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Supplementary Information to the Manuscript

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Content:

Supplementary Table S1. *F. acidiphilum* Y^T genes examined by real-time reverse-transcription PCR in this study.

Locus tag	Gene	Name	Forward primer / Reverse primer	TaqMan®
FAD_0161	***rpl2***	**50S ribosomal protein L2**	F: 5'-ATACAGGAGCCCGAGTCACA-3' R: 5'-TGTTGTGGTAGTGCCGTTGT-3'	5'-CACCGGGTAGAAATGCACCGTT-3'
FAD_0374	***gyrB***	**DNA gyrase B**	F: 5'-GCAGAGCTTCAAGGGGAGTT-3' R: 5'-CGCCAACAGGTAATGCAGTA-3'	5'-CTAAACGGTGCTGAAGTAATCA-3'
FAD_0567	*porA*	2-oxoacid--ferredoxin oxidoreductase, α subunit	F: 5'-CAACCGGGCTTCCAACAAAGA-3' R: 5'-GAGCACTATTCTTGGCATGT-3'	5'-AGATCTCAACCAGGTGCTTGGT-3'
FAD_0703	*morME*	malate oxidoreductase (malic enzyme)	F: 5'- ATGGCCAGGTGATGCAAAGA-3' R: 5'- TGCATAGGAAGCAGCAACCA-3'	5'- CCAATCAGATAAATAATAGCAT-3'
FAD_0712	*korA*	2-oxoacid--ferredoxin oxidoreductase, α subunit	F: 5'- AGCAGGGCGTATCAGCTAAC-3' R: 5'- AGGGTCATATGCCTGCCATT-3'	5'- AATGTTCGAGCCTTTCCCTGCA-3'
FAD_0713	*korB*	2-oxoacid--ferredoxin oxidoreductase, β subunit	F: 5'- AGATTGGTGCCCTGGATGTG-3' R: 5'- AGCTGACAAAGCCTGTGTTA-3'	5'- TGACTTTGGTATAGTAAGTGCG-3'
FAD_0714	*sdhD*	Succinate dehydrogenase subunit D	F: 5'- AGCCCCATTTGCCACTGAAA-3' R: 5'-TCAACCATAGTGCCGGAGAA-3'	5'- AATTAAACAATGCTATGTACCT-3'
FAD_0717	*sdhA*	Succinate dehydrogenase subunit A	F: 5'- TTCAACGCGCTGGAAACAAG-3' R: 5'- TGAAGTGTGCACCCCTTGTT-3'	5'- ATATGCTATGGCTACTGGAGCA-3'
FAD_0718	*mdh*	Malate dehydrogenase	F: 5'- TTTGCACCCAGGACCCTAA-3' R: 5'- CTGCAATGCGTATGCCATTA-3'	5'- AACAGATTAAGAAATATTCACC-3'
FAD_1044	*pepc*	phosphoenol pyruvate carboxylase	F: 5'- AGAAGGGAGCGGAAATTGCA-3' R: 5'- GCACGTGGCAAACTAGCTTT-3'	5'- CTATTTGGATACTCCCGTAGTA-3'

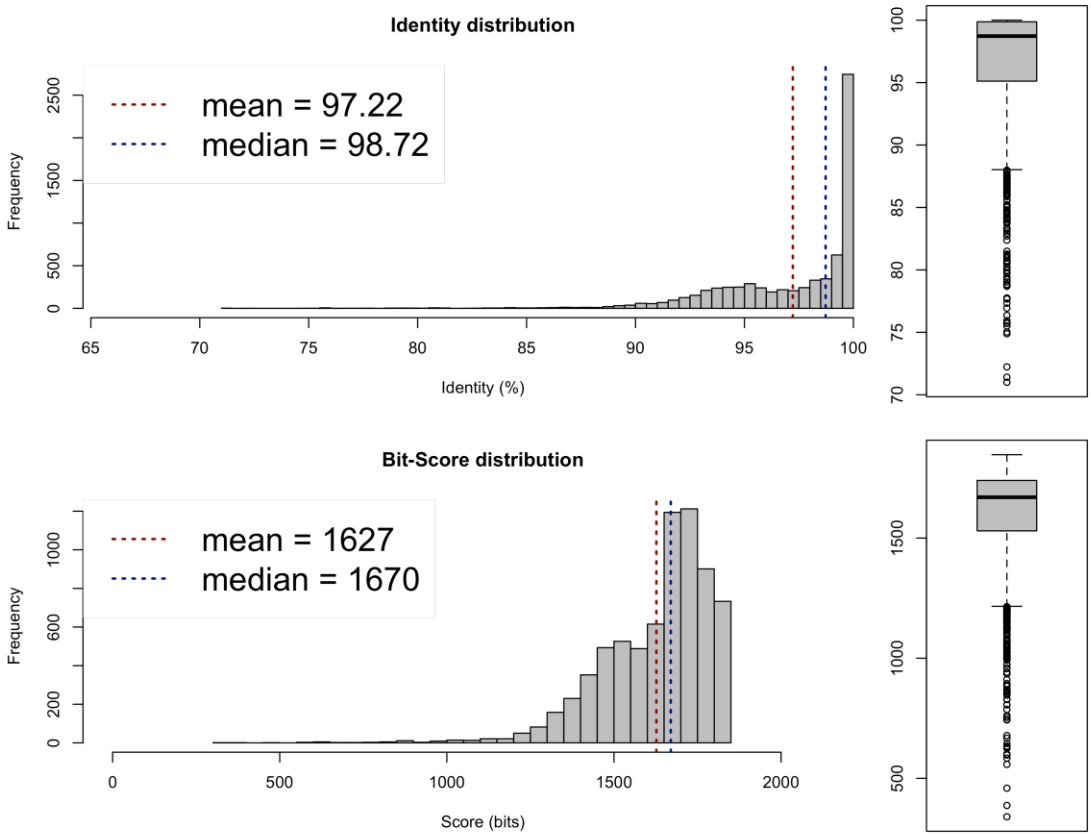
Supplementary Table S2. 116 single-nucleotide substitutions (separate Excel file).

