

Supplementary information

Physiological and ecological implications of an iron- or hydrogen-oxidizing member of the Zetaproteobacteria, *Ghiorsea bivora*, gen. nov., sp. nov.

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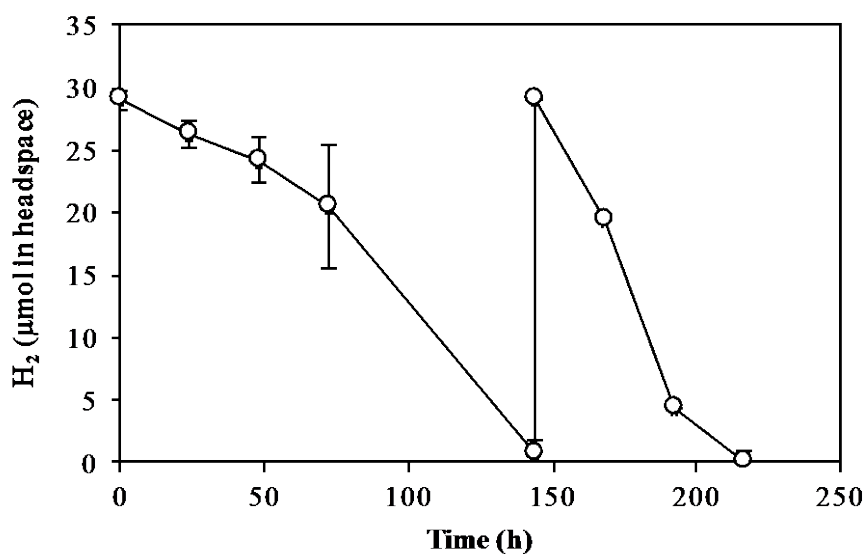


Figure S1. Monitoring of hydrogen amount in headspace of strain TAG-1 cultures. Error bars indicate SD (n=3).

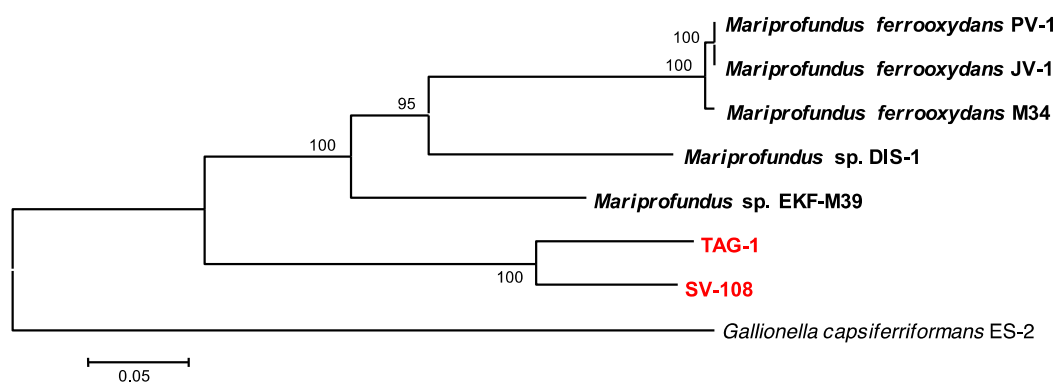


Figure S2. Maximum-likelihood trees of concatenated sequence of functional genes (*gyrB-ileS-leuS-efp-lepA-pyrG-recA-rpoA-rpoD-dnaJ-smpB*) of TAG-1, SV-108 and other Zetaproteobacteria isolates. Isolated strains of Zetaproteobacteria are in bold. The tree was rooted with genes of *G. capsiferiformans* ES-2. The tree was created with 1000 bootstrap iteration and the values below 50 are not reported.

