

Supplementary information for

A Novel Organotrophic Nitrate-reducing Fe(II)-oxidizing Bacterium Isolated from a Paddy Soil and Draft Genome Sequencing Indicates its Metabolic Versatility

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Running title: Nitrate-reducing Fe(II)-oxidizer from paddy soil

Table S1. Genes involved in ferrous iron oxidation of different physiological groups of the Fe(II) oxidizers.

Species	Physiology	Genes	Reference
<i>Acidithiobacillus ferrooxidans</i>	Acidophilic	<i>Cyc2, RusA, CycI, CycA1</i>	1
<i>Leptospirillum ferrooxidans</i>	Acidophilic	<i>Cyt572, Cyt579, CytC</i>	2
<i>Sideroxydans lithotrophicus</i>	Micro-aerobic	<i>MtoB, MtoA, CymA</i>	3
<i>Rhodopseudomonas palustris</i>	Phototrophic	<i>PioB, PioA, PioC</i>	4
<i>Rhodobacter ferrooxidans</i>	Phototrophic	<i>FoxE, FoxY</i>	5

Table S2. Predicted cytochrome in *Rhodocyclaceae* sp. strain Paddy-1 from genome annotation.

gene	Gene Length	AA length	No. COG	No. Pfam	No. GO
ORF_0007	594	197	NA	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_0030	2091	696	COG3258	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_0340	423	140	COG3245	PF00034	NA
ORF_0414	927	308	COG2010	PF00034	NA
ORF_0454	573	190	NA	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_0621	432	143	COG2010	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_0861	327	108	COG4654	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_0875	309	102	COG4654	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_0877	678	225	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1199	645	214	COG3245	NA	NA
ORF_1372	438	145	COG3245	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1447	678	225	NA	PF00034	NA
ORF_1451	1080	359	COG4654	PF00034	NA
ORF_1454	324	107	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1457	615	204	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1471	1719	572	COG2010	PF00034	NA
ORF_1632	429	142	COG2010	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1636	315	104	COG4654	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1687	720	239	COG2857	PF02167	GO:0009055, GO:0020037, GO:0005506
ORF_2104	978	325	COG1858	PF00034	GO:0055114, GO:0016491
ORF_2420	639	212	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_2425	411	136	COG3474	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_2539	642	213	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_2641	474	157	NA	PF01322	GO:0009055, GO:0020037, GO:0005506
ORF_2653	267	88	NA	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_2757	1260	419	COG2863	PF00034	GO:0005507, GO:0004129, GO:0016020
ORF_2759	624	207	COG3175	PF04442	GO:0005507
ORF_2862	459	152	COG3909	PF01322	GO:0009055, GO:0020037, GO:0005506
ORF_2876	1698	565	COG2010	PF00034	NA
ORF_2882	399	132	COG2010	NA	NA
ORF_2894	1617	538	COG2010	PF00034	NA

Description:

COG1858: Cytochrome c peroxidase; COG2010: Cytochrome c, mono- and diheme variants; COG2857: Cytochrome c1; COG2863: Cytochrome c553; COG3175: Cytochrome oxidase assembly factor; COG3245: Cytochrome c5; COG3258: Cytochrome c, mono- and diheme variants; COG3474: Cytochrome c2; COG3909: Cytochrome c556; COG4235: Cytochrome c biogenesis factor; COG4654: Cytochrome c551/c552.

PF00034: Cytochrome c; PF02167: Cytochrome C1 family; PF01322: Cytochrome C; PF04442: Cytochrome c

oxidase assembly protein CtaG/Cox11; PF07719: Tetratricopeptide repeat.

GO0004129: cytochrome-c oxidase activity; GO0005506: iron ion binding; GO0005507: copper ion binding; GO0009055: electron carrier activity; GO0016020: membrane; GO0016491: oxidoreductase activity; GO0020037: heme binding; GO0055114: oxidation reduction.

Table S3. Predicted Fe ion homeostasis systems in *Rhodocyclaceae* sp. strain Paddy-1 from genomic annotation.

