	1	0	1	1	0	0	1	1	1	0	0	0	0	0	PB 25
0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	PB 29
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 22
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 24
0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	PB 28
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 19
0	1	0	1	1	0	0	1	1	1	0	0	0	0	0	PB 21
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 23
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 27
0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	PB 31
0	1	0	1	1	0	0	1	1	1	0	0	0	0	0	PB 33
0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	PB 32
0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	PB 34
0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	PB 30
0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	PB 26
0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	PB 53
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB152
0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	PB153
0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	PB154
0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	PB156
0	1	1	1	0	0	0	1	1	1	0	0	0	1	0	PB109
0	1	1	1	0	0	0	1	1	1	0	0	0	1	0	PB110
0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	PB143
0	1	1	1	1	0	0	1	1	0	0	0	0	1	0	PB169

Table A.III.a continued

Characters															OTU
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	PB 7
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 80
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 81
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 78
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 11
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 13
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 12
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 83
0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	PB 9
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	PB175
0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	PB176
0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	PB189
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 92
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB 99
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB100
0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	PB 51
0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	PB 52
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 59
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 82
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 8
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 58
0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	PB 76
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB198
0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	PB202
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB199
0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	PB200
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB171
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB172
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB173
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	PB174
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 48
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 93
0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	PB 94
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 95
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 96
0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	PB197
0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	PB130
0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	PB131
0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	PB193
0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	PB194
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB196
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB195
0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	PB107
0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	PB148
0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	PB149
0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	PB201
0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	PB 35
0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	PB 36
0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	PB 37
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 38

Table A.III.a continued

Characters														OTU
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB 39
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 84
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB124
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB151
0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	PB 49
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 71
0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	PB 43
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB170
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 46
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 89
0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	PB101
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 90
0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	PB 91
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 98
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB150
0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	PB 67
0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	PB 68
0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	PB146
0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	PB145
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB102
0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	PB115
0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	PB123
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB 47
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB157
0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	PB177
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB178
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB179
0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	PB187
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB188
0	0	0	0	0	0	1	0	1	1	0	1	0	1	1	PB180
0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	PB182
0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	PB190
0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	PB181
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB185
0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	PB186
0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	PB183
0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	PB184
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 56
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB 57
0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	PB144
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 60
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB 70
0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	PB 66
0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	PB161
0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	PB165
0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	PB162
0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	PB163
0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	PB164
0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	PB166
0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	PB167

Table A.III.a continued

Characters															OTU
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	PB168
0	1	1	1	0	0	0	0	1	0	0	0	0	1	0	PB 40
0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	PB 72
0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	PB 75
0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	PB 73
0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	PB 74
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 54
0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	PB 55
0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	PB 61
0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	PB 62
0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	PB 64
0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	PB 65
0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	PB 41
0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	PB 63
0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	PB 44
0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	PB103
0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	PB104
0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	PB132
0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	PB120
0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	PB121
0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	PB105
0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	PB155
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB 45
0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	PB142
0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	PB127
0	1	1	0	0	0	0	1	1	0	0	0	1	1	0	PB139
0	1	1	0	0	0	0	1	1	0	0	0	1	1	0	PB141
0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	PB133
0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	PB134
0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	PB137
0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	PB138
0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	PB147
0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	PB135
0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	PB136
0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	PB 42
0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	PB122
0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	PB191
0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	PB 50
0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	PB118
0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	PB119
0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	PB106
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	PB117
0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	PB116
0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	PB158
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PB159
0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	PB160
0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	PB192
0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	PB108
0	1	1	1	1	0	0	1	0	1	0	0	0	1	0	PB111
0	1	1	0	0	0	0	0	0	1	0	1	0	1	0	PB113
0	1	1	1	1	0	0	1	1	0	0	1	1	1	0	PB112
0	1	1	1	1	0	0	1	1	0	0	1	0	1	0	PB114

Table A.III.a continued

Characters														OTU
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	
0	0	0	0	3	0	0	0	0	0	5	5	5	5	0	PB 1
0	0	0	0	0	5	3	0	0	0	5	5	5	5	0	PB 2
0	0	0	0	3	0	4	0	0	0	5	5	5	5	0	PB 3
0	0	0	0	4	0	3	0	0	0	5	5	5	5	4	PB 4
0	0	0	0	1	0	2	0	0	0	5	5	5	5	0	PB 79
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB126
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB128
0	0	0	0	3	0	3	0	0	0	5	5	5	5	0	PB 77
0	0	0	0	0	5	0	0	0	0	5	5	5	5	0	PB129
0	0	0	0	0	3	0	3	0	0	5	5	5	5	0	PB 85
0	0	0	0	2	5	0	0	0	0	5	5	5	5	0	PB 5
0	0	0	0	3	5	0	0	0	0	5	5	5	5	0	PB 6
0	0	0	0	1	1	0	0	0	0	5	5	5	5	0	PB 10
0	0	0	0	3	0	0	0	0	0	5	5	5	5	0	PB 86
0	0	0	0	0	5	0	0	3	0	5	5	5	5	0	PB 97
0	0	0	0	2	5	1	0	0	0	5	5	5	5	0	PB 87
0	0	0	0	3	0	0	2	0	0	5	5	5	5	1	PB 16
0	0	0	3	3	0	0	0	0	0	5	5	5	5	0	PB125
0	0	1	0	3	0	2	0	0	0	5	5	5	5	0	PB140
0	0	0	0	4	5	5	4	5	0	5	5	5	5	5	PB 14
0	0	0	0	4	5	4	4	5	0	5	5	5	5	5	PB 15
0	0	0	0	3	5	5	3	5	0	5	5	5	5	5	PB 17
0	0	1	0	4	5	4	4	5	0	5	5	5	5	5	PB 88
0	0	0	0	3	0	4	4	0	0	5	5	5	5	5	PB 69
0	0	0	0	2	5	5	0	0	0	5	5	5	5	0	PB 18
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 20
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 25
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 29
0	0	1	0	3	5	5	0	0	0	5	5	5	5	0	PB 22
0	0	1	0	0	5	5	3	0	0	5	5	5	5	0	PB 24
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 28
0	0	0	0	0	5	5	2	0	0	5	5	5	5	0	PB 19
0	0	1	0	0	5	5	0	0	0	5	5	5	5	2	PB 21
0	0	0	0	0	5	5	0	0	0	5	5	5	5	0	PB 23
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 27
0	0	1	0	2	5	5	2	0	0	5	5	5	5	0	PB 31
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 33
0	0	1	0	0	5	5	4	0	0	5	5	5	5	0	PB 32
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 34
0	0	0	1	1	5	5	3	0	3	5	5	5	5	2	PB 30
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB 26
0	0	0	0	0	5	5	0	0	4	5	5	5	5	0	PB 53
0	0	0	0	0	5	5	0	0	0	5	5	5	5	0	PB152
0	0	0	1	0	5	5	0	0	0	5	5	5	5	0	PB153
0	0	0	0	2	5	5	0	0	0	5	5	5	5	0	PB154
0	0	0	0	0	5	5	0	0	0	5	5	5	5	0	PB156
0	1	1	0	3	0	5	0	0	0	5	5	5	5	0	PB109
0	0	1	0	4	5	1	0	0	0	5	5	5	5	0	PB110
0	0	1	2	5	5	5	0	0	0	5	5	5	5	0	PB143
0	0	1	4	0	5	5	0	0	0	5	5	5	5	4	PB169

Table A.III.a continued

Characters														OTU
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	
0	0	1	0	0	5	0	0	0	0	5	5	5	5	0	PB 7
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 80
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 81
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 78
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 11
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 13
0	0	1	0	0	0	0	0	0	0	4	5	5	5	0	PB 12
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 83
0	0	1	0	0	0	0	0	0	0	0	5	5	5	0	PB 9
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB175
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB176
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB189
0	0	1	0	2	0	0	0	0	0	5	5	5	5	0	PB 92
0	0	1	0	2	0	0	0	0	0	5	5	5	5	0	PB 99
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB100
0	0	0	0	0	0	0	0	0	2	5	5	5	5	0	PB 51
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 52
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 59
0	0	1	0	0	0	0	0	0	0	5	5	4	5	0	PB 82
0	0	1	0	0	0	5	0	0	0	5	5	5	5	0	PB 8
0	0	0	0	0	0	4	0	0	0	5	5	5	5	0	PB 58
0	0	0	0	0	0	1	1	0	0	5	5	5	5	0	PB 76
0	0	0	0	0	0	5	0	0	0	5	5	5	5	0	PB198
0	0	0	0	0	5	5	0	0	0	5	5	5	5	0	PB202
0	0	0	0	0	0	5	0	0	0	5	5	5	5	0	PB199
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB200
0	0	1	0	0	5	5	2	0	0	5	5	5	5	0	PB171
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB172
0	0	1	0	0	0	4	0	0	0	5	5	5	5	0	PB173
0	0	1	0	3	0	2	0	0	3	5	5	5	5	0	PB174
0	0	0	0	0	0	5	0	0	0	5	5	5	5	0	PB 48
0	0	0	0	0	0	5	0	0	0	5	5	5	5	0	PB 93
0	0	0	0	0	0	0	0	2	2	5	5	5	5	0	PB 94
0	0	0	0	0	0	5	0	4	0	5	5	5	5	0	PB 95
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 96
0	0	0	0	0	0	3	0	0	0	5	5	5	5	0	PB197
0	0	0	0	0	0	0	0	0	0	5	5	5	5	4	PB130
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB131
0	0	0	0	0	1	0	0	3	0	5	5	5	5	1	PB193
0	0	0	0	0	5	0	0	0	0	5	5	5	5	0	PB194
0	0	1	1	0	0	0	0	0	0	5	5	5	5	0	PB196
0	0	0	0	0	5	5	0	0	4	5	5	5	5	0	PB195
0	0	1	0	4	5	0	0	0	0	5	5	5	5	0	PB107
0	0	1	0	0	0	5	0	0	0	5	5	5	5	0	PB148
0	0	1	0	0	0	3	0	0	0	5	5	5	5	0	PB149
0	0	1	1	0	5	0	0	0	0	5	5	5	5	0	PB201
0	0	0	0	0	0	0	0	0	0	5	5	5	0	0	PB 35
0	0	1	2	0	0	0	?	0	0	0	5	5	5	0	PB 36
0	0	1	0	0	0	0	2	0	0	5	5	5	4	0	PB 37
0	0	1	0	0	0	0	0	0	0	0	5	5	5	0	PB 38

Table A.III.a continued

Characters														OTU
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	
0	0	1	0	0	0	0	0	0	0	0	5	5	5	3	PB 39
0	0	1	0	0	0	3	0	0	0	0	5	5	5	0	PB 84
0	0	0	0	0	0	0	0	0	0	0	5	5	5	2	PB124
0	0	0	0	0	3	0	0	0	0	5	5	5	5	0	PB151
0	0	0	0	0	0	3	0	3	0	5	5	5	5	0	PB 49
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 71
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 43
0	0	1	0	2	2	2	0	0	0	5	5	5	5	0	PB170
0	0	0	2	0	0	0	0	0	0	5	5	5	5	2	PB 46
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 89
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB101
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 90
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 91
0	0	1	0	0	0	2	0	2	2	5	5	5	5	0	PB 98
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB150
0	0	0	0	0	0	0	0	0	0	5	5	5	5	5	PB 67
0	0	0	3	0	0	0	0	0	0	5	5	5	5	0	PB 68
0	0	0	3	0	4	0	0	0	0	5	5	5	5	0	PB146
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB145
0	0	1	0	0	0	0	0	0	0	4	5	5	5	0	PB102
1	1	1	0	1	0	0	0	0	0	5	5	5	5	0	PB115
0	1	1	0	0	0	0	0	0	0	5	5	5	5	0	PB123
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 47
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB157
1	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB177
1	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB178
1	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB179
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB187
1	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB188
1	0	0	0	3	0	0	0	0	0	5	5	5	5	0	PB180
1	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB182
1	0	0	0	0	1	0	0	0	0	5	5	5	5	0	PB190
1	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB181
1	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB185
1	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB186
1	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB183
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB184
0	0	0	0	0	1	0	0	0	0	5	5	5	5	0	PB 56
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 57
1	0	0	0	1	0	0	0	0	0	5	5	5	5	0	PB144
0	0	0	0	0	0	3	0	0	0	5	5	5	5	0	PB 60
0	0	0	0	0	1	1	0	0	0	5	5	5	5	0	PB 70
0	0	0	0	0	0	0	3	3	0	5	5	5	5	0	PB 66
1	0	1	0	0	0	0	0	0	0	0	5	5	5	0	PB161
0	0	0	0	0	4	0	0	0	0	5	5	1	2	2	PB165
0	0	0	0	0	5	0	0	0	0	5	5	5	5	0	PB162
0	0	0	0	2	5	2	0	0	0	5	5	5	5	2	PB163
0	0	0	0	0	5	0	0	0	0	5	5	5	5	0	PB164
0	0	0	0	0	0	3	0	0	0	5	5	5	5	0	PB166
0	0	0	1	0	5	0	0	0	0	5	5	5	5	0	PB167

Table A.III.a continued

Characters															OTU
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	
1	0	1	0	0	4	0	0	0	0	5	5	0	0	0	PB168
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 40
0	0	0	2	2	2	0	0	0	3	5	5	5	5	0	PB 72
0	1	0	0	3	0	0	0	0	0	5	5	5	5	5	PB 75
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 73
0	1	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 74
0	1	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 54
0	1	0	0	0	0	0	3	0	0	5	5	5	5	0	PB 55
0	1	0	0	0	0	1	1	1	0	5	5	5	5	0	PB 61
0	1	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 62
0	1	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 64
0	1	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 65
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 41
0	0	0	0	0	0	2	0	0	0	5	5	5	5	0	PB 63
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 44
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB103
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB104
0	0	0	0	0	2	3	0	0	0	5	5	5	5	2	PB132
0	0	1	0	0	0	0	0	0	2	5	5	5	5	0	PB120
0	1	1	0	0	0	0	0	0	0	5	5	5	5	0	PB121
0	1	0	0	0	1	0	0	0	0	5	5	5	5	0	PB105
0	0	0	0	2	0	0	0	0	0	5	5	5	5	0	PB155
0	0	0	0	0	0	0	0	0	0	5	5	5	5	0	PB 45
0	1	1	0	1	0	0	0	0	0	5	5	5	5	0	PB142
0	1	0	3	4	3	4	4	0	0	5	5	5	5	0	PB127
0	0	1	3	3	0	0	0	0	1	5	5	5	5	0	PB139
0	0	1	4	4	4	3	4	0	0	5	5	5	5	0	PB141
0	1	1	0	0	0	0	0	0	0	5	5	5	5	0	PB133
0	0	1	0	3	2	1	1	0	0	5	5	5	5	0	PB134
0	0	1	2	2	2	0	0	0	0	5	5	5	5	0	PB137
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB138
0	1	1	0	3	3	1	1	0	0	5	5	5	5	0	PB147
0	1	1	0	2	2	2	2	0	0	5	5	5	5	0	PB135
0	1	0	0	2	2	1	1	0	0	5	5	5	5	0	PB136
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 42
0	1	1	0	1	0	4	0	0	0	5	5	5	5	0	PB122
0	1	1	0	0	0	0	0	0	0	5	5	5	5	0	PB191
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB 50
0	0	1	0	2	0	0	0	0	0	5	5	5	5	0	PB118
0	0	1	0	0	0	5	0	0	0	5	5	5	5	0	PB119
0	1	1	0	0	0	0	0	0	0	5	5	5	5	0	PB106
0	1	1	0	0	0	0	0	0	0	5	5	5	5	0	PB117
0	0	1	0	0	5	0	0	0	0	5	5	5	5	0	PB116
1	1	0	2	2	2	4	0	0	0	5	5	5	5	0	PB158
0	0	1	0	0	2	0	0	0	0	5	5	5	5	0	PB159
1	1	1	0	0	0	0	0	0	0	5	5	5	5	0	PB160
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB192
0	0	1	0	0	5	0	0	0	0	5	5	5	5	0	PB108
0	0	1	0	0	5	5	0	0	0	5	5	5	5	0	PB111
0	0	0	0	0	5	2	0	0	0	5	5	5	5	0	PB113
0	0	1	0	0	0	0	0	0	0	5	5	5	5	0	PB112
0	0	1	3	3	0	0	0	0	0	5	5	5	5	0	PB114

Table A.III.a continued

Characters															OTU
76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	
0	0	3	5	5	1	5	5	5	5	5	5	5	0	5	PB 1
4	0	4	5	5	5	5	5	5	5	5	5	5	0	5	PB 2
5	0	5	5	5	1	5	5	5	5	5	5	5	0	5	PB 3
5	0	5	5	5	1	5	5	5	5	5	5	5	0	5	PB 4
0	0	5	5	5	1	5	5	5	5	5	5	5	0	5	PB 79
0	0	0	5	5	2	5	5	5	5	5	5	5	0	5	PB126
5	0	5	5	5	0	5	5	5	5	5	5	5	0	5	PB128
3	0	0	5	5	1	5	5	5	5	5	5	5	0	0	PB 77
0	0	0	5	4	0	5	5	5	5	5	5	5	0	0	PB129
0	0	5	5	5	4	5	5	5	5	5	5	5	0	5	PB 85
0	4	0	5	4	1	5	5	5	5	5	5	5	5	5	PB 5
0	0	0	5	4	1	5	5	5	5	5	5	5	5	5	PB 6
0	0	0	5	5	0	5	5	5	5	5	5	5	0	5	PB 10
0	0	0	0	0	0	5	5	5	5	5	5	5	0	0	PB 86
1	0	0	5	4	0	5	5	5	5	5	5	5	5	0	PB 97
5	0	0	5	4	1	5	5	5	5	5	5	5	0	5	PB 87
0	0	0	5	5	2	5	5	5	5	5	5	5	0	5	PB 16
5	0	4	5	5	5	5	5	5	5	5	5	5	0	5	PB125
5	0	0	5	5	2	5	5	5	5	5	5	5	0	0	PB140
5	0	0	5	5	5	5	5	5	5	5	5	5	0	5	PB 14
5	0	0	5	5	5	5	5	5	5	5	5	5	0	0	PB 15
5	0	0	5	5	5	5	5	5	5	5	5	5	5	5	PB 17
5	0	0	5	5	5	5	5	5	5	5	5	5	0	1	PB 88
5	0	0	5	5	3	5	5	5	5	5	5	5	5	5	PB 69
0	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 18
4	0	4	5	4	5	5	5	5	5	5	5	5	5	5	PB 20
3	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 25
1	0	0	5	5	4	5	5	5	5	5	5	5	5	5	PB 29
0	0	0	5	5	5	5	5	5	5	5	5	5	5	5	PB 22
0	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 24
1	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 28
0	0	4	5	4	5	5	5	5	5	5	5	5	5	5	PB 19
2	2	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 21
0	0	0	5	5	5	5	5	5	5	5	5	5	5	5	PB 23
0	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 27
2	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 31
0	0	0	5	3	5	5	5	5	5	5	5	5	5	5	PB 33
2	2	0	5	5	5	5	5	5	5	5	5	5	5	5	PB 32
1	0	0	5	5	5	5	5	5	5	5	5	5	5	5	PB 34
2	2	2	5	4	5	5	5	5	5	5	5	5	5	5	PB 30
0	3	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 26
5	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB 53
0	0	0	5	5	5	5	5	5	5	5	5	5	5	5	PB152
5	0	0	5	5	5	5	5	5	5	5	5	5	5	5	PB153
5	0	3	5	5	5	5	5	5	5	5	5	5	5	5	PB154
5	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB156
4	0	0	5	5	3	5	5	5	5	5	5	5	0	5	PB109
3	0	0	5	4	0	5	5	5	5	5	5	5	5	0	PB110
3	0	5	5	5	0	5	5	5	5	5	5	5	0	5	PB143
5	0	4	5	4	0	5	5	5	5	5	5	5	5	5	PB160

Table A.III.a continued

Characters															OTU
76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	
2	0	0	3	0	4	5	5	5	5	5	5	5	5	5	PB 7
0	0	0	1	1	0	5	5	5	5	5	5	5	5	5	PB 80
0	0	0	0	0	0	5	0	0	5	3	5	5	4	5	PB 81
0	0	0	3	0	0	5	5	5	5	5	5	5	5	5	PB 78
3	3	0	0	0	0	5	5	5	5	5	5	0	0	0	PB 11
0	0	0	0	0	0	5	5	5	5	5	5	0	0	5	PB 13
0	0	3	3	0	0	5	5	5	5	5	5	2	0	0	PB 12
3	0	0	5	0	0	5	5	5	5	5	5	0	0	5	PB 83
0	0	4	3	0	3	5	5	5	5	5	5	4	4	5	PB 9
0	0	0	0	0	1	5	5	5	5	5	5	5	0	5	PB175
0	0	0	0	0	0	5	5	5	5	5	5	5	0	5	PB176
0	0	0	0	0	5	3	5	5	5	5	5	5	0	5	PB189
0	0	0	0	0	0	5	5	5	5	5	5	5	0	5	PB 92
3	3	0	3	3	0	5	5	5	5	5	5	5	4	5	PB 99
0	0	0	0	0	0	5	5	5	5	5	5	5	5	5	PB100
0	0	0	4	3	4	5	5	5	0	5	5	5	0	5	PB 51
0	0	0	4	4	3	5	4	0	4	0	5	5	5	5	PB 52
0	0	0	5	5	0	3	5	0	2	3	5	5	0	5	PB 59
0	0	0	5	5	5	5	0	0	5	5	5	5	5	5	PB 82
1	0	5	5	4	3	5	5	5	5	5	5	5	5	5	PB 8
0	0	0	5	5	4	5	5	5	5	5	5	5	5	5	PB 58
0	0	0	5	5	3	5	5	5	5	5	5	5	5	5	PB 76
3	0	0	5	5	5	5	5	5	5	5	5	5	5	5	PB198
0	0	0	5	4	3	5	5	5	5	5	5	5	5	5	PB202
0	0	0	5	4	4	5	0	5	5	5	5	5	5	5	PB199
0	0	0	5	5	5	5	5	5	5	5	5	5	0	5	PB200
4	0	0	5	0	0	5	5	5	5	5	5	1	2	5	PB171
0	0	0	5	5	0	5	5	5	5	5	5	5	5	5	PB172
0	0	0	5	5	0	5	5	5	5	5	5	5	5	5	PB173
5	4	5	5	5	0	5	5	5	5	5	5	5	4	5	PB174
0	0	0	0	0	2	5	5	5	5	5	5	0	0	0	PB 48
0	0	0	0	2	0	5	5	5	5	5	5	3	0	0	PB 93
0	0	0	2	0	0	5	5	5	5	5	5	0	0	5	PB 94
0	0	0	0	0	4	5	5	5	5	5	5	5	0	0	PB 95
0	0	0	0	0	1	5	0	0	4	0	5	0	0	0	PB 96
0	0	0	0	0	4	5	5	5	5	5	5	5	0	0	PB197
0	0	0	0	0	0	5	5	5	5	5	5	5	0	0	PB130
0	2	2	2	0	4	5	5	5	5	5	5	5	0	5	PB131
1	0	0	0	0	4	5	5	5	5	5	5	5	0	0	PB193
0	0	0	0	0	0	5	5	5	5	5	5	5	0	0	PB194
0	0	0	5	4	5	5	5	5	5	5	5	5	4	0	PB196
2	0	0	5	4	5	5	5	5	5	5	5	5	5	5	PB195
0	0	0	0	0	3	5	5	5	5	5	5	0	0	5	PB107
0	0	0	5	0	0	5	0	0	0	5	5	5	0	0	PB148
0	0	0	0	0	0	5	5	5	5	5	5	5	5	5	PB149
0	0	0	0	0	2	5	5	5	5	5	5	5	5	5	PB201
0	1	0	1	0	1	0	4	0	0	0	0	5	2	5	PB 35
0	0	0	0	0	0	0	5	0	0	0	0	5	3	5	PB 36
0	0	0	0	0	0	0	0	0	0	0	0	5	5	5	PB 37
0	0	0	0	0	0	0	0	0	0	0	5	5	0	5	PB 38

Table A.III.a continued

Characters														OTU
76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
0	0	0	0	0	0	0	0	0	0	0	3	5	0	5 PB 39
0	0	0	4	4	0	0	0	0	0	0	5	5	0	5 PB 84
0	0	3	0	0	1	5	3	0	2	0	0	0	0	0 PB124
0	0	5	5	0	5	0	5	0	0	5	0	0	0	0 PB151
0	0	5	5	5	5	5	0	0	5	0	5	5	0	5 PB 49
0	0	0	5	5	5	5	5	5	5	5	5	5	0	5 PB 71
0	0	0	0	0	0	5	0	0	5	0	5	0	0	5 PB 43
4	0	0	0	0	0	5	0	0	1	5	5	0	0	0 PB170
3	3	0	0	0	3	5	0	0	0	0	5	5	0	5 PB 46
0	0	0	0	0	3	5	0	0	4	0	5	0	4	5 PB 89
4	2	0	0	0	5	5	0	0	0	3	5	5	5	5 PB101
0	0	0	0	0	3	3	0	0	5	0	5	0	0	0 PB 90
0	0	0	5	4	0	5	0	0	0	4	5	5	0	5 PB 91
0	0	2	0	2	0	5	0	0	5	0	5	5	5	5 PB 98
1	0	0	1	2	1	5	0	0	5	0	5	5	5	5 PB150
1	0	0	0	0	1	5	0	0	0	0	5	5	0	5 PB 67
0	0	0	0	0	0	5	5	5	5	5	5	5	0	5 PB 68
0	0	0	0	0	3	5	0	5	5	5	5	5	0	5 PB146
0	0	4	0	0	2	5	0	0	4	0	5	5	0	5 PB145
0	0	0	2	0	0	5	4	5	5	5	5	5	0	5 PB102
0	0	0	0	1	2	5	3	5	5	5	5	5	0	5 PB115
0	0	0	0	0	2	5	0	5	5	5	5	5	0	5 PB123
0	0	0	0	0	5	5	0	0	0	0	5	5	0	5 PB 47
0	0	0	0	0	0	5	0	0	0	0	5	5	0	5 PB157
0	0	0	0	0	0	5	5	5	5	5	5	5	5	5 PB177
3	0	4	3	0	1	5	5	2	5	5	5	5	5	5 PB178
0	0	3	3	0	0	5	5	5	5	5	5	5	5	5 PB179
0	0	0	0	0	4	5	5	2	5	5	5	5	5	5 PB187
0	0	3	3	0	0	5	0	0	5	0	5	5	3	5 PB188
0	0	0	3	0	3	5	0	0	0	0	5	5	3	5 PB180
0	0	0	0	0	5	0	0	0	0	0	5	5	0	5 PB182
0	0	3	0	3	3	5	0	0	4	0	5	5	0	5 PB190
0	0	0	0	4	2	5	4	0	0	0	5	5	5	5 PB181
0	0	0	2	0	1	5	0	0	0	0	5	5	5	5 PB185
2	0	0	0	0	4	5	0	0	4	0	5	5	5	5 PB186
0	0	0	0	0	1	5	0	0	0	0	5	5	0	5 PB183
0	0	0	4	4	1	5	0	0	0	0	5	5	5	5 PB184
0	0	0	0	0	0	5	4	0	5	5	5	5	0	5 PB.56
1	1	0	0	0	0	5	0	0	5	0	5	5	2	5 PB 57
0	0	0	0	0	2	5	0	0	2	2	5	5	5	5 PB144
0	1	0	5	5	4	4	0	0	0	4	5	5	1	5 PB 60
0	0	0	0	0	4	5	0	0	5	4	5	5	0	5 PB 70
0	3	0	5	5	5	5	0	0	5	3	5	5	5	5 PB 66
0	0	0	0	0	1	5	0	0	0	0	5	0	0	5 PB161
0	2	2	3	0	0	2	0	0	0	0	5	5	0	5 PB165
0	0	0	5	0	5	5	0	0	0	0	5	0	5	5 PB162
0	0	0	3	0	5	5	0	0	0	0	5	4	5	5 PB163
0	0	0	3	0	5	5	0	0	0	0	5	0	5	5 PB164
0	0	0	0	0	5	5	0	0	5	5	5	5	4	5 PB166
0	0	0	0	0	5	5	5	5	0	0	5	0	0	5 PB167

Table A.III.a continued

Characters															OTU
76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	
0	0	0	0	0	0	0	0	0	0	0	5	5	4	5	PB168
2	2	2	0	1	2	5	0	0	5	2	5	5	4	5	PB 40
0	0	0	0	0	1	5	5	0	5	5	5	5	0	5	PB 72
0	0	0	0	0	2	5	0	0	5	2	5	5	0	5	PB 75
0	0	0	0	0	1	5	0	0	0	0	5	5	0	5	PB 73
0	0	0	0	0	0	5	0	0	5	0	5	5	0	5	PB 74
0	0	0	0	0	1	5	0	0	5	0	5	5	5	5	PB 54
0	0	0	0	0	4	5	0	5	5	5	5	5	0	5	PB 55
0	0	2	2	0	1	5	0	0	5	5	5	5	1	5	PB 61
0	0	0	0	0	1	5	0	0	5	5	5	5	0	5	PB 62
0	0	0	5	4	4	5	0	5	5	5	5	5	0	5	PB 64
0	0	0	5	4	4	5	0	0	5	4	5	5	0	5	PB 65
0	0	0	0	0	3	5	0	3	5	5	5	0	0	5	PB 41
0	0	0	0	0	4	5	0	5	5	5	5	0	0	5	PB 63
0	0	0	0	0	3	5	5	5	5	5	5	5	0	5	PB 44
0	0	0	0	0	3	5	0	0	3	5	5	5	0	5	PB103
0	0	0	0	0	3	5	0	0	5	5	5	5	0	5	PB104
2	0	2	2	3	3	5	5	0	5	0	5	0	0	5	PB132
0	0	0	1	5	4	5	0	0	5	3	5	5	3	5	PB120
0	0	0	1	5	1	5	0	0	5	5	5	5	0	5	PB121
0	0	0	0	0	3	5	0	0	0	0	5	0	0	5	PB105
0	0	0	0	0	4	5	0	0	4	0	5	0	0	5	PB155
0	0	0	0	0	1	5	0	5	5	5	5	5	5	5	PB 45
0	0	0	0	0	1	5	5	5	5	5	5	5	5	5	PB142
3	0	4	0	0	4	5	0	0	5	0	5	5	2	5	PB127
0	0	0	0	0	5	5	3	0	5	4	5	0	4	5	PB139
0	0	0	5	0	5	5	5	5	5	5	5	5	5	5	PB141
0	3	0	0	0	1	5	0	1	5	5	5	5	0	5	PB133
0	0	0	3	3	1	5	0	0	5	0	5	0	0	5	PB134
0	0	3	3	3	5	5	5	0	5	0	5	3	0	5	PB137
0	0	0	0	0	1	5	3	0	5	0	5	0	3	5	PB138
0	0	0	0	0	4	5	4	0	5	0	5	0	0	5	PB147
0	0	0	2	2	4	5	4	0	5	0	5	5	0	5	PB135
0	0	0	0	0	3	5	1	0	5	0	5	5	2	5	PB136
0	0	0	5	4	1	5	5	5	5	5	5	5	0	5	PB 42
0	0	0	5	5	4	5	5	5	5	5	5	0	0	5	PB122
0	0	0	5	5	2	5	5	5	5	5	5	5	0	5	PB191
0	0	3	5	5	2	5	5	5	5	5	5	5	0	5	PB 50
0	0	0	5	5	3	5	5	5	5	5	5	5	0	5	PB118
0	3	0	5	5	1	5	5	5	5	5	5	5	4	5	PB119
0	4	0	5	5	5	5	0	0	5	5	5	5	5	5	PB106
3	0	0	5	5	5	5	3	0	5	5	5	5	5	5	PB117
3	0	0	5	5	4	5	3	5	5	5	5	5	5	5	PB116
0	3	0	5	5	2	5	5	5	5	5	5	5	0	5	PB158
0	0	0	0	0	4	5	5	5	5	5	5	5	5	5	PB159
0	0	0	0	4	4	5	0	5	5	5	5	5	0	5	PB160
0	0	0	4	4	4	5	5	5	5	5	5	5	5	5	PB192
5	0	5	0	0	3	5	5	5	5	5	5	5	5	5	PB108
4	0	0	0	0	0	5	2	5	3	5	3	3	0	0	PB111
1	0	0	0	0	0	5	5	5	5	5	5	0	2	0	PB113
0	0	0	3	0	2	3	0	0	0	0	5	5	3	5	PB112
3	0	3	0	0	3	3	0	0	0	4	5	5	0	5	PB114

Table A.III.a continued

Characters															OTU
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	
5	0	0	3	0	5	0	2	0	0	0	3	0	0	1	PB 1
5	0	0	5	0	5	5	0	5	0	0	5	0	0	1	PB 2
5	5	2	5	0	5	0	4	0	0	0	4	0	0	1	PB 3
5	0	0	4	0	5	0	3	0	0	0	1	0	0	1	PB 4
5	0	0	4	0	5	0	4	0	0	0	0	0	0	1	PB 79
5	0	0	5	0	5	0	4	0	0	0	0	0	0	1	PB126
5	0	0	0	0	5	0	4	0	0	0	0	0	0	1	PB128
5	0	0	5	0	5	0	4	0	3	0	3	0	0	1	PB 77
5	0	0	4	0	5	0	4	0	0	0	0	0	0	1	PB129
5	0	0	4	0	5	0	4	0	0	0	5	0	0	1	PB 85
5	0	0	2	0	0	0	4	0	0	0	0	0	0	1	PB 5
5	0	0	1	0	0	0	3	0	0	0	0	0	0	1	PB 6
5	5	2	2	0	5	0	3	0	0	0	0	0	0	1	PB 10
0	0	0	3	0	4	0	3	0	0	0	0	0	0	1	PB 86
5	0	0	4	0	5	0	4	0	0	0	0	0	0	1	PB 97
5	0	0	3	0	5	0	5	0	2	0	0	0	0	1	PB 87
5	0	0	4	0	5	0	4	0	4	0	0	0	0	1	PB 16
5	0	0	4	0	0	0	5	0	0	0	0	0	0	1	PB125
5	1	0	5	0	5	0	4	0	1	0	0	0	0	1	PB140
5	0	0	3	0	0	0	4	0	0	0	0	0	0	1	PB 14
5	0	0	3	0	2	0	4	0	0	0	0	0	0	1	PB 15
5	5	5	4	0	5	0	4	0	0	0	0	0	0	1	PB 17
5	0	2	2	0	0	0	4	0	0	0	0	0	0	1	PB 88
5	0	5	0	0	0	0	5	0	3	0	0	0	0	1	PB 69
5	5	5	4	0	5	0	4	0	0	0	0	3	0	1	PB 18
5	5	5	5	0	5	0	3	0	0	0	0	0	0	1	PB 20
5	5	5	4	0	5	0	4	0	0	0	0	0	0	1	PB 25
5	5	5	5	0	5	0	5	0	0	0	0	0	0	1	PB 29
5	5	5	5	0	5	0	0	0	0	0	0	5	0	1	PB 22
5	5	5	4	0	5	0	4	0	1	0	0	3	0	1	PB 24
5	5	5	4	0	5	0	4	0	0	0	0	3	0	1	PB 28
5	5	5	5	3	5	0	4	0	3	0	0	4	0	1	PB 19
5	5	5	4	0	5	0	4	0	0	0	0	2	0	1	PB 21
5	5	5	5	0	5	5	5	0	0	0	0	0	0	1	PB 23
5	5	5	5	0	5	0	5	0	0	0	0	3	0	1	PB 27
5	0	5	4	0	5	0	4	0	0	0	0	2	0	1	PB 31
5	0	5	4	0	5	0	5	0	0	0	0	0	0	1	PB 33
5	0	5	4	0	5	0	3	0	0	0	0	3	0	1	PB 32
5	0	5	3	0	5	0	5	0	0	0	0	0	0	1	PB 34
5	5	5	5	3	5	0	3	0	0	0	0	2	0	1	PB 30
5	5	5	4	0	5	0	3	0	3	0	0	4	0	1	PB 26
5	5	5	4	0	5	0	5	0	0	0	0	0	0	1	PB 53
5	5	5	5	0	5	5	5	0	0	0	0	0	0	1	PB152
5	0	5	5	0	5	0	4	0	0	0	0	0	0	1	PB153
5	3	5	4	0	5	0	4	0	0	0	0	0	0	1	PB154
5	0	5	5	0	5	5	5	0	4	0	0	0	0	1	PB156
5	0	0	5	0	5	5	5	0	0	0	0	0	0	1	PB109
5	0	0	3	0	4	0	3	0	0	0	0	0	0	1	PB110
5	0	0	5	0	5	4	3	0	0	0	0	0	0	1	PB143
5	3	0	5	0	5	0	5	0	0	0	0	0	0	1	PB169