Supplementary Table S3. Transporters in *F. acidiphilum* Y^T

Sugar & polysaccharide transporters	
FAD_1026	ABC-type sugar transporter, ATPase component
FAD_1027	ABC-type sugar transporter, permease component
FAD_1028	ABC-type sugar transporter, permease component
FAD_1029	ABC-type sugar transporter, extracellular component
FAD_1459	sugar ABC transporter 1, permease protein
FAD_1460	sugar ABC transporter 1, ATP binding protein
AA transporters	
FAD_0093	ABC-type peptide transporter, permease component
FAD_0094	oligopeptide ABC transporter Dpp2, permease protein
FAD_0126	6TMS neutral amino acid family transporter
FAD_0228	amino acid transporter
FAD_0377	amino acid transporter related protein
FAD_0539	cationic amino acid transporter
FAD_0636	amino acid transporter
FAD_0655	ABC transporter peptide-binding protein
FAD_0659	ABC transporter permease. dipeptide/oligopeptide/nickel transport system
FAD_0802	amino acid/polyamine/organocation superfamily transporter
FAD_0803	aspartate/glutamate family transporter
FAD_0804	aspartate/glutamate family transporter
FAD_0805	amino acid/polyamine/organocation superfamily transporter

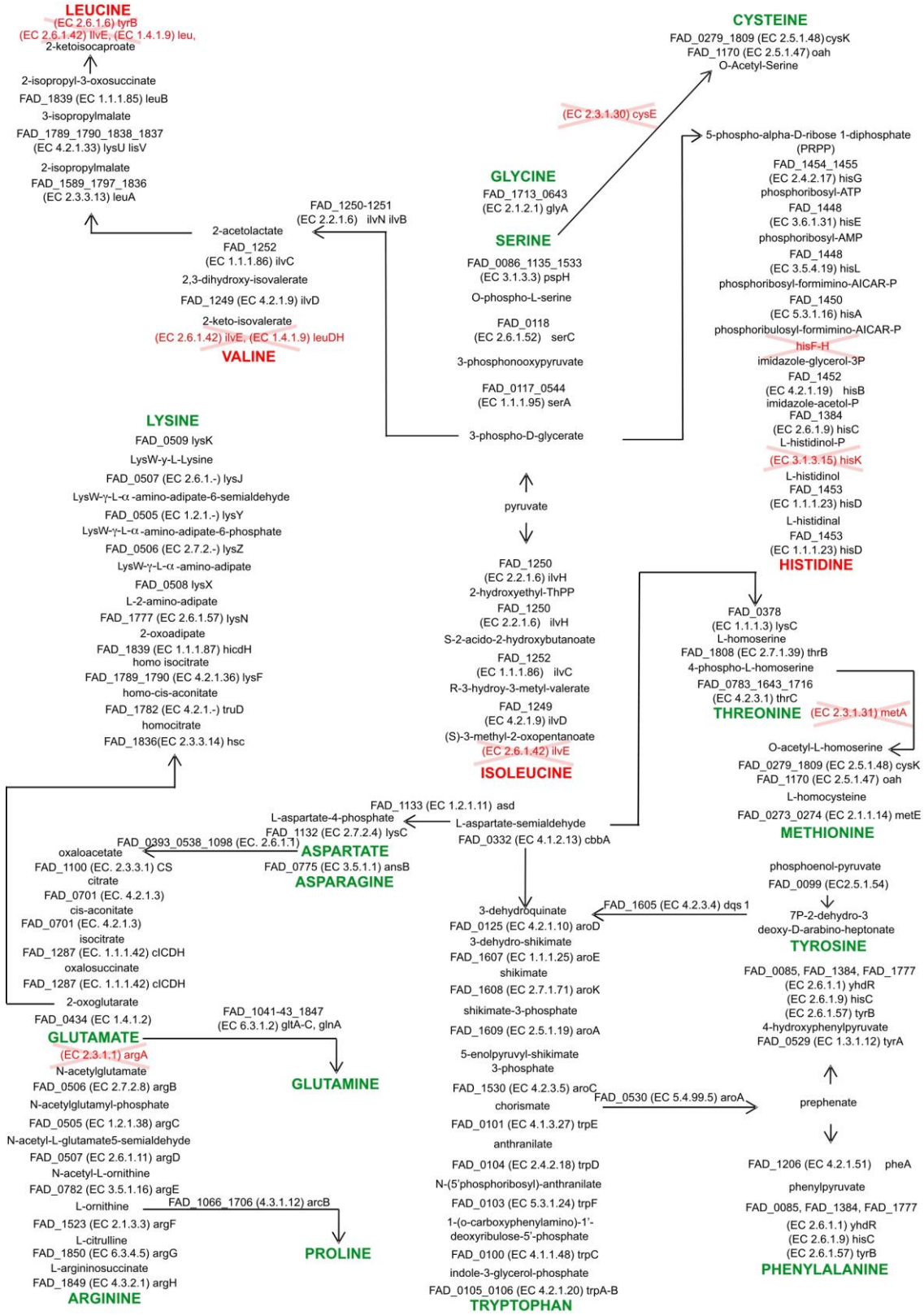
FAD_0853	ABC transporter peptide-binding protein
FAD_0914	amino acid/polyamine/organocation superfamily transporter
FAD_0915	amino acid/polyamine/organocation superfamily transporter
FAD_0919	ABC transporter substrate-binding protein (C-terminal fragment) peptide
FAD_1013	amino acid/polyamine/organocation superfamily transporter
FAD_1014	ABC-type peptide/opine/nickel family transporter ATPase
FAD_1015	ABC-type peptide/opine/nickel family transporter ATPase
FAD_1016	ABC-type peptide/opine/nickel family transporter
FAD_1017	ABC-type peptide/opine/nickel family transporter
FAD_1018	ABC-type peptide/opine/nickel family transporter
FAD_1069	amino acid transporter
FAD_1371	6TMS neutral amino acid family transporter
FAD_1571	amino acid transporter
FAD_1737	transporter probably aa
Major facilitator superfamily MFS multidrug efflux pumps and major facilitator superfamily permeases	
FAD_0778 _0801 _0803 _0815 _0839 _1106 FAD_1033 _1118 _1278 _1296 _1750_0006 _0143 _0213 _0472 _0497 _0561 _0684 _0768 _0796 _0822 _0844_0849 _0979 _0994 _0996 _1000 _1011 _1018 _1038 _1040_1047 _1048 _1129_1272 _1349 _1380 _1407 _1408 _1445 _1484 _1504 _1544 _1555 _1591 _1599 _1720 _1788 _1791	