	PV-1	JV-1	M34	EKF-M39	DIS-1	TAG-1	SV-108
<i>M. ferrooxydans</i> PV-1	100	(16S) 99.9 (ANI) 99.8 (AAI) 99.9	(16S) 99.8 (ANI) 97.7 (AAI) 98.3	(16S) 96.3 (ANI) 71.5 (AAI) 69.8	(16S) 95.4 (ANI) 71.0 (AAI) 70.3	(16S) 92.6 (ANI) 66.4 (AAI) 62.2	(16S) 92.6 (ANI) 66.6 (AAI) 62.3
<i>M. ferrooxydans</i> JV-1	(16S) 99.9 (ANI) 99.9 (AAI) 99.9	100	(16S) 99.9 (ANI) 97.8 (AAI) 98.3	(16S) 96.3 (ANI) 71.8 (AAI) 69.9	(16S) 95.3 (ANI) 71.1 (AAI) 70.3	(16S) 92.5 (ANI) 66.4 (AAI) 62.3	(16S) 92.5 (ANI) 66.5 (AAI) 62.2
<i>M. ferrooxydans</i> M34	(16S) 99.8 (ANI) 97.6 (AAI) 98.3	(16S) 99.9 (ANI) 97.6 (AAI) 98.3	100	(16S) 96.1 (ANI) 70.7 (AAI) 69.3	(16S) 94.9 (ANI) 71.1 (AAI) 70.6	(16S) 92.1 (ANI) 66.5 (AAI) 62.3	(16S) 92.1 (ANI) 66.4 (AAI) 62.2
<i>Mariprofundus</i> sp. EKF-M39	(16S) 96.3 (ANI) 71.3 (AAI) 69.8	(16S) 96.3 (ANI) 71.3 (AAI) 69.9	(16S) 96.1 (ANI) 70.6 (AAI) 69.3	100	(16S) 93.8 (ANI) 70.8 (AAI) 70.6	(16S) 92.3 (ANI) 66.4 (AAI) 61.6	(16S) 92.3 (ANI) 66.7 (AAI) 61.8
<i>Mariprofundus</i> sp. DIS-1	(16S) 95.4 (ANI) 70.9 (AAI) 70.3	(16S) 95.3 (ANI) 70.9 (AAI) 70.3	(16S) 94.9 (ANI) 71.0 (AAI) 70.6	(16S) 93.8 (ANI) 70.8 (AAI) 70.6	100	(16S) 93.9 (ANI) 67.2 (AAI) 62.4	(16S) 94.0 (ANI) 67.4 (AAI) 62.7
TAG-1	(16S) 92.6 (ANI) 66.5 (AAI) 62.2	(16S) 92.5 (ANI) 66.5 (AAI) 62.3	(16S) 92.1 (ANI) 66.3 (AAI) 62.3	(16S) 92.3 (ANI) 66.5 (AAI) 61.6	(16S) 93.9 (ANI) 67.3 (AAI) 62.4	100	(16S) 98.6 (ANI) 82.0 (AAI) 86.2
SV-108	(16S) 92.6 (ANI) 66.6 (AAI) 62.3	(16S) 92.5 (ANI) 66.5 (AAI) 62.2	(16S) 92.1 (ANI) 66.4 (AAI) 62.2	(16S) 92.3 (ANI) 66.7 (AAI) 61.8	(16S) 94.0 (ANI) 67.5 (AAI) 62.7	(16S) 98.6 (ANI) 82.1 (AAI) 86.2	100

Figure S3. Percentage similarities of SSU rRNA gene, average nucleic acid identity (ANI), and average amino acid identity (AAI) among isolates of Zetaproteobacteria (*Mariprofundus* PV-1, JV-1, M34, EKF-M39, DIS-1, strain TAG-1 and SV-108). Heat map is prepared by using ANI values.