Table A.III.a continued

Characters															OTU
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	
0	5	5	5	0	5	5	3	0	3	0	0	0	0	1	PB 7
0	5	5	5	0	5	5	3	0	0	0	0	0	0	1	PB 80
0	0	5	5	0	5	5	3	0	2	0	0	0	0	1	PB 81
5	0	5	1	0	3	0	4	0	0	0	0	0	0	1	PB 78
0	0	0	3	0	0	0	4	0	0	0	0	0	0	1	PB 11
5	0	0	2	0	0	0	0	4	0	0	0	0	0	1	PB 13
0	0	5	0	4	0	0	3	0	0	0	0	0	0	1	PB 12
0	5	0	0	0	0	0	3	0	0	0	0	0	0	1	PB 83
5	5	5	4	0	3	0	0	0	1	0	0	0	0	1	PB 9
5	5	5	0	0	0	0	4	0	0	0	0	0	0	1	PB175
5	0	5	5	0	2	0	4	0	0	0	0	0	0	1	PB176
5	3	3	3	0	3	0	4	0	0	0	0	0	0	1	PB189
5	0	0	3	0	0	0	0	0	0	0	0	0	0	1	PB 92
5	5	5	4	0	4	0	0	0	0	0	0	0	0	1	PB 99
5	0	5	0	0	0	0	3	0	0	0	0	0	0	1	PB100
5	0	0	5	0	5	0	3	0	0	0	0	0	0	1	PB 51
5	0	5	5	0	5	0	3	0	0	0	0	0	0	1	PB 52
5	0	0	4	0	4	0	5	0	0	0	0	0	0	1	PB 59
5	0	5	4	0	5	0	5	0	0	0	0	0	0	1	PB 82
5	5	5	5	0	5	5	5	0	0	0	0	0	0	1	PB 8
5	5	5	4	0	4	0	5	0	0	0	0	0	0	1	PB 58
5	5	5	4	0	2	0	3	0	1	0	0	0	0	1	PB 76
5	5	5	5	0	5	0	5	0	0	0	0	0	0	1	PB198
5	5	5	5	0	5	0	5	0	0	0	0	0	0	1	PB202
5	5	5	2	0	0	0	5	0	0	0	0	0	0	1	PB199
5	0	0	1	0	5	0	2	0	0	0	0	0	0	1	PB200
5	0	5	4	0	0	0	5	0	0	0	0	0	0	0	PB171
5	5	5	5	0	5	0	5	0	0	0	0	0	0	0	PB172
5	5	5	5	0	0	0	5	0	0	0	0	0	0	0	PB173
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB174
5	0	0	4	0	5	0	5	0	0	0	0	0	0	1	PB 48
5	4	0	4	0	5	0	5	0	0	0	0	0	0	1	PB 93
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB 94
5	0	0	5	0	5	0	3	0	0	0	0	0	0	1	PB 95
5	0	0	5	0	5	0	3	0	0	0	0	0	0	1	PB 96
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB197
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB130
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB131
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB193
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB194
5	0	0	5	0	5	0	5	0	0	0	0	0	0	1	PB196
5	5	5	5	0	5	5	5	0	0	0	0	0	0	1	PB195
5	0	0	5	0	5	2	3	0	0	0	0	0	0	1	PB107
5	0	3	5	0	5	0	4	0	0	0	0	0	0	1	PB148
5	5	5	4	0	5	0	4	0	0	0	0	0	0	1	PB149
5	5	5	5	0	5	5	5	0	0	0	0	0	0	1	PB201
5	0	0	0	0	0	0	3	0	0	0	0	0	0	1	PB 35
5	0	0	0	0	0	0	3	0	0	0	0	0	0	1	PB 36
0	0	5	5	0	4	0	4	0	0	0	0	0	0	1	PB 37
0	0	0	0	0	0	0	4	0	0	0	0	0	0	1	PB 38

Table A.III.a continued

Characters															OTU
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	
0	0	0	0	0	0	0	5	0	0	0	0	0	0	1	PB 39
0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	PB 84
0	0	2	0	0	4	0	5	0	0	0	0	0	0	1	PB124
0	5	0	0	0	3	0	4	0	0	0	0	0	0	1	PB151
5	0	0	4	0	5	0	5	0	3	0	0	0	0	1	PB 49
5	0	0	1	0	0	0	5	0	0	0	0	0	0	1	PB 71
5	0	0	1	0	0	0	0	0	0	0	0	0	0	1	PB 43
5	3	0	0	0	0	0	4	0	0	0	0	0	0	1	PB170
0	0	0	4	0	1	1	0	0	0	0	0	0	0	1	PB 46
0	0	0	3	0	0	0	0	0	0	0	0	0	0	1	PB 89
0	0	5	4	4	0	0	3	0	0	0	0	0	0	1	PB101
0	0	5	4	0	0	0	0	0	2	0	0	0	0	1	PB 90
0	3	0	5	0	4	0	3	0	0	0	0	0	0	1	PB 91
0	0	5	4	0	4	0	3	0	0	0	0	0	0	0	PB 98
3	0	5	4	0	4	0	0	3	0	0	0	0	0	1	PB150
5	5	0	5	0	4	3	0	0	0	0	0	0	0	1	PB 67
5	5	0	4	2	2	0	0	0	0	0	0	0	0	1	PB 68
5	5	0	2	0	0	0	3	0	0	0	0	0	0	1	PB146
0	0	0	3	0	0	0	0	0	0	0	0	0	0	1	PB145
5	0	0	4	3	3	0	0	0	0	0	0	0	0	1	PB102
5	0	4	5	0	5	5	0	0	0	0	0	0	0	1	PB115
5	5	0	5	0	5	5	0	2	0	0	0	0	0	1	PB123
4	0	0	5	0	0	0	0	0	0	0	0	0	0	1	PB 47
0	0	0	5	0	5	5	0	0	0	0	0	0	0	1	PB157
5	5	5	3	0	0	3	4	0	0	0	0	0	0	1	PB177
5	5	5	4	0	0	0	4	0	0	0	0	0	0	1	PB178
4	5	5	5	0	0	0	4	0	0	0	0	0	0	1	PB179
5	0	5	4	0	0	0	4	0	0	0	0	0	0	1	PB187
0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	PB188
5	0	0	0	0	4	5	0	0	0	0	0	0	0	1	PB180
5	0	0	4	0	2	0	0	0	2	0	0	0	0	0	PB182
5	0	0	2	0	0	0	4	0	1	0	0	0	0	1	PB190
0	0	5	4	0	4	0	0	0	0	0	0	0	0	1	PB181
0	0	5	4	0	3	0	0	0	0	0	0	0	0	1	PB185
5	4	5	4	0	0	0	0	0	1	0	0	0	0	1	PB186
5	0	0	4	0	0	3	0	0	0	0	0	0	0	1	PB183
5	0	5	4	0	4	0	0	0	0	0	0	0	0	0	PB184
5	2	0	0	0	0	0	0	2	0	0	0	0	0	1	PB 56
5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB 57
5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB144
5	0	0	2	0	0	0	3	0	0	0	0	0	0	1	PB 60
5	0	0	0	0	0	0	3	0	0	0	0	0	0	0	PB 70
5	0	5	5	3	5	5	5	0	0	0	0	0	0	1	PB 66
0	0	0	3	0	0	0	0	0	0	0	0	0	0	1	PB161
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB165
5	0	5	3	0	0	0	0	0	0	0	0	0	0	1	PB162
5	5	5	3	0	3	0	0	0	0	0	0	0	0	1	PB163
5	0	4	3	0	4	0	0	0	0	0	0	0	0	1	PB164
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB166
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	PB167

Table A.III.a continued

91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	OTU
3	0	0	0	0	2	0	0	0	0	0	0	0	0	1	PB168
5	0	5	0	0	3	0	0	0	0	0	0	0	0	1	PB 40
5	0	0	0	0	0	0	2	0	0	0	0	0	0	1	PB 72
5	0	0	3	0	2	0	3	0	0	0	0	0	0	1	PB 75
5	0	0	4	0	0	0	5	0	0	0	0	0	0	1	PB 73
5	0	0	1	0	0	0	0	0	0	0	0	0	0	1	PB 74
5	0	0	5	0	5	5	0	3	0	0	0	0	0	1	PB 54
5	0	1	5	0	4	0	1	3	0	0	0	0	0	1	PB 55
5	0	0	5	0	5	5	3	3	0	0	0	0	0	1	PB 61
5	0	0	5	0	5	5	2	0	0	0	0	0	0	1	PB 62
5	0	0	4	0	0	0	2	3	0	0	0	0	0	1	PB 64
5	0	3	5	0	4	0	3	0	0	0	0	0	0	1	PB 65
0	2	5	0	0	5	5	4	0	0	0	0	0	0	1	PB 41
0	0	0	0	0	5	5	4	0	0	0	0	0	0	1	PB 63
5	5	0	5	0	5	4	3	0	1	0	0	0	0	1	PB 44
5	1	0	5	0	5	4	4	0	0	0	0	0	0	1	PB103
5	0	0	5	4	5	5	3	0	0	0	0	0	0	1	PB104
5	0	2	5	0	5	5	4	0	0	0	0	0	0	1	PB132
2	2	0	5	0	5	5	3	0	0	0	0	0	0	1	PB120
0	0	0	5	0	5	5	4	0	0	0	0	0	0	1	PB121
5	0	0	5	0	5	0	0	0	0	0	0	0	0	1	PB105
5	0	0	5	0	5	5	4	3	0	0	0	0	0	1	PB155
5	5	0	4	0	2	0	3	0	0	0	0	0	0	1	PB 45
5	5	5	3	2	2	2	4	0	0	0	0	0	0	1	PB142
5	0	0	5	0	3	0	4	0	0	0	0	0	0	1	PB127
5	0	0	5	0	3	0	5	0	0	0	0	0	0	1	PB139
5	5	0	5	0	5	0	5	0	0	0	0	0	0	1	PB141
5	0	0	5	0	5	5	3	0	0	0	0	0	0	1	PB133
5	0	0	5	0	5	0	4	0	0	0	0	0	0	1	PB134
5	0	0	5	0	5	0	4	0	0	0	0	0	0	1	PB137
5	0	0	4	3	2	0	4	0	0	0	0	0	0	1	PB138
5	0	0	5	0	4	0	4	0	2	0	0	0	0	1	PB147
5	0	0	4	0	5	0	4	0	0	0	0	0	0	1	PB135
5	0	0	3	0	0	0	0	3	0	0	0	0	0	1	PB136
5	2	0	5	0	5	5	3	0	2	0	0	0	0	1	PB 42
5	0	0	3	0	0	0	3	0	1	0	0	0	0	1	PB122
5	0	0	3	0	0	0	0	3	0	0	0	0	0	1	PB191
5	5	5	4	0	0	0	3	0	0	0	0	0	0	1	PB 50
5	5	0	3	0	0	0	0	0	0	0	0	0	0	1	PB118
5	5	0	5	0	5	5	4	0	0	0	0	0	0	1	PB119
5	0	5	5	0	5	4	5	0	4	0	0	0	0	1	PB106
5	0	0	5	0	5	5	5	0	0	0	0	0	0	1	PB117
5	0	5	5	0	5	5	5	0	0	0	0	0	0	1	PB116
5	4	0	5	0	5	3	4	0	0	0	0	0	0	1	PB158
5	5	5	5	0	5	5	3	0	1	0	0	0	0	1	PB159
5	5	5	5	0	5	5	0	4	0	0	0	0	0	1	PB160
5	5	5	5	2	5	5	0	4	0	0	0	0	0	1	PB192
5	0	5	0	5	5	0	0	0	0	0	0	0	0	1	PB108
5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	PB111
3	0	0	0	0	0	0	5	0	0	0	0	0	0	1	PB113
0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	PB112
0	0	5	5	0	0	0	0	0	0	0	0	0	0	1	PB114

Table A.III.a continued

Characters															OTU
106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	PB 1
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	PB 2
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	PB 3
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 4
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 79
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB126
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB128
1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	PB 77
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB129
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 85
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 5
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	PB 6
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 10
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 86
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 97
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 87
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	PB 16
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB125
1	1	0	1	0	1	1	1	1	1	1	0	1	0	1	PB140
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 14
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 15
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 17
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 88
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 69
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 18
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 20
0	0	0	1	0	1	1	1	1	1	1	0	1	0	1	PB 25
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 29
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 22
0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	PB 24
1	0	0	0	0	1	1	1	1	1	0	0	1	0	1	PB 28
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 19
1	0	0	0	0	1	1	1	1	1	0	0	1	1	1	PB 21
1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	PB 23
1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	PB 27
1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	PB 31
1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	PB 33
1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	PB 32
0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	PB 34
1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	PB 30
0	0	0	1	0	1	1	1	1	1	0	0	1	0	1	PB 26
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 53
1	0	0	1	0	1	1	1	1	1	1	0	1	0	1	PB152
1	0	0	1	0	1	1	1	1	1	1	0	1	0	1	PB153
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB154
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB156
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB109
1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	PB110
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB143
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB169

Table A.III.a continued

Characters														OTU
106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1 PB 7
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 80
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 81
0	0	0	1	0	1	1	1	1	1	1	0	1	0	1 PB 78
1	1	1	0	0	1	1	1	1	1	0	0	1	1	1 PB 11
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1 PB 13
1	1	1	0	0	1	1	1	1	1	1	0	1	0	1 PB 12
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 83
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 9
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB175
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB176
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB189
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 92
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 99
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB100
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 51
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 52
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 59
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 82
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 8
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 58
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 76
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB198
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB202
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB199
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB200
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB171
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB172
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB173
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB174
0	0	0	0	0	1	1	1	1	1	1	0	1	1	1 PB 48
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 93
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 94
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 95
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 96
1	1	0	1	0	1	1	1	1	1	1	1	1	1	1 PB197
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB130
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB131
1	0	0	1	0	1	1	1	1	0	1	0	1	1	1 PB193
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB194
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB196
1	0	0	1	0	0	1	1	1	1	1	1	1	1	1 PB195
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB107
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB148
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB149
1	1	1	1	0	0	1	1	1	1	1	0	1	1	1 PB201
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 35
1	1	0	1	0	1	1	1	1	1	1	1	1	1	1 PB 36
1	1	0	1	0	1	1	1	1	1	1	1	1	1	1 PB 37
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1 PB 38

Table A.III.a continued

Characters														OTU
106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	pB 39
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	pB 84
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB124
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	pB151
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	pB 49
0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	PB 71
1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	PB 43
1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	PB170
1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	PB 46
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 89
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB101
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 90
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 91
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 98
1	1	0	1	0	1	1	1	1	1	1	0	1	0	1	PB150
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 67
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 68
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB146
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB145
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB102
1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	PB115
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB123
0	0	0	1	0	1	1	1	1	1	1	0	1	0	1	PB 47
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB157
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB177
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB178
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB179
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB187
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB188
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB180
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB182
0	0	0	1	0	0	1	1	1	1	1	0	1	1	1	PB190
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB181
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB185
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB186
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB183
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB184
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 56
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 57
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB144
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 60
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 70
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 66
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB161
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB165
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB162
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB163
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB164
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB166
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB167

Table A.III.a continued

Characters															OTU
106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB168
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 40
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 72
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 75
1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	PB 73
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 74
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 54
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 55
0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	PB 61
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 62
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 64
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 65
0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	PB 41
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 63
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 44
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB103
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB104
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB132
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB120
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB121
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB105
1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	PB155
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 45
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB142
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB127
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB139
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB141
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB133
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB134
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB137
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB138
1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	PB147
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	PB135
1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	PB136
0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	PB 42
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB122
0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB191
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB 50
1	0	0	1	0	1	1	1	1	0	1	0	1	1	1	PB118
1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	PB119
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB106
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB117
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB116
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB158
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB159
1	0	0	1	0	1	1	1	1	1	1	0	1	1	1	PB160
0	0	0	1	0	0	1	1	1	1	1	0	1	1	1	PB192
1	1	0	1	0	1	1	1	1	1	1	0	1	0	1	PB108
1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	PB111
1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	PB113
1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	PB112
1	0	0	1	0	1	1	1	1	0	1	0	1	1	1	PB114

Table A.III.a continued

Characters														OTU
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	
1	1	0	0	1	5	5	2	5	5	4	4	5	1	0	PB 1
1	1	0	0	1	5	5	0	5	4	0	0	5	1	0	PB 2
1	1	0	0	1	5	5	0	5	5	4	2	5	1	1	PB 3
1	1	0	0	1	5	5	0	5	5	0	1	5	1	0	PB 4
1	1	0	1	0	5	5	0	5	5	4	1	5	1	1	PB 79
1	1	0	0	1	5	5	0	5	5	4	3	0	1	0	PB126
1	1	0	0	1	5	5	3	5	5	4	0	0	1	1	PB128
1	1	0	1	0	5	5	0	5	0	4	3	5	1	0	PB 77
1	1	0	0	1	4	5	5	3	0	0	0	0	1	1	PB129
1	1	0	1	0	5	5	0	5	5	4	4	5	1	1	PB 85
1	1	0	0	1	5	5	0	5	2	0	3	5	1	0	PB 5
1	1	0	1	0	5	5	0	5	1	0	3	5	1	0	PB 6
1	1	0	1	0	5	5	0	5	2	2	3	5	1	0	PB 10
1	1	0	1	0	5	5	0	5	0	2	2	5	1	0	PB 86
1	1	0	1	0	5	5	0	5	0	5	3	5	1	0	PB 97
1	1	0	1	0	5	5	0	5	1	5	2	5	1	0	PB 87
1	1	0	1	0	5	5	0	5	5	5	4	5	1	0	PB 16
1	1	0	1	0	5	5	4	5	3	1	3	5	1	0	PB125
0	0	0	1	0	1	5	2	5	1	5	5	5	1	0	PB140
1	1	0	1	0	3	5	5	5	5	5	4	5	1	0	PB 14
1	1	0	1	0	5	5	2	5	5	5	3	5	1	0	PB 15
1	1	0	0	1	5	5	0	5	5	5	4	5	1	0	PB 17
1	1	0	1	0	4	5	5	5	5	2	3	5	1	0	PB 88
1	1	0	1	0	4	5	5	5	2	4	3	5	1	0	PB 69
1	1	0	1	0	4	5	3	5	3	5	4	5	1	0	PB 18
1	1	0	1	0	4	5	0	5	0	5	3	5	1	0	PB 20
1	1	0	1	0	4	5	0	5	3	5	3	5	1	0	PB 25
1	1	0	1	0	5	5	0	5	2	5	4	5	1	0	PB 29
1	1	0	1	0	5	5	0	5	3	5	3	5	1	0	PB 22
1	1	0	1	0	4	5	4	5	5	5	2	5	1	0	PB 24
1	1	0	1	0	5	5	3	5	3	5	3	5	1	0	PB 28
1	1	0	1	0	5	5	0	5	3	5	3	5	1	0	PB 19
1	1	0	0	1	5	5	0	5	3	5	4	5	1	0	PB 21
1	1	0	0	1	5	5	0	5	5	5	3	0	1	0	PB 23
1	1	0	0	1	4	5	0	5	3	5	3	0	1	0	PB 27
1	1	0	1	0	4	5	0	5	2	5	4	5	1	0	PB 31
1	1	0	1	0	4	5	0	5	0	5	1	0	1	0	PB 33
1	1	0	0	1	5	5	0	5	2	5	4	5	1	0	PB 32
1	1	0	1	0	3	5	5	5	0	5	4	5	1	0	PB 34
1	1	0	1	0	3	5	0	5	0	5	4	5	1	0	PB 30
1	1	0	1	0	4	5	0	5	3	5	3	5	1	0	PB 26
1	1	0	1	0	5	5	5	5	4	0	5	0	1	0	PB 53
1	1	0	1	0	5	5	5	5	5	5	5	0	1	0	PB152
1	1	1	0	0	5	5	4	5	5	5	0	0	1	0	PB153
1	1	1	0	0	5	5	0	5	5	5	3	0	1	0	PB154
1	1	0	1	0	5	5	3	5	4	5	0	0	1	0	PB156
1	1	0	1	0	5	5	0	5	1	3	5	0	0	0	PB109
1	1	0	0	1	5	5	0	5	0	5	5	5	1	0	PB110
1	1	0	0	1	5	5	5	5	5	5	5	0	1	0	PB143
1	1	0	1	0	5	5	5	5	5	3	5	0	0	0	PB169

Table A.III.a continued

Characters														OTU
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	
1	1	0	1	0	0	5	0	5	0	0	3	5	1	0	PB 7
1	1	0	0	1	1	5	0	5	0	0	4	5	1	0	PB 80
1	1	0	1	0	0	5	0	0	0	2	2	5	1	0	PB 81
1	1	1	0	0	3	5	0	5	1	1	1	5	1	0	PB 78
1	1	0	1	0	0	5	0	5	3	0	4	5	1	0	PB 11
1	1	0	1	0	1	5	2	5	5	0	4	5	1	0	PB 13
1	1	0	1	0	0	5	0	5	0	0	3	5	1	0	PB 12
1	1	0	1	0	0	5	0	5	0	5	5	5	1	0	PB 83
1	1	0	1	0	0	5	0	5	0	1	3	5	0	0	PB 9
1	1	0	1	0	0	5	1	0	3	2	4	5	0	0	PB175
1	1	0	1	0	0	5	0	5	0	1	1	5	0	0	PB176
1	1	0	1	0	0	5	0	5	4	1	4	5	0	0	PB189
1	1	0	1	0	1	1	0	5	3	2	2	2	1	0	PB 92
1	1	0	1	0	1	2	0	5	0	3	3	0	0	0	PB 99
1	1	0	1	0	1	5	0	0	0	1	4	5	1	0	PB100
1	1	0	1	0	0	2	3	5	3	0	4	5	1	0	PB 51
1	1	0	1	0	4	2	4	3	3	0	4	5	1	0	PB 52
1	1	0	1	0	0	2	3	3	3	3	4	5	1	0	PB 59
1	1	0	1	0	0	5	5	0	3	4	4	5	1	0	PB 82
1	1	1	0	0	4	5	3	5	0	0	0	0	1	0	PB 8
1	1	1	0	0	0	5	5	5	3	0	5	5	1	0	PB 58
1	1	1	0	0	0	5	0	5	0	2	3	5	1	0	PB 76
1	1	0	1	0	3	0	0	5	5	3	2	0	1	0	PB198
1	1	0	1	0	5	5	3	5	5	0	2	3	1	0	PB202
1	1	1	0	0	0	1	0	0	5	0	0	3	1	0	PB199
1	1	1	0	0	0	1	0	0	0	0	0	0	1	0	PB200
1	1	1	0	0	3	5	2	0	0	1	4	0	0	0	PB171
1	1	0	1	0	5	5	0	5	5	5	2	0	0	0	PB172
1	1	1	0	0	5	4	0	5	5	4	0	0	0	0	PB173
1	1	1	0	0	5	5	2	5	5	5	4	0	0	0	PB174
1	1	0	1	0	2	5	0	4	0	0	0	0	1	0	PB 48
1	1	0	1	0	2	5	0	4	0	0	0	1	1	0	PB 93
1	1	0	1	0	2	5	2	1	0	2	3	5	1	0	PB 94
1	1	0	1	0	0	5	0	5	0	0	2	5	1	0	PB 95
1	1	0	0	1	0	5	0	0	0	0	4	5	1	0	PB 96
1	1	1	0	0	0	3	0	5	0	0	3	5	1	0	PB197
1	1	0	1	0	0	5	0	0	0	4	5	5	1	0	PB130
1	1	0	1	0	0	5	0	0	0	4	4	0	1	0	PB131
1	1	0	1	0	1	5	0	5	0	5	4	0	1	0	PB193
1	1	0	1	0	2	5	0	5	2	5	3	0	1	0	PB194
1	1	0	1	0	1	5	0	5	0	5	1	0	1	0	PB196
1	1	0	1	0	2	5	0	5	2	5	4	0	1	0	PB195
1	1	0	1	0	5	5	3	0	2	1	2	0	0	0	PB107
1	1	0	1	0	1	0	3	0	1	5	1	0	0	0	PB148
1	1	0	1	0	0	1	0	5	0	0	0	0	1	0	PB149
1	1	0	0	1	0	1	0	5	0	0	3	3	0	0	PB201
1	1	0	1	0	0	5	0	0	4	0	4	5	1	0	PB 35
1	1	1	0	0	3	5	0	0	4	3	4	5	1	0	PB 36
1	1	0	1	0	3	5	0	0	3	4	2	5	1	0	PB 37
1	1	0	1	0	2	1	0	0	2	4	3	0	1	0	PB 38

Table A.III.a continued

Characters														OTU
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	
1	1	0	1	0	0	1	0	0	0	0	1	0	1	0	PB 39
1	1	0	1	0	2	5	0	0	3	1	3	5	1	0	PB 84
1	1	0	1	0	0	3	0	0	0	0	3	4	1	0	PB124
1	1	0	1	0	0	5	5	0	5	0	5	5	1	0	PB151
1	1	0	1	0	0	5	0	0	2	3	3	5	1	0	PB 49
1	1	0	1	0	2	5	1	5	5	1	3	0	1	0	PB 71
1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	PB 43
1	1	1	0	0	2	0	0	0	0	1	2	5	0	0	PB170
1	1	0	1	0	5	1	0	0	2	0	3	0	1	0	PB 46
1	1	0	0	1	0	1	3	0	0	0	1	0	0	0	PB 89
1	1	1	0	0	0	3	0	0	1	0	1	0	1	0	PB101
1	1	0	1	0	0	5	0	0	3	3	4	5	1	0	PB 90
1	1	0	1	0	2	1	0	0	1	0	2	2	1	0	PB 91
1	1	0	1	0	2	2	0	0	3	0	2	0	1	0	PB 98
1	1	0	1	0	1	5	0	0	3	0	4	0	1	0	PB150
1	1	0	1	0	4	5	0	0	1	1	0	5	1	0	PB 67
1	1	0	1	0	5	1	0	5	0	1	0	0	0	0	PB 68
1	1	1	0	0	4	5	2	5	2	0	0	0	1	0	PB146
1	1	1	0	0	0	5	0	0	1	0	0	5	1	0	PB145
1	1	1	0	0	0	5	0	5	4	1	3	5	0	0	PB102
1	1	0	1	0	1	5	1	3	2	1	2	5	0	0	PB115
1	1	0	1	0	2	5	0	0	0	2	3	5	1	0	PB123
1	1	0	1	0	0	1	0	0	5	0	0	3	1	0	PB 47
1	1	1	0	0	1	5	0	0	2	0	0	5	1	0	PB157
1	1	1	0	0	3	5	0	5	0	1	1	5	0	0	PB177
1	1	0	1	0	4	5	1	0	0	0	0	5	0	0	PB178
1	1	0	1	0	5	3	0	5	0	1	1	0	0	0	PB179
1	1	0	1	0	0	5	0	0	3	1	3	5	0	0	PB187
1	1	1	0	0	3	5	0	0	3	0	4	5	0	0	PB188
1	1	0	1	0	5	5	3	0	5	3	5	5	0	0	PB180
1	1	0	1	0	2	5	0	0	3	1	2	5	0	0	PB182
1	1	0	1	0	1	0	3	0	2	1	3	5	0	0	PB190
1	1	1	0	0	3	5	0	0	4	0	3	5	0	0	PB181
1	1	1	0	0	1	5	0	0	4	0	5	5	1	0	PB185
1	1	1	0	0	3	5	0	0	4	3	5	5	0	0	PB186
1	1	0	1	0	2	5	0	0	3	0	0	5	0	0	PB183
1	1	0	1	0	2	5	0	0	3	1	5	5	1	0	PB184
1	1	1	0	0	0	5	0	0	0	0	1	5	0	1	PB 56
1	1	1	0	0	0	5	1	0	0	1	3	5	0	1	PB 57
1	1	1	0	0	0	5	0	0	0	0	3	3	0	1	PB144
1	1	1	0	0	0	5	0	0	0	0	3	5	1	0	PB 60
1	1	1	0	0	2	5	3	0	0	1	3	5	0	0	PB 70
1	1	1	0	0	5	5	3	0	2	3	4	5	1	0	PB 66
1	1	1	0	0	0	5	0	0	3	1	5	5	1	0	PB161
1	1	0	1	0	0	5	2	0	0	1	4	5	1	0	PB165
1	1	1	0	0	0	5	0	0	1	5	1	0	1	0	PB162
1	1	0	1	0	0	5	3	0	2	5	5	5	1	0	PB163
1	1	0	1	0	0	5	0	0	0	5	1	4	1	0	PB164
1	1	0	1	0	0	3	0	4	0	5	4	0	1	0	PB166
1	1	0	1	0	0	3	0	0	0	5	0	0	1	0	PB167

Table A.III.a continued

Characters														OTU
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	
1	1	0	1	0	0	5	3	0	0	4	3	5	1	0	PB168
1	1	0	0	1	0	5	1	5	0	0	5	5	1	0	PB 40
1	1	0	0	1	0	5	1	0	2	1	0	5	1	0	PB 72
1	1	0	0	1	3	5	0	0	0	0	2	5	1	0	PB 75
1	1	0	0	1	2	5	0	0	1	0	0	0	1	0	PB 73
1	1	0	1	0	0	5	0	0	0	0	0	5	1	0	PB 74
1	1	0	1	0	0	0	0	0	2	1	1	5	0	0	PB 54
1	1	0	1	0	0	0	0	0	1	1	1	5	0	0	PB 55
1	1	0	1	0	3	0	0	0	1	0	2	5	0	0	PB 61
1	1	0	1	0	0	0	0	0	0	0	0	5	0	1	PB 62
1	1	0	1	0	0	0	0	0	1	0	1	5	0	1	PB 64
1	1	0	1	0	1	0	0	0	3	0	0	5	0	0	PB 65
1	1	0	1	0	2	5	0	0	0	0	0	5	0	0	PB 41
1	1	0	1	0	4	5	0	1	2	0	0	5	1	0	PB 63
1	1	0	0	1	5	5	0	5	3	0	2	5	0	0	PB 44
1	1	0	1	0	4	5	0	0	4	0	0	5	1	0	PB103
1	1	0	0	1	4	5	0	0	3	1	1	5	1	0	PB104
1	1	0	1	0	4	5	1	0	2	2	0	5	1	0	PB132
1	1	0	1	0	3	5	1	0	0	0	3	5	1	0	PB120
1	1	0	1	0	0	5	1	0	0	0	2	5	1	0	PB121
1	1	0	1	0	4	5	0	1	0	0	0	5	1	0	PB105
1	1	0	1	0	5	5	0	0	2	1	4	5	0	0	PB155
1	1	0	1	0	5	5	0	5	2	0	4	5	0	0	PB 45
1	1	0	1	0	2	5	0	5	3	3	2	0	1	0	PB142
1	1	0	1	0	4	5	0	0	3	3	4	5	1	0	PB127
1	1	0	1	0	5	5	0	3	2	2	4	0	1	0	PB139
1	1	1	0	0	5	5	5	5	3	4	3	0	1	0	PB141
1	1	0	0	1	0	1	0	0	1	1	2	0	0	1	PB133
1	1	0	1	0	0	5	0	0	1	1	3	5	0	0	PB134
1	1	0	1	0	1	5	0	0	3	3	4	5	0	0	PB137
1	1	0	1	0	1	5	0	0	1	1	2	0	1	0	PB138
1	1	0	1	0	2	5	0	0	3	3	2	5	1	0	PB147
1	1	0	0	1	0	5	0	0	2	3	2	5	1	0	PB135
1	1	0	0	1	0	5	0	0	3	3	4	5	1	0	PB136
1	1	0	1	0	5	5	0	5	2	5	4	5	1	0	PB 42
1	1	0	1	0	5	5	0	4	4	2	2	5	0	0	PB122
1	1	0	1	0	2	5	0	2	2	1	3	5	1	0	PB191
1	1	0	1	0	3	5	0	5	0	0	3	5	1	0	PB 50
1	1	0	1	0	3	5	3	5	0	1	3	5	1	0	PB118
1	1	0	1	0	3	4	0	5	0	3	3	2	1	0	PB119
1	1	1	0	0	5	5	0	5	3	3	4	5	0	0	PB106
1	1	1	0	0	5	5	4	4	0	3	4	5	0	0	PB117
1	1	1	0	0	4	5	4	0	3	0	3	5	0	0	PB116
1	1	1	0	0	5	5	0	5	3	0	0	5	1	0	PB158
1	1	0	1	0	0	5	0	0	3	0	3	5	0	0	PB159
1	1	0	1	0	0	5	0	0	0	0	2	5	1	0	PB160
1	1	0	1	0	0	5	4	4	2	1	4	5	1	0	PB192
1	1	1	0	0	3	5	4	5	5	5	5	0	1	0	PB108
1	1	0	0	1	5	5	1	1	1	0	1	0	0	0	PB111
1	1	0	1	0	3	5	0	3	0	1	0	0	0	0	PB113
1	1	0	0	1	5	5	2	0	5	3	5	5	0	0	PB112
1	1	0	0	1	3	5	0	3	5	5	5	5	0	0	PB114