Other transporters	
FAD_0049	stomatin family transporter
FAD_0249	cation diffusion facilitator family transporter, that increase tolerance to divalent metal ions such as cadmium, zinc, and cobalt

FAD_0250	cation diffusion facilitator family transporter, that increase tolerance to divalent metal ions such as cadmium, zinc, and cobalt
FAD_1045	ammonium transporter
FAD_1137	C4-dicarboxylate transporter
FAD_1177	ABC-2-type family permease
FAD_1178	ABC-2-type family permease
FAD_0071	VIT family Fe2+/Mn2+ transporter
FAD_1261	VIT family Fe2+/Mn2+ transporter
FAD_0016	transporter of sulfur-containing compounds
FAD_0264	transporter of sulfur-containing compounds
FAD_0848	transporter of sulfur-containing compounds
FAD_1341	transporter of sulfur-containing compounds
FAD_1460	nitrate/sulfonate/bicarbonate ABC transporter ATP-binding protein
FAD_1459	nitrate/sulfonate/bicarbonate ABC transporter ATP-binding protein
FAD_1458	nitrate/sulfonate/bicarbonate ABC transporter ATP-binding protein
FAD_1494	monovalent cation:proton antiporter-2 family transporter
FAD_1510	sodium-dependent phosphate transporter
FAD_1593	manganese/divalent cation transporter
FAD_1667	multidrug ABC transporter ATP-binding protein
FAD_1771	daunorubicin resistance ABC transporter
FAD_1800	Kef-type potassium transporter NAD-binding component



Supplementary Figure S1. Average Nucleotide Identity (ANI) analysis (http://enve-omics.ce.gatech.edu/ani/[1]) of genomes "*F. acidarmanus* fer1" and *F. acidiphilum* Y^T suggests ANI values above 95 %, which is the accepted cut off for separation of two species based on the whole-genome comparisons, suggesting that on the basis of their genomic data, *F. acidiphilum* Y^T and "*F. acidarmanus*" fer1 belong to the same species.

1. Goris, J., Konstantinidis, K. T., Klappenbach, J. A., Coenye, T., Vandamme, P. & Tiedje, J. M. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol.* **57**, 81-91 (2007).



Supplementary Figure S2. Overview of amino acid biosynthesis pathways in the *F. acidiphilum* Y^T genome. Missing genes and reaction are indicated by red colour and crossed out. All amino acids with incomplete synthesis pathways are indicated in red. EC numbers are shown in parentheses.