

Supplementary Material

Shedding Light on Microbial ‘Dark Matter’: Insights into Novel Cloacimonadota and Omnitrophota from an Antarctic Lake

Timothy J. Williams, Michelle A. Allen, Jonathan F. Berengut and Ricardo Cavicchioli

1 Supplementary Figures

Figure S1 | Expanded phylogeny of phylum *Candidatus* Cloacimonadota.

Figure S2 | Expanded phylogeny of phylum *Candidatus* Omnitrophota.

2 Supplementary Tables

Table S1 | Description of all Ace Lake Cloacimonadota and Omnitrophota MAGs. (see accompanying excel file)

Table S2 | Etymologies and metadata for proposed *Candidatus* genus and species Cloacimonadota and Omnitrophota from Ace Lake. (see accompanying excel file)

Table S3 | Proteins and IMG Gene IDs for the ten Ace Lake Cloacimonadota MAGs chosen for analysis in this study. (see accompanying excel file)

Table S4 | Metabolic traits inferred from analysis of Ace Lake Cloacimonadota MAGs.

Table S5 | Proteins and IMG Gene IDs for the 14 Ace Lake Omnitrophota MAGs chosen for analysis in this study. (see accompanying excel file)

Table S6 | Metabolic traits inferred from analysis of Ace Lake Omnitrophota MAGs.