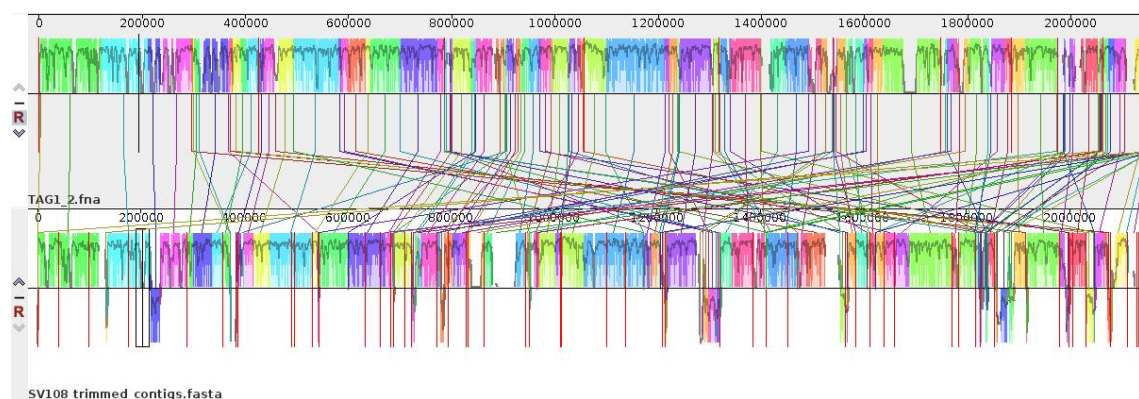


Figure S4. Pairwise genome alignment of TAG-1 and SV-108 on Mauve. Regions with each color indicate sequences that have high homology with those in another genome.