(A) Ferredoxin Ferritin

Gene	Gene Length	AA length	No. COG	No. Pfam	No. GO
ORF_0190	353	118	COG3411	PF01257	GO0051287, GO0055114, GO0016491
ORF_0267	1436	479	COG0716	PF00258	NA
ORF_0269	386	129	COG2146	NA	GO0051537, GO0055114, GO0016491
ORF_0415	1451	484	COG0348	PF11614	NA
ORF_0547	470	157	COG0783	PF00210	GO0006879, GO0008199
ORF_0627	1025	342	COG0348	NA	NA
ORF_0865	1163	388	COG0633	PF00111	GO0055114, GO0016491
ORF_1063	875	292	COG0348	NA	NA
ORF_1248	308	103	COG3411	PF01257	GO0051287, GO0055114, GO0016491
ORF_1394	1490	497	COG0348	PF11614	NA
ORF_1575	773	258	COG1018	NA	GO0055114, GO0016491
ORF_1672	1049	350	COG0633	PF00970	GO0009055, GO0051536
ORF_1696	371	124	COG2146	PF00355	GO0051537, GO0055114, GO0016491
ORF_2327	1112	371	COG0276	PF00762	GO0004325, GO0006783
ORF_2240	866	289	COG1018	PF00970	GO0055114, GO0016491
ORF_2262	323	108	COG1146	PF00037	NA
ORF_3077	473	158	COG2193	PF00210	GO0006879, GO0008199
ORF_3169	341	114	COG0633	PF00111	GO0009055, GO0051536
ORF_3264	1370	457	COG0348	NA	NA

(B) Transporters of ferrous and ferric ion

Gene	Gene Length	AA length	No. COG	No. Pfam	No. GO
ORF_0158	1013	338	NA	PF02421	GO0005525, GO0005622
ORF_0363	1109	370	COG1840	PF01547	GO0005215
ORF_0364	1709	570	COG1178	PF00528	NA
ORF_0385	1346	449	COG1160	PF02421	GO0005525, GO0005622
ORF_0387	1268	423	COG2262	PF02421	GO0005525, GO0005622
ORF_0429	785	262	COG0672	FTR1	GO0016020, GO0055085
ORF_0568	1130	377	COG0536	PF02421	GO0005525
ORF_0728	1052	351	COG1840	PF01547	GO0006810, GO0005215
ORF_0731	1685	562	COG1178	PF00528	NA
ORF_0844	929	310	COG0614	PF01497	GO0006827, GO0005381
ORF_0936	944	315	COG1161	PF02421	NA
ORF_0995	542	181	COG0735	PF01475	GO0006355, GO0003700
ORF_1126	860	287	COG0614	PF01497	GO0006827, GO0005381

ORF_1211	1343	448	COG4097	PF01794	GO0009055,GO0050660,GO0016021
ORF_1315	1352	451	COG0486	PF02421	NA
ORF_1744	977	326	COG0609	PF01032	GO0016020,GO0006810,GO0005215
ORF_1788	1091	364	COG0012	PF02421	NA
ORF_2331	458	153	COG0735	PF01475	GO0006355,GO0003700
ORF_2503	1319	440	COG4097	PF01794	GO0009055,GO0050660,GO0016021
ORF_2543	674	225	COG2717	PF01794	GO0009055,GO0050660,GO0016021
ORF_3088	2831	944	COG3064	PF02421	NA
ORF_3260	557	186	COG3470	PF10634	NA

Description:

COG0012: Predicted GTPase, probable translation factor; COG0276: Protoheme ferro-lyase (ferrochelataase); COG0348: Polyferredoxin; COG0486: Predicted GTPases; COG0536: Predicted GTPases; COG0609: ABC-type Fe³⁺-siderophore transport system, permease component; COG0614: ABC-type Fe³⁺-hydroxamate transport system, periplasmic component; COG0633: Ferredoxin; COG0672: High-affinity Fe²⁺/Pb²⁺ permease; COG0716: Flavodoxins; COG0735: Fe²⁺/Zn²⁺ uptake regulation proteins; COG0783: DNA-binding ferritin-like protein (oxidative damage protectant); COG1018: Flavodoxin reductases (ferredoxin-NADPH reductases) family 1; COG1146: Ferredoxin; COG1160: Predicted GTPases; COG1161: Predicted GTPases; COG1178: ABC-type Fe³⁺ transport system, permease component; COG1840: ABC-type Fe³⁺ transport system, periplasmic component; COG2146: Ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenases; COG2193: Bacterioferritin (cytochrome b1); COG2262: Predicted GTPases; COG2717: Predicted membrane protein; COG3064: Membrane protein involved in colicin uptake; COG3411: Ferredoxin; COG3470: Uncharacterized protein probably involved in high-affinity Fe²⁺ transport; COG4097: Predicted ferric reductase.