Table A.III.a continued

Characters														OTU
136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
0	0	1	2	3	0	5	0	1	0	5	5	5	0	4 PB 1
0	0	1	2	4	0	5	0	2	0	5	5	5	0	3 PB 2
0	0	3	3	5	0	4	0	2	0	5	5	5	0	3 PB 3
0	0	3	3	5	0	4	0	1	0	5	5	5	0	5 PB 4
0	0	2	2	4	0	3	0	2	0	5	5	5	0	4 PB 79
0	1	2	2	4	0	3	0	1	0	4	4	3	0	1 PB126
0	0	3	3	4	0	4	0	3	0	5	4	5	0	5 PB128
0	0	1	2	3	0	2	0	2	0	3	3	4	0	3 PB 77
0	0	2	3	4	0	3	0	2	0	4	4	5	0	3 PB129
0	0	1	1	3	0	2	0	0	0	0	1	5	0	5 PB 85
0	0	2	3	5	0	3	0	1	0	0	1	2	0	0 PB 5
0	0	1	3	5	0	4	0	1	0	0	1	3	0	0 PB 6
0	0	1	2	2	0	4	0	1	0	1	0	1	0	3 PB 10
0	0	1	1	3	0	3	0	0	0	0	0	2	0	1 PB 86
0	0	1	3	3	0	2	0	1	0	1	1	2	0	3 PB 97
0	0	1	2	4	0	2	0	0	0	2	1	1	0	3 PB 87
0	0	5	3	3	0	5	0	2	0	0	5	2	0	0 PB 16
0	0	2	3	4	0	4	0	2	0	2	4	5	0	5 PB125
1	0	5	1	3	0	3	0	2	0	5	5	4	0	2 PB140
1	0	1	2	3	0	2	0	1	0	0	2	3	0	1 PB 14
1	0	1	2	3	0	3	0	2	0	0	1	5	0	2 PB 15
1	0	1	2	4	0	2	0	2	0	0	1	5	0	3 PB 17
1	0	1	2	4	0	2	0	0	0	0	1	3	0	3 PB 88
0	0	1	2	3	0	4	0	1	0	0	2	2	0	3 PB 69
1	0	1	2	4	0	3	0	1	0	5	5	4	2	5 PB 18
1	0	1	3	4	0	4	0	0	0	5	4	3	1	4 PB 20
1	0	2	1	4	0	3	0	0	0	5	4	4	3	3 PB 25
1	0	1	2	4	0	5	0	1	0	5	3	4	3	3 PB 29
1	0	1	2	3	0	4	0	1	0	5	4	5	1	5 PB 22
1	0	1	2	4	0	4	0	1	0	5	3	4	3	4 PB 24
1	0	2	2	4	0	5	0	1	0	5	4	4	3	5 PB 28
1	0	1	2	3	0	3	0	0	0	4	3	3	0	4 PB 19
1	0	1	1	5	0	5	0	1	0	5	4	5	2	5 PB 21
1	0	1	2	3	0	4	0	1	0	5	4	4	3	5 PB 23
1	0	1	2	4	0	5	0	1	0	5	3	4	3	4 PB 27
1	0	1	2	4	0	4	0	2	0	5	4	4	1	5 PB 31
1	0	2	3	4	0	5	0	2	0	5	5	5	3	5 PB 33
1	0	2	4	4	0	5	0	1	0	5	4	4	0	2 PB 32
1	0	1	3	5	0	5	0	2	0	5	4	4	3	3 PB 34
1	0	2	2	4	0	4	0	2	0	5	4	4	5	4 PB 30
1	0	1	2	4	0	5	0	1	0	5	4	4	1	5 PB 26
0	0	1	2	4	0	5	0	3	0	5	4	5	0	4 PB 53
0	0	1	2	3	0	5	0	1	0	0	2	1	0	1 PB152
0	0	1	3	4	0	4	0	0	0	5	3	4	0	5 PB153
0	1	1	2	3	2	5	0	0	0	5	3	4	0	4 PB154
0	1	5	2	3	0	5	0	1	0	1	5	5	0	0 PB156
0	0	1	2	3	0	1	0	0	0	0	1	1	0	0 PB109
1	0	1	2	2	0	1	0	0	0	0	1	1	0	0 PB110
0	0	4	2	1	0	3	0	0	0	0	4	1	0	0 PB143
1	0	1	3	3	0	5	5	0	0	0	1	3	0	3 PB169

Table A.III.a continued

Characters														OTU
136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	
0	0	1	3	3	0	5	0	0	0	0	5	5	1	0	PB 7
0	0	1	2	3	0	3	0	0	0	0	3	1	1	0	PB 80
1	0	1	2	3	0	5	0	0	0	0	2	1	0	0	PB 81
0	0	3	2	2	0	5	0	0	0	0	5	1	0	4	PB 78
0	0	1	2	3	0	3	0	0	0	0	1	1	0	0	PB 11
0	0	1	2	2	0	3	0	0	0	0	1	2	0	0	PB 13
0	0	1	2	3	0	3	0	0	0	0	1	1	0	0	PB 12
0	0	1	3	3	0	3	1	1	0	0	1	1	0	0	PB 83
0	0	2	2	2	0	3	0	0	0	0	3	3	0	0	PB 9
1	0	1	2	2	0	5	5	1	0	0	1	3	0	3	PB175
0	0	1	3	2	0	1	0	0	0	0	2	1	0	1	PB176
0	0	1	3	3	0	3	0	0	0	0	0	2	0	2	PB189
0	0	3	2	2	0	4	1	2	0	0	5	3	0	2	PB 92
0	0	1	1	3	0	5	0	1	0	1	3	3	0	4	PB 99
0	0	1	3	1	0	5	0	0	0	0	4	3	1	5	PB100
0	0	2	3	2	0	1	0	0	0	0	2	2	0	0	PB 51
0	0	1	2	2	0	4	0	0	0	1	0	2	0	0	PB 52
0	0	1	3	3	0	2	0	1	0	0	0	2	0	0	PB 59
0	0	1	2	3	0	5	0	0	0	0	1	1	0	0	PB 82
0	0	1	1	3	0	5	0	0	0	0	5	2	0	0	PB 8
0	0	2	3	3	0	4	0	3	0	0	5	3	0	0	PB 58
0	0	1	1	3	0	3	0	0	0	0	0	2	0	4	PB 76
0	0	2	3	3	0	4	0	2	0	0	4	4	0	0	PB198
0	0	1	2	3	0	5	0	2	0	0	5	4	0	0	PB202
0	0	1	2	3	0	1	0	0	0	0	4	2	0	0	PB199
0	0	2	3	4	0	2	0	1	0	0	5	4	0	0	PB200
0	0	1	3	1	0	0	0	0	0	0	0	1	0	0	PB171
0	0	3	3	4	0	0	0	0	0	1	3	1	0	0	PB172
0	0	1	3	3	0	1	0	0	0	0	1	1	0	0	PB173
0	0	1	2	3	0	5	1	1	0	0	3	3	0	0	PB174
0	0	1	1	2	0	1	0	0	0	0	2	2	0	0	PB 48
0	0	1	2	3	0	4	0	2	0	2	4	4	0	0	PB 93
0	0	1	2	3	0	4	0	0	0	2	4	3	0	0	PB 94
0	0	1	2	3	0	1	0	0	0	0	4	1	0	0	PB 95
0	0	1	2	3	0	2	0	0	0	1	4	3	0	0	PB 96
0	0	2	3	3	0	4	0	2	0	0	4	4	0	0	PB197
0	0	1	2	4	0	3	0	1	0	1	1	2	0	3	PB130
0	0	1	2	3	0	2	0	1	0	0	3	2	0	0	PB131
0	0	2	2	4	0	2	0	0	0	0	5	3	0	0	PB193
0	0	1	2	2	0	3	0	0	0	1	4	2	0	0	PB194
0	0	2	4	4	0	2	0	0	0	0	3	3	0	0	PB196
0	0	2	3	3	0	4	0	2	0	1	5	5	0	0	PB195
0	0	3	2	2	0	2	0	0	0	0	4	1	0	0	PB107
0	0	1	2	2	0	0	0	0	0	0	4	2	0	0	PB148
0	0	3	1	1	0	3	0	0	0	0	4	1	0	1	PB149
0	0	2	4	3	0	1	0	0	0	0	2	4	0	0	PB201
0	0	1	1	1	0	1	0	0	0	0	0	1	0	4	PB 35
0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	PB 36
0	0	1	1	2	0	1	0	0	0	0	0	1	0	2	PB 37
0	0	1	1	2	0	0	0	0	0	0	1	1	0	0	PB 38

Table A.III.a continued

Characters															OTU
136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	
0	0	1	2	2	0	0	0	0	0	0	0	1	0	0	
0	0	1	2	3	0	1	0	0	0	0	0	1	0	0	PB 39
0	0	2	2	2	0	3	0	0	0	0	4	1	0	0	PB 84
0	0	1	2	1	0	0	0	0	0	0	0	1	0	0	PB124
0	0	2	2	3	0	1	0	0	0	0	1	1	0	0	PB151
															PB 49
0	0	1	2	2	0	0	0	0	0	0	2	2	0	0	
0	0	4	2	2	0	5	0	0	0	0	5	2	0	0	PB 71
0	0	1	2	4	0	3	1	1	0	0	1	1	0	1	PB 43
0	0	4	2	2	0	5	1	0	0	1	5	1	0	4	PB170
0	0	1	3	3	0	5	2	0	0	1	0	1	0	0	PB 46
															PB 89
0	0	1	2	3	0	2	0	0	0	0	1	1	0	1	
0	0	1	3	2	0	3	0	0	0	0	0	2	0	0	PB101
0	0	1	2	3	0	3	0	0	0	1	0	2	0	0	PB 90
0	0	1	2	3	0	4	1	0	0	0	0	2	0	0	PB 91
0	0	2	2	3	0	4	0	0	0	1	0	3	0	0	PB 98
															PB150
0	0	1	2	3	0	1	0	0	0	0	2	2	0	0	
0	0	1	2	2	0	3	0	0	0	0	1	1	0	0	PB 67
0	0	1	1	3	0	4	0	1	0	0	0	1	0	0	PB 68
0	0	3	1	1	0	2	0	0	0	1	5	2	0	2	PB146
0	0	4	1	1	0	5	1	1	0	0	5	2	0	0	PB145
															PB102
0	0	5	1	3	0	5	1	0	0	0	5	1	0	0	
0	1	1	1	3	0	5	0	0	0	2	1	1	0	3	PB115
0	0	1	2	3	0	1	0	0	0	0	1	1	0	0	PB123
0	1	1	3	3	0	2	0	0	0	0	0	1	0	0	PB 47
0	0	1	2	3	0	5	1	0	0	1	1	1	0	1	PB157
															PB177
0	0	1	2	2	0	5	1	0	0	1	1	1	0	1	
0	0	4	1	2	0	5	1	0	0	0	5	1	0	1	PB178
0	0	5	2	3	0	5	2	0	0	1	5	1	1	2	PB179
0	0	1	3	3	0	4	1	0	0	2	1	1	0	4	PB187
0	0	4	1	2	1	5	1	0	0	0	5	1	0	1	PB188
															PB180
0	0	4	1	3	1	5	0	0	0	0	4	2	0	0	
0	0	1	2	2	0	0	0	0	0	0	0	2	0	0	PB182
0	0	1	3	3	1	1	0	0	0	0	0	2	0	0	PB190
0	0	1	2	3	0	1	0	0	0	0	1	2	0	1	PB181
0	0	1	3	3	0	1	0	0	0	0	0	2	0	0	PB185
															PB186
0	0	1	2	5	1	2	0	0	0	0	4	2	0	0	
0	0	1	2	5	1	1	0	0	0	0	3	2	0	0	PB183
0	0	4	2	2	0	5	0	1	0	0	4	3	0	4	PB184
0	0	5	3	3	0	4	0	3	0	1	5	3	0	4	PB 56
0	0	1	2	4	0	3	0	2	0	2	3	3	0	1	PB 57
															PB144
0	0	1	3	3	0	3	0	0	0	3	2	2	0	0	
0	0	1	2	3	0	4	0	0	0	5	2	2	0	2	PB 60
0	0	4	1	2	0	4	0	0	0	1	4	1	0	2	PB 70
1	0	5	1	5	0	0	0	0	0	1	5	1	0	0	PB 66
1	1	1	2	2	0	1	0	0	0	0	1	1	4	0	PB161
															PB165
1	0	1	2	2	0	1	0	0	0	0	1	1	1	1	
1	0	1	2	2	0	1	0	0	0	0	1	1	1	1	PB162
1	0	3	2	3	0	0	0	0	0	0	1	1	0	0	PB163
1	0	1	3	2	0	1	0	0	0	0	1	1	4	4	PB164
1	0	1	2	3	0	1	0	0	0	0	1	1	0	0	PB166
															PB167

Table A.III.a continued

Characters															OTU
136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	
1	0	1	1	2	0	5	1	0	0	0	1	1	5	5	PB168
0	1	5	1	3	0	1	0	1	0	0	5	3	0	0	PB 40
0	1	3	2	3	0	1	0	1	0	0	4	2	0	0	PB 72
0	1	4	1	2	0	2	0	1	0	0	5	3	0	0	PB 75
0	1	4	2	2	0	2	0	1	0	0	5	3	0	0	PB 73
0	1	3	2	2	0	1	0	0	0	0	4	2	0	0	PB 74
0	0	5	3	3	0	5	2	2	0	1	5	3	0	0	PB 54
0	0	5	3	3	0	5	1	3	0	0	5	3	0	0	PB 55
0	0	5	2	2	0	4	2	2	0	0	5	3	0	0	PB 61
0	0	5	3	3	0	5	1	1	0	0	5	3	0	0	PB 62
0	0	5	3	2	0	2	0	2	0	0	5	3	0	0	PB 64
0	0	5	1	1	0	3	0	0	1	0	5	3	0	0	PB 65
0	0	5	3	3	0	3	0	0	0	0	5	3	0	0	PB 41
0	0	5	2	2	0	4	0	0	0	0	5	2	0	0	PB 63
0	0	5	2	3	0	5	2	2	0	0	5	5	0	2	PB 44
0	0	5	3	3	1	5	1	1	0	1	5	3	0	0	PB103
0	0	5	2	2	0	5	1	3	0	1	5	4	0	1	PB104
0	0	1	1	4	0	3	0	2	0	5	5	5	0	3	PB132
0	0	5	2	3	0	5	3	1	0	0	5	3	0	0	PB120
0	0	3	2	2	0	4	0	0	0	0	5	2	0	0	PB121
0	0	5	2	2	0	5	0	2	0	1	5	3	0	0	PB105
0	1	5	3	3	0	5	0	2	0	2	5	5	0	0	PB155
0	0	5	2	2	0	5	2	0	0	0	5	2	0	0	PB 45
0	0	5	3	3	0	4	0	2	0	0	5	4	0	0	PB142
0	1	5	2	3	0	3	0	2	0	0	5	5	0	0	PB127
0	1	4	2	3	0	2	0	2	0	0	5	3	0	0	PB139
0	0	4	1	2	0	1	0	2	0	0	5	3	0	0	PB141
0	0	5	3	3	0	5	1	1	0	1	5	4	0	0	PB133
0	0	5	3	4	0	1	0	2	0	0	5	4	0	0	PB134
0	1	4	2	3	0	2	0	2	0	0	5	4	0	0	PB137
0	1	5	2	3	0	3	0	2	0	0	5	4	0	0	PB138
0	0	4	2	3	0	4	0	2	0	0	5	5	0	0	PB147
0	0	5	2	2	0	2	0	3	0	0	5	3	0	0	PB135
0	1	5	2	3	0	4	0	4	0	0	5	5	0	0	PB136
1	0	3	2	5	0	5	0	3	0	5	5	3	0	4	PB 42
0	1	3	2	2	0	4	0	0	0	0	5	3	0	0	PB122
0	0	4	2	2	0	1	0	0	0	0	5	3	0	0	PB191
0	0	4	2	3	0	5	1	0	0	2	4	3	0	0	PB 50
0	0	3	3	2	0	3	1	0	0	0	3	3	0	0	PB118
0	0	4	1	2	0	5	2	0	0	0	5	2	0	0	PB119
0	0	5	3	2	0	5	1	1	0	1	5	5	0	0	PB106
0	0	5	2	2	0	5	1	1	0	0	5	1	0	0	PB117
0	0	5	2	2	0	5	1	1	0	0	5	1	0	0	PB116
0	1	5	1	3	0	5	0	1	0	1	5	5	0	2	PB158
0	0	1	2	3	0	5	0	0	0	0	1	1	0	0	PB159
0	0	1	2	3	0	5	1	0	0	2	1	1	0	3	PB160
0	0	1	3	3	0	4	0	0	0	2	0	1	0	3	PB192
1	0	1	1	3	0	4	3	0	0	0	0	1	0	0	PB108
1	0	1	1	1	0	4	1	0	0	0	1	1	0	1	PB111
1	0	1	1	2	0	2	0	0	0	0	0	2	0	0	PB113
0	0	4	2	4	0	0	0	0	0	0	4	1	0	0	PB112
1	0	4	2	2	0	0	0	0	0	0	5	1	0	0	PB114

Table A.III.a continued

Characters							OTU
151	152	153	154	155	156	157	
0	3	4	0	0	0	1	PB 1
0	5	3	0	0	0	1	PB 2
0	3	3	0	0	0	1	PB 3
0	3	5	0	0	0	1	PB 4
0	4	4	3	0	0	1	PB 79
0	5	4	1	0	0	1	PB126
0	4	5	4	0	0	1	PB128
0	4	4	1	0	0	1	PB 77
0	4	5	4	0	0	1	PB129
0	4	4	0	0	0	1	PB 85
0	0	2	0	0	0	1	PB 5
0	0	5	0	0	0	1	PB 6
0	0	4	1	0	0	1	PB 10
0	0	2	0	0	0	1	PB 86
0	0	2	0	0	0	1	PB 97
0	0	3	0	0	0	1	PB 87
5	4	1	0	0	0	1	PB 16
0	5	5	0	2	0	1	PB125
0	4	4	3	0	0	1	PB140
0	0	2	0	0	0	1	PB 14
0	0	1	0	0	0	1	PB 15
0	1	1	0	0	0	1	PB 17
0	3	2	0	0	0	1	PB 88
0	0	5	0	0	0	1	PB 69
2	0	2	4	0	0	1	PB 18
2	0	2	4	0	0	1	PB 20
4	0	5	3	0	0	1	PB 25
4	0	4	5	0	0	1	PB 29
1	0	4	5	0	0	1	PB 22
4	0	5	5	0	0	1	PB 24
4	0	4	5	0	0	1	PB 28
1	0	2	4	0	0	1	PB 19
3	0	3	5	0	0	1	PB 21
3	0	4	5	0	0	1	PB 23
4	0	5	5	0	0	1	PB 27
4	0	5	5	0	0	1	PB 31
5	0	5	5	0	0	1	PB 33
2	0	3	2	0	0	1	PB 32
3	0	4	5	0	0	1	PB 34
4	0	4	5	0	0	1	PB 30
3	0	2	3	0	0	1	PB 26
2	0	5	3	0	0	1	PB 53
1	1	0	0	0	0	0	PB152
0	5	5	5	0	0	0	PB153
0	4	4	5	0	0	1	PB154
4	4	0	0	0	0	1	PB156
0	1	0	0	0	0	0	PB109
0	0	4	0	0	0	0	PB110
0	1	0	0	0	0	0	PB143
0	0	3	0	0	0	0	PB169

Table A.III.a continued

Characters							OTU
151	152	153	154	155	156	157	
0	5	5	0	0	0	1	PB 7
0	4	1	0	0	0	1	PB 80
0	5	0	0	0	0	1	PB 81
0	4	2	0	0	0	1	PB 78
0	3	4	0	0	0	1	PB 11
0	4	3	0	0	0	1	PB 13
0	3	4	0	0	0	1	PB 12
0	2	3	0	0	0	1	PB 83
0	3	0	0	0	0	0	PB 9
0	0	4	0	0	0	0	PB175
0	1	0	0	0	0	0	PB176
0	4	0	0	0	0	0	PB189
0	4	4	0	0	0	1	PB 92
0	5	4	0	0	0	0	PB 99
0	3	1	0	0	0	0	PB100
0	0	1	0	0	0	1	PB 51
0	0	0	0	0	0	1	PB 52
0	5	0	0	0	0	1	PB 59
0	1	0	0	0	0	1	PB 82
0	5	5	0	0	0	1	PB 8
0	5	4	0	0	0	1	pB 58
0	0	4	0	0	0	0	pB 76
0	5	4	0	0	0	0	PB198
0	5	5	0	0	0	0	PB202
0	4	3	0	0	0	0	PB199
0	4	5	0	0	0	0	PB200
0	0	0	0	0	0	0	PB171
0	1	0	0	0	0	0	PB172
0	1	1	0	0	0	0	PB173
0	3	4	0	0	0	0	PB174
0	4	4	0	0	0	1	PB 48
0	5	5	0	0	0	1	PB 93
0	3	5	0	0	0	1	PB 94
0	0	0	0	0	0	0	PB 95
0	3	5	0	0	0	0	PB 96
0	5	4	0	0	0	0	PB197
0	0	5	0	0	0	1	PB130
0	2	4	0	0	0	1	PB131
0	5	5	0	0	0	0	PB193
0	3	5	0	0	0	0	PB194
0	5	5	0	0	0	0	PB196
0	4	5	0	0	0	0	PB195
0	0	1	0	0	0	0	PB107
0	1	1	0	0	0	0	PB148
0	2	1	1	0	0	0	PB149
0	4	4	0	0	0	0	PB201
0	0	0	0	0	0	1	PB 35
0	0	0	0	0	0	1	PB 36
0	0	0	0	0	0	1	PB 37
0	0	0	0	0	0	1	PB 38

Table A.III.a continued

Characters							OTU
151	152	153	154	155	156	157	
0	0	0	0	0	0	1	PB 39
0	0	0	0	0	0	1	PB 84
1	0	0	0	0	0	1	PB124
0	0	0	0	0	0	1	PB151
0	2	0	0	0	0	1	PB 49
0	0	0	0	0	0	0	PB 71
0	2	0	0	0	0	0	PB 43
0	0	0	0	0	0	0	PB170
0	3	0	2	0	0	1	PB 46
0	4	0	0	0	0	0	PB 89
0	4	3	0	0	0	0	PB101
0	1	0	0	0	0	1	PB 90
0	0	0	0	0	0	1	PB 91
0	2	0	0	0	0	0	PB 98
0	0	0	0	0	0	0	PB150
0	2	0	0	0	0	1	PB 67
0	1	0	0	0	0	0	PB 68
0	0	0	0	0	0	1	PB146
0	2	0	2	0	0	0	PB145
0	1	1	0	0	0	0	PB102
1	3	1	0	0	0	0	PB115
0	2	1	4	0	0	1	PB123
0	1	0	0	0	0	1	PB 47
0	1	0	0	0	0	1	PB157
0	0	0	0	0	0	0	PB177
0	0	0	0	0	0	0	PB178
0	0	1	3	0	0	0	PB179
0	4	1	0	0	0	0	PB187
0	4	0	1	0	0	0	PB188
0	3	0	0	0	0	0	PB180
0	2	0	0	0	0	0	PB182
0	0	0	0	0	0	0	PB190
0	0	0	0	0	0	0	PB181
0	0	0	0	0	0	0	PB185
0	0	0	0	0	0	0	PB186
0	3	0	0	0	0	0	PB183
0	1	0	0	0	0	0	PB184
0	4	0	0	0	0	0	PB 56
0	4	0	0	0	0	0	PB 57
0	4	3	1	0	0	0	PB144
0	0	2	0	0	0	1	PB 60
2	0	1	0	0	0	0	PB 70
0	1	0	0	0	0	1	PB 66
0	0	0	0	0	0	1	PB161
0	0	0	0	0	0	1	PB165
0	0	0	0	0	0	0	PB162
0	1	0	0	0	0	0	PB163
0	0	0	0	0	0	1	PB164
0	4	0	0	0	0	0	PB166
0	4	1	0	0	0	0	PB167

Table A.III.a continued

Characters							OTU
151	152	153	154	155	156	157	
0	5	0	0	0	0	0	PB168
4	1	0	0	0	0	1	PB 40
3	2	0	0	0	0	1	PB 72
3	3	0	0	0	0	1	PB 75
3	3	0	0	0	0	1	PB 73
1	1	0	0	0	0	1	PB 74
2	5	0	0	0	0	0	PB 54
0	4	0	0	0	0	0	PB 55
0	5	0	0	0	0	0	PB 61
0	5	0	0	0	0	0	PB 62
0	5	0	0	0	0	0	PB 64
0	5	0	0	0	0	0	PB 65
4	1	0	0	0	0	0	PB 41
5	3	0	0	0	0	0	PB 63
5	5	0	0	0	0	0	PB 44
3	3	0	0	0	0	0	PB103
3	4	0	0	0	0	0	PB104
0	5	5	3	0	0	0	PB132
5	4	0	0	0	0	0	PB120
2	2	0	0	0	0	0	PB121
4	3	0	0	0	0	0	PB105
5	5	0	0	0	0	0	PB155
0	1	2	0	0	0	0	PB 45
4	4	0	0	0	0	0	PB142
5	5	5	0	0	0	1	PB127
2	4	0	0	0	0	0	PB139
3	3	0	0	0	0	0	PB141
0	4	0	0	0	0	0	PB133
4	4	1	0	0	0	0	PB134
3	4	1	0	0	0	0	PB137
2	4	1	0	0	0	1	PB138
5	5	1	0	0	0	1	PB147
4	4	0	0	0	0	1	PB135
5	4	1	0	0	0	1	PB136
0	5	3	3	0	0	0	PB 42
3	3	0	0	0	0	0	PB122
1	4	0	0	0	0	0	PB191
5	4	1	0	0	0	0	PB 50
1	1	0	0	0	0	1	PB118
1	3	0	0	0	0	1	PB119
5	5	0	0	0	0	0	PB106
4	4	0	0	0	0	0	PB117
4	4	0	0	0	0	0	PB116
2	0	0	0	0	5	0	PB158
1	1	1	1	0	0	0	PB159
0	2	2	5	0	0	0	PB160
3	3	0	3	0	0	0	PB192
0	0	2	2	0	0	0	PB108
0	0	1	0	0	0	0	PB111
0	0	0	0	0	0	0	PB113
0	2	0	0	0	0	0	PB112
0	1	0	0	0	0	0	PB114

Appendix IV

Similarity matrix appendix

Figure A.IV.1 $\underline{S}_G$ average linkage matrix

Figure A.IV.2 $\underline{S}_G$ single linkage matrix

Figure A.IV.3 $\underline{D}_P$ average linkage matrix

Figure A.IV.4 $\underline{S}_G$ average linkage matrix from identification matrix PDBSTP2

See back pocket

Appendix V

Vigour appendix

Table A.V.a List of vigour values for all OTUs

Table A.V.a Vigour values.

Strain.	Received as.	Vigour.
PB 1	*S. faecalis*	0.573
PB 2	*S. faecalis*	0.592
PB 3	"*S. faecalis* subsp. *liquefaciens*"	0.618
PB 4	"*S. faecalis* subsp. *zymogenes*"	0.580
PB 5	*S. faecium*	0.490
PB 6	*S. faecium*	0.497
PB 7	*S. bovis*	0.484
PB 8	*S. bovis*	0.465
PB 9	*S. salivarius*	0.427
PB 10	"*S. durans*"	0.490
PB 11	*S. equinus*	0.382
PB 12	*S. equinus*	0.376
PB 13	*S. equinus*	0.382
PB 14	"*S. avium*"	0.561
PB 15	"*S. avium*"	0.554
PB 16	"*S. avium*"	0.548
PB 17	"*S. avium*"	0.586
PB 18	"*Streptococcus* sp. (D)"	0.599
PB 19	"*Streptococcus* sp. (D)"	0.611
PB 20	"*Streptococcus* sp. (D)"	0.586
PB 21	"*Streptococcus* sp. (D)"	0.561
PB 22	"*Streptococcus* sp. (D)"	0.580
PB 23	"*Streptococcus* sp. (D)"	0.586
PB 24	"*Streptococcus* sp. (D)"	0.599
PB 25	"*Streptococcus* sp. (D)"	0.573

Table A.V.a Vigour values continued.

Strain.	Received as.	Vigour.
PB 26	"Streptococcus sp. (D)"	0.561
PB 27	"Streptococcus sp. (D)"	0.573
PB 28	"Streptococcus sp. (D)"	0.580
PB 29	"Streptococcus sp. (D)"	0.573
PB 30	"Streptococcus sp. (D)"	0.586
PB 31	"Streptococcus sp. (D)"	0.580
PB 32	"Streptococcus sp. (D)"	0.586
PB 33	"Streptococcus sp. (D)"	0.567
PB 34	"Streptococcus sp. (D)"	0.580
PB 35	S. thermophilus	0.325
PB 36	S. thermophilus	0.376
PB 37	S. thermophilus	0.382
PB 38	S. thermophilus	0.312
PB 39	S. thermophilus	0.299
PB 40	S. agalactiae	0.414
PB 41	S. equi	0.369
PB 42	"S. infrequens"	0.465
PB 43	"Streptococcus sp. (F)"	0.350
PB 44	"Streptococcus sp. (G)"	0.452
PB 45	"Streptococcus sp. (H)"	0.401
PB 46	"Streptococcus sp. (K)"	0.357
PB 47	"Streptococcus sp. (M)"	0.344
PB 48	"Streptococcus sp. (N)"	0.376
PB 49	"Streptococcus sp. (O)"	0.439
PB 50	S. uberis	0.465
PB 51	"S. faecium subsp. casseliflavus"	0.433

Table A.V.a Vigour values continued.

Strain.	Received as.	Vigour.
PB 52	"S. faecium subsp. casseliflavus"	0.439
PB 53	"S. faecium subsp. mobilis"	0.548
PB 54	S. pyogenes	0.382
PB 55	S. pyogenes	0.395
PB 56	"S. milleri"	0.376
PB 57	"S. milleri"	0.369
PB 58	S. rattus	0.471
PB 59	"S. sobrinus"	0.420
PB 60	"S. sobrinus"	0.395
PB 61	S. pyogenes	0.401
PB 62	S. pyogenes	0.401
PB 63	S. equi	0.376
PB 64	S. pyogenes	0.414
PB 65	S. pyogenes	0.389
PB 66	S. sanguis	0.452
PB 67	S. sanguis	0.376
PB 68	S. sanguis	0.344
PB 69	"S. faecalis subsp. malodoratus"	0.561
PB 70	S. pneumoniae	0.344
PB 71	"Streptococcus sp. (N)"	0.389
PB 72	S. agalactiae	0.408
PB 73	S. agalactiae	0.395
PB 74	S. agalactiae	0.395
PB 75	S. agalactiae	0.420
PB 76	S. mutans	0.446
PB 77	S. faecalis	0.573

Table A.V.a Vigour values continued.

Strain.	Received as.	Vigour.
PB 78	*S. bovis*	0.420
PB 79	"*S. faecalis* subsp. *zymogenes*"	0.567
PB 80	*S. bovis*	0.427
PB 81	*S. bovis*	0.401
PB 82	*S. equinus*	0.420
PB 83	*S. equinus*	0.382
PB 84	*S. thermophilus*	0.344
PB 85	"*S. faecalis* subsp. *liquefaciens*"	0.592
PB 86	"*S. durans*"	0.471
PB 87	*S. faecium*	0.535
PB 88	"*S. avium*"	0.561
PB 89	*Streptococcus* sp.	0.344
PB 90	"*Streptococcus* sp. (O)"	0.357
PB 91	"*Streptococcus* sp. (O)"	0.369
PB 92	"*Streptococcus* sp. (strain MG)"	0.420
PB 93	*S. lactis*	0.446
PB 94	*S. lactis*	0.433
PB 95	*S. cremoris*	0.382
PB 96	*S. cremoris*	0.357
PB 97	"*S. durans*"	0.535
PB 98	*S. mitis*	0.318
PB 99	*S. salivarius*	0.459
PB 100	*S. salivarius*	0.395
PB 101	*S. mitis*	0.389
PB 102	*Streptococcus* sp.	0.389
PB 103	"*S. equisimilis*"	0.427

Table A.V.a Vigour values continued.

Strain.	Received as.	Vigour.
PB 104	"S. equisimilis"	0.459
PB 105	"S. equisimilis"	0.382
PB 106	"S. dysgalactiae"	0.478
PB 107	A. viridans	0.459
PB 108	P. pentosaceus	0.465
PB 109	A. catalyticus	0.503
PB 110	A. catalyticus	0.516
PB 111	P. acidilacti	0.408
PB 112	A. viridans	0.471
PB 113	P. halophilus	0.389
PB 114	P. damnosus	0.497
PB 115	"S. suis"	0.395
PB 116	"S. dysgalactiae"	0.497
PB 117	"S. dysgalactiae"	0.459
PB 118	S. uberis	0.465
PB 119	S. uberis	0.497
PB 120	"S. zooepidemicus"	0.433
PB 121	"S. zooepidemicus"	0.414
PB 122	"Streptococcus sp. (E)"	0.471
PB 123	"S. suis"	0.427
PB 124	"S. cremoris subsp. alactosus"	0.338
PB 125	S. faecalis	0.586
PB 126	"S. faecalis subsp. zymogenes"	0.548
PB 127	"Streptococcus sp. (B)"	0.516
PB 128	"S. faecalis subsp. liquefaciens"	0.586
PB 129	S. faecium	0.522

Table A.V. a Vigour values continued.

Strain.	Received as.	Vigour.
PB 130	"S. lactis subsp. diacetylactis"	0.439
PB 131	"S. lactis subsp. diacetylactis"	0.484
PB 132	"S. equisimilis"	0.433
PB 133	"Streptococcus sp. (B)"	0.408
PB 134	"Streptococcus sp. (B)"	0.427
PB 135	"Streptococcus sp. (B)"	0.471
PB 136	"Streptococcus sp. (B)"	0.452
PB 137	"Streptococcus sp. (B)"	0.484
PB 138	"Streptococcus sp. (B)"	0.439
PB 139	"Streptococcus sp. (B)"	0.490
PB 140	"Streptococcus sp. (D)"	0.548
PB 141	"Streptococcus sp. (B)"	0.510
PB 142	"Streptococcus sp. (G)"	0.459
PB 143	S. salivarius	0.465
PB 144	"S. milleri"	0.369
PB 145	"S. mitior"	0.344
PB 146	S. sanguis	0.363
PB 147	"Streptococcus sp. (B)"	0.452
PB 148	Streptococcus sp.	0.401
PB 149	Streptococcus sp.	0.414
PB 150	Streptococcus sp.	0.376
PB 151	"S. mutans subsp. mutans"	0.395
PB 152	Streptococcus sp.	0.484
PB 153	"Streptococcus sp. (D)"	0.522

Table A.V. a Vigour values continued.

Strain.	Received as.	Vigour.
PB 154	"Streptococcus sp. (K)"	0.541
PB 155	"Streptococcus sp. (L)"	0.420
PB 156	"Streptococcus sp. (L)"	0.529
PB 157	"Streptococcus sp. (M)"	0.357
PB 158	"Streptococcus sp. (P)"	0.516
PB 159	"Streptococcus sp. (R)"	0.408
PB 160	"Streptococcus sp. (S)"	0.439
PB 161	Gemella haemolysans	0.344
PB 162	Leuconostoc paramesenteroides	0.338
PB 163	L. oenos	0.389
PB 164	L. cremoris	0.401
PB 165	L. lactis	0.306
PB 166	L. dextranicum	0.357
PB 167	L. mesenteroides	0.331
PB 168	P. halophilus	0.376
PB 169	P. acidilacti	0.554
PB 170	Streptococcus sp.	0.325
PB 171	Streptococcus sp.	0.331
PB 172	Streptococcus sp.	0.395
PB 173	Streptococcus sp.	0.363
PB 174	Streptococcus sp.	0.452
PB 175	Streptococcus sp.	0.408
PB 176	Streptococcus sp.	0.382
PB 177	Streptococcus sp.	0.382

Table A.V. a Vigour values continued.