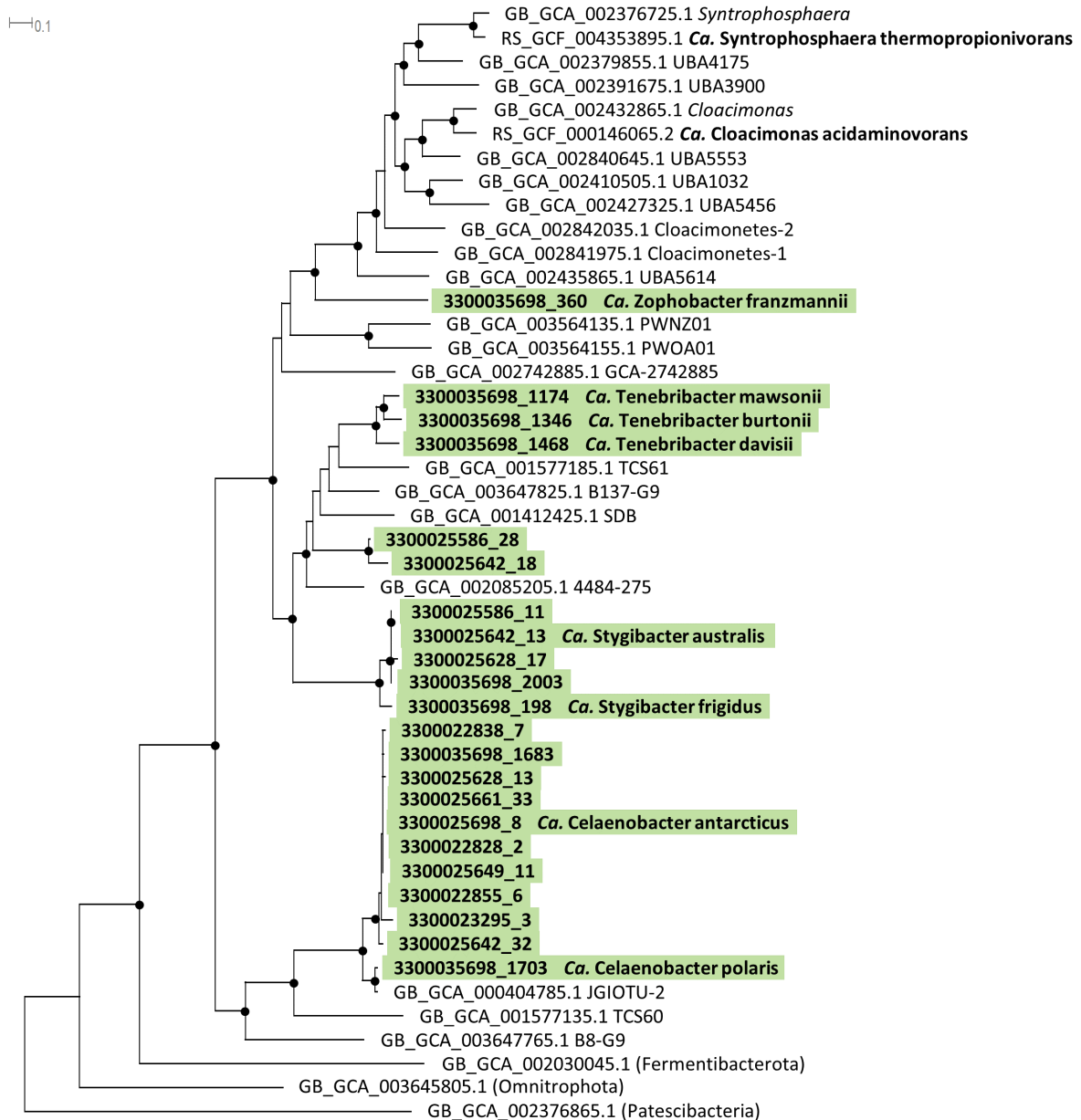


Figure S1 | Expanded phylogeny of phylum *Candidatus* Cloacimonadota. Maximum likelihood tree constructed in IQ-Tree with autoselection of the best-fit model (LG+F+I+G4) and 1000 ultra-fast bootstraps. UFBootstraps ≥ 95% (black dot); Metagenome-assembled genomes (MAGs) featured in this study (green) with their IMG MAG ID and proposed *Candidatus* genus and species name. Reference Cloacimonadota MAGs are shown with their Genome Taxonomy Database (GTDB) accession and GTDB taxonomy, except for *Candidatus* Cloacimonas acidaminovorans and *Candidatus* Syntrophosphaera thermopropionivorans, which already have names. The tree is rooted using a representative of the Patescibacteria.