PF00037: 4Fe-4S binding domain; PF00111: 2Fe-2S iron-sulfur cluster binding domain; PF00175: Oxidoreductase NAD-binding domain; PF00210: Ferritin-like domain; PF00258: Flavodoxin; PF00355: Rieske [2Fe-2S] domain; PF00528: Binding-protein-dependent transport system inner membrane component; PF00762: Ferrochelataase; PF00970: Oxidoreductase FAD-binding domain; PF01032: FecCD transport family; PF01257: Respiratory-chain NADH dehydrogenase 24 Kd subunit; PF01475: Ferric uptake regulator family; PF01497: Periplasmic binding protein; PF01547: Bacterial extracellular solute-binding protein; PF01794: Ferric reductase like transmembrane component; PF02421: Ferrous iron transport protein B; PF10634: Fe²⁺ transport protein; PF11614: Ubp3 associated protein Bre5.

GO0003700: transcription factor activity ; GO0004325: ferrochelataase activity ; GO0005215: transporter activity ; GO0005381: iron ion transmembrane transporter activity ; GO0005525: GTP binding ; GO0005622: intracellular ; GO0006355: regulation of transcription, DNA-dependent ; GO0006783: heme biosynthetic process ; GO0006810: transport ; GO0006827: high-affinity iron ion transport ; GO0006879: cellular iron ion homeostasis ; GO0008199: ferric iron binding ; GO0009055: electron carrier activity ; GO0016020: membrane ; GO0016021: integral to membrane ; GO0016491: oxidoreductase activity ; GO0050660: FAD binding ; GO0051287: NAD or NADH binding ; GO0051536: iron-sulfur cluster binding ; GO0051537: 2 iron, 2 sulfur cluster binding ; GO0055085: transmembrane transport ; GO0055114: oxidation reduction.

Table S4. Predicted iron oxidase and related electron transfer system in *Rhodocyclaceae* sp. strain Paddy-1 by comparing to other oxidizing bacteria.

gene	Gene Length	AA length	Most related protein	Similarity (E value)	Score (Bits)	No. COG	No. Pfam	No. GO
ORF_0385	1346	449	feoB (<i>S. oneidensis</i> MR-1)	8.00E-08	103	COG1160	PF02421	GO0005525, GO0005622
ORF_0877	678	225	cyc1 (<i>A. ferrooxidans</i> 23270)	2.00E-19	181	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1061	674	225	cymA (<i>S. oneidensis</i> MR-1)	7.00E-23	203	COG3005	PF03264	NA
ORF_1454	324	107	cycA-2 (<i>A. ferrooxidans</i> 23270)	2.00E-11	115	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1457	615	204	cyc1 (<i>A. ferrooxidans</i> 23270)	9.00E-32	265	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_1788	1091	364	feoB (<i>S. oneidensis</i> MR-1)	1.00E-07	100	COG0012	PF02421	NA
ORF_2420	639	212	cyc1 (<i>A. ferrooxidans</i> 23270)	1.00E-39	319	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_2421	1457	486	cyc2 (<i>A. ferrooxidans</i> 23270)	3.00E-33	306	NA	NA	NA
ORF_2539	642	213	cycA-1 (<i>A. ferrooxidans</i> 23270)	1.00E-28	245	COG2863	PF00034	GO:0009055, GO:0020037, GO:0005506
ORF_2877	623	208	cymA (<i>S. oneidensis</i> MR-1)	1.00E-31	262	COG3005	PF03264	NA

Description

COG0012: Predicted GTPase, probable translation factor; COG1160: Predicted GTPases; COG2863: Cytochrome c553; COG3005: Nitrate/TMAO reductases, membrane-bound tetraheme cytochrome c subunit.