Strain.	Received as.	Vigour.
PB 178	Streptococcus sp.	0.376
PB 179	Streptococcus sp.	0.408
PB 180	Streptococcus sp.	0.433
PB 181	Streptococcus sp.	0.389
PB 182	Streptococcus sp.	0.344
PB 183	Streptococcus sp.	0.331
PB 184	Streptococcus sp.	0.344
PB 185	Streptococcus sp.	0.331
PB 186	Streptococcus sp.	0.369
PB 187	Streptococcus sp.	0.414
PB 188	Streptococcus sp.	0.382
PB 189	Streptococcus sp.	0.408
PB 190	Streptococcus sp.	0.357
PB 191	"Streptococcus sp. (M)"	0.395
PB 192	"Streptococcus sp. (T)	0.471
PB 193	"S. lactis subsp. diacetylactis"	0.446
PB 194	"S. lactis subsp. diacetylactis"	0.446
PB 195	"S. lactis subsp. diacetylactis"	0.503
PB 196	S. lactis	0.439
PB 197	S. cremoris	0.401
PB 198	Streptococcus sp.	0.439
PB 199	S. raffinolactis	0.395
PB 200	S. raffinolactis	0.369
PB 201	Streptococcus sp.	0.427
PB 202	Streptococcus sp.	0.471

Appendix VI

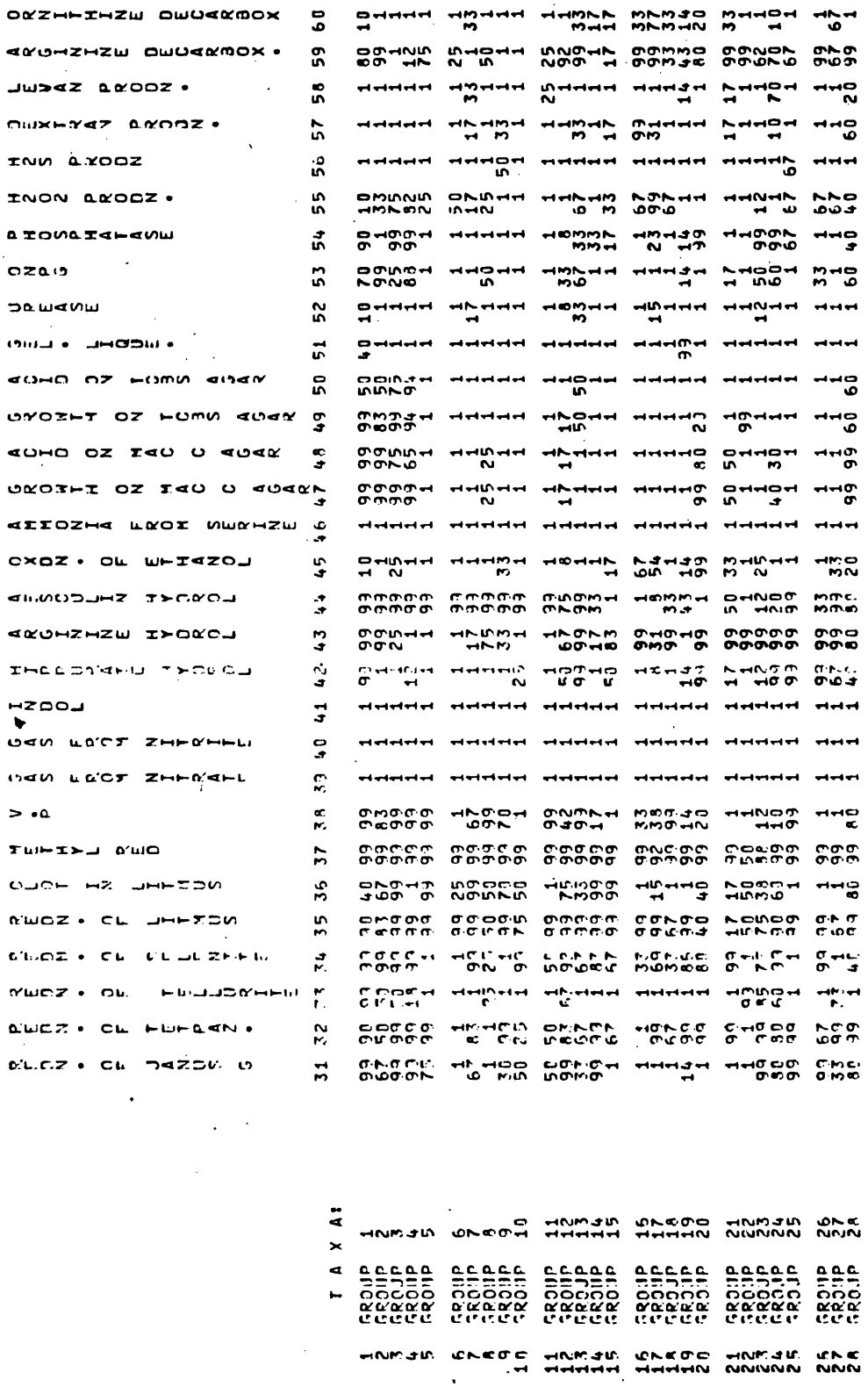
Integer groups appendix

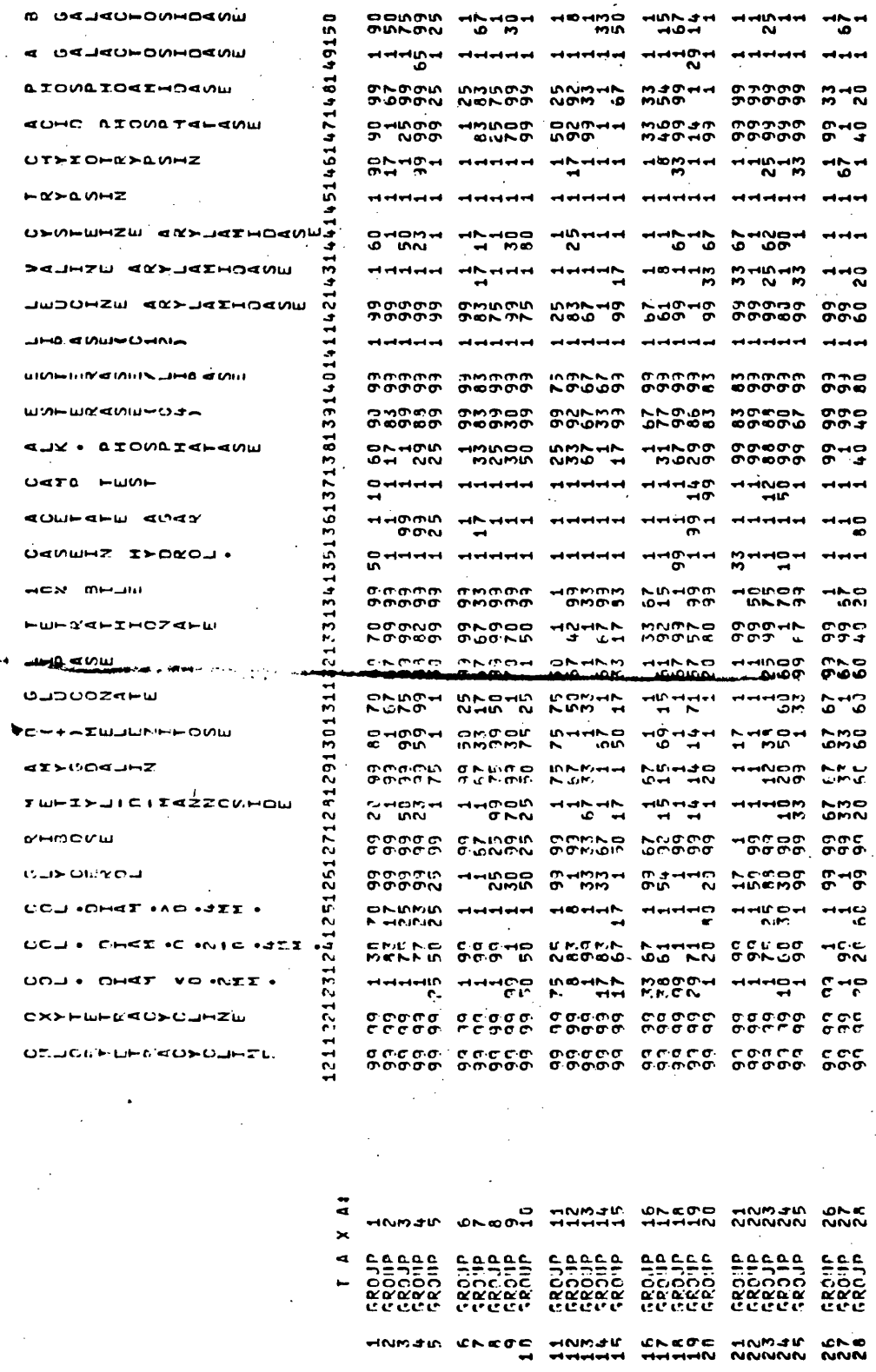
Table A.VI.a Percent positive results for each subphenon.

Table A.VI.b OVCLUST results for adjacent subphenons.

Table A.VI.c OVCLUST results for some other pairs of subphenons.

	TAXA1		GRAM +VE	COLONY ENTIRE	COLONY MUCOID	COLONY CONVEX	COLONY GREY/WHITE	COLONY YELLOW	OXIDASE	CATALASE	ALPHA HAEM.	BETA HAEM.	NO HAEM.	FINAL PH 4.25-4.75	FINAL PH 5.4-5.75	GROWTH AT 4 C	GROWTH AT 10 C	GROWTH AT 25 C	GROWTH AT 45 C	TOLERANCE OF 3%NACL	TOLERANCE OF 4%NACL	TOLERANCE OF 6.5%NAC	GROWTH WITH NA AZIDE	THALLOUS ACETATE	0.0002% CRYST.VIOL.	0.0004% CRYST.VIOL.	BILE SOLUBILITY	GROWTH AT PH 9.6	REDN. OF NITRATE	REDN. OF NITRITE	REDN. OF METH.BLUE
			CHARACTERS																												
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	GROUP	1	99	99	1	99	99	1	1	1	40	1	60	99	1	1	10	99	99	99	99	99	99	99	99	20	10	1	99	1	99	99
2	GROUP	2	99	99	1	99	99	1	1	1	83	1	17	99	1	1	99	99	99	99	99	99	99	99	99	17	1	1	99	17	99	67
3	GROUP	3	99	99	1	99	99	1	1	1	99	1	1	99	1	1	1	99	99	99	99	99	99	99	99	75	25	1	99	1	75	25
4	GROUP	4	99	99	1	99	99	1	1	1	99	1	1	99	1	1	6	99	99	99	99	99	99	99	99	53	12	1	99	47	99	1
5	GROUP	5	99	99	1	99	99	1	1	1	50	1	50	99	1	1	1	1	99	99	99	99	1	75	99	1	1	1	1	1	99	1
6	GROUP	6	99	99	1	99	99	1	1	1	25	1	75	99	1	1	1	1	99	99	99	99	1	99	99	1	1	1	1	1	99	1
7	GROUP	7	99	99	1	99	99	1	1	1	67	1	33	99	1	1	1	17	99	83	67	50	1	83	99	67	50	1	1	1	50	33
8	GROUP	8	99	99	1	99	99	1	1	1	75	1	25	75	25	1	1	25	99	99	99	99	1	99	99	25	1	1	50	50	99	1
9	GROUP	9	99	99	1	99	99	1	1	1	1	1	99	99	1	1	1	1	67	99	67	67	1	99	99	99	1	1	1	70	99	70
10	GROUP	10	99	99	1	99	99	1	1	1	1	1	99	99	1	1	1	75	99	1	75	75	25	99	99	75	1	1	1	1	1	1
11	GROUP	11	99	99	1	99	99	1	1	1	25	1	75	99	1	1	1	1	25	25	1	1	1	1	99	99	75	1	1	1	99	25
12	GROUP	12	99	99	1	99	99	1	1	1	67	1	33	92	8	1	1	92	99	1	99	75	1	83	99	25	1	1	1	8	25	75
13	GROUP	13	99	99	1	99	99	1	1	1	67	1	33	99	1	1	1	99	99	99	67	33	33	1	99	67	33	1	33	1	33	99
14	GROUP	14	99	99	1	99	99	1	1	1	67	1	33	99	1	1	1	1	99	99	99	17	1	83	99	99	1	1	1	1	99	1
15	GROUP	15	99	99	1	99	99	1	1	1	83	17	1	33	67	1	1	33	83	99	99	33	1	33	99	17	1	1	1	17	1	99
16	GROUP	16	99	99	1	99	99	1	1	1	99	1	1	67	33	1	33	1	99	99	99	1	1	1	99	33	1	1	1	1	67	1
17	GROUP	17	99	99	1	99	99	1	1	1	99	1	1	92	8	1	1	15	23	38	1	1	1	54	77	99	99	1	1	8	92	8
18	GROUP	18	99	99	1	99	99	1	1	1	33	33	33	99	1	1	1	1	99	33	67	67	1	99	99	99	33	1	1	67	67	67
19	GROUP	19	99	99	1	99	99	1	1	1	99	1	1	14	1	85	14	43	99	1	29	1	1	14	57	57	99	1	1	24	99	99
20	GROUP	20	99	99	1	99	99	1	1	1	20	80	1	99	1	1	1	1	99	20	99	99	1	99	99	99	99	1	1	1	1	1
21	GROUP	21	99	99	1	99	99	1	1	1	1	99	1	1	99	1	1	1	99	1	99	1	1	99	99	99	99	1	1	1	83	1
22	GROUP	22	99	99	1	99	99	1	1	1	1	99	1	50	50	1	1	1	99	1	99	1	1	99	99	1	1	1	1	99	99	50
23	GROUP	23	99	99	1	99	99	1	1	1	12	88	1	50	50	1	1	38	99	1	88	62	1	75	99	1	1	1	1	25	75	12
24	GROUP	24	99	99	1	99	99	1	1	1	60	30	10	70	30	1	1	1	88	70	99	99	1	40	99	10	1	1	1	1	50	70
25	GROUP	25	99	99	1	99	99	1	1	1	99	1	1	1	99	1	1	99	99	1	99	99	1	67	99	99	99	1	1	1	1	1
26	GROUP	26	99	99	1	99	99	1	1	1	99	1	1	33	67	1	1	1	99	1	99	67	1	99	99	99	33	1	1	1	67	1
27	GROUP	27	99	99	1	99	99	1	1	1	1	1	99	1	99	1	1	67	67	33	33	33	1	67	99	33	99	1	1	1	67	67
28	GROUP	28	99	99	1	99	99	1	1	1	40	1	60	60	40	1	1	60	99	80	99	80	50	60	99	99	80	1	20	1	60	40





T A X A:		B GLUCORONIDASE	A GLUCOSIDASE	B GLUCOSIDASE	N ACETYL B GLUCOSAMI	A MANNOSIDASE	A FUCOSIDASE	40% BILE
		151	152	153	154	155	156	157
1	GROUP 1	1	99	99	30	1	1	99
2	GROUP 2	1	1	99	1	1	1	99
3	GROUP 3	1	25	50	1	1	1	99
4	GROUP 4	88	1	99	99	1	1	99
5	GROUP 5	1	99	50	1	1	1	99
6	GROUP 6	1	99	99	1	1	1	99
7	GROUP 7	1	67	50	1	1	1	17
8	GROUP 8	1	25	1	1	1	1	99
9	GROUP 9	1	70	99	1	1	1	70
10	GROUP 10	1	99	99	1	1	1	1
11	GROUP 11	1	25	25	1	1	1	1
12	GROUP 12	1	83	92	1	1	1	42
13	GROUP 13	1	33	1	1	1	1	1
14	GROUP 14	1	1	1	1	1	1	99
15	GROUP 15	1	1	17	1	1	1	33
16	GROUP 16	1	33	1	1	1	1	67
17	GROUP 17	1	38	1	8	1	1	1
18	GROUP 18	1	99	33	1	1	1	1
19	GROUP 19	1	29	1	1	1	1	43
20	GROUP 20	17	1	1	1	1	99	0
21	GROUP 21	17	99	1	1	1	1	1
22	GROUP 22	99	50	1	1	1	1	1
23	GROUP 23	88	99	12	12	1	1	1
24	GROUP 24	90	99	10	1	1	1	50
25	GROUP 25	33	67	1	1	1	1	67
26	GROUP 26	99	99	1	1	1	1	1
27	GROUP 27	33	67	33	67	1	1	1
28	GROUP 28	1	20	20	20	1	1	1

Table A.VI.b OVCLUST results for adjacent subphenons.

Subphenon 1 and Subphenon 2.

S. faecalis and S. faecium

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.345	0.586	0.232	10	6

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	F
4.0158	5.92522E-5	16.0635	12.7502

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
2.51293	1.19734E-5	10.0517

$T_{(O)},P=0.90$	$T_{(O)},P=0.95$	$T_{(O)},P=0.99$
12.5649	13.8418	16.8313

Subphenon 2 and subphenon 3.

S. faecium and "S. avium"

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,MO}$	$N_{(L)}$	$N_{(M)}$
4.03	0.268	0.15	6	4

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	F
9.60732	2.09359E-18	30.381	7.89689

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
8.75217	2.09359E-18	27.6768

$T_{(O)},P=0.90$	$T_{(O)},P=0.95$	$T_{(O)},P=0.99$
11.1106	12.6546	16.5433

Table A.VI.b continued.

Subphenon 3 and Subphenon 4

S. avium and Streptococcus sp. (chicken)

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.801	3.588	0.284	4	17

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
2.74686	6.01690E-3	12.5877	3.33649

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
0.262824	0.792686	1.20441

$T_{(O)},P=0.90$	$T_{(O)},P=0.95$	$T_{(O)},P=0.99$
22.0598	27.4951	48.2814

Subphenon 4 and Subphenon 5

Streptococcus sp. (chicken) and S. bovis

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
5.146	0.246	0.395	17	4

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
5.44288	5.25498E-8	24.9424	3.56698

Correction for error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
4.70624	2.52617E-6	21.5667

$T_{(O)},P=0.90$	$T_{(O)},P=0.95$	$T_{(O)},P=0.99$
21.2683	26.2625	39.3013

Table A.VI.b continued.

Subphenon 5 and Subphenon 6

S. bovis and S. equinus

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.509	0.187	0.221	4	4

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	F
8.57078	1.02826E-17	24.2418	5.84001

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
7.33712	2.81360E-13	20.7525

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
10.8927	12.7563	17.6931

Subphenon 6 and Subphenon 7

S. equinus and S. salivarius

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.726	0.395	0.467	4	6

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	F
4.29228	1.76970E-5	13.5734	7.36101

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
1.65037	9.88682E-2	5.21891

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
11.3111	12.9546	17.1454

Table A.VI.b continued.

Subphenon 7 and Subphenon 8

S. salivarius and S. casseliflavus

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.748	0.448	0.404	6	4

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	F
4.34948	1.36563E-5	13.7543	7.08001

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
1.79388	7.28321E-2	5.67275

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
11.4293	13.132	17.5037

Subphenon 8 and Subphenon 9

S. casseliflavus and S. mutans

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.918	0.196	0.097	4	3

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	F
13.1197	2.53863E-39	34.7116	4.55127

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
12.2351	2.01773E-34	32.3712

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
11.1901	13.4813	19.7351

Table A.VI.b continued.

Subphenon 10 and Subphenon 11

S. raffinolactis and "Oral I"

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
4.085	0.318	0.347	4	4

$V_{(0)} = 0.025$

$W_{(0)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
6.13702	8.41429E-10	17.3581	5.95487

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
4.24712	2.16676E-5	12.0127

$T_{(0)}$,P=0.90	$T_{(0)}$,P=0.95	$T_{(0)}$,P=0.99
10.8257	12.6538	17.4814

Subphenon 11 and Subphenon 12

"Oral I" and S. lactis

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
4.544	0.582	0.421	4	12

$V_{(0)} = 0.025$

$W_{(0)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
3.60225	3.15551E-4	14.409	4.10374

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
1.77868	7.52928E-2	7.1147

$T_{(0)}$,P=0.90	$T_{(0)}$,P=0.95	$T_{(0)}$,P=0.99
17.3824	21.0954	31.1069

Table A.VI.b continued.

Subphenon 9 and Subphenon 10

S. mutans and S. raffinolactis

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.467	0.33	0.342	3	4

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
5.11857	3.08413E-7	13.5425	4.55114

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
1.94194	5.21438E-2	5.1379

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
11.1903	13.4815	19.7355

Subphenon 17 and Subphenon 19

"Oral II" and Leuconostoc sp.

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.718	0.303	0.441	13	17

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
4.99041	6.03464E-7	27.3336	27.751

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
4.43518	9.20731E-6	24.2925

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
15.3042	16.2907	18.4094

Table A.VI.b continued.

Subphenon 14 and Subphenon 15

S. thermophilus and S. mitis

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
4.028	0.554	0.296	6	6

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	F
4.53454	5.77843E-6	15.7081	7.63961

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{W,EST)}$
2.73473	6.24332E-3	9.47337

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
12.21	13.9269	18.2836

Subphenon 19 and Subphenon 20

Leuconostoc sp. and S. agalactiae

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
5.101	0.304	0.364	7	5

$V_{(O)}$ = 0.025

$W_{(O)}$ = 2.24138

W	$V_{(G)}$	$T_{(W)}$	G
7.39029	1.46590E-10	25.6007	7.70378

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
6.44461	1.15976E-10	22.3248

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
12.1839	13.888	18.2059

Table A.VI.b continued.

Subphenon 20 and Subphenon 21

S. agalactiae and S. pyogenes

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
4.242	0.239	0.228	5	6

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
9.0241	1.81246E-19	29.9295	8.47033

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
8.19522	2.50246E-16	27.1805

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
11.4256	12.9377	16.7031

Subphenon 23 and Subphenon 24

Streptococcus sp. (Group B) and S. equisimilis

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
2.831	0.364	0.438	8	10

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
5.52929	4.16759E-4	14.9735	15.9568

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
1.93227	5.33259E-2	8.19793

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
12.8258	13.9682	16.5632

Table A.VI.c OVCLUST results for some other pairs of subphenons.

Subphenon 10 and Subphenon 12

S. raffinolactis and S. lactis

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.379	0.264	0.599	4	12

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
3.88318	1.03141E-4	15.5327	12.2734

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
2.2949	2.17389E-2	9.17959

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
12.6481	13.9618	17.0553

Subphenon 7 and Subphenon 11

S. salivarius and "Oral I"

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.569	0.71	0.642	6	4

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
2.60951	9.06730E-3	8.25199	7.0675

Correction for sampling error makes $D_{(L,M)}$ negative.

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
11.4348	13.1403	17.5204

Table A.VI.c continued.

Subphenon 2 and Subphenon 8

S. faecium and S. casseliflavus

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.99	0.221	0.3	6	4

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
7.20821	5.67246E-13	22.7944	5.15828

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
6.02148	1.72955E-9	19.0416

$T_{(O)}$,P=Q.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
12.6263	14.9546	21.2233

Subphenon 1 and Subphenon 12

S. faecalis and S. lactis

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
4.141	0.498	0.514	10	12

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
4.08031	4.9991E-5	19.1384	19.4991

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
3.08425	2.04082E-3	14.4664

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
13.7317	14.8153	17.2233

Table A.VI.c continued.

Subphenon 11 and Subphenon 17

"Oral I" and "Oral II"

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.753	0.342	0.554	4	13

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
3.95942	7.51639E-5	16.3251	8.42669

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
2.53806	0.011147	10.4647

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
14.0702	15.9008	20.4838

Subphenon 15 and Subphenon 17

S. mitis and "Oral II"

$D_{(L,M)}$	$S_{(Q,L)}$	$S_{(Q,M)}$	$N_{(L)}$	$N_{(M)}$
3.803	0.286	0.396	6	13

$V_{(O)} = 0.025$

$W_{(O)} = 2.24138$

W	$V_{(G)}$	$T_{(W)}$	F
5.44279	5.25761E-8	23.7246	13.3937

Correction for sampling error.

$W_{(EST)}$	$V_{(G,EST)}$	$T_{(W,EST)}$
4.62177	3.80876E-6	20.1458

$T_{(O)}$,P=0.90	$T_{(O)}$,P=0.95	$T_{(O)}$,P=0.99
13.5212	14.8438	17.9258

Appendix VII

Identification matrix appendix

Table A.VII.a Variances and standard deviations of groups in identification matrix PDBSTP.

Table A.VII.b Variances and standard deviations of groups in identification matrix PDBSTP2.

Table A.VII.c MOSTTYP results for identification matrix PDBSTP

Table A.VII.d MOSTTYP results for identification matrix PDBSTP2

Table A.VII.e Literature results on which the matrix PDBSTP is based

Table A.VII.f OVERMAT results for identification matrix PDBSTP

Table A.VII.g OVERMAT results for identification matrix PDBSTP2

Table A.VII.a Variances and standard deviations of groups in identification matrix PDBSTP.

Taxon.	No.	Variance.	Std. Deviation.
S. faecalis	15	4.23938E-2	0.205898
S. faecium	15	9.34836E-2	0.305751
"S. avium"	15	7.34044E-2	0.270933
"Strep. sp. (chicken)"	15	5.04324E-2	0.224572
S. bovis	15	6.79923E-2	0.260753
S. equinus	15	7.42934E-2	0.272568
S. salivarius	15	8.91834E-2	0.298636
"S. casseliflavus"	15	0.132675	0.364245
S. mutans	15	9.84961E-2	0.313841
S. raffinolactis	15	0.107273	0.327526
"Oral I"	15	8.42519E-2	0.290262
S. lactis	15	0.133795	0.36578
A. viridans	15	0.130943	0.361861
S. thermophilus	15	6.36863E-2	0.252361
S. mitis	15	0.118891	0.344806
S. sanguis	15	9.77027E-2	0.312574
"Oral II"	15	0.126255	0.355324
"S. milleri"	15	8.45357E-2	0.29075
Leuconostoc sp.	15	0.113629	0.33709
S. agalactiae	15	6.39093E-2	0.252803
S. pyogenes	15	3.89653E-2	0.197396
S. equi	15	0.050027	0.223667
"S. equisimilis"	15	0.106598	0.326494
"Strep. sp. (B) clin."	15	0.110285	0.332091
S. uberis	15	7.69836E-2	0.277459
"S. dysgalactiae"	15	9.22268E-2	0.303689
"Strep.sp. (R,S & T)"	15	8.67249E-2	0.294491

Table A.VII.b Variances and standard deviations of groups in identification matrix PIBSTP2.

Taxon.	No.	Variance.	Std. deviation.
S. faecalis	10	7.08574E-2	0.266191
S. faecium	6	0.082672	0.287527
"S. avium"	4	9.35222E-2	0.305814
"Strep. sp. (chicken)"	17	4.94665E-2	0.222411
S. bovis	4	0.117642	0.34299
S. equinus	4	6.72844E-2	0.259392
S. salivarius	7	0.150755	0.388271
"S. casseliflavus"	4	0.135987	0.368764
S. mutans	3	0.105727	0.325158
S. raffinolactis	4	0.112527	0.33545
"Oral I"	4	0.121589	0.348696
S. lactis	12	0.129985	0.360535
A. viridans	3	0.163412	0.404243
S. thermophilus	6	0.09348	0.305745
S. mitis	6	0.146036	0.382147
S. sanguis	3	0.15213	0.390038
"Oral II"	13	0.139802	0.373902
"S. milleri"	3	0.12573	0.354584
Leuconostoc sp.	7	0.124575	0.352951
S. agalactiae	5	8.78375E-2	0.296374
S. pyogenes	6	0.076402	0.276409
S. equi	2	0.09183	0.303035
"S. equisimilis"	8	0.113811	0.337359
"Strep. sp. (B) clin."	10	0.134222	0.366364

Table A.VII.b continued.

Taxon.	No.	Variance.	Std. deviation.
S. uberis	3	0.09933	0.315167
"S. dysgalactiae"	3	0.08877	0.297943
"Strep. sp. (R, S & T)"	3	0.14157	0.376258
Pediococcus sp.	5	0.202681	0.450201

Table A.VII.c MOSTTYP results for identification matrix PDBSTP.

```
PROGRAM MOSTTYP FOR CALCULATING IDENTIFICATION
SCORES OF MOST TYPICAL OTU'S
(FILE NAME PHB7M21).

THIS IS A PROGRAM TO EVALUATE AN IDENTIFICATION
MATRIX BY CALCULATING IDENTIFICATION SCORES
OF THE MOST TYPICAL OTU AGAINST THE TAXA

THIS PROGRAM CALCULATES IDENTIFICATION STATISTICS
OF THE MOST TYPICAL OTU OF A TAXON THAT IS
POSSIBLE (HYPOTHETICAL MEDIAN ORGANISM, MMO)
WHEN COMPARED WITH THE CLOSEST TAXA,
HOPEFULLY INCLUDING ITS OWN TAXON.

IDENTIFICATION MATRIX BY FUNTHAN PROGRAM MJSS6ER.

THE PROGRAM COMPARES THE MMO #, WHOSE VALUE
M(I,K) FOR CHARACTER I, TAXON K, IS
0 IF P(I,K) < 0.5, ELSE 1, AGAINST THE TAXA.
IT THEN CALCULATES THE FOLLOWING STATISTICS

  (1) WILLCOX'S PROBABILITIES, = LL(K)/SUM
LL(K) FOR K = 1 TO G, WHERE L(K) = PRODUCT
ABS(M(I,K) - P(I,J) - J).
  (2) TAXONOMIC DISTANCE, D, = SUM1 (SUM
(M(I,K) - P(I,J))*(M(I,K) - P(I,J))/N).
  (3) STANDARD ERROR SCORE OF D, WHICH
IS C IN D = MEAN D(J) + C*S(D(J)).
WHERE MEAN D(J) IS APPROXIMATED BY SUM1(MEAN
D(J) SQUARED * (2N - 1)/ 2N), AND MEAN
D(J) SQUARED IS SUM(P(I,J)*(1 - P(I,J)))/N
MULTIPLIED BY THE CONNECTION FACTOR IN
D(J), (THIS IS N(J)/(N(J) - 1) IF N>1)
IS GIVEN, OTHERWISE B(J) = 1.  S(D(J))
IS APPROXIMATED BY SUM1 (MEAN D
SQUARED /2N).
  (4) GAUSSIAN INTEGRAL OF STANDARD
ERROR SCORE OF D.

NUMBERS OF OTU'S, N(J), ON WHICH EACH
TAXON J IS BASED, MAY BE GIVEN AS
DATA AT 9001 COMMANDS, AND THESE OVERWRITE
A ZERO VALUE AT 9001 WHICH OTHERWISE
SETS DEGREES OF FREEDOM CONNECTION FACTOR
B(J) TO UNITY.  NUMBERS MUST BE OVER
1, ELSE PROGRAM FAILS.

PDBSTP IS A MATRIX OF 37 TESTS FOR 27 GROUPS OF STREPTOCOCCI

 M              0
 N             27
TAXON NUMBER  TAXON NAME      N(J)
 1             STREP. FAECALIS          15
 2             STREP. FAECIUM           15
 3             STREP. AVIUM             15
 4             STREP. SP.(CHICKEN)      15
 5             STREP. BOVIS             15
 6             STREP. EQUINUS           15
 7             STREP. SALIVARIUS        15
 8             STREP. CASSELIFLAVU      15
 9             STREP. MUTANS            15
 10            STREP. RAFFINOLACTI      15
 11            UNAL STREPTOCOCCI 1      15
 12            STREP. LACTIS            15
 13            AEROCOCCUS VIRIDANS      15
 14            STREP. THERMOPHILUS      15
 15            STREP. MITIS             15
 16            STREP. SANGUIS           15
 17            UNAL STREPTOCOCCI 2      15
 18            STREP. MILLERI           15
 19            LEUCONOSTOC/GEMELLA      15
 20            STREP. AGALACTIAE        15
 21            STREP. PYOGENES          15
 22            STREP. EQUI              15
 23            STREP. SANGUISIMILUS     15
 24            STREP. SP B              15
 25            STREP. UBERIS            15
 26            STREP. DYSGALACTIAE      15
 27            STREP. SP K,S,I          15

IDENTIFICATION SCORES OF MMO'S TO TAXA

MMO            1              STREP. FAECALIS

TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 1            1.             .110093        -3.61912       .999847
 2            2.779425E-10   .419036        3.59811        3.41397E-4
 6            2.693194E-12   .477841        2.7411         3.06174E-3

MMO            2              STREP. FAECIUM
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 2            .995872        .248126        -2.60849       .997452
 3            4.495764E-3    .31467         -1.10776       .866018
 5            3.18238E-5     .369627        3.19196        7.06622E-4

MMO            3              STREP. AVIUM
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 3            1.             .100867        -3.18253       .999264
 6            1.81093E-7     .416759        1.14577        8.91883E-2
 4            1.560452E-7    .371934        5.70312        5.88648E-4

MMO            4              STREP. SP.(CHICKEN)
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 4            .999993        .135407        -2.35716       .999606
 6            6.951181E-6    .427777        2.99819        5.84155E-3
 8            7.13501E-9     .437876        1.77834        3.76758E-2

MMO            5              STREP. BOVIS
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 5            .999999        .182475        -2.52409       .9942
 4            5.876408E-7    .419788        3.54818        1.98019E-4
 6            1.60417E-9     .485969        2.58408        4.88146E-3

MMO            6              STREP. EQUINUS
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 6            1.             .144127        -2.73291       .998861
 15           1.05462E-8     .421295        1.98435        2.47445E-2
 5            6.30986E-10    .42081         5.53864        4.69248E-6

MMO            7              STREP. SALIVARIUS
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 7            .999803        .167456        -3.1442        .999167
 4            1.942942E-4    .319126        -8.349438E-2   .517346
 17           1.69979E-4     .396607        1.05779        .145075

MMO            8              STREP. CASSELIFLAVU
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 8            .994897        .478427        -1.96844       .975491
 2            4.93077E-3     .333762        .8464427       .198657
 5            1.72364E-4     .39644         4.04329        2.03661E-5

MMO            9              STREP. MUTANS
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 9            .999843        .193827        -3.25316       .999256
 7            1.066491E-4    .344864        1.26705        8.58804E-2
 11           2.99244E-7     .36036         2.37268        8.82507E-3

MMO            10             STREP. RAFFINOLACTI
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 10           .999999        .248536        -2.81631       .976116
 11           2.62935E-7     .401366        3.35105        4.82584E-4
 4            8.881421E-8    .435536        3.39347        3.44487E-4

MMO            11             UNAL STREPTOCOCCI 1
TO TAXON      WILLCOX PROB   TAX DIST       S.E.(D)        GAU S.E.
 11           .999402        .174022        -3.3666        .9996646
 4            5.558408E-4    .335253        .6852231       .2549391
 17           6.12538E-5     .3741166       .5144888       .3603455
```

HMO	12	STREP. LACTIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
12	.999999	.261988	-2.38264	.991405
20	8.05223E-7	.376793	4.27744	9.45950E-6
24	1.22234E-8	.452665	3.18159	7.32410E-4

HMO	13	AEROCOCCUS VIRIDANS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
13	.999998	.260358	-2.35464	.99073
6	1.79655E-6	.441995	1.89452	2.90781E-2
2	8.81958E-8	.428123	3.50127	2.31569E-4

HMO	14	STREP. THERMOPHILUS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
14	.999536	.144325	-3.62435	.999855
15	4.64449E-4	.317264	-.628784	.735255
17	3.91466E-9	.425536	1.75816	3.93602E-2

HMO	15	STREP. MITIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
15	.999997	.257026	-2.13164	.983482
26	1.74854E-6	.409519	3.05607	1.12136E-3
16	6.46644E-7	.423816	3.11984	9.04799E-4

