D-1284 Rapid Identification of Veterinary Enterococci by MALDI-ToF MS

QUOTIENT BIORESEARCH

D. R. Knight[1], K. Maher[1]. A. de Jong[2]. J. Blackman Northwood[1]. I. Morrissey[1] and the EASSA Study Group[2].

[1]Quotient Bioresearch Ltd., Microbiology, Newmarket Road, Fordham, Cambridgeshire, CB7 5WW, UK , [2]*EASSA Study Group, CEESA, Belgium*

51st ICAAC, Sept 17-20, 2011

Introduction

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF-MS) is a rapid method for identification of bacteria and is fast becoming the preferred method in clinical laboratories. Correct identification of enterococci is traditionally based on phenotypic characterization by biochemical tests (API), 16S rRNA sequencing and more recently by genotyping (PCR). These techniques are both expensive and time consuming and can often lead to ambiguous results.

A need has arisen for a low-cost, fast and reproducible method for enterococci speciation. The purpose of the study was to evaluate the performance of the MALDI-ToF-MS Biotyper library v2.0 on a range of genotypically identified enterococci.

Methodology

Isolates

One hundred and ninety-seven European isolates of enterococci collected during 2008-2009 from ten countries across the EU with the following origins were analysed: 60 isolates were from cattle, 34 from pig and 103 from broiler chickens. Isolates were sourced from healthy food producing animals at slaughter. Three *Enterococcus* ATCC reference strains were also included, these being ATCC 19434 *Enterococcus faecium*, ATCC 29212 *Enterococcus faecalis* and ATCC 700425 *Enterococcus gallinarum*.

Identification by PCR

Test strains were subcultured from –70°C storage on to horse blood agar and incubated at 35°C, aerobically, overnight. Nucleic acid extraction was performed as described previously [1] and used as a template for a 7-group multiplex PCR assay [1]. Briefly, the 16S rRNA gene was used as a target for genus-specific PCR primers and the superoxide dismutase gene (*sodA*) for species differentiation of 23 species of enterococci [1].

PCR amplification product was run on 2% agarose gel in 0.5 TBE buffer, stained using ethidium bromide and visualized under UV light. All test isolates were assayed with all seven multiplex groups. An isolate showing a genus specific band at 737bp was reported as enterococci. Isolates with a species specific band visible were reported as positive for the corresponding species.

Identification by MALDI-ToF MS

Mass spectra were acquired using a Microflex MALDI-ToF (Bruker Daltonik GmbH) mass spectrometer. Measured mass range of spectra was from 2000 to 20,000 Da. Spectra acquisition was performed using Bruker Biotyper data library v2.0 build 223.8 (3741 entries).

Results were reported as the primary ID e.g. best match to the Bruker database with corresponding score value. Score values are ranked on an exponential scale from 0 – 3 with the following ID confidence acceptance criteria; Log Score ≥2.20 (Very good ID), 2.00 - 2.19 (Good ID), 1.70 - 1.99 (Acceptable ID) and ≤1.70 (Poor ID).

Analysis

Genus and species level concordance between the MALDI-ToF method and the PCR method was determined for all isolates. Cross reactivity between primer pairs sometimes produces ambiguous bands with the PCR assay, in these instances, the assay was repeated from a pure single colony and if the same ambiguous band was seen again, the stronger band was selected as previously described [1].

Results

MALDI-ToF MS

The MALDI-ToF Biotyper successfully speciated all 200 isolates. Mean identification log score values for all isolates was 2.312 (Table 1). Good consistency was seen between the primary and secondary identifications at both genus and species level. Overall ten species of enterococci were identified by the Bruker Biotyper data library v2.0.

PCR

In total, only 197 (98.5%) isolates covering seven different species were identified by this method. Low % matches were observed between MALDI-ToF and PCR for *E. faecium, E. gallinarum and E. hirae*. This was mainly due to primer cross reactivity resulting in ambiguous PCR results. For these strains a faint band could be seen corresponding with *E. hirae* or *E. faecium* and a stronger band for some of the rarer *Enterococcus* spp. as shown in Figure 1.