PF00034: Cytochrome c; PF02421: Ferrous iron transport protein B; PF03264: NapC/NirT cytochrome c family, N-terminal region.

GO0005506: iron ion binding ; GO0005525: GTP binding ; GO0005622: intracellular ; GO0009055: electron carrier activity ; GO0020037: heme binding.

Supplementary Figures

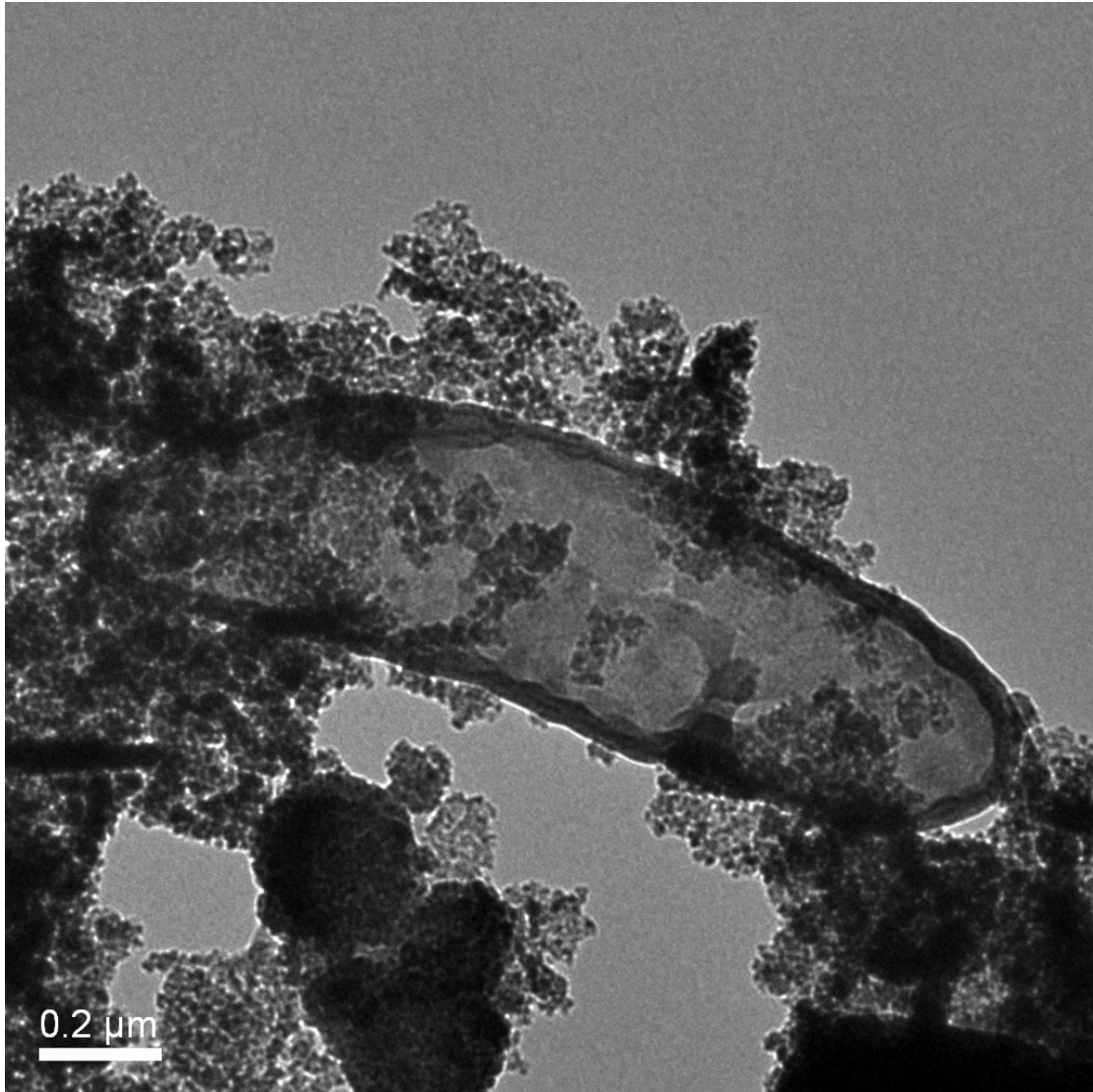


Fig. S1 Transmission electron micrographs of a single cell of *Rhodocyclaceae* sp. strain Paddy-1 in the presence of 10 mM nitrate, 5 mM FeCl_2 and 5 mM acetate at day 12 of incubation time.