HMO	16	STREP. SANGUIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
16	.999978	.20834	-2.81031	.997525
7	2.07887E-5	.380618	2.41986	7.76332E-3
15	9.14708E-7	.395389	1.32029	.093369

HMO	17	ORAL STREPTOCOCCI 2		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
17	.999832	.235957	-2.83152	.997684
18	1.57581E-4	.35224	1.87759	3.02182E-2
15	3.64045E-6	.393333	1.269	.102221

HMO	18	STREP. MILLERI		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
18	.999649	.174813	-3.37188	.999627
17	3.49193E-4	.339316	-.329226	.629008
23	8.44170E-7	.397927	1.9404	2.61653E-2

HMO	19	LEUCONOSTOC/GEMELLA		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
19	1.	.239143	-2.44123	.992661
15	7.18686E-10	.479423	3.41679	3.16873E-4
17	6.90301E-10	.466152	2.74145	3.05849E-3

HMO	20	STREP. AGALACTIAE		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
20	.999992	.16709	-2.85831	.99787
24	7.50583E-6	.360986	.806787	.209894
23	2.01223E-8	.391767	1.77809	3.76944E-2

HMO	21	STREP. PYOGENES		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
21	.996408	.11082	-3.71456	.999898
18	3.02951E-3	.264611	-.715055	.762713
23	3.07217E-4	.301295	-.605629	.727619

HMO	22	STREP. EQUI		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
22	.99994	.144484	-2.98709	.998592
24	5.97364E-5	.30751	-.441876	.67071
17	2.00278E-9	.4287	1.83476	3.32709E-2

HMO	23	STREP. EQUISIMILIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
23	.999985	.222929	-2.67037	.996212
17	1.51932E-5	.41655	1.54059	.061708
24	5.23340E-8	.408739	2.04376	2.04886E-2

HMO	24	STREP. SP B		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
24	1.	.234895	-2.4594	.993042
20	5.37998E-9	.416322	5.62253	9.41655E-9
23	1.61817E-10	.456745	3.49012	2.41448E-4

HMO	25	STREP. UBERIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
25	1.	.189701	-2.66253	.996122
8	2.37593E-10	.485139	2.91344	1.78744E-3
13	7.83681E-11	.460555	2.40453	8.09659E-3

HMO	26	STREP. DYSGALACTIAE		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
26	.999983	.186366	-3.26497	.999453
17	1.71493E-5	.375608	.549345	.291367
15	2.04841E-7	.402838	1.50613	.066017

HMO	27	STREP. SP R,S,T		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
27	.999998	.16709	-3.07895	.998961
17	1.79686E-6	.369039	.874567	.190905
7	1.11096E-8	.436825	4.03892	2.68625E-5

Table A.VII.d MOSTTYP results for identification matrix PDBSTP2.

```
PROGRAM MOSTTYP FOR CALCULATING IDENTIFICATION
SCORES OF MOST TYPICAL OTU'S
(FILE NAME PHS7921).

THIS IS A PROGRAM TO EVALUATE AN IDENTIFICATION
MATRIX BY CALCULATING IDENTIFICATION SCORES
OF THE MOST TYPICAL OTU AGAINST THE TAXA

THIS PROGRAM CALCULATES IDENTIFICATION STATISTICS
OF THE MOST TYPICAL OTU OF A TAXON THAT IS
POSSIBLE (HYPOTHETICAL MEDIAN ORGANISM, MMO)
WHEN COMPARED WITH THE CLOSEST TAXA,
HOPEFULLY INCLUDING ITS OWN TAXON.

NUMBERS OF OTU'S, N(J), ON WHICH EACH
TAXON J IS BASED, MAY BE GIVEN AS
DATA AT 9001 ONWARDS, AND THESE OVERWRITE
A ZERO VALUE AT 9001 WHICH OTHERWISE
SETS DEGREES OF FREEDOM CORRECTION FACTOR
B(J) TO UNITY.  NUMBERS MUST BE OVER
1, ELSE PROGRAM FAILS.
PDBSTP2 IS A MATRIX OF 60 DIAGNOSTIC TESTS FOR THE STREPTOCOCCACEAE
 M              N
 60             28
IF OUTPUT AT THE TERMINAL IS TO BE SUPPRESSED,
ENTER 1, ELSE ENTER ZERO
? 0
TAXON NUMBER  TAXON NAME      N(J)
 1             S.FAECALIS                  10
 2             S.FAECIUM                   6
 3             S.AVIUM                     4
 4             S.SP.(CHICKEN)              17
 5             S.BOVIS                     4
 6             S.EQUINUS                   4
 7             S.SALIVARIUS                7
 8             S.CASSELIFLAVUS             4
 9             S.MUTANS                    3
 10            S.RAFFINOLACTIS             4
 11            ORAL 1                      4
 12            S.LACTIS                    12
 13            A.VIRIDANS                  3
 14            S.THERMOPHILUS              6
 15            S.MITIS                     6
 16            S.SANGUIS                   3
 17            ORAL 2                      13
 18            S.MILLERI                   3
 19            LEUCONOSTOC                 7
 20            S.AGALACTIAE                5
 21            S.PYOGENES                  6
 22            S.EQUI                      2
 23            S.EQUISIMILIS               8
 24            STREP.SP.B                  10
 25            S.UBERIS                    3
 26            S.DYSGALACTIAE              3
 27            STREP.SP.R,S,T              3
 28            PEDIOCOCCUS                 5
IDENTIFICATION SCORES OF MMO'S TO TAXA

MMO            1          S.FAECALIS
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 1              1            .14965      -4.75023       .999999
 2              1.12587E-16  .431014      5.51242       1.77115E-8
 12             1.75055E-21  .523304      4.99128       3.00369E-7

MMO            2          S.FAECIUM
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 2              1.           .180111     -4.04669       .999974
 1              1.51500E-9   .372015      4.40069       5.39979E-6
 3              3.78572E-10  .373753      2.47937       6.58069E-3

MMO            3          S.AVIUM
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 3              1.           .155        -5.35651       1.
 4              1.52182E-11  .35418       6.53581       3.16547E-11
 2              2.21197E-16  .411226      4.7585        9.76382E-7

MMO            4          S.SP.(CHICKEN)
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 4              1.           .114499     -5.26926       1.
 3              1.17662E-8   .326637      .791627       .214289
 1              3.06972E-24  .506355      9.92913       1.55521E-23

MMO            5          S.BOVIS
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 5              1.           .216645     -3.98949       .999967
 7              1.63576E-12  .426554      1.12582       .130121
 12             3.09655E-14  .471713      3.42377       3.08850E-4

MMO            6          S.EQUINUS
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 6              1            .140961     -4.95576       1.
 7              2.11536E-16  .476041      2.52201       5.83428E-3
 5              2.74270E-19  .450483      3.47886       2.51822E-4

MMO            7          S.SALIVARIUS
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 7              1.           .256804     -3.6634        .999876
 5              3.37836E-13  .431974      2.88772       1.94030E-3
 12             2.62786E-16  .490761      4.00252       3.13502E-5

MMO            8          S.CASSELIFLAVUS
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 8              1            .221382     -4.33236       .999993
 5              3.93192E-17  .459639      3.77129       8.12279E-5
 12             1.25657E-17  .513335      4.68841       1.37824E-6

MMO            9          S.MUTANS
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 9              1.           .167377     -5.26984       1.
 5              6.93130E-14  .423007      2.60133       4.64321E-3
 7              7.27217E-16  .463985      2.18186       1.45598E-2

MMO            10         S.RAFFINOLACTIS
TO TAXON       WILLCOX PROB  TAX DIST     S.E.(D)       GAU S.E.
 10             1.           .226064     -3.52636       .999789
 12             3.42109E-14  .480465      3.68968       1.12299E-4
 7              1.05880E-14  .470051      2.35303       9.31059E-3
```

HMO	11	ORAL 1		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
11	1	.219032	-4.02772	.999972
7	9.32339E-19	.503271	3.29026	5.00526E-4
17	4.40987E-20	.518766	4.28994	8.94247E-6

HMO	12	S.LACTIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
12	1.	.217976	-4.28577	.999991
7	8.57487E-13	.463985	2.18186	1.45598E-2
24	1.40437E-13	.450777	2.56975	5.08866E-3

HMO	13	A.VIRIDANS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
13	1	.230705	-4.6569	.999998
12	5.30741E-16	.488378	3.93012	4.24704E-5
7	9.58751E-18	.514731	3.6136	1.51024E-4

HMO	14	S.THERMOPHILUS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
14	1	.159478	-5.19481	1.
15	1.24250E-15	.444938	1.8457	.032468
7	1.76604E-16	.457473	1.99815	.02285

HMO	15	S.MITIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
15	1	.244888	-3.88886	.99995
24	4.02001E-18	.478052	3.38527	3.55596E-4
14	1.17554E-20	.5001	7.00923	1.19888E-12

HMO	16	S.SANGUIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
16	1	.217343	-4.80395	.999999
17	9.20253E-17	.485577	3.31757	4.54076E-4
15	5.53843E-19	.497631	3.35617	3.95207E-4

HMO	17	ORAL 2		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
17	1.	.249903	-3.58712	.999833
19	1.07061E-12	.428428	2.39448	8.32198E-3
28	1.33929E-18	.552891	2.54442	5.47306E-3

HMO	18	S.MILLERI		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
18	1	.193397	-4.87216	.999999
17	4.77321E-16	.460925	2.59532	4.72519E-3
19	1.15459E-23	.540729	5.87375	2.13188E-9

HMO	19	LEUCONOSTOC		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
19	1.	.22373	-3.96488	.999963
17	5.96212E-11	.415474	1.26958	.102117
15	6.55480E-15	.442569	2.35109	9.35919E-3

HMO	20	S.AGALACTIAE		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
20	1	.150765	-5.33621	1.
24	5.70841E-17	.473955	3.32258	4.45995E-4
28	4.86164E-21	.540699	2.24775	.012296

HMO	21	S.PYOGENES		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
21	1	.180273	-3.76426	.999916
17	4.06114E-18	.477966	3.09459	9.85486E-4
28	1.07064E-19	.52504	1.86723	3.09345E-2

HMO	22	S.EQUI		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
22	1	.193869	-3.90053	.999952
23	1.49964E-17	.437319	3.29156	4.98235E-4
12	1.12139E-24	.557835	6.04048	7.68841E-10

HMO	23	S.EQUISIMILIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
23	1.	.197269	-4.50317	.999997
24	9.61557E-11	.408493	1.30544	9.58722E-2
22	2.46174E-17	.429634	4.62218	1.90062E-6

HMO	24	STREP.SP.B		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
24	1.	.232809	-3.94761	.999961
12	1.85878E-13	.459906	3.06501	1.08838E-3
23	3.78520E-14	.457072	3.93295	4.19727E-5

HMO	25	S.UBERIS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
25	1	.170626	-4.97814	1.
12	1.69249E-21	.518183	4.83569	6.64312E-7
17	1.63923E-23	.569021	5.76229	4.15228E-9

HMO	26	S.DYSGALACTIAE		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
26	1	.159445	-5.03904	1.
17	1.25297E-21	.528001	4.5605	2.55418E-6
24	1.95763E-22	.528394	4.89053	5.03550E-7

HMO	27	STREP.SP.R,S,T		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
27	1	.208854	-4.82809	.999999
17	4.42314E-21	.551771	5.25491	7.33932E-8
19	2.47868E-24	.554426	6.29885	1.50036E-10

HMO	28	PEDIOCOCCUS		
TO TAXON	WILLCOX PROB	TAX DIST	S.E.(D)	GAU S.E.
28	1	.288713	-3.88366	.999949
2	4.18787E-23	.543237	9.78798	6.34216E-23
7	9.05753E-24	.569165	5.14935	1.30936E-7

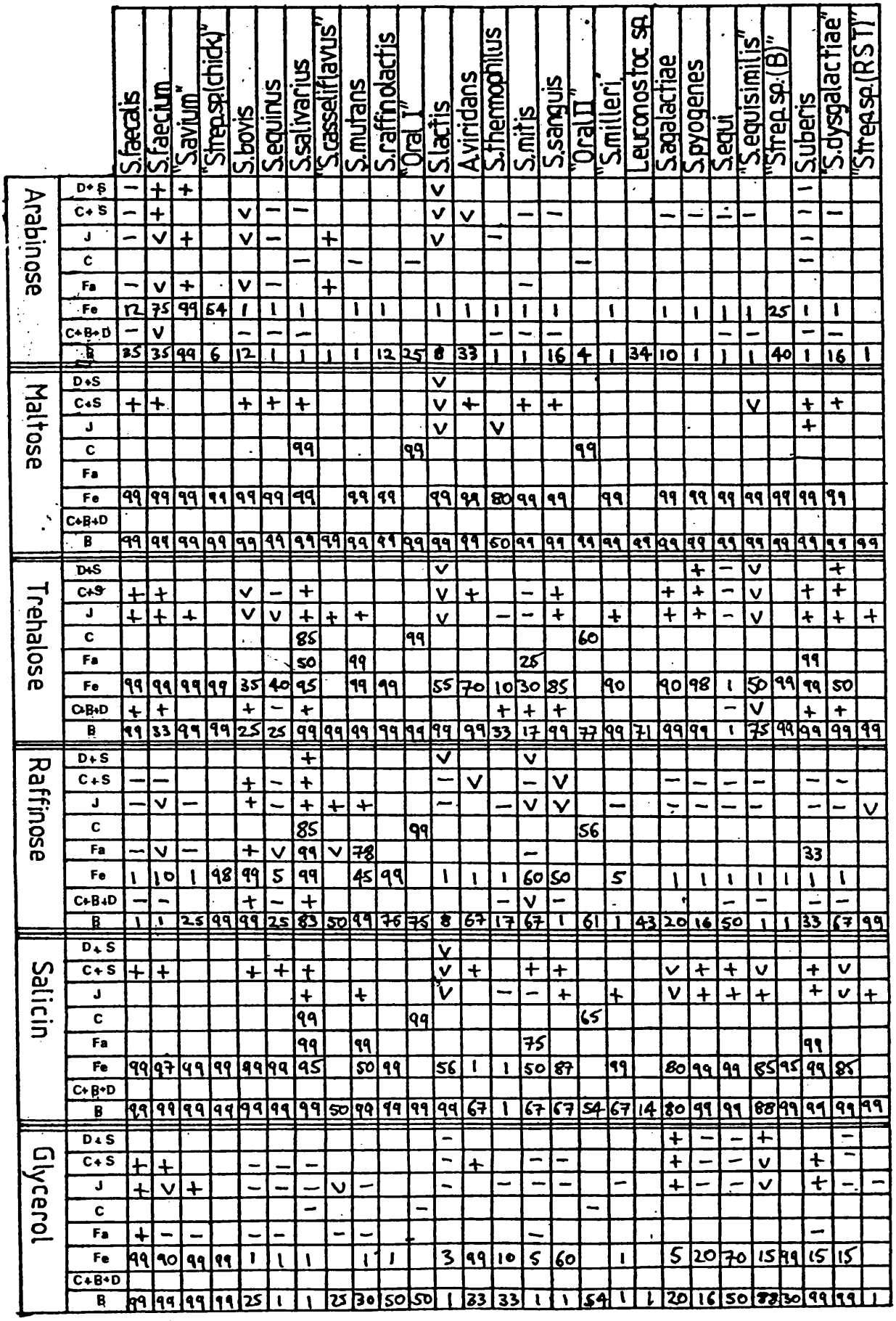
Table A.VII.e Literature results on which the matrix PDBSTP is based.

Study.	Designated by.	Positive and negative result range.
Diebel & Seeley (1974)	D+S	+; between 90 and 100% -; between 0 and 10%*
Cowan & Steel (1974)	C+S	+; between 85 and 100% -; between 0 and 85%*
Jones (1978)	J	Range of results not given
Carlsson (1968	C	% results given[1]
Fackl am (1972; 1977)	Fa	Range not given for 1972 study; % results given for 1977 study[1]
Feltham (1979)	Fe	% results given[1]
Crowley, Bradley & Darrel (1969)	C+B+D	Range not given
Bridge This study (1981)	B	% results given[1]

*V used to signify variable results.

[1]In cases where all results positive 99% is given;

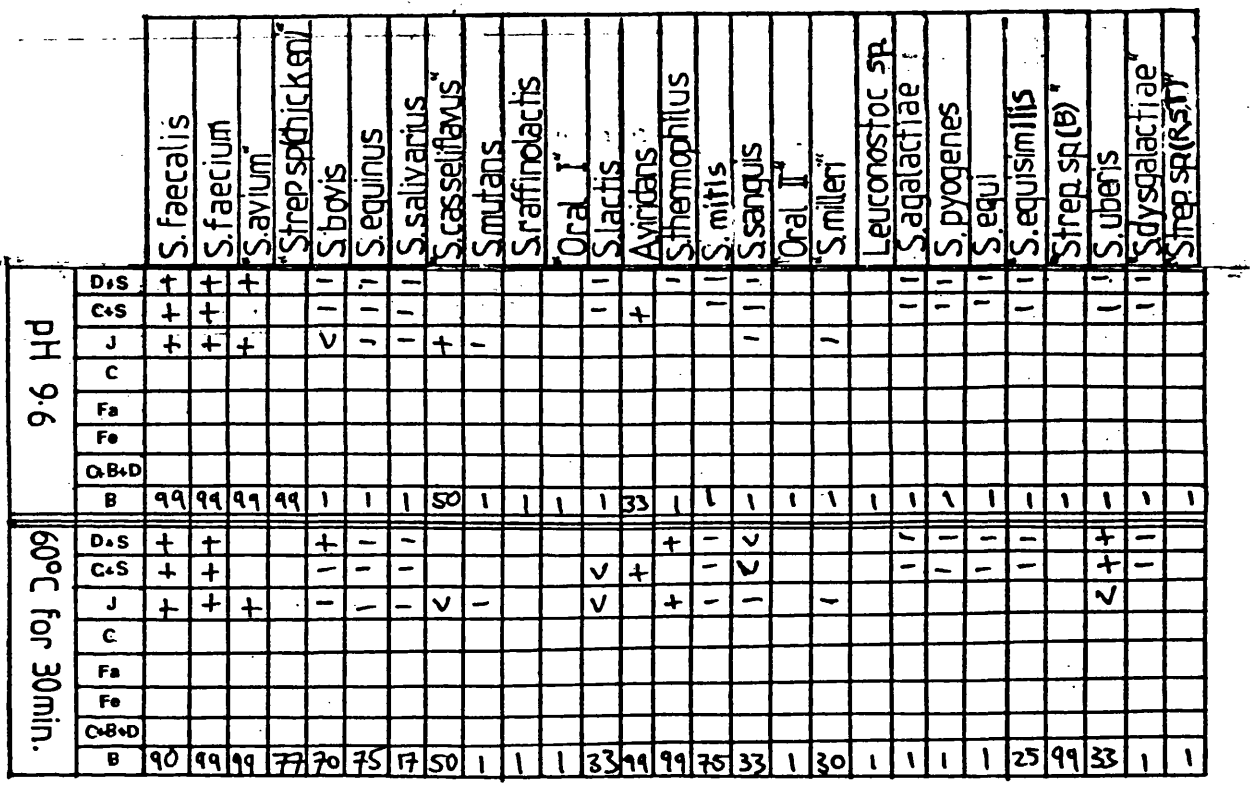
In cases where all results negative 1% is given.



		S.faecalis	S.faecium	"S.avium"	"Strep.sp.(chick)"	S.bovis	S.equinus	S.salivarius	"S.casseliflavus"	S.mutans	S.raffinolactis	"Oral I"	S.lactis	A.viridans	S.thermophilus	S.mitis	S.sanquis	"Oral II"	"S.milleri"	Leuconostoc sp.	S.agalactiae	S.pyogenes	S.equi	"S.equisimilis"	"Strep.sp.(B)"	S.uberis	"S.dysgalactiae"	Strep.sp.(RST)
Mannitol	D+S																											
	C+S	+	V			V	-	-					V	V		-	-				-	-	-	-		+	-	
	J	+	V	+		V	-	-	+	+			V		-	-	-		-		-	-	-	-		+	-	-
	C							-				89						-										
	Fa	+	V	+		V	-	-	+	99						-									99			
	Fe	99	99	99	99	15	10	1		99	1		10	99	1	1	10		5		1	20	1	10	1	99	10	
	C+β+D	+	V			+	-	-							-	-	-						-	-		+	-	
	β	99	83	99	99	50	60	33	99	99	99	99	17	33	17	17	1	38	1	57	1	33	1	1	1	99	99	33
Sorbitol	D+S	+	-	+																		-	-	V		+	V	
	C+S	+	-			-	-	-					-	V		-	-				-	-	-	V		+	V	
	J	+	V	+		V	-	-	V	+			-		-	-	-		-		-	-	-	V		+	V	-
	C							-				99						20										
	Fa	+	-	+		-	-	-	-	91						-									99			
	Fe	99	56	99	99	1	1	1		97	1		1	20	1	1	10		1		1	20	1	55		99	55	
	C+β+D	+	-			-	-	-															-	V		+	V	
	β	99	83	99	99	1	1	17	99	99	99	75	17	1	17	17	1	23	1	1	1	33	1	38	1	99	99	67
Lactose	D+S					+							+															
	C+S																											
	J	+	+	+		+	-	+	+	+			+		+	+	+		+		V	+	-	V		+	V	+
	C							85				99						99										
	Fa	+	+	+		+	-	75	+	97						99									99			
	Fe	99	99	99	99	99	1	99		98	10		90	99	99	90	95		85		95	98	1	75	20	99	75	
	C+β+D	+	+			+	-	+							+	+	+						-	V		+	+	
	β	99	99	99	99	99	1	1	99	99	99	75	75	67	99	67	99	99	99	43	99	99	1	62	60	99	99	99
Inulin	D+S							+					-			-	+					-		-				
	C+S																											
	J	-	-	V		+	V	+	V	+			-		-	-	+		-		-	-	-	-		+	-	+
	C							38				89						38										
	Fa	-	-	-		V	-	99	V	99						-									99			
	Fe	1	10	1	80	55	5	10		13	1		3	1	1	1	20		5		1	1	1	1	3		1	
	C+β+D																											
	β	10	17	25	77	50	25	50	1	99	75	50	5	33	1	17	99	31	1	14	1	1	1	12	10	99	1	99
Sucrose	D+S												V															
	C+S												V															
	J	+	V	+			+	+	+	+					+	V	+		+							+		
	C																											
	Fa	+	V	+		+	+	99	+	99						99									99			
	Fe	90	55	99	99	99	70	99		99	99		5	99	99	50	99		99		99	99	99	99	99	99	99	
	C+β+D																											
	β	80	67	50	99	99	50	99	+	99	99	99	25	67	99	83	99	99	99	99	99	99	99	99	99	99	99	99
Melibiose	D+S																											
	C+S																											
	J	-	+	V		+	-		+																			
	C							-				89						55										
	Fa							-		33						33												
	Fe	5	48	1	99	99	1	20		45	1		5	1	1	65	48		20		5	5	1	3		1	3	
	C+β+D																											
	β	1	50	25	99	99	1	50	50	99	75	75	17	33	33	67	1	77	33	57	20	7	1	12	30	33	99	67

		S. faecalis	S. faecium	"S. avium"	"Strep. sp. (chick)"	S. bovis	S. equinus	S. salivarius	"S. casseliflavus"	S. mutans	S. raffinolactis	Oral I	S. lactis	A. viridans	S. thermophilus	S. mitis	S. sanguis	"Oral II"	"S. milleri"	Leuconostoc sp.	S. agalactiae	S. pyogenes	S. equi	"S. equisimilis"	"Strep. sp. (B)"	S. uberis	"S. dysgalactiae"	Strep. sp. (RS T)
Melezitose	D+S																											
	C+S																											
	J	+	−	+		−	−		−																			
	C							−				−						−										
	Fa																											
	Fe	85	1	99	50	1	5	1		1	1		1	50	1	50	20		1		1	5	1	1	5	35	1	
	C+B+D																											
	B	80	1	99	59	1	60	33	99	30	75	75	1	1	67	1	1	69	1	14	1	1	1	38	50	1	67	38
Glucose	D+S	+	+	+		+	+	+					+	+	+	+	+				+	+	+	+		+	+	
	C+S																											
	J												+		+											+		
	C											99																
	Fa																											
	Fe	99	99	99	99	99	99	99		99	99		99	99	99	99	99		99		99	99	99	99	99	99	99	
	C+B+D																											
	B	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
Xylose	D+S			+		V	−	−					V		V	−	V				−			V		−		
	C+S																											
	J												V		−											−		
	C							−				−						−										
	Fa																											
	Fe	26	20	55	55	1	1	1		1	20		30	1	1	1	1		1		1	1	1	5	1	1	1	
	C+B+D																											
	B	15	1	99	59	1	1	1	1	35	35	25	20	33	8	1	1	1	1	14	1	8	1	6	20	16	1	1

		S.faecalis	S.faecium	"S.avium"	"Strep.sp.(chick)"	S.bovis	S.equinus	S.salivarius	"S.casseliflavus"	S.mutans	S.raffinolactis	"Oral I"	S.lactis	A.viridans	S.thermophilus	S.mitis	S.sanguis	"Oral II"	"S.milleri"	Leuconostoc sp.	S.agalactiae	S.pyogenes	S.equi	"S.equisimilis"	"Strep sp. (B)"	S.uberis	"S.dysgalactiae"	"Strep.sp.(RS T)"
Aesculin	D+S	+	+	+		+	+	+					<		-		+			<	-	+	-	<		+	<	
	C+S	+	+			+	+	+					<	<		-	+				-	-	-	<		+	-	
	J							<		<			<		-	-	+		+		-	<	-	<		+		+
	C							+				99						35										
	Fa	+	+	+		+	+	+	+	88						-										+		
	Fe	98	99	99	99	99	99	95		50	99		50		1	1	40		99		1	40	60	10	1	99	10	
	C+B+D																											
	B	99	99	99	99	99	99	99	99	99	99	99	75	99	33	1	1	8	33	43	1	50	1	12	20	99	33	99
Arginine	D+S	+	+	-		-	-	-					+	-	-	<	+			-	+	+	+	+		+	+	
	C+S	+	+			-	-	-					<	-		<	+				+	+	+	+		+	+	
	J	+	+	-		-	-	-	<	-			<			-	+		+									
	C							-				11						50										
	Fa							-		-						25										+		
	Fe																											
	C+B+D																										-	
	B	99	99	25	1	1	1	17	75	33	1	1	97	99	17	83	99	31	99	1	99	99	99	99	99	99	99	99
Hippurate	D+S	+		-		<	-	-					<		-	-	-				+	-	-	-		+	<	
	C+S	<	<			-	-	-		-			<	+		-	-				+	-	-	-		+	<	
	J	+	<	-		-	-	-	<	-			<		-	-			-		+	-	-	-		+		-
	C							-				-						-										
	Fa							-								-										+		
	Fe																											
	C+B+D																											
	B	90	1	1	12	1	1	1	1	1	25	1	50	99	1	50	1	8	1	14	99	16	1	12	99	99	99	1
Starch	D+S	-	-	-		+	+	-					-		+		-				-	-		<		<		
	C+S																											
	J	-	-	-		+	<		-	-					+											-		
	C																											
	Fa	-	-	-		+	-	25	-	-						50										33		
	Fe																											
	C+B+D																											
	B	1	1	25	77	99	99	83	26	33	50	99	1	99	83	83	1	46	1	14	1	1	50	62	80	99	99	99
Gelatin	D+S	<	-	-		-	-	-					-		-		-				-	<	-	-		-	-	
	C+S	<	-			-	-	-					-			-	-				-	-	-	-		-	-	
	J																											
	C																											
	Fa	<	-	-		-	-		-																			
	Fe	35	1	1		1	1	1			1		1	1	1	1	1		1		1	1	1	1	1	1	1	
	C+B+D																											
	B	40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	1	1	1	1	1	1	1	1


S.faecalis
S.faecium
S.avium
Strep sp(chicken)
S.bovis
S.equinus
S.salivarius
S.casseliflavus
S.mutans
S.raffinolactis
Oral I
S.lactis
Aviridans
S.thermophilus
S.mitis
S.sanguis
Oral II
S.milleri
Leuconostoc sp
S.agalactiae
S.pyogenes
S.equi
S.equisimilis
Strep sp(B)
S.uberis
S.dysgalactiae
Strep sp(R&I)
pH 9.6
60°C for 30min.
D+S
C+S
J
C
Fa
Fe
C+B+D
B

		S.faecalis	S.faecium	"S.avium"	"Strep.sp(chick)"	S.bovis	S.equinus	S.salivarius	"S.casseliflavus"	S.mutans	S.raffinolactis	"Oral I"	S.lactis	A.viridans	S.thermophilus	S.mitis	S.sanguis	"Oral II"	"S.milleri"	Leuconostoc sp.	S.agalactiae	S.pyogenes	S.equi	"S.equisimilis"	"Strep.sp.(B)"	S.uberis	"S.dysgalactiae"	"Strep.sp.(RS T)"
10% Bile	D+S	+	+			+	+	-					+			-	+				+						-	
	C+S	+	+			+	+	+					+	+		-	+				+	-	v	v		+	-	
	J																											
	C																											
	Fa							50		34						75										99		
	Fe																											
	C+B+D																											
	B	99	99	99	99	99	99	33	99	99	99	1	99	1	99	83	67	16	1	99	99	1	50	75	70	99	1	67
40% Bile	D+S	+	+			+	+	-					+		-	-	+				+	-	-	-		-	-	
	C+S	+	+			+	+	-					+	+		-	+				+	-	-	-		v	-	
	J																											
	C																											
	Fa							25		22						-										22		
	Fe																											
	C+B+D																											
	B	99	99	99	99	99	99	17	99	70	1	1	42	1	99	35	67	1	1	43	99	1	1	1	60	67	1	1
Tellurite	D+S																											
	C+S																											
	J	+	-	-		-	-		v										v									
	C							38				-						60										
	Fa	+	-	-		-	-	-	v	-																-		
	Fe															-												
	C+B+D																											
	B	99	60	50	18	1	1	1	25	1	1	1	67	1	1	1	1	1	1	1	1	1	99	85	60	1	1	33
Tetrazolium	D+S																											
	C+S																											
	J	+	-	v		v	-		v																			
	C																											
	Fa	+	-	-		v	-	25	v	37						-										56		
	Fe																											
	C+B+D																											
	B	90	50	99	99	99	1	83	1	99	25	50	83	67	99	67	1	99	67	99	99	99	1	99	60	99	67	99

IDEN %67	S.faecalis	S.faecium	"S.avium"	"Strep.sp(chick)"	S.bovis	S.equinus	S.salivarius	"S.casseliflavus"	S.mutans	S.raffinolactis	"Oral I"	S.lactis	A.viridans	S.thermophilus	S.mitis	S.sanguis	"Oral II"	"S.milleri"	Leuconostoc.sp	S.agalactiae	S.pyogenes	S.equi	"S.equisimilis"	"Strep.sp. (B)"	S.uberis.	"S.dysgalactiae"	"Strep.sp(RST)"
D+S																											
C+S																											
J						V	-		+						+			V									
C							38				64	V					28										
Fa							-		50						-										+		
Fe																											
C+B+D																											
B	99	99	99	99	99	99	50	99	67	75	1	75	33	17	33	1	1	67	1	99	1	1	62	99	99	67	33

IDEN %5·9	S.faecalis	S.faecium	"S.avium"	"Strep.sp(chick)"	S.bovis	S.equinus	S.salivarius	"S.casseliflavus"	S.mutans	S.raffinolactis	"Oral I"	S.lactis	A.viridans	S.thermophilus	S.mitis	S.sanguis	"Oral II"	"S.milleri"	Leuconostoc.sp	S.agalactiae	S.pyogenes	S.equi	"S.equisimilis"	"Strep.sp. (B)"	S.uberis.	"S.dysgalactiae"	"Strep.sp(RST)"
D + S	+	+	+		-	-	-					-		-	-	-				-	-	-	-		-	-	
C + S	+	+			-	-	-					-	+		-	-				-	-	-	-		-	-	
J	+	+	+		-	-		+							-												
C							-				11						-										
Fa	+	+	+		-	-	-	+	16						-										67		
Fe																											
C+B+D																											
B	99	99	99	99	1	1	1	99	1	25	1	1	33	1	1	1	1	1	1	1	1	1	1	1	1	1	1

		S.faecalis	S.faecium	"S.avium"	"Strep.sp(chick)"	S.bovis	S.equinus	S.salivarius	"S.casseliflavus"	S.mutans	S.raffinolactis	"Oral I"	S.lactis	A.viridans	S.thermophilus	S.mitis	S.sanguis	"Oral II"	"S.milleri"	Leuconostoc sp.	S.agalactiae	S.pyogenes	S.equi	"S.equisimilis"	"Strep.sp.(B)"	S.uberis	"S.dysgalactiae"	"Strep.sp.(RST)"
Growth at 10°C	D+S	+	+	+		-	/		+				+	+	-		-			+	-	-	-	-		+	/	
	C+S																											
	J	+	+	v		-	/		+	-			+		-	-	-		-							+		
	C																											
	Fa	+	+	-		-	/														-							
	Fe																											
	C+B+D																											
	B	99	99	99	99	1	1	17	25	1	75	1	99	99	1	33	1	15	1	43	1	1	1	28	1	99	1	67
Growth at 45°C	D+S	+	+	+		+	+	+					-	-	+	v	v			-	-	-	-	-		+	-	
	C+S	+	v			+	+	+					-	-		v	v	•			-	-	-	-		+	-	
	J	+	+	+		+	+	-	v	v					+	-	v		-							v		
	C							-				11						14										
	Fa	+	+	+		+			+																			
	Fe																											
	C+B+D																											
	B	99	99	99	99	99	99	83	99	99	1	25	1	99	99	99	99	38	33	1	20	1	1	1	70	1	1	33

		S.faecalis	S.faecium	"S.avium"	"Strep sp.(chick)"	S.bovis	S.equinus	S.salivarius	"S.casseliflavus"	S.mutans	S.raffinolactis	"Oral I"	S.lactis	A.viridans	S.thermophilus	S.mitis	S.sanguis	"Oral II"	"S.milleri"	Leuconostoc sp.	S.agalactiae	S.pyogenes	S.equi	"S.equisimilis"	"Strep.sp.(B)"	S.uberis	"S.dysgalactiae"	"Strep.sp.(RST)"
Methylene blue	D+S	+	+	-		-	-	+					V			-	-				-	-	-	-		-	-	
	C+S																					V						
	J																											
	C																											
	Fa	+	+	-		V	-	-	+	-						-	-									-		
	Fe																											
	C+B+D																											
	B	99	99	25	1	1	1	33	1	70	1	25	75	99	1	99	1	1	8	67	1	1	50	12	70	1	1	67
Acid from litmus	D+S																											
	C+S	+	+			+	-	+					+	+		+	+				+	+	+	+		+	+	
	J																											
	C																											
	Fa							+		+						+	+									+		
	Fe																											
	C+B+D																											
	B	99	83	99	99	99	99	99	50	99	75	99	99	99	99	99	99	99	99	67	40	17	50	75	99	99	99	67
Clot in litmus	D+S																											
	C+S	+	-			-	-	99					+	-		+	+				+	-	-	V		+	V	
	J																											
	C																											
	Fa	+	V	-		99	-		V																			
	Fe																											
	C+B+D																											
	B	40	67	99	1	99	25	99	50	70	1	1	75	33	99	99	99	1	15	1	40	17	50	38	60	1	1	1