Initially the stronger band and corresponding ID was accepted (Table 1 - 1º Speciation). However; if the second weaker band was selected then 28 (48.3%) of these mismatched isolates correctly matched with the result obtained by the MALDI-ToF (Table 1 - 2º Speciation).

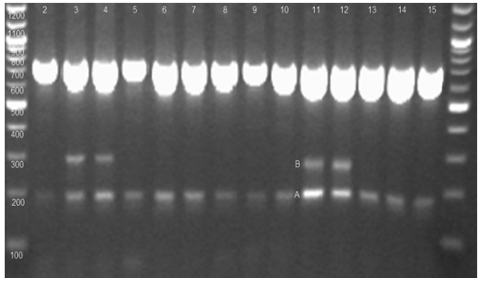

Figure 1. Example gel image showing 14 isolates of Enterococci assayed with Group 1 PCR primers. For the isolates in wells 3, 4, 11 and 12 a strong DNA band can be seen corresponding to 215bp (*E. faecium*, Figure 1, A) and a faint DNA band can be seen corresponding to 295bp (*E. durans*, Figure 1, B). The bright bands at 737bp correspond to enterococci genus bands. 100bp ladder is shown in wells 1 and 16.

MALDI-ToF ID			PCR ID - ID Matches	
Species	N	Mean ID LogScore	1º Speciation N (%)	2º Speciation N (%)
E. faecium	63	2.448	41 (65.1)	53 (84.1)
E. faecalis	55	2.253	51 (92.7)	52 (94.5)
E. hirae	45	2.314	23 (51.1)	37 (82.2)
E. durans	21	2.134	19 (90.5)	19 (90.5)
E. gallinarum	9	2.214	5 (55.5)	6 (66.6)
E. casseliflavus	2	2.156	2 (100.0)	2 (100.0)
E. mundtii	2	2.126	1 (50.0)	1 (50.0)
E. avium	1	2.166	0 (0.0)	0 (0.0)
E. cecorum	1	2.348	0 (0.0)	0 (0.0)
E. villorum	1	2.281	0 (0.0)	0 (0.0)
Grand Total	200	2.312	142 (71.0)	170 (85.0)

Table 1. *Enterococcus* spp. concordance results between MALDI-ToF and PCR methodologies. 1º speciation shows concordance for PCR ID to MALDI ID. 2º speciation shows concordance for PCR ID to MALDI ID taking the weaker second band where primer cross reactivity occurs. Also shown is the mean ID score value obtained for each species identification by MALDI-ToF.

Discussion

Enterococci are important not only because they are a leading cause of nosocomial infections, but also because they may have a significant role in dissemination and persistence of antimicrobial resistance. For epidemiology and chemotherapy purposes correct identification of enterococci is highly important.

Many of the identification mismatches obtained by the PCR and MS methodologies could be due to a combination of ambiguous PCR identifications and the current Biotyper library v2.0 being under-represented with less common strains of enterococci.

Discussion

- Analysis of the results from the PCR assay can be subjective due to ambiguous bands but is still superior to most other identification methods [1].
- MALDI-ToF is a promising and reproducible technique for speciation of veterinary enterococci.
- The addition of more *Enterococcus* species to the Bruker database will enhance the utility of the MALDI-ToF further.
- MALDI-ToF may well in the future replace PCR as the method of choice for identification of veterinary enterococci in clinical reference laboratories.

References

[1] Jackson *et al.* Use of a genus- and species-specific multiplexes PCR for identification of enterococci. J Clin Microbiol. 2004; 42(8):3558-65.

Acknowledgements

We are grateful to the Quotient Bioresearch Ltd. Microbiology team for their laboratory support. The EASSA Study Group consists of: K. Smets (Alpharma), A. de Jong (Bayer), P. Butty (Ceva), S. Simjee (Elanco), U. Klein (Novartis), K. Godinho (Pfizer), V. Thomas (Intervet/Schering Plough), M. Valle (Vetoquinol) and D. Rigaut (Virbac).

Tel: +44 (0) 1638 720500 Email: info@quotientbioresearch.com www.quotientbioresearch.com