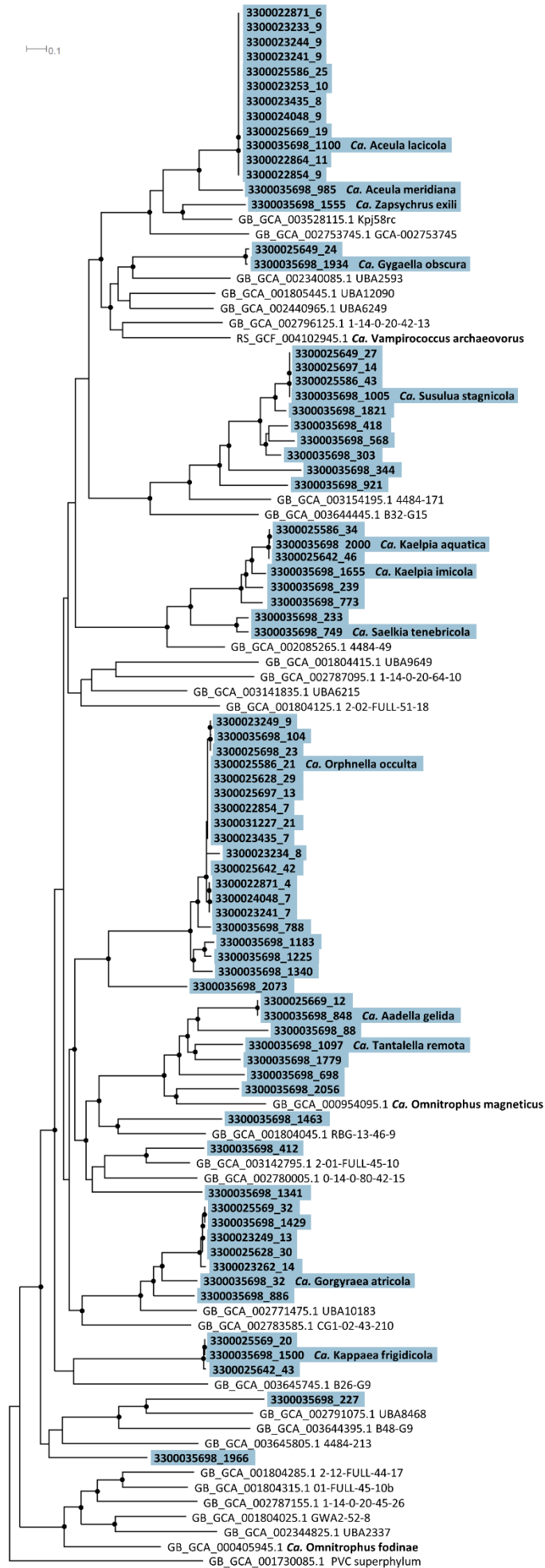


Figure S2 | Expanded phylogeny of phylum *Candidatus* Omnitrophota. Maximum likelihood tree constructed in IQ-Tree with autoselection of the best-fit model (LG+F+I+G4) and 1000 ultra-fast bootstraps. UFBootstraps $\geq 95\%$ (black dot); Metagenome-assembled genomes (MAGs) featured in this study (blue) with their IMG MAG ID and proposed *Candidatus* genus and species names. Reference Omnitrophota MAGs are shown with their Genome Taxonomy Database (GTDB) accession and GTDB taxonomy, except for *Candidatus* Omnitrophus fodinae and *Candidatus* Omnitrophus magneticus, which already have names. The tree was rooted with a basal representative of the 'Planctomycetes-Verrucomicrobia-Chlamydiae' (PVC) superphylum.

Table S4 | Metabolic traits inferred from analysis of Ace Lake Cloacimonadota MAGs.