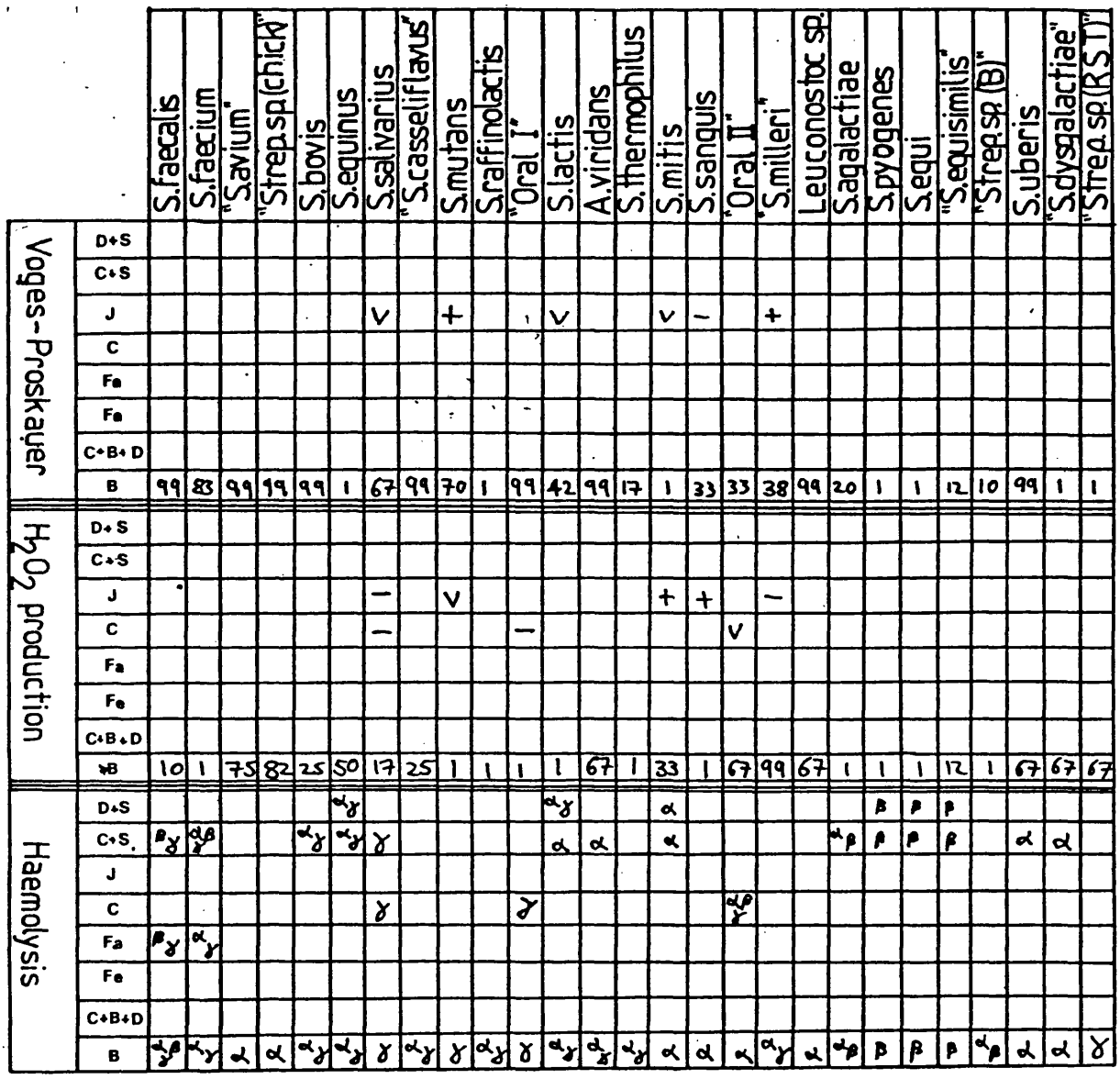

Voges-Proskauer
H2O2 production
Haemolysis
D+S
C+S
J
C
Fa
Fe
C+B+D
B
S.faecalis
S.faecium
"S.avium"
"Strep.sp(chick)"
S.bovis
S.equinus
S.salivarius
"S.casseliflavus"
S.mutans
S.raffinolactis
"Oral I"
S.lactis
A.viridans
S.thermophilus
S.mitis
S.sanguis
"Oral II"
"S.milleri"
Leuconostoc sp.
S.agalactiae
S.pyogenes
S.equi
"S.equisimilis"
"Strep.sp.(B)"
S.uberis
"S.dysgalactiae"
"Strep.sp.(RST)"

Table A.VII.f OVERMAT results for identification matrix PDBSTP.

```
PROGRAM OVERMAT FOR OVERLAP STATISTICS BETWEEN
TAXA IN AN IDENTIFICATION MATRIX
(FILE NAME PH57811), UPDATED NOVEMBER 1976
THIS CALCULATES OVERLAP BETWEEN TAXA FROM A
MATRIX NAMED A GENERATED BY FORTRAN
PROGRAM MJS54ER FROM AN IDENTIFICATION
MATRIX OF PERCENT POSITIVE CHARACTERS.
MATRIX A HAS W ROWS OF TAXA FOR M
CHARACTERS. THE NUMBER OF STRAINS, N(J),
ON WHICH EACH TAXON J WAS BASED, MUST BE
GIVEN AS DATA AT 9001 ONWARDS.
THESE NUMBERS MUST BE INTEGERS OVER 1.

THE OVERLAP STATISTICS OF SNEATH (1977)
ARE CALCULATED BETWEEN EACH PAIR OF TAXA
AND OUTPUT IF W IS UNDER A CUTOFF LEVEL
C (C >= 0; FOR COMPLETE OVERLAP W = 0).
IF C IS NEGATIVE THE LEVEL IS SET TO THAT
EXPECTED FOR A RECTANGULAR DISTRIBUTION,
SQRT 3 * (3/(N(L) * N(M) - 1)).

IF THE INTERCENTROID DISTANCE IS TO BE CORRECTED
FOR THE ESTIMATED SAMPLING ERROR, INPUT 1, ELSE
INPUT ZERO.  NOTE THAT THIS MAY GIVE NEGATIVE
DISTANCES WHICH WILL BE TREATED AS ZEROS.

OPTION = 0:  INTERCENTROID DISTANCE IS
NOT CORRECTED FOR ESTIMATED SAMPLING ERROR


** EOR **


PROGRAM OVERMAT FOR OVERLAP STATISTICS BETWEEN
TAXA IN AN IDENTIFICATION MATRIX
(FILE NAME PH57811), UPDATED NOVEMBER 1976
THIS CALCULATES OVERLAP BETWEEN TAXA FROM A
MATRIX NAMED A GENERATED BY FORTRAN
PROGRAM MJS54ER FROM AN IDENTIFICATION
MATRIX OF PERCENT POSITIVE CHARACTERS.
MATRIX A HAS W ROWS OF TAXA FOR M
CHARACTERS. THE NUMBER OF STRAINS, N(J),
ON WHICH EACH TAXON J WAS BASED, MUST BE
GIVEN AS DATA AT 9001 ONWARDS.
THESE NUMBERS MUST BE INTEGERS OVER 1.

THE OVERLAP STATISTICS OF SNEATH (1977)
ARE CALCULATED BETWEEN EACH PAIR OF TAXA
AND OUTPUT IF W IS UNDER A CUTOFF LEVEL
C (C >= 0; FOR COMPLETE OVERLAP W = 0).
IF C IS NEGATIVE THE LEVEL IS SET TO THAT
EXPECTED FOR A RECTANGULAR DISTRIBUTION,
SQRT 3 * (3/(N(L) * N(M) - 1)).

IF THE INTERCENTROID DISTANCE IS TO BE CORRECTED
FOR THE ESTIMATED SAMPLING ERROR, INPUT 1, ELSE
INPUT ZERO.  NOTE THAT THIS MAY GIVE NEGATIVE
DISTANCES WHICH WILL BE TREATED AS ZEROS.

OPTION = 0:  INTERCENTROID DISTANCE IS
NOT CORRECTED FOR ESTIMATED SAMPLING ERROR

INPUT THE CUTOFF OVERLAP V(G) DESIRED WHICH
WILL BE CONVERTED TO W TO GIVE THE CUTOFF
LEVEL C.  V(G) MUST BE <= 1; IF NEGATIVE
THEN W WILL BE THAT EXPECTED FOR A
RECTANGULAR DISTRIBUTION. IF CUTOFF W INSTEAD
OF V(G) IS PREFERRED, INPUT ZERO, AND INPUT
THE DESIRED W AT THE NEXT INPUT.
CUTOFF LEVEL OF V(G) =                .00001

IF THE CUTOFF V(G) PREVIOUSLY INPUT IS TO BE
USED, INPUT ZERO, ELSE INPUT THE DESIRED CUTOFF
LEVEL OF W ITSELF (WHICH OVERWRITES THE
PREVIOUS CUTOFF VALUE, EVEN IF NEGATIVE).

PDBSTP IS A MATRIX OF 37 TESTS FOR 27 GROUPS OF STREPTOCOCCI
 M              G                C
 37             27               4.41745

IF T(U) FOR PROBABILITIES P = 0.90,
0.95, 0.99 ARE REQUIRED, INPUT THE CHOSEN
CRITICAL OVERLAP AS A PROPORTION V(U),
OR INPUT -1 FOR RECTANGULAR CASE, ELSE INPUT ZERO.
IF CALCULATED, T(U) ARE PRINTED ON THE
FOURTH LINE
CRITICAL OVERLAP V(G) FOR W0 = .025
CORRESPONDING TO W(G) OF                      2.24136

OVERLAP STATISTICS ARE PRINTED AS FOLLOWS
     ON FIRST LINE NAMES OF TAXA BEING COMPARED
     ON SECOND LINE
TAXON NO.    TAXON NO.    N(L)     N(M)     D(L,M)
     ON THIRD LINE
   W              V(G)             T(W)             F
     ON FOURTH LINE
   T(U), P = 0.90   T(U), P = 0.95   T(U), P = 0.99

 STREPTOCOCCUS FAECA              STREPTOCOCCUS FAECI
 1                2               15               15               .35933
 4.19515          2.78655E-5      22.9778          24.3318
 15.3007          16.3632         18.8744

 STREPTOCOCCUS FAECA              STREPTOCOCCUS CASSE
 1                6               15               13               .42001
 4.31759          1.57860E-5      23.0484          22.118
 15.0825          16.8105         19.3711

 STREPTOCOCCUS FAECI              STREPTOCOCCUS AVIUM
 2                3               15               15               .354157
 3.77288          1.92453E-4      20.4235          27.0005
 15.3124          16.3021         18.4280

 STREPTOCOCCUS FAECI              STREPTOCOCCUS CASSE
 2                8               15               15               .27194
 2.43953          1.39118E-2      13.4714          27.1637
 15.3357          16.3343         18.4035

 STREPTOCOCCUS FAECI              STREPTOCOCCUS LACTI
 2                12              15               15               .441279
 3.98125          6.83651E-5      21.8062          27.140
 15.3378          16.3372         18.4085

 STREPTOCOCCUS FAECI              AEROCOCCUS VIRIDANS
 2                13              15               15               .367423
 3.33596          8.50290E-4      18.2710          27.2411
 15.3324          16.3298         18.4756

 STREPTOCOCCUS AVIUM              STREPTOCOCCUS [illegible]
 3                4               15               15               .299033
 3.00474          2.47075E-4      20.0725          27.0005
 15.3422          16.3433         18.4909

 STREPTOCOCCUS AVIUM              STREPTOCOCCUS CASSE
 3                8               15               13               .32033
 3.09379          1.97028E-3      16.9454          25.0008
 15.4141          16.4429         18.0000
```

STREPTOCOCCUS AVIUM		AEROCOCCUS VIRIDANS		.423212
3	13	15	15	
4.02607	5.03713E-5	22.0330	23.9431	
15.409	16.4359	18.0303		
STREPTOCOCCUS SP. (CRICK)		STREPTOCOCCUS CASSE		.354405
4	8	15	15	
3.50232	3.07074E-4	19.3110	23.2997	
15.5891	16.0803	19.0801		
STREPTOCOCCUS SP. (CRICK)		AEROCOCCUS VIRIDANS		.404343
4	13	15	15	
4.08567	4.39727E-5	22.3701	23.3911	
15.5823	16.0767	19.0090		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS EQUIN		.37001
5	6	15	15	
4.28751	1.80815E-5	23.4657	27.9452	
15.2937	16.2701	18.3847		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS SALIV		.352005
5	7	15	15	
3.82820	1.27U78E-4	20.9082	27.5001	
15.316	16.3097	18.4418		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS CASSE		.399171
5	8	15	15	
3.83272	1.20778E-4	20.9927	23.3040	
15.4454	16.4804	18.743		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS MUTAN		.355353
5	9	15	15	
3.74588	1.79819E-4	20.317	27.0906	
15.341	16.3416	18.4959		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS RAFFI		.420496
5	10	15	15	
4.32016	1.56015E-5	23.0026	28.0000	
15.3658	16.376	18.3543		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS LACTI		.44542
5	12	15	15	
4.24575	2.10U1UE-5	23.4344	25.3080	
15.449	16.4914	18.7515		
STREPTOCOCCUS BOVIS		AEROCOCCUS VIRIDANS		.421032
5	13	15	15	
4.06017	4.9U614E-5	22.6385	25.4515	
15.4399	16.4787	18.7297		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS MITIS		.370445
5	15	15	15	
3.66574	2.2e1U1E-4	20.1076	26.0005	
15.4014	16.4253	18.6388		
STREPTOCOCCUS BOVIS		STREPTOCOCCUS SANGU		.41364
5	16	15	15	
4.3663	1.26349E-5	23.9163	27.1278	
15.3589	16.3380	18.4909		
STREPTOCOCCUS BOVIS		ORAL STREPTOCOCCI 2		.427507
5	17	15	15	
4.17207	3.02U25E-5	22.8514	25.0009	
15.4248	16.4578	18.094		
STREPTOCOCCUS EQUIN		STREPTOCOCCUS SALIV		.41U027
6	7	15	15	
4.30825	1.23344E-5	23.9259	27.7090	
15.3032	16.2893	18.407		
STREPTOCOCCUS EQUIN		STREPTOCOCCUS RAFFI		.392142
6	10	15	15	
3.95833	7.55U95E-5	21.6807	27.1057	
15.3401	16.3404	18.4939		
STREPTOCOCCUS EQUIN		STREPTOCOCCUS LACTI		.445737
6	12	15	15	
4.20281	2.03787E-5	23.0197	25.0030	
15.4127	16.441	18.0052		
STREPTOCOCCUS EQUIN		STREPTOCOCCUS MITIS		.375026
6	15	15	15	
3.6699[illegible]	2.42636E-4	20.1013	26.5833	
15.3704	16.3823	18.5652		

STREPTOCOCCUS EQUIN		STREPTOCOCCUS SANGU		.362253
6	16	15	15	
3.75697	1.74U34E-4	20.3778	27.4906	
15.3185	16.3105	18.443		
STREPTOCOCCUS EQUIN		ORAL STREPTOCOCCI 2		.450807
6	17	15	15	
4.32976	1.49361E-5	23.7133	28.2380	
15.3907	16.4108	18.0439		
STREPTOCOCCUS SALIV		STREPTOCOCCUS CASSE		.402166
7	8	15	15	
2.40276	1.U6872E-5	24.116	28.9038	
15.3682	16.3516	18.513		
STREPTOCOCCUS SALIV		STREPTOCOCCUS MUTAN		.273661
7	9	15	15	
2.71919	6.54433E-3	14.8936	27.9312	
15.2944	16.2772	18.3865		
STREPTOCOCCUS SALIV		STREPTOCOCCUS RAFFI		.524016
7	10	15	15	
3.14427	1.66517E-3	17.2219	27.7040	
15.3034	16.2896	18.4077		
STREPTOCOCCUS SALIV		ORAL STREPTOCOCCI 1		.535128
7	11	15	15	
3.40121	5.37849E-4	18.9578	27.9774	
15.2919	16.2737	18.3807		
STREPTOCOCCUS SALIV		STREPTOCOCCUS LACTI		.382912
7	12	15	15	
3.48781	4.87U80E-4	19.1035	28.9223	
15.3500	16.3549	18.5180		
STREPTOCOCCUS SALIV		STREPTOCOCCUS THERM		.400548
7	14	15	15	
4.40410	1.U6284E-5	24.1660	27.2422	
15.3524	16.3297	18.4757		
STREPTOCOCCUS SALIV		STREPTOCOCCUS MITIS		.528103
7	15	15	15	
3.09378	1.97U32E-3	16.9452	27.4408	
15.3213	16.3143	18.4490		
STREPTOCOCCUS SALIV		STREPTOCOCCUS SANGU		.296575
7	16	15	15	
2.95082	3.36U79E-3	16.0528	27.9419	
15.2939	16.2704	18.3652		
STREPTOCOCCUS SALIV		ORAL STREPTOCOCCI 2		.30926
7	17	15	15	
2.866	4.157U7E-3	15.0977	28.1940	
15.3351	16.3334	18.482		
STREPTOCOCCUS SALIV		STREPTOCOCCUS MILLE		.334046
7	18	15	15	
3.44721	5.00302E-4	18.8812	27.98	
15.2918	16.2735	18.3604		
STREPTOCOCCUS SALIV		LEUCONOSTOC/GEMELLA		.422583
7	19	15	15	
4.03601	5.43938E-5	22.1006	27.599	
15.3125	16.3022	18.429		
STREPTOCOCCUS SALIV		STREPTOCOCCUS EQUISIM		.419814
7	23	15	15	
4.07890	4.52610E-5	22.3414	27.7802	
15.3020	16.2905	18.4057		
STREPTOCOCCUS SALIV		STREPTOCOCCUS BYSSA		.429012
7	26	15	15	
4.33630	1.47U30E-5	23.7693	27.9961	
15.2912	16.2728	18.3700		
STREPTOCOCCUS SALIV		STREPTOCOCCUS SP. K-		.361905
7	27	15	15	
3.71201	4.U30E-4	20.3313	27.9965	
15.291	16.2725	18.3785		
STREPTOCOCCUS CASSE		STREPTOCOCCUS MUTAN		.414720
8	9	15	15	
3.71003	2.U7275E-4	20.3208	27.4U1	
15.3235	16.3174	18.4547		

STREPTOCOCCUS CASSE		STREPTOCOCCUS RAFFI		
8	10	15	15	.434017
5.616643	1.455555E-4	20.9023	27.6697	
15.3075	16.2933	18.4173		
STREPTOCOCCUS CASSE		STREPTOCOCCUS LACTI		
8	12	15	15	.424003
3.553791	4.033794E-4	19.3779	27.9993	
15.2908	16.2721	18.3779		
STREPTOCOCCUS CASSE		AEROCOCCUS VIRIDANS		
8	13	15	15	.355439
2.960193	2.873604E-3	16.3673	27.9980	
15.2908	16.2721	18.376		
STREPTOCOCCUS CASSE		STREPTOCOCCUS MITIS		
8	15	15	15	.478304
4.102222	4.096227E-3	22.4068	27.9102	
15.2952	16.2783	18.3804		
STREPTOCOCCUS CASSE		STREPTOCOCCUS SANGU		
8	16	15	15	.452091
4.205128	5.009644E-3	22.1898	27.3093	
15.3252	16.3198	18.4589		
STREPTOCOCCUS CASSE		ORAL STREPTOCOCCI 2		
8	17	15	15	.302182
4.246479	2.109746E-3	23.2497	27.9826	
15.2917	16.2733	18.38		
STREPTOCOCCUS CASSE		LEUCONOSTOC/GEMELLA		
8	19	15	15	.473535
4.103393	4.006379E-3	22.4785	27.8336	
15.2997	16.2845	18.3984		
STREPTOCOCCUS CASSE		STREPTOCOCCUS SP B		
8	24	15	15	.500524
4.256761	1.257113E-3	23.9224	27.7042	
15.3035	16.2897	18.4077		
STREPTOCOCCUS CASSE		STREPTOCOCCUS UBERI		
8	25	15	15	.402056
3.777673	1.509933E-4	20.686	28.1546	
15.396	16.4179	18.6259		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS RAFFI		
9	10	15	15	.351902
3.33367	8.478978E-4	18.6754	27.9491	
15.2955	16.2758	18.3842		
STREPTOCOCCUS MUTAN		ORAL STREPTOCOCCI 1		
9	11	15	15	.274369
2.760154	5.771064E-3	15.1201	27.6309	
15.2998	16.2847	18.3992		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS LACTI		
9	14	15	15	.453172
3.866571	1.198088E-4	21.1734	27.366	
15.3253	16.3199	18.4591		
STREPTOCOCCUS MUTAN		AEROCOCCUS VIRIDANS		
9	15	15	15	.469203
4.239282	1.119882E-3	24.0004	27.451	
15.3207	16.3135	18.4482		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS MITIS		
9	15	15	15	.413204
3.811183	1.137987E-4	20.6783	27.7557	
15.3039	16.2903	18.4088		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS SANGU		
9	16	15	15	.369748
3.53904	3.302461E-4	19.6835	27.9995	
15.2908	16.2721	18.3779		
STREPTOCOCCUS MUTAN		ORAL STREPTOCOCCI 2		
9	17	15	15	.357446
3.246789	1.186455E-3	17.7060	27.5793	
15.3136	16.3037	18.4315		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS MILLE		
9	18	15	15	.397903
4.000037	6.327333E-3	21.9109	27.838	
15.2995	16.2841	18.3983		

STREPTOCOCCUS MUTAN		LEUCONOSTOC/GEMELLA		
9	19	15	15	.42745
3.499160	6.558860E-5	21.8043	27.8582	
15.2984	16.2826	18.3957		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS [redacted]		
9	23	15	15	.461209
4.238032	1.185977E-3	23.992	27.9564	
15.2931	16.2753	18.3853		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS UBERI		
9	25	15	15	.400901
4.116036	3.851177E-5	22.348	27.5854	
15.3133	16.3052	18.4307		
STREPTOCOCCUS MUTAN		STREPTOCOCCUS SP R		
9	27	15	15	.38105
3.814422	1.366000E-4	20.6914	27.8874	
15.2968	16.2804	18.3921		
STREPTOCOCCUS RAFFI		ORAL STREPTOCOCCI 1		
10	11	15	15	.327571
3.219143	1.286600E-3	17.0353	27.6012	
15.3124	16.304	18.4287		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS LACTI		
10	14	15	15	.391922
3.433333	5.903643E-4	18.8051	27.6651	
15.3089	16.2971	18.4204		
STREPTOCOCCUS RAFFI		AEROCOCCUS VIRIDANS		
10	15	15	15	.457315
4.003008	5.578515E-3	22.0737	27.7203	
15.3055	16.2925	18.4126		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS MITIS		
10	15	15	15	.384436
3.322635	4.278831E-4	19.6727	27.9263	
15.2967	16.2775	18.3871		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS SANGU		
10	16	15	15	.390755
3.712220	2.053674E-4	20.3324	27.9391	
15.294	16.2788	18.3853		
STREPTOCOCCUS RAFFI		ORAL STREPTOCOCCI 2		
10	17	15	15	.347494
3.009734	1.952281E-3	16.9046	27.8102	
15.3008	16.2858	18.4011		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS MILLE		
10	18	15	15	.407073
3.997842	6.395795E-5	21.897	27.812	
15.3118	16.3012	18.4273		
STREPTOCOCCUS RAFFI		LEUCONOSTOC/GEMELLA		
10	19	15	15	.411051
3.767717	1.031062E-4	20.9336	27.9708	
15.292	16.2736	18.3808		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS [redacted]		
10	23	15	15	.419088
3.990315	9.699855E-5	21.5765	27.9997	
15.2908	16.2721	18.3779		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS SP B		
10	24	15	15	.478208
4.240975	1.035777E-3	24.1336	27.9968	
15.291	16.2726	18.3745		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS UBERI		
10	25	15	15	.425326
4.220581	2.411044E-3	23.3556	27.6033	
15.3312	16.326	18.4729		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS PYOGA		
10	26	15	15	.389703
3.723276	1.744933E-4	20.3367	27.6618	
15.2993	16.2838	18.3979		
STREPTOCOCCUS RAFFI		STREPTOCOCCUS SP R		
10	27	15	15	.341174
3.331166	8.033338E-4	18.4484	27.8874	
15.3078	16.2933	18.4173		

ORAL STREPTOCOCCI 1		STREPTOCOCCUS MITIS		
11	15	15	15	.44655
4.26124	2.03433E-5	23.3396	27.2089	
15.3343	10.3323	18.4601		
ORAL STREPTOCOCCI 1		ORAL STREPTOCOCCI 2		
11	17	15	15	.524000
3.03799	2.36174E-3	10.0396	26.9279	
15.3503	10.3545	18.5179		
ORAL STREPTOCOCCI 1		STREPTOCOCCUS MILLE		
11	18	15	15	.39673
4.15346	3.27874E-5	22.7495	27.9999	
15.2907	10.2721	18.3778		
ORAL STREPTOCOCCI 1		LEUCONOSTOC/GEMELLA		
11	19	15	15	.448219
4.33383	1.40658E-5	23.7373	27.3902	
15.3237	10.3177	18.4554		
ORAL STREPTOCOCCI 1		STREPTOCOCCUS DYSGA		
11	20	15	15	.599188
4.06714	4.36992E-5	22.3661	27.9429	
15.2936	10.2703	18.385		
ORAL STREPTOCOCCI 1		STREPTOCOCCUS SP N/		
11	27	15	15	.568965
3.63796	1.44097E-4	21.0413	27.9961	
15.2911	10.2725	18.3788		
STREPTOCOCCUS LACTI		AEROCOCCUS VIRIDANS		
12	13	15	15	.590400
3.31375	9.20702E-4	18.1301	27.9968	
15.2909	10.2723	18.3782		
STREPTOCOCCUS LACTI		STREPTOCOCCUS THERM		
12	14	15	15	.420046
4.06357	4.77393E-5	22.2681	24.866	
15.4781	10.3318	18.8206		
STREPTOCOCCUS LACTI		STREPTOCOCCUS MITIS		
12	15	15	15	.558031
3.06349	2.16787E-3	16.7796	27.9029	
15.296	10.2793	18.3901		
STREPTOCOCCUS LACTI		STREPTOCOCCUS SANGU		
12	16	15	15	.578169
3.38081	7.22621E-4	18.5175	27.3355	
15.3271	10.3224	18.4634		
STREPTOCOCCUS LACTI		ORAL STREPTOCOCCI 2		
12	17	15	15	.594373
3.32632	8.80123E-4	18.219	27.9763	
15.292	10.2738	18.3806		
STREPTOCOCCUS LACTI		STREPTOCOCCUS MILLE		
12	18	15	15	.577119
3.47142	5.17812E-4	19.0137	26.6437	
15.3668	10.3774	18.5568		
STREPTOCOCCUS LACTI		LEUCONOSTOC/GEMELLA		
12	19	15	15	.587682
3.35226	8.01607E-4	18.3612	27.0152	
15.3007	10.2858	18.4012		
STREPTOCOCCUS LACTI		STREPTOCOCCUS ANGLA		
12	20	15	15	.541222
3.30076	9.64337E-4	18.079	24.8899	
15.4765	10.3296	18.8168		
STREPTOCOCCUS LACTI		STREPTOCOCCUS EQUISIM		
12	23	15	15	.579252
3.3427	8.77981E-4	18.2227	27.0461	
15.3099	10.2966	18.4229		
STREPTOCOCCUS LACTI		STREPTOCOCCUS SP B		
12	24	15	15	.57927
3.30193	9.6U321E-4	18.0854	27.7426	
15.3046	10.2913	18.4105		
STREPTOCOCCUS LACTI		STREPTOCOCCUS UBERI		
12	25	15	15	.528085
4.01053	6.U61U3E-5	21.9666	26.1036	
15.3491	10.4222	18.6332		

STREPTOCOCCUS LACTI		STREPTOCOCCUS DYSGA		
12	26	15	15	.45325
3.91966	8.87U95E-5	21.4089	27.0639	
15.3413	10.3421	18.4966		
STREPTOCOCCUS LACTI		STREPTOCOCCUS SP N/		
12	27	15	15	.447392
4.09779	4.17340E-5	22.4463	26.7799	
15.3589	10.3604	18.5381		
AEROCOCCUS VIRIDANS		STREPTOCOCCUS MITIS		
13	15	15	15	.442432
3.80721	1.40593E-4	20.8529	27.935	
15.2942	10.2769	18.366		
AEROCOCCUS VIRIDANS		STREPTOCOCCUS SANGU		
13	16	15	15	.460836
4.14525	3.39634E-5	22.7045	27.4205	
15.3224	10.3159	18.4522		
AEROCOCCUS VIRIDANS		ORAL STREPTOCOCCI 2		
13	17	15	15	.475636
4.03392	5.48816E-5	22.0967	27.9907	
15.2912	10.2727	18.379		
AEROCOCCUS VIRIDANS		LEUCONOSTOC/GEMELLA		
13	19	15	15	.418695
3.6415	2.71127E-4	19.9453	27.8604	
15.2983	10.2824	18.3955		
AEROCOCCUS VIRIDANS		STREPTOCOCCUS SP B		
13	24	15	15	.417697
3.65907	2.52611E-4	20.0446	27.7901	
15.3017	10.2873	18.4036		
AEROCOCCUS VIRIDANS		STREPTOCOCCUS UBERI		
13	25	15	15	.368553
3.67071	5.U8037E-4	19.04	26.2333	
15.3913	10.4113	18.6146		
AEROCOCCUS VIRIDANS		STREPTOCOCCUS DYSGA		
13	26	15	15	.473526
4.30952	1.63729E-5	23.0042	27.1819	
15.3358	10.3344	18.4837		
AEROCOCCUS VIRIDANS		STREPTOCOCCUS SP N/		
13	27	15	15	.475633
4.38491	1.10166E-5	24.0172	26.8903	
15.3525	10.3575	18.523		
STREPTOCOCCUS THERM		STREPTOCOCCUS MITIS		
14	15	15	15	.496908
3.00885	2.62256E-3	16.4801	25.0340	
15.427	10.4608	18.8992		
STREPTOCOCCUS THERM		ORAL STREPTOCOCCI 2		
14	17	15	15	.370004
3.71934	1.99802E-4	20.3717	25.2341	
15.4522	10.4939	18.7391		
STREPTOCOCCUS THERM		STREPTOCOCCUS SP B		
14	24	15	15	.411041
4.24489	8.18046E-5	23.2502	26.1250	
15.3978	10.4203	18.6301		
STREPTOCOCCUS MITIS		STREPTOCOCCUS SANGU		
15	16	15	15	.520500
2.96284	3.U6869E-3	16.2281	27.7340	
15.3051	10.2919	18.4115		
STREPTOCOCCUS MITIS		ORAL STREPTOCOCCI 2		
15	17	15	15	.400083
2.53U68	1.43685E-2	13.7073	27.9748	
15.2921	10.2739	18.361		
STREPTOCOCCUS MITIS		STREPTOCOCCUS MILLE		
15	18	15	15	.545918
3.2988	9.71121E-4	18.0083	27.2233	
15.3334	10.3311	18.4762		
STREPTOCOCCUS MITIS		LEUCONOSTOC/GEMELLA		
15	19	15	15	.403776
3.00116	3.10433E-4	19.7488	27.9857	
15.2915	10.2731	18.3796		

STREPTOCOCCUS MITIS		STREPTOCOCCUS AGALA		.391667
15	20	15	15	
3.94217	6.07807E-5	21.3922	25.0771	
15.4256	16.4568	18.6936		
STREPTOCOCCUS MITIS		STREPTOCOCCUS [illegible]		.392909
15	22	15	15	
3.53943	3.71736E-4	19.4938	27.917	
15.2952	16.2702	18.3683		
STREPTOCOCCUS MITIS		STREPTOCOCCUS SP_G		.382455
15	24	15	15	
3.43621	3.90012E-4	18.8204	27.9608	
15.2929	16.275	18.3828		
STREPTOCOCCUS MITIS		STREPTOCOCCUS DYSGA		.293396
15	26	15	15	
2.74049	6.02306E-3	15.0431	27.5604	
15.3146	16.3051	18.434		
STREPTOCOCCUS MITIS		STREPTOCOCCUS SP_R/		.4046U2
15	27	15	15	
3.83080	1.24653E-4	21.0154	27.3311	
15.3274	16.3228	18.4039		
STREPTOCOCCUS SANGU		ORAL STREPTOCOCCI 2		.337615
16	17	15	15	
3.07031	2.13832E-3	18.8188	27.5522	
15.3151	16.3057	18.435		
STREPTOCOCCUS SANGU		STREPTOCOCCUS MILLE		.319243
16	18	15	15	
3.21653	1.27764E-3	17.6177	27.6546	
15.2986	16.2829	18.3982		
STREPTOCOCCUS SANGU		LEUCONOSTOC/GEMELLA		.446494
16	19	15	15	
4.17752	2.94882E-3	22.8812	27.8419	
15.2993	16.2838	18.3976		
STREPTOCOCCUS SANGU		STREPTOCOCCUS AGALA		.36858
16	20	15	15	
4.15748	3.41972E-3	22.7714	26.827	
15.3561	16.3626	18.5516		
STREPTOCOCCUS SANGU		STREPTOCOCCUS [illegible]		.421208
16	23	15	15	
4.00818	6.12177E-5	21.9537	27.947	
15.2936	16.276	18.3645		
STREPTOCOCCUS SANGU		STREPTOCOCCUS SP_G		.422115
16	24	15	15	
3.98105	6.866414E-5	21.8051	27.8979	
15.2962	16.2796	18.3907		
STREPTOCOCCUS SANGU		STREPTOCOCCUS DYSGA		.394732
16	26	15	15	
3.89576	9.79301E-5	21.538	27.9767	
15.292	16.2736	18.3808		
STREPTOCOCCUS SANGU		STREPTOCOCCUS SP_R/		.380352
16	27	15	15	
3.80942	1.39343E-4	20.865	27.9011	
15.2961	16.2794	18.3905		
ORAL STREPTOCOCCI 2		STREPTOCOCCUS MILLE		.24846
17	18	15	15	
2.32765	1.99308E-2	12.749	26.9445	
15.3493	16.3532	18.5156		
ORAL STREPTOCOCCI 2		LEUCONOSTOC/GEMELLA		.355462
17	19	15	15	
3.12178	1.79772E-3	17.0967	27.9227	
15.2949	16.2778	18.3876		
ORAL STREPTOCOCCI 2		STREPTOCOCCUS AGALA		.405483
17	20	15	15	
3.99939	6.35352E-5	21.9056	25.2825	
15.4507	16.4938	18.7555		
ORAL STREPTOCOCCI 2		STREPTOCOCCUS PYOGE		.291501
17	21	15	15	
3.08657	2.03859E-3	18.8949	21.89	
15.7018	16.8431	19.3574		