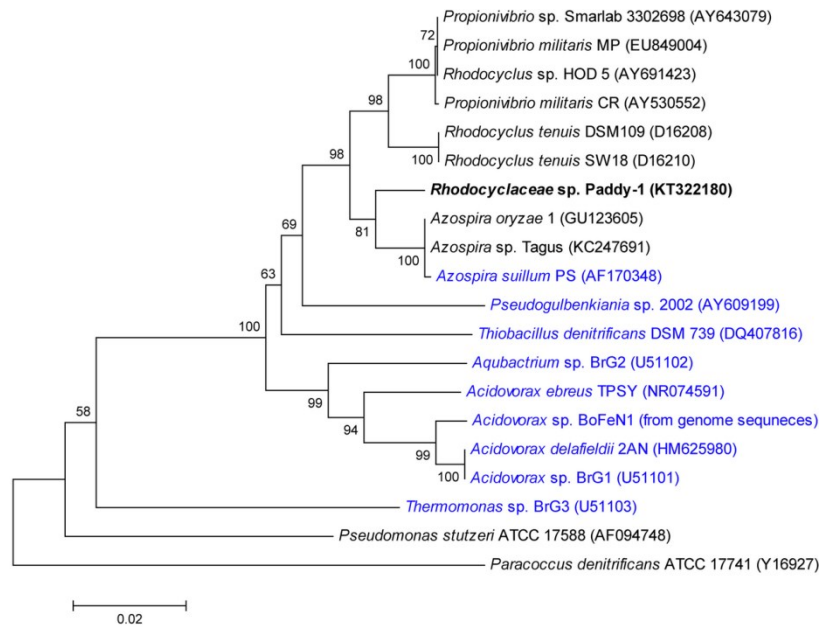


Fig. S2 Neighbor-joining tree based on 16S rRNA sequences showing the phylogenetic affiliation of *Rhodocyclaceae* sp. strain Paddy-1 and evolutionary distance to other nitrate-reducing Fe(II)-oxidizing bacteria. Bootstrap values were determined on the basis of results of 1,000 replicates. Accession numbers are listed after the bacterium. The scale bar represents evolutionary distance as 0.02 change per nucleotide position. The numbers given are bootstrap values. Identified nitrate-dependent Fe(II)-oxidizing bacteria are shown in blue color of the font.

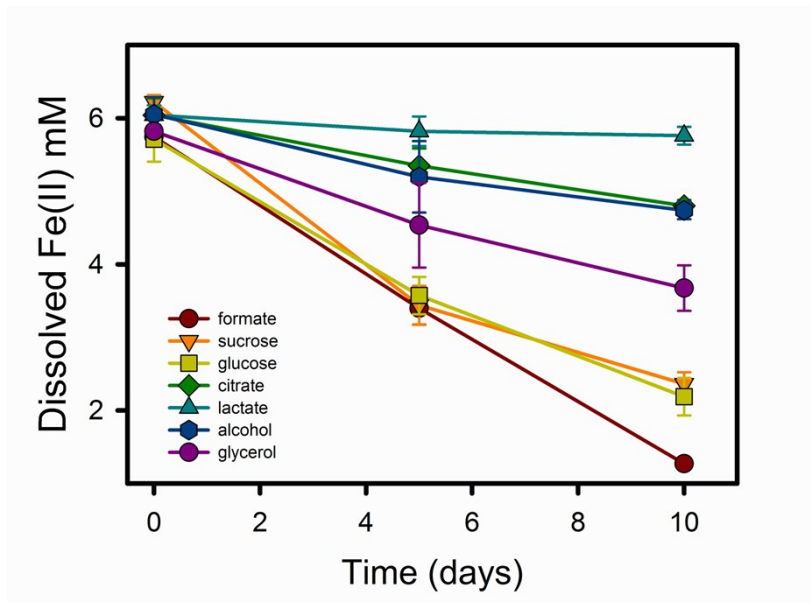


Fig. S3 Oxidation of dissolved Fe(II) in cultures of the nitrate-reducing Fe(II)-oxidizing *Rhodocyclaceae* sp. strain Paddy-1 using different organic carbon (5 mM) as an electron donor. The prestarved cell ($\sim 10^9$ cells mL⁻¹) were cultured in the basal medium containing 5 mM Fe(II) and 10 mM of nitrate. Data were all presented as means \pm standard deviations (SD) of triplicate. When not shown, error bars are smaller than the symbol size.