Class (GTDB)	Cloacimonadia									
Order (GTDB)	Cloacimonadales						JGIOTU-2			
Family (GTDB)	TCS61			--			JGIOTU-2			
<i>Candidatus</i> genus	Tenebriabacter			Stygibacter			Zophobacter	Celaenobacter		
<i>Candidatus</i> species	burtonii (type)	mawsonii	davisii	australis (type)		frigidus	franzmannii (type)	antarcticus (type)		polaris
MAG ID	3300035698_1346 (type)	3300035698_1174 (type)	3300035698_1468 (type)	3300025642_13 (type)	3300035698_2003	3300035698_198 (type)	3300035698_360 (type)	3300025698_8 (type)	3300035698_1683	3300035698_1703 (type)
Genome completeness	97%	91%	97%	88%	69%	58%	80%	92%	65%	91%
Genome contamination	0%	0%	1.7%	3.3%	2.8%	0%	1.3%	4.4%	1.3%	1.1%
Cell surface	PGA	PGA	PGA	PGA, cellulosome-like, halomucin-like*	PGA, cellulosome-like, halomucin-like*	PGA, cellulosome-like	PGA			
rTCA cycle	ACL	TFR		TFR	TFR	ACL, TFR		ACL, TFR	ACL	TFR
Hydrogenases	FeFe Group C1	NiFe Group 4g (Mbh)	FeFe Group C1	NiFe Group 4g (Mbh), FeFe Group A3 (Hnd), FeFe Group C1	NiFe Group 4g (Mbh), FeFe Group A3 (Hnd), FeFe Group C1	FeFe Group A3 (Hnd)		NiFe Group 4g (Mbh), NiFe Group 3c (Mvh)		NiFe Group 4g (Mbh), NiFe Group 3c (Mvh)
Sulfur metabolism	TST	Hdr, TST, TusA	TST	Hdr, TST, TusA	Hdr, TST, TusA	Hdr, TusA		Hdr, Sat+CysC	Hdr, Sat+CysC	Hdr, CysC
Ferredoxin oxidoreductases	POR, OGOR, IOR, AOR	POR, OGOR, VOR, IOR, AOR	POR, VOR, IOR, AOR	POR, OGOR, VOR, AOR	POR, OGOR, IOR, AOR	OGOR, VOR, IOR	OGOR, VOR, IOR	POR, OGOR, IOR	POR, OGOR, IOR	POR, OGOR, IOR
SCFA catabolism	Ptb+Buk, Fdh	Ptb+Buk	Ptb+Buk, Fdh	Ptb+Buk, Pta+Ack, Fdh	Ptb+Buk, Pta+Ack, Fdh	Ptb, Fdh	Ptb+Buk, Pta+Ack,	Ptb+Buk	Ptb	Ptb+Buk
Energy conservation	Rnf, Mrp, HppA, Nqr, Sud	Rnf, HppA, Nqr, Sud	Rnf, HppA, Nqr, Sud	Rnf, HppA, Nqr,	Rnf, Mrp, HppA, Nqr, Sud	Mrp, HppA, Nqr, Sud	Rnf, HppA, Nqr, Sud	Rnf, Mrp, HppA,	Rnf, Mrp	Rnf, Mrp, Sud
Glycerol catabolism	GlpK	GlpK	GlpK	GlpK			GlpK			
Alcohol catabolism	Adh			Adh	Adh	Adh				
Poly/ oligosaccharide degradation (glycoside hydrolases)	GH18, GH38	GH13	GH16, GH3, GH30, GH38	GH16, GH2, GH13, GH3, GH30, GH31, GH38 GH57*, GH18*	GH16, GH2, GH18, GH13, GH38, GH57*, GH18*	GH16, GH2, GH13, GH3, GH30, GH57*, GH18*	GH16, GH18, GH3, GH27, GH30, GH31, GH92	GH64, GH53	GH64	GH13
Polypeptide degradation (proteases/ peptidases)	S1, S9A, S49, M16, M18, M20, M24, M42	S1, S9A, S49, M16, M18, M20, M24, M42	S1, S9A, S49, M16, M18, M20, M24, M42	S1, S9A, S49, S8/S53*, M16, M18, M20, M24, M42	S1, S9A, S49, S8/S53*, M16, M18, M20, M24, M42	S1, S9A, S49, S8/S53*, M16, M18, M20, M24, M42	S1, S9A, S49, M16, M18, M20, M24, M42	S1, S9A, S49, M18, M20, M24, M42	S1, S9A, M18, M24, M42	S1, S49, M16, M18, M24, M42
Citrate catabolism	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
Transporters	ABC (sugars, phosphonate, Fe), sugar-phosphate permease, glucose:Na ⁺ symporter, amino acid permease, amino acid/polyamine transporter, uracil permease, PO ₄ ³⁻ transporter (PiT), Fe ³⁺ transporter (Feo), Zn transporter (ZupT), Mg/Co transporter	ABC (sugars, phosphonate, Fe), sugar-phosphate permease, glucose:Na ⁺ symporter, uracil permease	ABC (sugars, phosphonate, Fe), glucose:Na ⁺ symporter, amino acid permease, uracil permease, PO ₄ ³⁻ transporter (PiT), Fe ³⁺ transporter (Feo), Mg/Co transporter	ABC (sugars, phosphonate, Fe), malto-oligosaccharide permease, amino acid/polyamine transporter, PO ₄ ³⁻ transporter (PiT), Fe ³⁺ transporter (Feo), Mg/Co transporter	ABC (sugars, phosphonate, Fe), malto-oligosaccharide permease, amino acid permease, amino acid/polyamine transporter, PO ₄ ³⁻ transporter (PiT), Fe ³⁺ transporter (Feo), Zn transporter (ZupT), Mg/Co transporter	ABC (sugars, phosphonate, Fe), malto-oligosaccharide permease, amino acid/polyamine transporter, PO ₄ ³⁻ transporter (PiT), Fe ³⁺ transporter (Feo), Zn transporter (ZupT), Mg/Co transporter	ABC (sugars, phosphonate, Zn, Mo), PO ₄ ³⁻ transporter (PiT), Fe ³⁺ transporter (Feo), Zn transporter (ZupT), Mg/Co transporter	ABC (sugars, BCAA, phosphonate, Zn, Mo), uracil permease, PO ₄ ³⁻ transporter (PiT), Na ⁺ :PO ₄ ³⁻ cotransporter, Fe ³⁺ transporter (Feo), Mg/Co transporter	ABC (sugars, BCAA, Fe, Zn), amino acid/polyamine transporter, PO ₄ ³⁻ transporter (PiT), Na ⁺ :PO ₄ ³⁻ cotransporter, Mg/Co transporter	ABC (sugars, BCAA, phosphonate, Fe, Zn), uracil permease, PO ₄ ³⁻ transporter (PiT), Na ⁺ :PO ₄ ³⁻ cotransporter, Mg/Co transporter
Ammonia assimilation	GS, GDH	GS, GDH	GS, GDH	GS, GDH	GS, GDH	GS, GDH	GS, GDH	GDH	GDH	GS
Oxidative stress	KatA, Dfr	KatA, Dfr	Dfr	Dfr	Dfr	Dfr	Dfr	KatA, Dfr	Dfr	Dfr