ORAL STREPTOCOCCI 2		STREPTOCOCCUS EQUI		.392504
17	22	15	15	
4.02093	5.27974E-5	22.0235	23.5891	
15.5676	16.6562	19.0344		
ORAL STREPTOCOCCI 2		STREPTOCOCCUS [illegible]		.313662
17	23	15	15	
2.7958	3.17725E-3	15.3132	27.8019	
15.3014	16.2868	18.4029		
ORAL STREPTOCOCCI 2		STREPTOCOCCUS SP_B		.422147
17	24	15	15	
3.73334	1.89013E-4	20.4484	27.8729	
15.2976	16.2815	18.3939		
ORAL STREPTOCOCCI 2		STREPTOCOCCUS DYSGA		.276479
17	26	15	15	
2.54414	1.04954E-2	13.9348	27.3309	
15.3271	16.3223	18.4032		
ORAL STREPTOCOCCI 2		STREPTOCOCCUS SP_R/		.3046U9
17	27	15	15	
2.83896	4.52643E-3	15.5496	27.0078	
15.3423	16.3434	18.499		
STREPTOCOCCUS MILLE		LEUCONOSTOC/GEMELLA		.414452
18	19	15	15	
4.00446	6.21826E-5	21.9335	27.4092	
15.323	16.3167	18.4537		
STREPTOCOCCUS MILLE		STREPTOCOCCUS AGALA		.375618
18	20	15	15	
4.19324	2.75106E-5	22.9675	27.4696	
15.3197	16.3121	18.4458		
STREPTOCOCCUS MILLE		STREPTOCOCCUS PYOGE		.241722
18	21	15	15	
2.95847	3.09181E-3	16.2042	24.0446	
15.493	16.3525	18.6561		
STREPTOCOCCUS MILLE		STREPTOCOCCUS [illegible]		.306529
18	23	15	15	
2.99602	4.73539E-3	16.4099	27.6318	
15.3107	16.2997	18.4247		
STREPTOCOCCUS MILLE		STREPTOCOCCUS SP_B		.4U39
18	24	15	15	
3.93588	8.29259E-5	21.5527	27.5193	
15.3169	16.3083	18.4393		
STREPTOCOCCUS MILLE		STREPTOCOCCUS DYSGA		.303694
18	26	15	15	
3.10894	1.07773E-3	17.0284	27.9471	
15.2936	16.276	18.3845		
STREPTOCOCCUS MILLE		STREPTOCOCCUS SP_R/		.373503
18	27	15	15	
3.88031	1.04277E-4	21.4344	27.9954	
15.291	16.2724	18.3784		
LEUCONOSTOC/GEMELLA		STREPTOCOCCUS SP_R/		.458594
19	27	15	15	
4.21433	4.50435E-5	23.0834	27.504	
15.3178	16.3094	18.4413		
STREPTOCOCCUS AGALA		STREPTOCOCCUS [illegible]		.351100
20	23	15	15	
3.84744	2.04437E-4	19.9778	26.3484	
15.3443	16.4017	18.5982		
STREPTOCOCCUS AGALA		STREPTOCOCCUS SP_B		.317730
20	24	15	15	
3.27466	1.03802E-3	17.958	26.1408	
15.3965	16.4166	18.627		
STREPTOCOCCUS AGALA		STREPTOCOCCUS DYSGA		.370130
20	26	15	15	
4.04963	4.43881E-5	22.4254	27.1083	
15.34	16.3402	18.4935		
STREPTOCOCCUS PYOGE		STREPTOCOCCUS ZOOEP		.260338
21	23	15	15	
3.10039	1.5757/1E-3	17.3102	23.0200	
15.6098	16.715	19.1357		

Streptococcus pyoge		Streptoococcus dysga		
21	26	15	15	.368287
4.37339	1.22429E-5	23.954	24.038	
15.5351	16.6111	18.9567		
Streptococcus equi		Streptoococcus equisim		
22	23	15	15	.26863
2.91951	.003506	15.9908	24.7687	
15.4846	16.5409	18.8361		
Streptococcus equisim		Streptococcus sp. B		
23	24	15	15	.347831
3.21249	1.31604E-3	17.5955	27.9919	
15.2912	16.2726	18.3789		
Streptococcus equisim		Streptococcus dysga		
23	26	15	15	.355205
3.42633	6.11907E-4	18.7668	27.8545	
15.2986	16.2829	18.3962		
Streptococcus equisim		Streptococcus sp. R,		
23	27	15	15	.395826
3.87211	1.07936E-4	21.2084	27.7072	
15.3066	16.294	18.415		
Streptococcus sp. B		Streptococcus dysga		
24	26	15	15	.371425
3.55003	3.85264E-4	19.4443	27.7791	
15.3027	16.2885	18.4058		
Streptococcus dysga		Streptococcus sp. R,		
26	27	15	15	.378932
3.85282	1.16808E-4	21.1028	27.9736	
15.2922	16.274	18.3812		

END OF CALCULATIONS AND OF RUN

Table A.VII.g OVERMAT results for identification matrix PDBSTP2.

```
PROGRAM OVERMAT FOR OVERLAP STATISTICS BETWEEN
TAXA IN AN IDENTIFICATION MATRIX
(FILE NAME PHS7B11), UPDATED NOVEMBER 1978
THIS CALCULATES OVERLAP BETWEEN TAXA FROM A
MATRIX NAMED A GENERATED BY FORTRAN
PROGRAM MJS54ER FROM AN IDENTIFICATION
MATRIX OF PERCENT POSITIVE CHARACTERS.
MATRIX A HAS Q ROWS OF TAXA FOR M
CHARACTERS. THE NUMBER OF STRAINS, N(J),
ON WHICH EACH TAXON J WAS BASED, MUST BE
GIVEN AS DATA AT 9001 ONWARDS.
THESE NUMBERS MUST BE INTEGERS OVER 1.

THE OVERLAP STATISTICS OF SNEATH (1977)
ARE CALCULATED BETWEEN EACH PAIR OF TAXA
AND OUTPUT IF W IS UNDER A CUTOFF LEVEL
C (C >= 0) FOR COMPLETE OVERLAP W = 0).
IF C IS NEGATIVE THE LEVEL IS SET TO THAT
EXPECTED FOR A RECTANGULAR DISTRIBUTION,
SQRT 3 * (3/(N(L) * N(M) - 1)).

IF THE INTERCENTROID DISTANCE IS TO BE CORRECTED
FOR THE ESTIMATED SAMPLING ERROR, INPUT 1, ELSE
INPUT ZERO.  NOTE THAT THIS MAY GIVE NEGATIVE
DISTANCES WHICH WILL BE TREATED AS ZEROS.

OPTION = 0:  INTERCENTROID DISTANCE IS
NOT CORRECTED FOR ESTIMATED SAMPLING ERROR

INPUT THE CUTOFF OVERLAP V(G) DESIRED WHICH
WILL BE CONVERTED TO W TO GIVE THE CUTOFF
LEVEL C.  V(G) MUST BE <= 1: IF NEGATIVE
THEN W WILL BE THAT EXPECTED FOR A
RECTANGULAR DISTRIBUTION. IF CUTOFF W INSTEAD
OF V(G) IS PREFERRED, INPUT ZERO, AND INPUT
THE DESIRED W AT THE NEXT INPUT.
CUTOFF LEVEL OF V(G) =                .00001

IF THE CUTOFF V(G) PREVIOUSLY INPUT IS TO BE
USED, INPUT ZERO, ELSE INPUT THE DESIRED CUTOFF
LEVEL OF W ITSELF (WHICH OVERWRITES THE
PREVIOUS CUTOFF VALUE, EVEN IF NEGATIVE).

PDBSTP2 IS A MATRIX OF 60 DIAGNOSTIC TESTS FOR THE STREPTOCOCCACEAE
 M              Q              C
 60             28             4.41745

IF T(0) FOR PROBABILITIES P = 0.90,
0.95, 0.99 ARE REQUIRED, INPUT THE CHOSEN
CRITICAL OVERLAP AS A PROPORTION V(0),
OR INPUT -1 FOR RECTANGULAR CASE, ELSE INPUT ZERO.
IF CALCULATED, T(0) ARE PRINTED ON THE
FOURTH LINE
-------------------------------------------------

CRITICAL OVERLAP V(0) FOR W0 = .025
CORRESPONDING TO W(0) OF                    2.24138

OVERLAP STATISTICS ARE PRINTED AS FOLLOWS
     ON FIRST LINE NAMES OF TAXA BEING COMPARED
     ON SECOND LINE
TAXON NO.   TAXON NO.    N(L)     N(M)     D(L,M)
     ON THIRD LINE
   W                V(G)             T(W)          F
     ON FOURTH LINE
   T(0), P = 0.90   T(0), P = 0.95   T(0), P = 0.99

S.FAECALIS                          A.VIRIDANS
 1                13                 10               3               .496085
 4.29559          1.74356E-5         15.488           2.54461
 28.8512          27.1297            38.832

S.FAECALIS                          PEDIOCOCCUS
 1                28                 10               5               .473786
 4.34219          1.41178E-5         16.8172          5.44666
 15.8409          17.672             24.7852

S.AVIUM                             S.SP.(CHICKEN)
 3                4                  4                17              .300787
 3.13482          1.71975E-3         14.3655          3.7822
 28.6385          25.2837            37.6141

S.SP.(CHICKEN)                      A.VIRIDANS
 4                13                 17               3               .565156
 4.88645          4.38250E-5         18.2752          2.21859
 28.0608          38.1069            48.6365

S.SP.(CHICKEN)                      PEDIOCOCCUS
 4                28                 17               5               .551765
 4.37163          1.23418E-5         20.5848          4.53896
 19.3397          23.1211            33.3338

S.BOVIS                             S.SALIVARIUS
 5                7                  4                7               .350281
 3.65014          2.62167E-4         11.5427          7.23628
 11.3623          13.0314            17.3084

S.BOVIS                             S.CASSELIFLAVUS
 5                8                  4                4               .398926
 4.33864          1.43478E-5         12.2715          5.95878
 18.8178          12.6417            17.4564

S.BOVIS                             S.MUTANS
 5                9                  4                3               .383884
 4.41889          1.83832E-5         11.6701          4.59674
 11.1456          13.4116            19.5929

S.BOVIS                             S.LACTIS
 5                12                 4                12              .418189
 4.8361           5.43747E-5         16.1444          5.41641
 15.5477          18.2725            25.5603

S.SALIVARIUS                        S.CASSELIFLAVUS
 7                8                  7                4               .432325
 4.32981          1.49889E-5         13.6895          6.90172
 11.5696          13.253             17.7467

S.SALIVARIUS                        S.MUTANS
 7                9                  7                3               .488712
 4.18615          2.83896E-5         12.5585          4.94586
 12.1986          14.5261            28.8227

S.SALIVARIUS                        S.RAFFINOLACTIS
 7                10                 7                4               .396266
 4.17796          2.94389E-5         13.2119          7.33144
 11.3231          12.9725            17.1816

S.SALIVARIUS                        ORAL 1
 7                11                 7                4               .41415
 4.27767          1.88994E-5         13.5272          7.16386
 11.3933          13.878             17.3944

S.SALIVARIUS                        S.LACTIS
 7                12                 7                12              .347565
 3.31349          9.21494E-4         14.858           9.32515
 14.127           15.8471            28.8847

S.SALIVARIUS                        A.VIRIDANS
 7                13                 7                3               .434189
 3.95582          7.63078E-5         11.8474          3.98844
```

13.3453	16.3148	24.3694		
S.SALIVARIUS		S.THERMOPHILUS		
7	14	14	6	.389117
4.27504	1.91234E-5	14.8092	9.42159	
11.6231	13.8574	16.568		
S.SALIVARIUS		STREP.MITIS		
7	15	15	6	.420387
4.1962	2.71685E-5	14.536	9.93182	
11.4411	12.449	16.1639		
S.SALIVARIUS		ORAL 2		
7	17	6	13	.395323
3.67218	2.48557E-4	16.8867	9.35222	
14.4493	16.2465	28.5756		
S.SALIVARIUS		S.MILLERI		
7	18	7	3	.441677
4.38119	1.18125E-5	13.1436	4.53701	
12.6173	15.1761	22.1384		
S.SALIVARIUS		PEDIOCOCCUS		
7	28	12	5	.426282
3.86419	1.11501E-4	12.8161	8.89421	
11.5499	13.1223	17.8693		
S.CASSELIFLAVUS		S.LACTIS		
8	12	4	12	.43491
3.97774	6.96045E-5	15.911	5.87577	
15.9185	18.8387	26.6978		
S.CASSELIFLAVUS		ORAL 2		
8	17	4	13	.466838
4.13478	3.49351E-5	17.8645	5.87128	
16.3987	19.4836	27.4898		
S.MUTANS		S.LACTIS		
9	12	3	12	.468979
4.36971	1.24588E-5	16.9238	3.36865	
18.6377	23.2471	35.8553		
S.RAFFINOLACTIS		S.LACTIS		
10	12	4	12	.393724
3.86257	1.12242E-4	15.4503	5.53143	
15.4341	18.8997	25.212		
ORAL 1		ORAL 2		
11	17	4	13	.428194
3.89144	9.96918E-5	16.8448	5.33137	
16.1811	18.9493	26.579		
S.LACTIS		A.VIRIDANS		
12	13	12	3	.389446
3.84797	2.38485E-3	11.8047	2.85401	
28.5982	26.3161	39.1584		
S.LACTIS		STREP.MITIS		
12	15	12	6	.448746
4.36863	1.25127E-5	16.5345	9.57844	
14.8442	15.726	19.8589		
S.LACTIS		S.SANGUIS		
12	16	12	3	.48573
3.91596	9.80814E-5	15.1665	2.92146	
28.283	25.8234	38.5975		
S.LACTIS		S.MILLERI		
12	18	12	3	.581651
4.3687	1.25044E-5	16.9199	3.12944	
19.4265	24.4826	36.8852		
S.LACTIS		STREP.SP.B		
12	24	12	18	.37151
3.93949	6.16861E-5	18.4779	19.1719	
13.7686	14.8566	17.2962		
S.LACTIS		STREP.SP.R,S,T		
12	27	12	3	.49962
4.14833	3.35118E-5	16.8664	2.99486	
19.9622	25.3213	37.9913		
S.LACTIS		PEDIOCOCCUS		
12	28	12	5	.448681
3.71913	1.99969E-4	15.3344	6.26082	

15.2613	17.677	24.818		
A.VIRIDANS		STREP.MITIS		
13	15	3	6	.426542
3.92386	8.74662E-5	11.7692	3.87702	
13.5253	16.5976	24.9833		
A.VIRIDANS		ORAL 2		
13	17	3	13	.488074
3.7888	2.14985E-4	14.8032	2.84916	
21.2822	27.1847	48.3786		
A.VIRIDANS		STREP.SP.B		
13	24	3	18	.41883
4.3873	7.05942E-4	12.2131	3.86572	
18.3482	23.2171	35.818		
A.VIRIDANS		PEDIOCOCCUS		
13	28	3	5	.426712
4.7913	1.49913E-4	10.7234	4.76492	
11.782	14.8062	28.266		
S.THERMOPHILUS		STREP.MITIS		
14	15	6	6	.388154
4.34487	1.39971E-5	15.8483	9.54864	
11.5919	13.8116	16.4791		
S.THERMOPHILUS		PEDIOCOCCUS		
14	28	6	5	.44383
4.37574	1.21117E-5	14.5127	6.8555	
12.8626	13.8895	18.6887		
STREP.MITIS		S.SANGUIS		
15	16	6	3	.413799
3.98886	9.62087E-5	11.7882	4.81182	
13.3111	16.261	24.267		
STREP.MITIS		ORAL 2		
15	17	6	13	.415285
3.93942	8.17125E-5	17.1715	9.61257	
14.4823	16.1192	28.3385		
STREP.MITIS		LEUCONOSTOC		
15	19	6	7	.383112
4.88963	6.88417E-5	14.4569	18.3664	
11.8338	13.2867	16.5173		
STREP.MITIS		STREP.SP.B		
15	24	6	18	.396169
3.94795	7.68581E-5	15.7918	18.2957	
13.8757	14.582	18.2284		
STREP.MITIS		PEDIOCOCCUS		
15	28	6	5	.471966
4.32762	1.50641E-5	14.3531	7.95285	
11.6882	13.1971	17.218		
S.SANGUIS		ORAL 2		
16	17	3	13	.379476
3.96487	3.03618E-3	11.8563	2.91636	
28.9564	26.6759	39.8127		
S.SANGUIS		LEUCONOSTOC		
16	19	3	7	.44687
4.17458	2.98714E-5	13.2812	3.58616	
14.9656	18.6871	28.1963		
S.SANGUIS		STREP.SP.B		
16	24	3	18	.496487
4.21118	2.54282E-5	15.1836	3.14982	
18.8489	22.747	34.3713		
S.SANGUIS		PEDIOCOCCUS		
16	28	3	5	.488554
4.35677	1.32896E-5	12.3228	4.98754	
11.5788	13.8824	19.8493		
ORAL 2		S.MILLERI		
17	18	13	3	.374186
4.15752	1.59131E-3	12.6381	3.1238	
28.8699	25.2898	38.8564		

ORAL 2		LEUCONOSTOC		
17	19	13	7	.33217
3.40498	6.61796E-4	15.2275	13.0582	
13.9155	15.2927	18.5163		
ORAL 2		S.EQUI		
17	22	13	2	.503485
4.23003	2.33815E-5	16.3828	1.51636	
39.6431	51.7828	31.3102		
ORAL 2		STREP.SP.R,S,T		
17	27	13	3	.4744
3.81642	1.35451E-4	15.2657	2.98954	
20.6244	26.1572	39.1916		
ORAL 2		PEDIOCOCCUS		
17	28	13	5	.43297
3.49044	4.82318E-4	14.8087	6.25708	
15.6919	18.1725	24.685		
S.EQUI		S.EQUISIM		
22	23	2	8	.391918
3.91457	9.06014E-5	12.379	1.69248	
27.7027	37.3426	30.3813		
S.EQUISIM		STREP.SP.B		
23	24	8	10	.327386
3.5947	3.24841E-4	15.251	15.6232	
12.8652	14.0243	16.6648		
STREP.SP.B		PEDIOCOCCUS		
24	28	10	5	.454476
3.91301	9.11897E-5	15.155	6.75817	
14.0435	16.1636	21.662		

END OF CALCULATIONS AND OF RUN

REFERENCES

Abd-el-Malek, Y. & Gibson, T. (1948) Studies in the bacteriology of milk. I The streptococci of milk. Journal of Dairy Research 15, 233-248.

Amstein, F.A. & Hartman, P.A. (1972) Differentiation of enterococci by gas chromatography. Journal of Bacteriology 113, 38-41.

Andrewes, F.A. & Horder, T.J. (1906) A study of the streptococci pathogenic for man. Lancet 2, 708-713, 775-782, 852-855.

Ayers, S.H. & Mudge, C.S. (1922) The streptococci of the bovine udder. Journal of Infectious Diseases 34, 29-48.

Balcke, J. (1884) Über faurigen geruch des bieres. Wochenschrift fur brauerei 1, 257.

Barnes, E.M., Mead, G.C., Impey, C.S. & Adams, B.W. (1978) The effect of dietary bacitracin on the incidence of Streptococcus faecalis subsp. liquefaciens and related streptococci in the intestines of young chicks. British Poultry Science 19, 713-723.

Barnes, I.J., Seeley, H.W. & van Demark, P.J. (1961) Nutrition of Streptococcus bovis in relation to dextran formation. Journal of Bacteriology 82, 85-93.

Beers, R.J. & Lockhart, W.R. (1962) Experimental methods in computer taxonomy. Journal of General Microbiology 28, 633-640.

Beers, R.J. Fisher, J., Megraw, S. & Lockhart, W.R. (1962) A comparison of methods for computer taxonomy.

Beighton, D., McIntosh, H.A. & McDougall, W.A. (1979) Bacteriological studies on the effects of cow's milk on dental plaque and dental caries in rats. Journal of Applied Bacteriology 47, 255-262.

Beighton, D., Russell, R.R.B. & Hayday, H. (1981) The isolation and characterisation of Streptococcus mutans serotype h from dental plaque of monkeys (Macaca fascicularis). Journal of General Microbiology 124, 271-279.

Belozersky, A. & Spirin, A.S. (1960) Chemistry of the nucleic acids of micro-organisms. In The Nucleic Acids, Vol. 3 Eds. Chargoff, E. & Davidson, J.N. New York & London: Academic Press.

Berger, U. (1961) A proposed new genus of gram-negative cocci: Gemella. International Bulletin of Bacteriological Nomenclature and Taxonomy 11, 17-19.

Bergey, D.H., Harrison, F.C., Breed, R.S., Hammer, B.W. & Huntoon, F.M. (1926) Bergey's Manual of Determinative Bacteriology 2nd edn., Baltimore: The Williams & Wilkins Company.

Brandis, H. (1978) Bacteriocins of streptococci and bacteriocin typing. In Methods in Microbiology, Vol.12, Eds. Bergan, T. & Norris, J.R. London: Academic Press.

Bryan-Jones, D.G. & Whittenbury, R. (1969) Haematin dependant oxidative phosphorylation in Streptococcus faecalis. Journal of General Microbiology 58, 247-260.

Buchanan, R.E. & Gibbons, N.E. (1974) Bergey's Manual of Determinative Bacteriology, 8th edn., Baltimore: The Williams & Wilkins Company.

Buchanan, R.E., Holt, J.G. & Lessel, E.F. (1966 Index Bergeyana London: Livingstone.

Buissière, J. (1972) Perfectionnement du tube d'Ivan Hall pour l'étude en série de la croissance et du la physiologie des bactéries. Comptes Rendus Academie Sciences Paris 274, 1426-1429.

Carlsson, J. (1968) A numerical study of human oral streptococci. Odontologisk Revy 19, 137-160.

Carlsson, J. (1973) Simplified gas chromatographic procedure for identification of bacterial metabolic products. Applied Microbiology 25, 287-289.

Chesbro, W.R. & Evans, J.B. (1959) Factors affecting the growth of enterococci in highly alkaline media. Journal of Bacteriology 78, 858-862.

Christie, R., Atkins, N.E. & Munch-Peterson, E. (1944) A note on a lytic phenomenon shown by group B streptococci. Australian Journal of Experimental Biology and Medical Science 22, 197-200.

Clarke, J.K. (1924) On the bacterial factor in the aetiology of dental caries. British Journal of Experimental Pathology 5, 141-147.

Clewell, D.B., Yagi, Y., Dunney, G.M. & Schultz, S.K. (1974) Characterisation of three plasmid deoxyribonucleic acid molecules in a strain of Streptococcus faecalis: Identification of a plasmid determining erythromycin resistance. Journal of Bacteriology 117, 283-289.

Cliffe, A.J. & Law, B.A. (1979) An electrophoretic study of peptidases in starter streptococci and in cheddar cheese. Journal of Applied Bacteriology 47, 65-73.

Cole, J.S. & Kolstad, R.A. (1974) Some atypical features of Streptococcus sanguis ATCC 10557. International Journal of Systematic Bacteriology 24, 370-372.

Collins, M.D. & Jones, D. (1979) The distribution of isoprenoid quinomes in streptococci of serological groups D and N. Journal of General Microbiology 114, 27-33.

Colman, G. (1968) The application of computers to the classification of streptococci. Journal of General Microbiology 50, 149-158.

Colman, G. & Williams, R.E.O. (1965) Cell walls of streptococci. Journal of General Microbiology 41, 375-387.

Colman, G. & Williams, R.E.O. (1972) Taxonomy of some human viridans streptococci. In Streptococci and Streptoccocal Diseases. eds Wannamaker, L.W. & Matsen, J.M. New York & London: Academic Press.

Colobert, L. & Blondeau, H. (1962) L'espèce Streptococcus faecalis. I Etudé de l'homogénéité par la methode Adansonnienne. Annals de l'Institut Pasteur, Paris, 103 345-362.

Cooper, K.E. & Ramadan, F.M. (1955) Studies in the differentiation between human and animal pollution by means of the faecal streptococci. Journal of General Microbiology 12, 180-190.

Cords, B.R., McKay, L.L. & Guerry, P. (1974) Extra-chromosomal elements in group N streptococci. Journal of Bacteriology 117, 1149-1152.

Cowan, S.T. & Steel, K.J. (1974) Cowan & Steel's Manual for the Identification of Medical Bacteria. 2nd. edn., Cambridge: Cambridge University Press.

Coykendall, A.L. (1974) Four types of Streptococcus mutans based on their genetic, antigenic and bio-chemical characteristics. Journal of General Microbiology 83, 327-338.

Coykendall, A.L. (1977) Proposal to elevate the sub-species of Streptococcus mutans to species status, based on their molecular composition. International Journal of Systematic Bacteriology 27, 26-30.

Crowley, N. Bradley, J.M. & Darrel, J.H. (1969) Practical Bacteriology London: Butterworths.

Cruickshank, R., Duguid, J.P., Marmion, B.P. & Swain, R.H.A. (1968) Medical Microbiology Vol.1. 11th edn. Edinburgh: Churchill Livingstone.

Cumming, G.C., Ross, P.W., Poxton, I.R. & McBridge, W.H. (1980) Grouping of β-haemolytic streptococci by enzyme linked immunosorbent assay. Journal of Medical Microbiology 13, 459-462.

Davis, G.H.G., Fomin, L., Wilson, E. & Newton, K.G. (1969) Numerical taxonomy of Listeria, streptococci and possibly related bacteria. Journal of General Microbiology 57, 333-348.

De Ley, J. (1970) Re-examination of the association between melting point, bouyant density and chemical base composition of deoxyribonucleic acid. Journal of Bacteriology 101, 738-754.

De Ley, J., Segers, P. & Gillis, M. (1978) Intra- and intergenic similarities of Chromobacterium and Janthinobacterium ribosomal ribonucleic acid cistrons. International Journal of Systematic Bacteriology 28, 154-168.

De Moor, C.E. and Thal, E. (1968) Beta haemolytic streptococci of the Lancefield groups E, P and U, Streptococcus infrequens. Antonie van Leeuwenhoek 34, 377-387

Dent, V.E., Hardie, J.M. & Bowden, G.H. (1978) Streptococci isolated from dental plaque of animals. Journal of Applied Bacteriology 44, 249-258.

Diebel, R.H. (1964) The group D streptococci. Bacteriological Reviews 28, 330-366.

Diebel, R.H., Lake, D.E. & Niven, C.F. Jnr. (1963) Physiology of the enterococci as related to their taxonomy. Journal of Bacteriology 86, 1275-1282.

Diebel, R.H. and Seeley, H.W. (1974) Streptococcaceae fam. nov. In Bergey's Manual of Determinative Bacteriology 8th edn, eds. Buchanan, R.E. & Gibbons, N.E. Baltimore: Williams and Wilkins Company.

Diebel, R.H., Yao, J., Jacobs, N.J. & Niven, C.F. (1964) Group E streptococci: I Physiological characterisation of strains isolated from swine cervical abcesses. Journal of Infectious Diseases 114, 327-332.

Diernhofer, K. (1932) Aesculinbouillon als Hilfsmittel fur die Differenzierung von Euten und Milchstreptokokken bei Massenuntersuchungen. Milchwirtschaftliche Forschungen 13, 368-374.

Dolezil, L. & Kirsop, B.H. (1977) The use of the API Lactobacillus system for the characterisation of the Pediococci. Journal of Applied Bacteriology 42,213-217.

Drucker, D.B. & Melville, T.H. (1969) Computer classification of streptococci, mostly of oral origin. Nature, London 221,664.

Dunney, G.M. & Clewell, D.B. (1975) Transmissible toxin (haemolysin) plasmid in Streptococcus faecalis and its mobilisation of a non-infectious drug resistance plasmid. Journal of Bacteriology 124, 784-790.

Elliot, S.D. (1966) Streptococcal infection in young pigs. I. An immunological study of the causative agent (PM streptococcus). Journal of Hygiene, Cambridge 64, 205-212

Facklam, R.R. (1972) Recognition of group D streptoccal species of human origin by biochemical and physiological tests. Applied Microbiology 113, 38-41.

Facklam, R.R. (1974) Characteristics of Streptococcus mutans isolated from human dental plaque and blood. International Journal of Systematic Bacteriology 24, 313-319.

Facklam, R.R. (1977) Physiological differentiation of viridans streptococci. Journal of Clinical Microbiology 5, 184-201

Facklam, R.R. & Moody, M.D. (1970) Presumptive identification of group D streptococci: The bile esculin test. Applied Microbiology 20, 245-250.

Facklam, R.R., Padula, J.F., Thacker, L.G., Worthman, E.C. & Sconyers, B.J. (1974) Presumptive identification of group A, B and D streptococci. Applied Microbiology 27, 107-113.

Falkow, S. (1958) Activity of lysine decarboxylase as an acid in the identification of Salmonellae and Shigellae. American Journal of Clinical Pathology 29, 598-600.

Farrow, J.A.E. (1980) Lactose hydrolysing enzymes in Streptococcus lactis and Streptococcus cremoris and also in some other species of streptococci. Journal of Applied Bacteriology 49, 493-503.

Feltham, R.K.A. (1975) A comparison of numerical and electrophoretic studies of the Enterobacteriaceae. Ph.D Thesis, University of Leicester, U.K.

Feltham, R.K.A. (1979) A taxonomic study of the genus Streptococcus. In Pathogenic Streptococci, ed. Parker, M. Surrey: Reedbooks Ltd.

Feltham, R.K.A., Power, A.K., Pell, P.A. & Sneath, P.H.A. (1978) A simple method for storage of bacteria at -76°C. Journal of Applied Bacteriology 44, 313-316.

Feltham, R.K.A. & Sneath, P.H.A. (1979) Quantitative comparison of electrophoretic traces of bacterial proteins. Computers and Biomedical Research 12, 247-263.

Fowler, R.C., Coble, D.W., Kramer, N.C. & Brown, T.McP. (1963) Starch gel electrophoresis of a fraction of certain of the pleuropneumonia like group of micro-organisms. Journal of Bacteriology 86, 1145-1151.

Frost, W.D. & Engelbrecht, M.A. (1936) A revision of the genus Streptococcus. Department of Agricultural Bacteriology, University of Wisconsin, Madison, pp 1-4.

Fuchs, P.G., Jocanta, Z. & Dobrzanski, W.T. (1975) Possible plasmid nature of the determinant for production of the antibiotic nisin in some strains of Streptococcus lactis. Journal of General Microbiology 88, 189-192.

Garvie, E.I. (1960) The genus Leuconostoc and its nomenclature. Journal of Dairy Research 27, 283-292.

Garvie, E.I. (1969) Lactic dehydrogenases of strains of the genus Leuconostoc. Journal of General Microbiology 58, 85-94.

Garvie, E.I. (1974) The genus Leuconostoc. In Bergey's Manual of Determinative Bacteriology, 8th edn, eds. Buchanan, R.E. & Gibbons, N.E. Baltimore: Williams and Wilkins Company.

Garvie, E.I. (1976) Hybridization between the deoxyribonucleic acids of some strains of heterofermentative lactic acid bacteria. International Journal of Systematic Bacteriology 26, 116-122.

Garvie, E.I. (1978) Streptococcus raffinolactis (Orla-Jensen & Hansen); a group N streptococcus found in raw milk. International Journal of Systematic Bacteriology 28, 190-193.

Garvie, E.I. (1979) A note on the preparation of deoxyribonucleic acid from Streptococcus bovis and variations in the melting temperature of different preparations. Journal of Applied Bacteriology (1979) 46, 553-555.

Garvie, E.I. & Bramley, A.J. (1979a) Streptococcus uberis; an approach to its classification. Journal of Applied Bacteriology 46, 295-304.

Garvie, E.I. & Bramley, A.J. (1979b) Streptococcus bovis; an approach to its classification and its importance as a cause of bovine mastitis. Journal of Applied Bacteriology 46, 557-566.

Gibbons, R.J., Kapsimalis, B. & Socransky, S.S. (1964) The source of salivary bacteria. Archives of Oral Biology 9, 101-103.

Gordon, A.H. (1969) Electrophoresis of Proteins in Polyacrylamide and Starch Gels. Vol. 1 Part 1 of Laboratory Techniques in Biochemistry and Molecular Biology. Eds. Work, T.S. & Work, E. London: North Holland Publishing Company.

Gordon, M.H. (1905) A ready method of differentiating streptococci and some results already obtained by its application. Lancet 2, 1400-1403.

Guthof, O. (1956) Über pathogene "vergrunende Streptokokken". Zentralblatt fur Bakteriologie, Parasitenkunde, Infectionskrankheiten und Hygiene (Abteilung 1), 553-564.

Gutteridge, C.S. & Norris, J.R. (1979) The application of pyrolysis techniques to the identification of micro-organisms. Journal of Applied Bacteriology 47, 5-44.

Hamada, S. & Mizuno, J. (1974) Iso-electric focusing in polyacrylamide gel of the membrane proteins of Streptococcus mutans and related streptococci. Journal of Dental Research 53, 547-553.

Harwood, C.R. (1980) Plasmids. In Microbiological Classification and Identification. Eds. Goodfellow, M. & Board, R.G. Society for Applied Bacteriology Symposium Series No.8. London: Academic Press.

Hess, E.L. & Slade, H.D. (1965) An electrophoretic examination of cell free extracts from various serological types of group A haemolytic streptococci. Biochemica et Biophysica Acta 16, 346-353.

Hill, L.R. (1966) An index to deoxyribonucleic acid base compositions of bacterial species. Journal of General Microbiology 44, 419-437.

Hill, L.R. (1968) The determination of deoxyribonucleic acid base compositions and its application to bacterial taxonomy. In Identification Methods for Microbiologists Part B. Eds. Gibbs, B.M. & Shapton, D.A. London: Academic Press.

Hitchcock, C.H. (1924) Precipitation and complement fixation reactions with residue antigens in the non-haemolytic streptococcus group. Journal of Experimental Medicine 40, 377-443.

Holdeman, L.V. & Moore, W.E.C. (1974) New genus Coprococcus, twelve new species and amended description of four previously described species of bacteria from human faeces. International Journal of Systematic Bacteriology 24, 260-277.

Humble, M.W., King, A. & Phillips, I. (1977) APIzym: A simple rapid system for the detection of bacterial enzymes. Journal of Clinical Pathology 30, 275-277.

Jelinková, J. & Kubin, V. (1974) Proposal of a new serological group ("V") of haemolytic streptococci isolated from swine lymph nodes. International Journal of Systematic Bacteriology 24, 434-437.

Jelinková, J. & Rotta, J. (1978) Identification and typing of enterococci. In Methods of Microbiology Vol.12. Eds. Bergan, T. & Norris, J.R. London: Academic Press.

Jokipii, A.M.M. & Jokipii, L. (1979) Presumptive identification and antibiotic susceptibility of group B streptococci. Journal of Clinical Pathology 29, 736-739.

Jones, D. (1978) Composition and differentiation of the genus Streptococcus. In Streptococci. Eds. Skinner, F.A. & Quesnel, L.B. London: Academic Press.

Jones, D. & Shattock, P.M.F. (1960) The location of the group antigen of group D streptococci. Journal of General Microbiology 23, 335-343.

Jones, D. & Sneath, P.H.A. (1970) Genetic transfer and bacterial taxonomy. Bacteriological Reviews 34, 40-81.

Jones, D., Sackin, M.J. & Sneath, P.H.A. (1972) A numerical taxonomic study of the streptococci of serological group D. Journal of General Microbiology 72, 439-450.

Kalina, A.P. (1970) The taxonomy and nomenclature of enterococci. International Journal of Systematic Bacteriology 20, 185-189.

Kandler, O., Schleifer, K.M. & Dandl, R. (1968) Differentiation of Streptococcus faecalis (Andrewes & Horder) and Streptococcus faecium (Orla-Jensen) based on the amino-acid composition of their murein. Journal of Bacteriology 96, 1935-1939.

Kersters, K. & De Ley, J. (1975) Identification and grouping of bacteria by numerical analysis of their electrophoretic protein patterns. Journal of General Microbiology 87, 333-342.