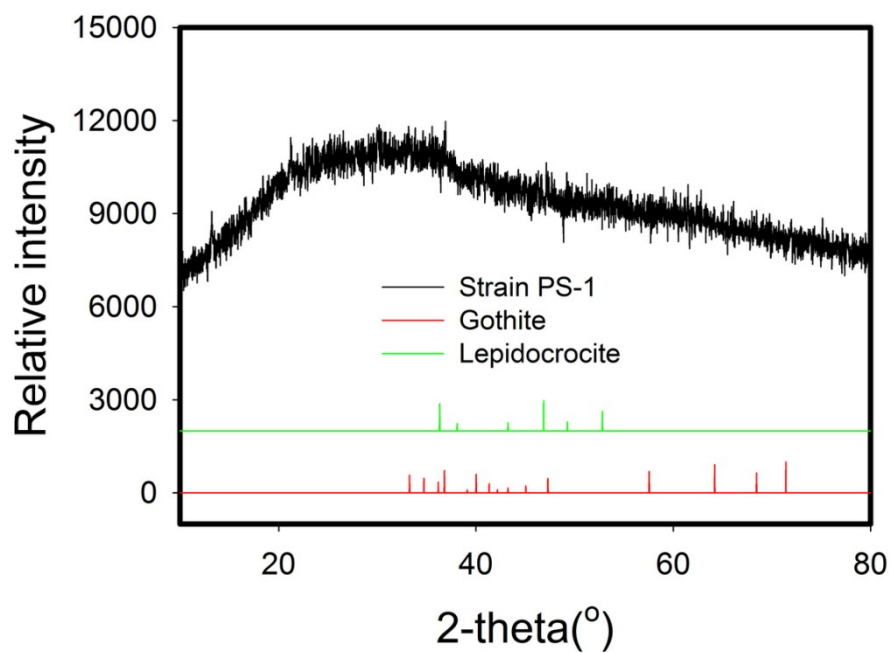


Fig. S4 X-ray diffraction (XRD) spectrum of the *Rhodocyclaceae* sp. strain Paddy-1 culture. No distinct diffractive acuti-apices implies the formation of amorphous iron oxides.



Fig. S5 Results of ANI calculations using JSpecies software of strain Paddy-1 with respect to the reported nitrate-reducing Fe(II)-oxidizing species.



Fig. S6 Egnog categories of the genes predicted from nitrate-reducing Fe(II)-oxidizers.