ABC, ATP-binding cassette transport system; Ack, acetate kinase; ACL, ATP-citrate lyase; Adh, alcohol dehydrogenase; AOR, aldehyde:ferredoxin oxidoreductase; BCAA, branched-chain amino acids; Buk, butyrate kinase; CL, citrate lyase; CysC, adenylylsulfate kinase; Dfr, desulfoferredoxin; GH, glycoside hydrolase; GlpK, glycerol kinase; GDH, glutamate dehydrogenase; GS, glutamine synthetase; Hdr, heterodisulfide reductase; HppA, pyrophosphate-energized sodium pump; IOR, indolepyruvate:ferredoxin oxidoreductase; KatA, catalase; Mrp, multicomponent Na⁺:H⁺ antiporter; Nqr, sodium-translocating NADH:quinone oxidoreductase; PGA, poly-γ-glutamate; POR, pyruvate:ferredoxin oxidoreductase; Pta, phosphate acetyltransferase; Ptb, phosphate butyryltransferase; Rnf, ferredoxin:NAD⁺-oxidoreductase complex; rTCA cycle, reverse tricarboxylic acid cycle; Sat, sulfate adenylyltransferase; SCFA, short-chain fatty acids; Sud, bifunctional sulfide dehydrogenase/ferredoxin:NADP oxidoreductase; TFR, thiol:fumarate reductase; TST, thiosulfate:sulfurtransferase (rhodanese-like); TusA, sulfur relay protein; VOR, 2-oxoisovalerate:ferredoxin oxidoreductase. * indicates protein has C-terminal dockerin domain.

Table S6 | Metabolic traits inferred from analysis of Ace Lake Omnitrophota MAGs.