Kersters, K. & De Ley, J. (1980) Classification and identification of bacteria by electrophoresis of their proteins. In Microbiological Classification and Identification. Eds. Goodfellow, M. & Board, R.G. Society for Applied Bacteriology Symposium Series No. 8. London: Academic Press.

Kiel, P. & Skadhauge, K. (1973) Studies in mannitol fermenting strains of Streptococcus bovis. Acta Pathologica et Microbiologica Scandanavica 81, 10-14.

Klein, E. (1884) Micro-organisms and disease. Practitioner 32, 321-352.

Kozak, W., Rajchert-Trzpil, M & Dobrzanski, W.T. (1974) The effect of proflavin, ethidium bromide and an elevated temperature on the appearance of nisin negative clones in nisin producing strains of Streptococcus lactis. Journal of General Microbiology 83, 295-302.

Krieg, R.E. & Lockhart, W.R. (1970) Analysis of the thermal transition curves of deoxyribonucleic acid from microorganisms. Canadian Journal of Microbiology 16, 989-995.

Lancefield, R.C. (1933) A serological differentiation of human and other groups of haemolytic streptococci. Journal of Experimental Medicine 57, 571-595.

Lancefield, R.C. (1934) A serological differentiation of specific types of bovine haemolytic streptococci (Group B). Journal of Experimental Medicine, 59, 441-458.

Langston, C.W., Guttierez, J. & Bouma, C. (1960) Motile enterococci (Streptococcus faecium var. mobilis var. N) isolated from grass silage. Journal of Bacteriology 80, 714-718.

Lapage, S.P., Bascomb, S., Willcox, W.R. & Curtis, M.A. (1970) Computer identification of bacteria. In Automation, Mechanization and Data Handling in Microbiology. Eds. Baillie, A. & Gilbert, R.J. Society of Applied Bacteriology Technical Series No.4. London: Academic Press.

Law, B.A. (1979) Extracellular peptidases in group N streptococci used as cheese starters. Journal of Applied Bacteriology 46, 455-463.

Lawrence, S.H., Melnick, P.J. & Weimer, H.E. (1960) A comparison of serum proteins and enzymes by starch gel electrophoresis. Proceedings for the Society for Experimental Biology and Medicine 105, 572-577.

Lehmann, K.B. & Neumann, R. (1896) Atlas and Grundriss der Bakteriologie und Lehrbuch der Speziellen Bakteriologisichen Diagnostik, 1st edn. J.F. Lehmann München.

Lockhart, W.R. & Hartman, P.A. (1963) Formation of monothetic groups in quantitiative bacterial taxonomy. Journal of Bacteriology 85, 68-77.

Logan, N.A. & Berkeley, R.C.W. (1981) Classification and identification of members of the genus Bacillus using API tests. In The Aerobic Endospore-Forming Bacteria. Special Publications for the Society of General Microbiology Vol.4. Eds. Berkeley, R.C.W. & Goodfellow, M. London: Academic Press.

Löhnis, F. (1909) Die Benennung der Milchsäurebakterien. Zentralblatt fur Bakteriologie, Parasitenkunde, Infectionskrankheiten und Hygiene Abteilung 2, 22. 553-555.

London, J. & Kline, K. (1973) Aldolase of lactic acid bacteria: a case history in the use of an enzyme as an evolutionary marker. Bacteriological Reviews 37, 453-478.

London, J., Chace, N.M. & Kline, K. (1975) Aldolase of lactic acid bacteria: immunological relationships among aldolases of streptococci and gram positive non spore forming anaerobes. International Journal of Systematic Bacteriology 25, 114-123.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

Lund, B.M. (1965) A comparison by the use of gel electrophoresis of soluble protein components and esterase enzymes of some group D streptococci. Journal of General Microbiology 40, 413-419.

Lund, B.M. (1967) A study of some motile group D streptococci. Journal of General Microbiology 49, 67-80.

Lütticken, R. (1978) Studies on streptococci resembling Streptococcus milleri and an associated surface protein antigen. Journal of Medical Microbiology 11, 419-431.

McKay, L.L., Cords, B.R. & Baldwin, K.A. (1973) Transduction of lactose metabolism in Streptococcus lactis C2. Journal of Bacteriology 115, 810-815.

Marmur, J. (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. Journal of Molecular Biology 3, 208-218.

Marmur, J. & Doty, P. (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. Journal of Molecular Biology 5, 109-118.

Medrek, T.F. & Barnes, E.M. (1962) The physiological and serological properties of Streptococcus bovis and related organisms isolated from cattle and sheep. Journal of Applied Bacteriology 25, 169-179.

Metcalf, R.H. & Diebel, R.H. (1969) Differential lytic response of enterococci associated with addition order of lysozyme and anions. Journal of Bacteriology 99, 674-680.

Michener, C.D. & Sokal, R.R. (1957) A quantitative approach to a problem in classification. Evolution 11, 130-162.

Mordarski, M., Goodfellow, M., Tkacz, A., Pulverer, G. & Schaal, K.P. (1980) Ribosomal ribonucleic acid similarities in the classification of Rhodococcus and related taxa. Journal of General Microbiology 118, 313-319.

Moreira-Jacob, M. (1956) The streptococci of Lancefield's group E. Biochemical and serological identification of the haemolytic strains. Journal of General Microbiology 14, 268-280.

Morris, J.A. (1973) The use of polyacrylamide gel electrophoresis in taxonomy of Brucella. Journal of General Microbiology 76, 231-237.

Mundt, J.O. (1963a) Occurrence of enterococci in animals in a wild environment. Applied Microbiology 11, 136-140.

Mundt, J.O. (1963b) Occurrence of enterococci in plants in a wild environment. Applied Microbiology 11, 141-144.

Mundt, J.O. (1975) Unidentified streptococci from plants. International Journal of Systematic Bacteriology 25, 281-285.

National Collection of Dairy Organisms (1974) Catalogue of Strains Maintained at the National Collection of Dairy Organisms. Reading: University of Reading.

National Collection of Type Cultures (1972) Catalogue of the National Collection of Type Cultures, Public Health Laboratory Service Board London; HMSO.

Norris, J.R. (1964) The classification of Bacillus thuringiensis. Journal of Applied Bacteriology 27, 439-447.

Nowlan, S.S. & Diebel, R.H. (1967a) Group Q streptococci. I Ecology, serology, physiology and relationship to established enterococci. Journal of Bacteriology 94, 291-296.

Nowlan, S.S. & Diebel, R.H. (1967b) Group Q streptococci. II Nutritional characteristics and growth relationship to thymine, folate and folinate. Journal of Bacteriology 94, 297-299.

Nozaki, M. & Hayaishi, O. (1971) Separation and purification of proteins. In Methods in Microbiology Vol.5B. Eds. Norris, J.R. & Ribbons, D.W. London: Academic Press.

Odds, F.C., Hall, C.A. & Abott, A.B. (1978) Peptones and mycological reproducibility. Sabouraudia 16, 237-246.

Orchard, V.A. & Goodfellow, M. (1980) Numerical classification of some named strains of Nocardia asteroides and related isolates from soil. Journal of General Microbiology 118, 295-312.

Orla-Jensen, S. (1919) The Lactic Acid Bacteria. Copenhagen: Adr. Fred Host & Son.

Orla-Jensen, A.D. & Hansen, P.A. (1932) The bacteriological flora of spontaneously soured milk and of commercial starters for butter making. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene Abteilung 2 86, 6-29.

Osborn, R.M., Lamberts, B.L., Meyer, T.S. & Roush, A.H. (1976) Acrylamide gel electrophoretic studies of extracellular sucrose metabolizing enzymes of Streptococcus mutans. Journal of Dental Research 55, 77-89.

Owen, R.J. & Lapage, S.P. (1976) The thermal denaturation of partly purified bacterial deoxyribonucleic acid and its taxonomic implications. Journal of Applied Bacteriology 41, 335-340.

Pier, G.B. & Madin, S.H. (1976) Streptococcus iniae sp. nov., a beta haemolytic streptococcus isolated from an Amazon fresh water dolphin Inia geoffrensis. International Journal of Systematic Bacteriology 26, 545-553.

Pier, G.B., Madin, S.H. & Al-Nakeer, S. (1978) Isolation and characterization of a second isolate of Streptococcus iniae. International Journal of Systematic Bacteriology 28, 311-314.

Power, A.K. (1978) Taxonomy of the genus Haemophilus. Ph.D Thesis, University of Leicester, U.K.

Pritchard, G.G. & Wimpenny, J.W.T. (1978) Cytochrome formation, oxygen induced protein extrusion and respiratory activity in Streptococcus faecalis var. zymogenes grown in the presence of haematin. Journal of General Microbiology 104, 15-22.

Raj, H. & Colwell, R.R. (1965) Taxonomy of enterococci by computer analysis. Canadian Journal of Microbiology 12, 353-362.

Reyn, A., Birch-Anderson, A. & Berger, U. (1970) Fine structure and taxonomic position of Neisseria haemolysans (Thjøtta & Bøe 1938) or Gemella haemolysans (Berger, 1960). Acta Pathologica et Microbiologica Scandinavica Section B 78B, 375-389.

Reyn, A. (1974) The genus Gemella. In Bergey's Manual of Determinative Bacteriology, 8th edn. Eds. Buchanan, R.E. & Gibbons, N.E. Baltimore: Williams & Wilkins Company.

Ritchey, T.W. & Seeley, H.W. Jnr. (1974) Cytochromes in Streptococcus faecalis var. zymogenes grown in a haematin containing medium. Journal of General Microbiology 85, 220–228.

Ritchey, T.W. & Seeley, H.W. Jnr. (1976) Distribution of cytochrome like respiration in streptococci. Journal of General Microbiology 93, 195–203.

Rogosa, M. (1974) Genus II Peptostreptococcus Kleyver & van Niel 1936. In Bergey's Manual of Determinative Bacteriology, 8th edn., eds. Buchanan, R.E. & Gibbons, N.E. Baltimore: Williams & Wilkins Company.

Roguiňsky, M. (1971) Caractères biochimiques et serologiques de Streptococcus uberis. Annals de l'Institut Pasteur, Paris 120, 154–163.

Roop, D.R., Mundt, J.O. & Riggsby, W.S. (1974) Deoxyribonucleic acid hybridization studies among some strains of group D and group N streptococci. International Journal of Systematic Bacteriology 24, 330–337.

Rosenbach, F.J. (1884) Micro-organismen bei den Wund-Infections-Krankheiten des Menschen. J.F. Bergmann, Wiesbaden.

Rotta, J. (1978) Group and type (groups A & B) identification of haemolytic streptococci. In Methods in Microbiology, Vol.12, eds. Bergan, T. & Norris, J.R. London: Academic Press.

Rouatt, J.W., Skyring, G.W, Purkayastha, V. & Quadling, C. (1970) Soil bacteria; a numerical analysis of electrophoretic protein patterns developed in acrylamide gels. Canadian Journal of Microbiology 16, 202-205.

Sackin, M.J. (1981) Vigour and pattern as applied to multistate quantitative characters in taxonomy. Journal of General Microbiology 122, 247-254.

Sand, C. & Jensen, C.O. (1888) Die Aetiologie der Druise. Deutsche Zeitschrift fur Tiermedizin und Vergleichende Pathologie, 13, 437-464.

Sargent, J.R. (1971) Zone electrophoresis of the separation of microbial cell components. In Methods in Microbiology, Vol.5B, eds. Norris, J.R. & Ribbons, D.W. London: Academic Press.

Scildkraut, C. & Lifson, S. (1965) Dependance of the melting temperature of DNA on salt concentration. Biopolymers 3, 195-208.

Schleifer, K.H. & Kandler, O. (1972) Peptidoglycan types of bacerial cell walls and their taxonomic implications. Bacteriological Reviews 36, 407-477.

Seeley, H. & Dain, J.A. (1960) Starch hydrolysing streptococci. Journal of Bacteriology 79, 230-235.

Seyfried, P.L. (1968) An approach to the classification of lactobacilli using computer aided numerical analysis. Canadian Journal of Microbiology 14, 313-318.

Sherman, J.M. (1937) The streptococci. Bacteriological Reviews 1, 3-97.

Sherman, J.M. (1938) The enterococci and related streptococci. Journal of Bacteriology 35, 81-95.

Skadhauge, K. (1950) Studies on the Enterococci with Special Reference to the Serological Properties. Copenhagen: Einar Munksgaard.

Skerman, V.B.D. (1969) Abstracts of Microbiological Methods. New York: John Wiley & Sons.

Skerman, V.B.D., McGowan, V. & Sneath, P.H.A. (1980) Approved lists of bacterial names. International Journal of Systematic Bacteriology 30, 225-420.

Slade, H.D. & Slamp, W.C. (1962) Cell wall composition and the grouping antigens of streptococci. Journal of Bacteriology 84, 345-351.

Slade, H.D. & Slamp, W.C. (1972) Peptidoglycan composition and taxonomy of group D, E and H streptococci and Streptococcus mutans. Journal of Bacteriology 109, 691-695.

Smith, P.B., Hancock, G.A. & Rhoden, D.L. (1969) Improved medium for detecting deoxyribonuclease producing bacteria. Applied Microbiology 18, 991-993.

Smith, D.G. & Shattock, P.M.F. (1964) The cellular locations of antigens in streptococci of groups D, N and Q. Journal of General Microbiology 34, 165-175.

Sneath, P.H.A. (1957) The application of computers to taxonomy. Journal of General Microbiology 17, 201-226.

Sneath, P.H.A. (1974) Test reproducibility in relation to identification. International Journal of Systematic Bacteriology 24, 508-523.

Sneath, P.H.A. (1977) A method for testing the distinctness of clusters: A test of the disjunction of two clusters in Euclidean space as measured by their overlap. Mathematical Geology 9, 123-143.

Sneath, P.H.A. (1979a) BASIC program for a significance test for two clusters in Euclidean space as measured by their overlap. Computers & Geosciences 5, 143-155.

Sneath, P.H.A. (1979b) BASIC program for identification of an unknown with presence absence data against an identification matrix of percent positive characters. Computers & Geosciences 5, 195-213.

Sneath, P.H.A. (1979c) BASIC program for character separation indices from an identification matrix of percent positive characters. Computers & Geosciences 5, 349-357.

Sneath, P.H.A. (1980a) BASIC program for determining the best identification scores possible from the most typical examples when compared with an identification matrix of percent positive characters. Computers & Geosciences 6, 27-34.

Sneath, P.H.A. (1980b) BASIC program for determining overlap between groups in an identification matrix of percent positive characters. Computers & Geosciences 6, 267-278.

Sneath, P.H.A. & Collins, V.G. (1974) A study in test reproducibility between laboratories: Report of a pseudomonas working party. Antonie van Leeuwenhoek 40, 481-527.

Sneath, P.H.A. & Johnson, R. (1972) The influence on numerical taxonomic similarities of errors in microbiological tests. Journal of General Microbiology 72, 377-392.

Sneath, P.H.A. & Skerman, V.B.D. (1966) A list of type and reference strains of bacteria. International Journal of Systematic Bacteriology 16, 1-133.

Sneath, P.H.A. & Sokal, R.R. (1973) Numerical Taxonomy, 2nd. edn., San Francisco: W.H.Freeman & Company.

Sørhaug, T. & Solberg, P. (1973) Fractionation of dipeptidase specificity of some lactic acid bacteria. Applied Microbiology 25, 388-395.

Stack, M.V., Donoghue, H.D. & Tyler, J.E. (1978) Discrimination between oral streptococci by pyrolysis gas liquid chromatography. Applied & Environmental Microbiology 35, 45-50.

van Tieghem, P. (1879) Sur la gomme du sucrerie (Leuconostoc mesenteroides). Annales des Sciences Naturelles Botanique 7, 180-203.

Vaughn, D.H., Riggsby, W.S. & Mundt, J.O. (1979) Deoxyribonucleic acid relatedness of strains of yellow pigmented, group D streptococci. International Journal of Systematic Bacteriology 29, 204-212.

Waitkins, S.A., Ball, L.C. & Fraser, C.A.M. (1980) Use of the APIzym system in rapid identification of α and non haemolytic streptococci. Journal of Clinical Pathology 33, 53-57.

Weissman, S.M. Reich, P.R., Somerson, N.L. & Cole, R.M. (1966) Genetic differentiation by nucleic acid homology. IV. Relationships among Lancefield groups and serotypes of streptococci. Journal of Bacteriology 92, 1372-1377.

White, J.C. & Niven, C.F. (1946) Streptococcus S.B.E.: a streptococcus associated with subacute bacterial endocarditis. Journal of Bacteriology 5, 717-722.

Whittenbury, R. (1963) The use of soft agar in the study of conditions affecting the utilization of fermentable substrates by lactic acid bacteria. Journal of General Microbiology 32, 375-384.

Whittenbury, R. (1964) Hydrogen peroxide formation and catalase activity in lactic acid bacteria. Journal of General Microbiology 35, 13-26.

Whittenbury, R. (1965a) The differentiation of Streptococcus faecalis and faecium. Journal of General Microbiology 38, 279-287.

Whittenbury, R. (1965b) A study of some pediococci and their relationship to Aerococcus viridans and the enterococci. Journal of General Microbiology 40, 97-106.

Willcox, W.R., Lapage, S.P., Bascomb, S. & Curtis, M.A. (1973) Identification of bacteria by computer: Theory and programming. Journal of General Microbiology 77, 317-330.

Williams, R.A.D. & Bowden, E. (1968) The starch gel electrophoresis of glucose-6-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenease of Streptococcus faecalis, S. faecium and S. durans. Journal of General Microbiology 50, 329-336.

Williams, R.E.O. (1956) Streptococcus salivarius (vel.hominis) and its relation to Lancefield's group K. Journal of Pathology and Bacteriology 72, 15-25.

Williams, R.E.O. (1979) Presidential address. In The Pathogenic Streptococci, ed. Parker, M. Surrey: Reedbooks Ltd.

Williams, R.E.O., Hirch, A. & Cowan, S.T. (1953) Aerococcus, a new bacterial genus. Journal of General Microbiology 8, 475–480.

Wilson, G.S. & Miles, A.A. (1975) Topley & Wilson's Principles of Bacteriology and Immunity, Vol.1, 6th edn. London: Arnold.

Woese, C.R., Fox, G.E., Zablen, L., Uchida, T., Bonen, L., Pechman, K., Lewis, B.J. & Stahl, D. (1975) Conservation of primary structure in 16s ribosomal RNA. Nature, London 254, 83–86.

Yawger, E.S. & Sherman, J.M. (1937a) Variants of Streptococcus lactis which do not ferment lactose. Journal of Dairy Science 20, 83–86.

Yawger, E.S. & Sherman, J.M. (1937b) Streptococcus cremoris. Journal of Dairy Science 20, 205–212.

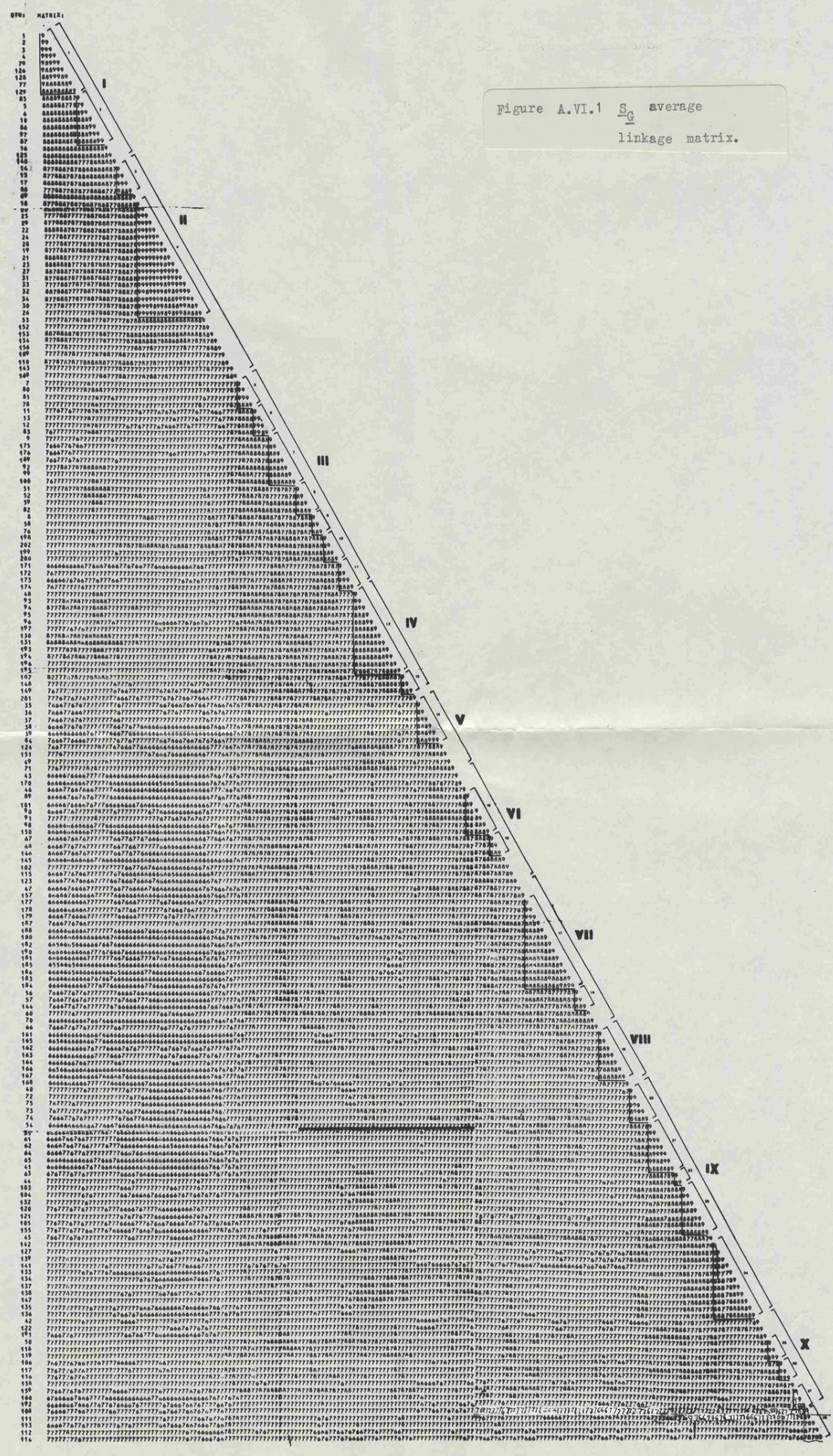


Figure A.VI.1 S_G average linkage matrix.

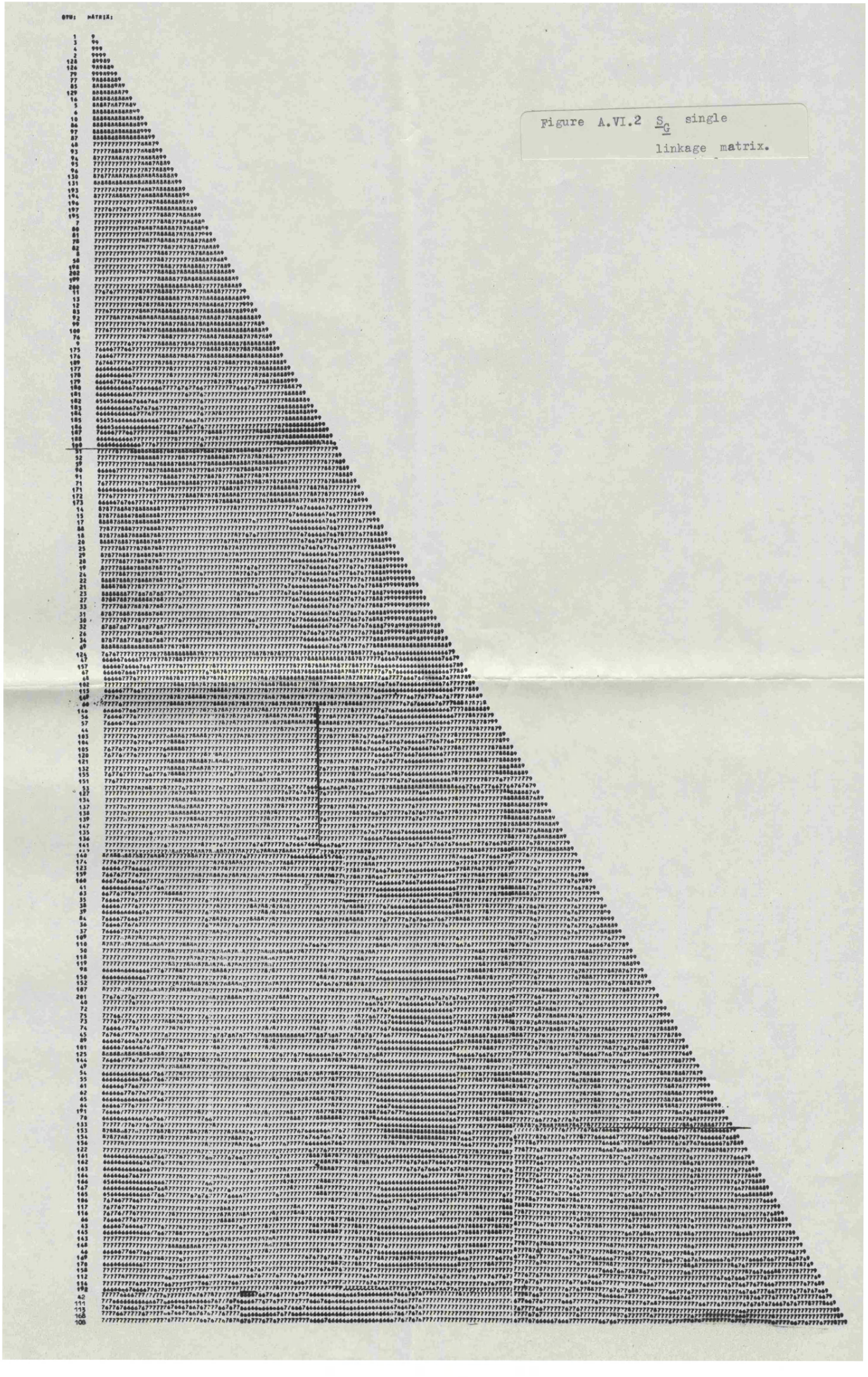


Figure A.VI.2 S_G single linkage matrix.

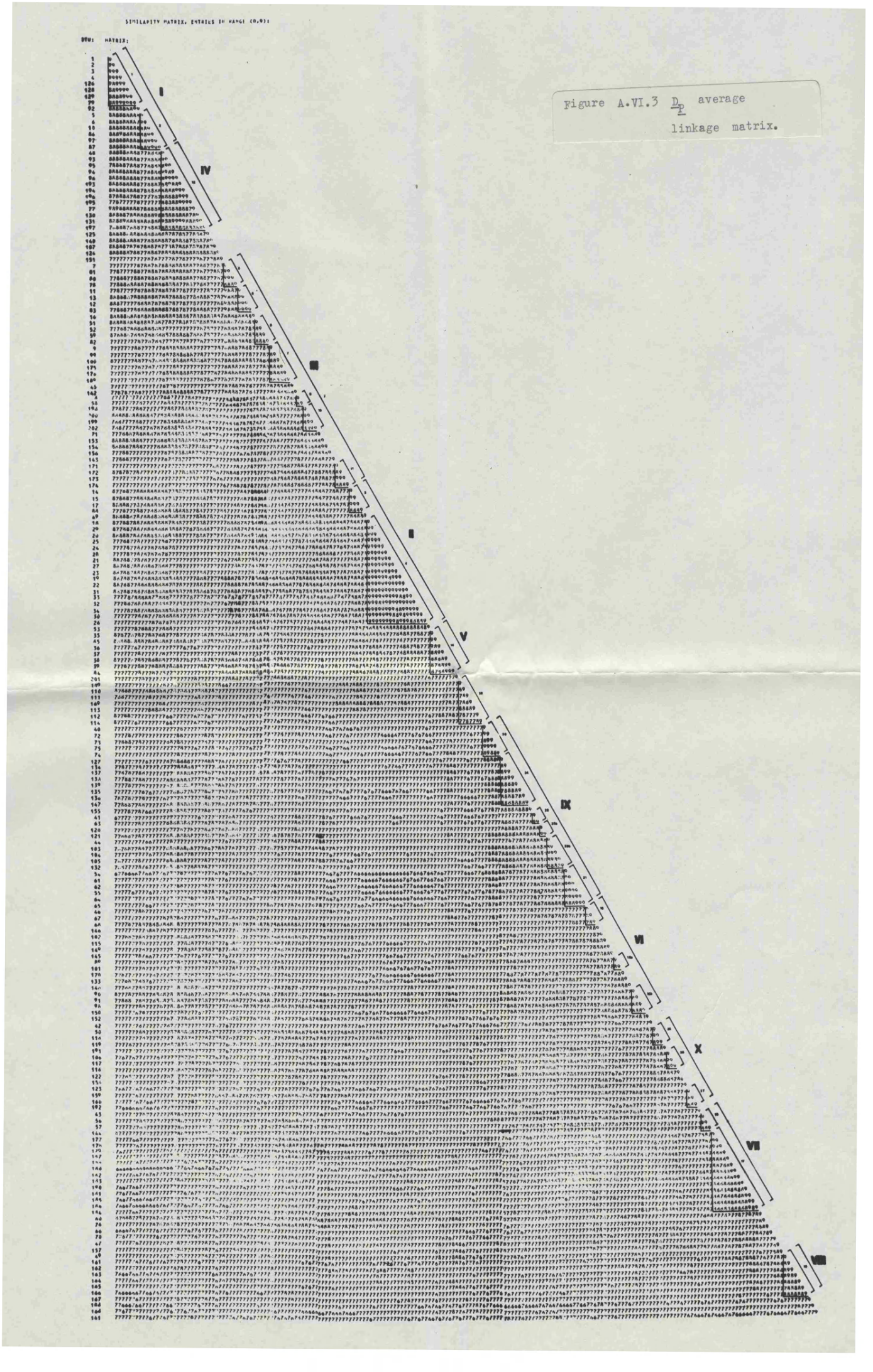


Figure A.VI.3 D_p average linkage matrix.

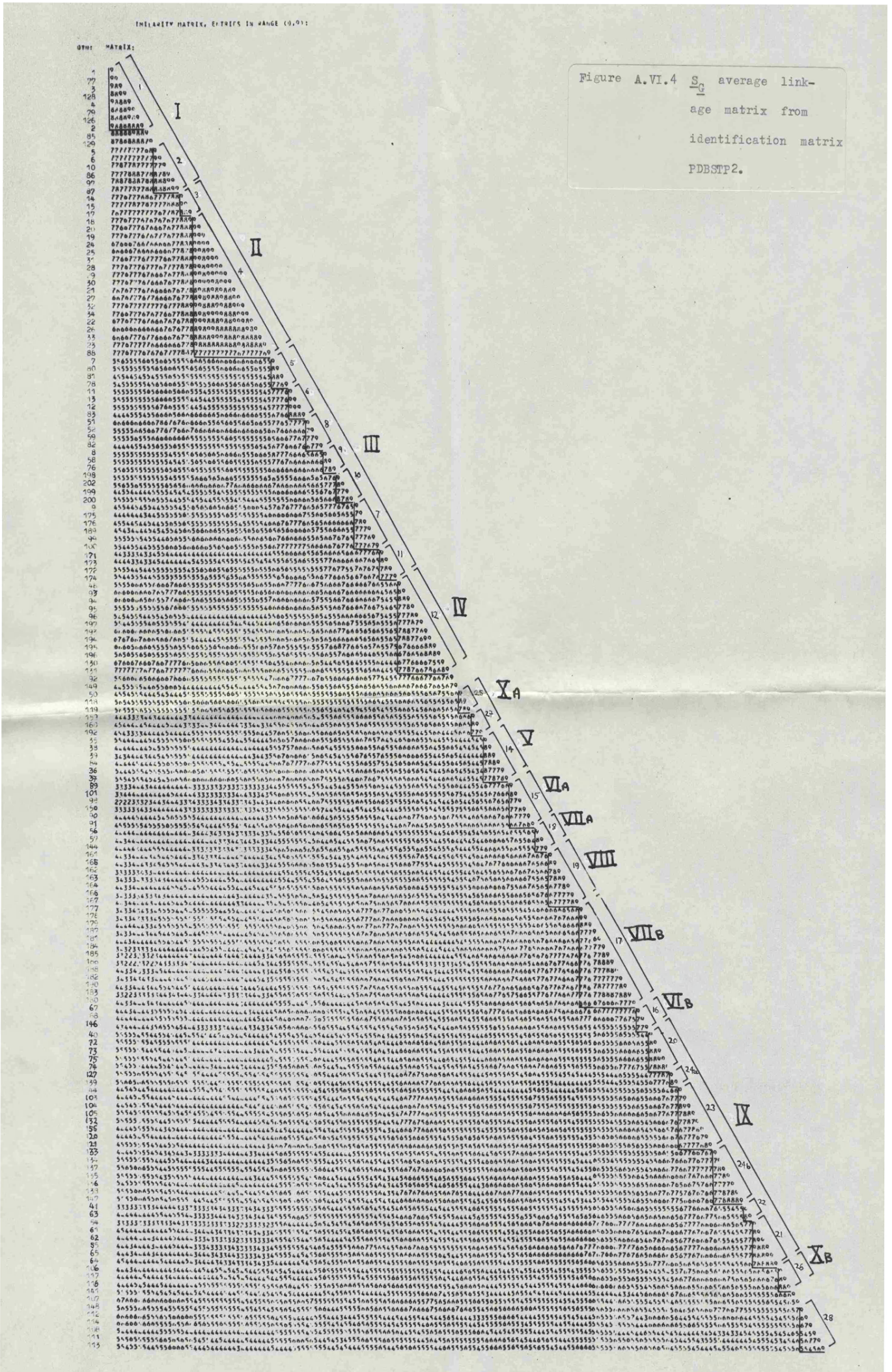


Figure A.VI.4 S_G average linkage matrix from identification matrix PDBSTP2.

A TAXONOMIC STUDY OF THE GENUS STREPTOCOCCUS.

PAUL DENNIS BRIDGE OCTOBER 1981.

ABSTRACT.

Two-hundred and two strains of streptococci and related organisms were used in a numerical taxonomy. Ten major phenons and one loosely linked subphenon were found using Gower's coefficient and UPGMA methods. The Simple Matching coefficient and the Pattern difference were also used and these gave findings in broad agreement with those of Gower's coefficient.

Nine of the ten phenons contained streptococci, the tenth containing members of the genera Leuconostoc and Gemella. Members of the genus Pediococcus appeared in the loosely linked subphenon. Overlap statistics were performed on the subphenons.

An identification matrix was made from the taxonomy and tested. A further dendrogram was produced from this matrix, and this proved similar to those seen earlier. A further identification matrix was constructed using both test results from this study and from the literature. Both of these matrices were tested for overlap. The former giving more distinct groups.

Further work was undertaken on representative strains from the subphenons. This involved the determination of DNA base ratios, detection of esterases in polyacrylamide gels and the numerical analysis of protein traces in polyacrylamide gels. This further work failed to group any of the organisms at anything other than species level. However, the results did not directly contradict the numerical taxonomy, and the groups from this were retained. These were eight species-groups, enterococcus,viridans,para-viridans, pyogenic, para-pyogenic, lactic, S. thermophilus and S. pneumoniae. The strains received as aerococci did not form a distinct cluster. They showed properties that may be intermediate between the streptococci and the pediococci.