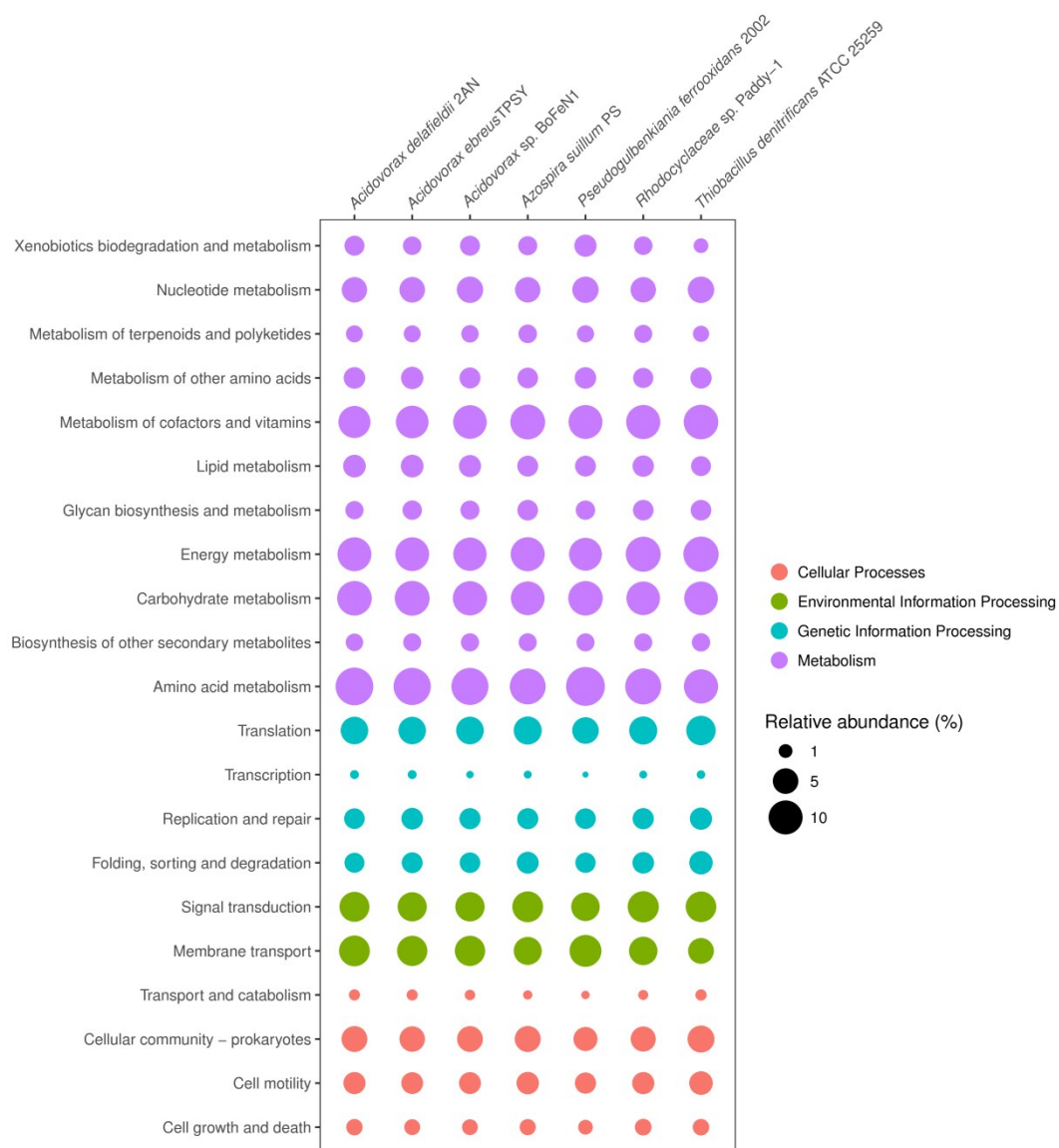


Fig. S7 KEGG categories of the genes predicted from nitrate-reducing Fe(II)-oxidizers.