Class (GTDB)	Koll11					4484-49					Koll11				
Order (GTDB)	UBA10015			GIF10	4484-171	4484-49			--		UBA1560		UBA10183	CG1-02-43-210	
Family (GTDB)	--		Kpj58rc	--	4484-171	4484-49	--		--		--		UBA10183	--	
<i>Candidatus</i> genus	Aceula		Zapsychnus	Gygaella	Susulua	Saelkia	Kaelpia		Kappaea		Tantalella	Aadella	Gorgyraea	Orphnella	
<i>Candidatus</i> species	laticola (type)	meridiana	exili (type)	obscura (type)	stagnicola (type)	tenebricola (type)	aquatica (type)	imicola	frigidicola (type)		remota (type)	gelida (type)	atricola (type)	occulta (type)	
MAG ID	3300035698 1100 (type)	3300035698 985 (type)	3300035698 1555 (type)	3300035698 1934 (type)	3300035698 1005 (type)	3300035698 749 (type)	3300035698 2000 (type)	3300035698 1655 (type)	3300035698 1500 (type)	3300035698 1097 (type)	3300035698 848 (type)	3300035698 32 (type)	3300025586 21 (type)	3300035698 104	
Genome completeness	92%	92%	89%	85%	82%	91%	93%	92%	76%	93%	91%	92%	85%	61%	
Hydrogenases	NiFe Group 3d	NiFe Group 3d	NiFe Group 3d	FeFe Group A3	FeFe Group A3	NiFe Group 4g	FeFe Group A3	FeFe Group A3	FeFe Group A3	NiFe Group 3b / Shy	NiFe Group 3b / Shy	NiFe Group 4g	NiFe Group 3b / Shy	NiFe Group 3b / Shy	
Energy conservation	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf, Mrp	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud, Etf, Mrp, WLP	Rnf, HppA, Sud, Etf	Rnf, HppA, Sud	Rnf, HppA, Sud
Ferredoxin oxidoreductases	POR (mon)	POR (mon)	POR (mon)	POR (multi), VOR	POR (multi), VOR	POR (multi), VOR	POR (multi), VOR	POR (multi), VOR	POR (multi), VOR	POR (mon), VOR	POR (mon), VOR	POR (mon), VOR	VOR	VOR	POR (multi), VOR
Citrate synthase	Si	Si	Si	Si	Si	Re	Si	Si	Re	Re	Re	Re	Re	Re	Re
SCFA catabolism				Pta+Ack	Pta+Ack	Pta+Ack	Pta+Ack	Pta+Ack	Pta+Ack	Pta+Ack	Pta+Ack	Pta+Ack	Pta+Ack		Pta+Ack
Glycerol catabolism	GlpK		GlpK								GlpK	GlpK			
Alcohol catabolism						Adh	Adh	Adh		Adh					
Poly/ oligosaccharide degradation (glycoside hydrolases)	GH2, GH5, GH94/GH36	GH2, GH94/GH36, GH65	GH2, GH5, GH94/36	GH5, GH94/GH36, GH65	GH1, GH2, GH94/GH36, GH65	GH94/GH36, GH65	GH94/GH36, GH65	GH94/GH36, GH65	GH2, GH35, GH94/GH36, GH65	GH1	GH1	GH2, GH94/GH36, GH65	GH2, GH94/GH36, GH65	GH2, GH94/GH36, GH65	
Glycogen/ α -glucan synthesis	GlgABC	GlgABC	GlgABC	GlgABC	GlgABC	GlgABC	GlgABC	GlgABC		GlgABC		GlgABC			
Polypeptide degradation (proteases/peptidases)	M17, M24, M48, M50, S1C	M17, M24, M48, M50, S1C	M16, M17, M24, M48, M50, S1C	M24, M48, M50, S1C	M16, M24, M50, S1C	M16, M17, M24, M50, S1C	M16, M17, M24, M50	M16, M17, M24, M50, S1C	M16, M24, M48, M50	M16, M24, M50	M16, M17, M48, M50, M50	M16, M24, M48, M50, S1C	M16, M48, S1C	M16, M24, M48, M50, S1C	
Transporters	ABC (sugar, oligopeptide), amino acid permease, PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo), NH ₄ ⁺ transporter (Amt)	ABC (sugar, oligopeptide), PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo), NH ₄ ⁺ transporter (Amt)	ABC (sugar, oligopeptide), amino acid permease, PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo), NH ₄ ⁺ transporter (Amt)	ABC (sugar, oligopeptide, Fe), amino acid permease, PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo)	ABC (sugar, oligopeptide), PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo)	ABC (sugar, oligopeptide, Fe), amino acid permease, PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo)	ABC (sugar, oligopeptide, Fe), amino acid permease, PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo), NH ₄ ⁺ transporter (Amt)	ABC (sugar, oligopeptide, Fe), amino acid permease, PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo)	ABC (sugar, Fe), sugar-proton symporter, Fe ³⁺ transporter (Feo)	ABC (sugar, oligopeptide), sugar-proton symporter, Fe ³⁺ transporter (Feo)	ABC (sugar, oligopeptide, Fe, Co/Ni), amino acid permease, Fe ³⁺ transporter (Feo), NH ₄ ⁺ transporter (Amt)	ABC (sugar, oligopeptide, Fe), PO ₄ ³⁻ :Na ⁺ symporter, Fe ³⁺ transporter (Feo)	ABC (sugar, oligopeptide, Fe), Fe ³⁺ transporter (Feo)	ABC (oligopeptide, Fe), sugar-proton symporter, PO ₄ ³⁻ :Na ⁺ symporter	
Ammonia assimilation	GS, GOGAT, GDH	GS	GS, GOGAT, GDH	GS	GS, GDH	GS, GDH	GS, GDH	GS, GDH		GS, GDH	GS, GDH	GS, GDH	GS, GDH	GS, GDH	GS, GDH
Oxidative stress	Dfr	Dfr	Dfr	Dfr		Dfr	Dfr	Dfr		Dfr	Dfr	Dfr	Dfr	Dfr	Dfr

ABC, ATP-binding cassette transport system; Ack, acetate kinase; AcsABCD, carbon monoxide dehydrogenase/acetyl-CoA synthase complex; Adh, alcohol dehydrogenase; Dfr, desulfoferredoxin; Etf, electron transfer flavoprotein; FocA, formate transporter; GH, glycoside hydrolase; GlgABC, glycogen storage proteins; GlpK, glycerol kinase; GDH, glutamate dehydrogenase; GOGAT, glutamine synthetase; GS, glutamine synthetase; HppA, pyrophosphate-energized sodium pump; mon, monomeric; multi, multimeric; POR, pyruvate:ferredoxin oxidoreductase; Pta, phosphate acetyltransferase; Rnf, ferredoxin:NAD⁺-oxidoreductase complex; SCFA, short-chain fatty acids; Shy, sulfhydrogenase; Sud, bifunctional sulfide dehydrogenase/ferredoxin:NADP oxidoreductase; VOR, 2-oxoisovalerate:ferredoxin oxidoreductase; WLP, Wood-Ljungdahl pathway.