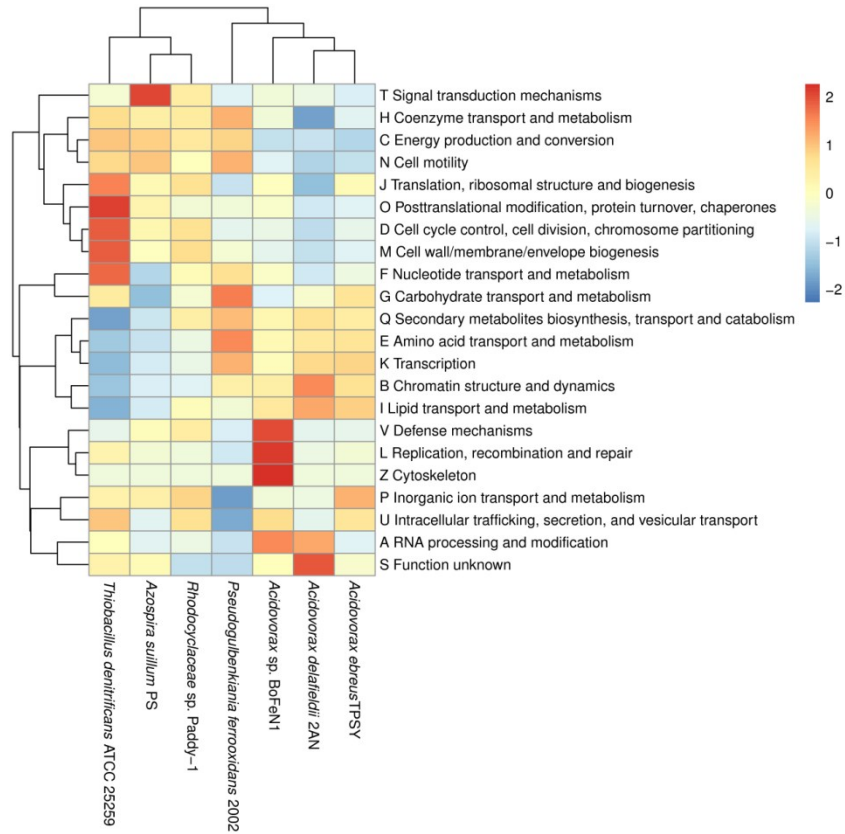


Fig. S8 Clustering of the nitrate-reducing Fe(II)-oxidizers based on functional annotation of the genes by eggNOG database on the gene abundance. The color code indicates the relative abundance of the genes after z-score normalization.

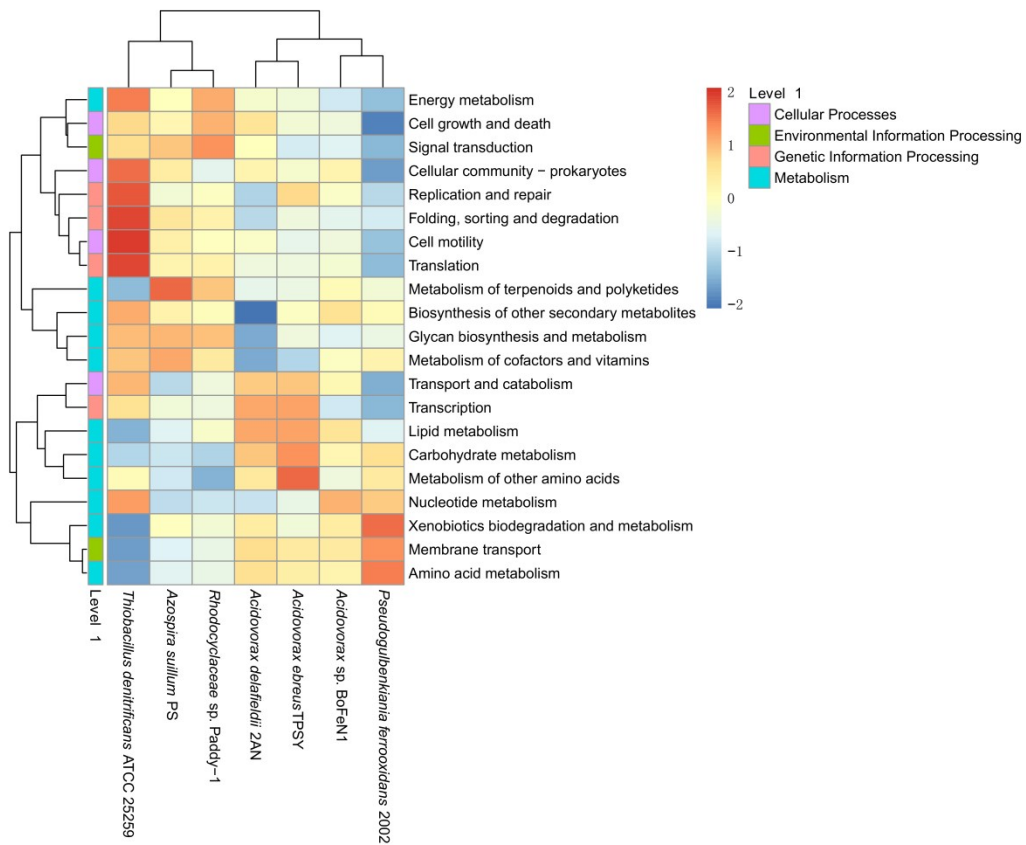


Fig. S9 Clustering of the nitrate-reducing Fe(II)-oxidizers based on functional annotation of the genes by KEGG pathways on the gene abundance. The color code indicates the relative abundance of the genes after z-score normalization.

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