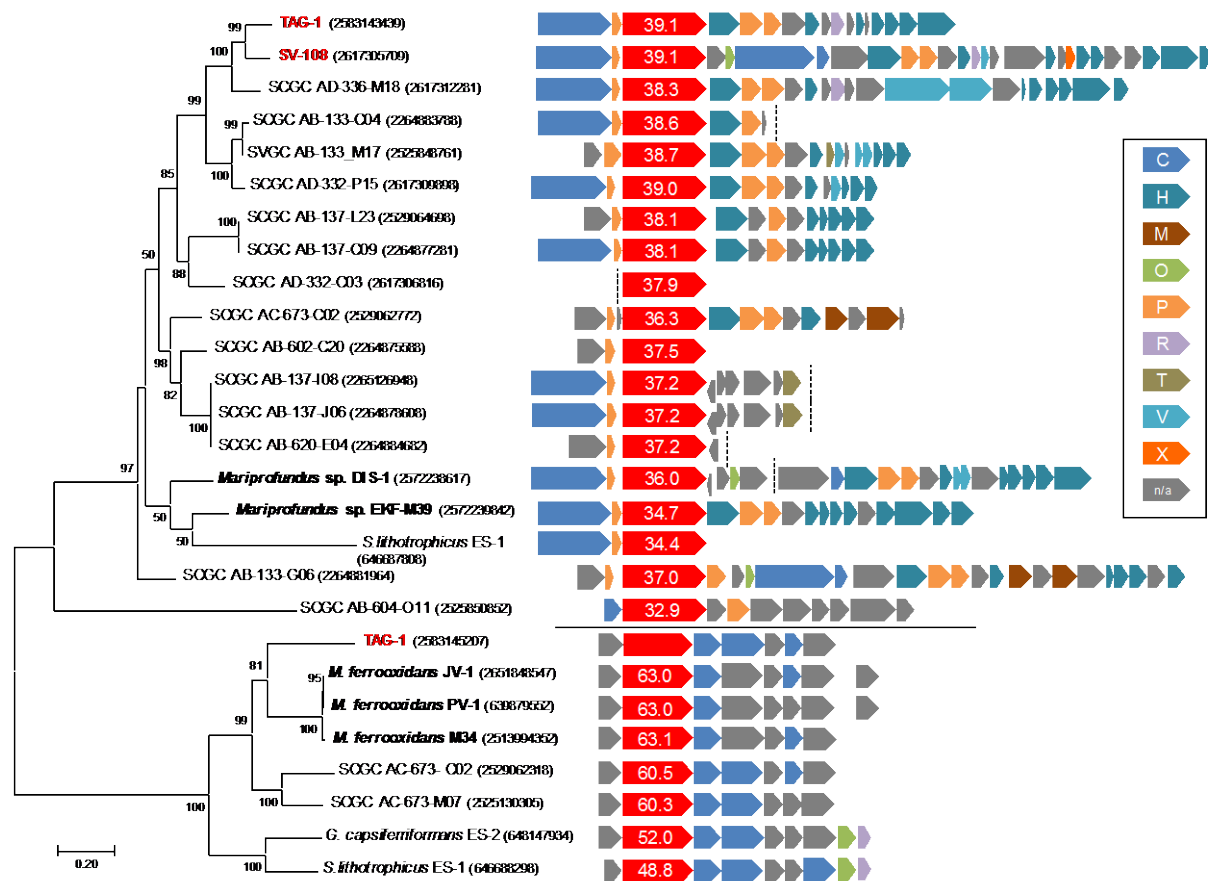


Figure S5. Maximum-likelihood phylogenetic tree and gene synteny of molybdopterin oxidoreductase (*actB*) of other Zetaproteobacteria isolates and SAGs. Genes of *G. capsiferiformans* ES-2 and *S. lithotrophicus* ES-1 are also shown as references. Isolated strains of Zetaproteobacteria are in bold and Gene IDs on IMG are given in parentheses. The tree was created with 1000 bootstrap iteration and the values below 50 are not reported. *actB* is shown as red arrows and nucleotide similarities (%) to TAG-1 are given. Genes except for *actB* are shown in different colors according to COG functional category as listed in a box. Dash lines indicate end of the contig.

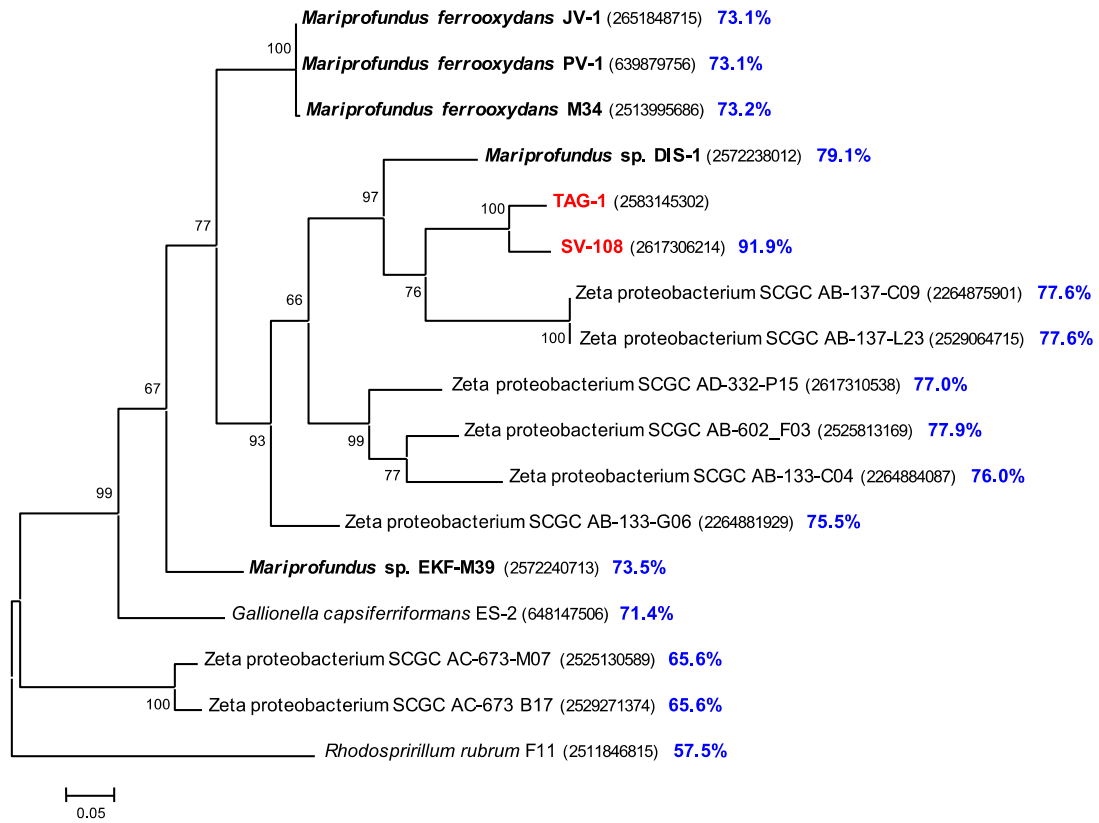


Figure S6. Maximum-likelihood phylogenetic tree of the Form II ribulose-1,5-bisphosphate carboxylase (RubisCO) large subunit (*cbbM*) gene of TAG-1, SV-108 and other Zetaproteobacteria isolates and SAGs. Isolated strains of Zetaproteobacteria are in bold and Gene IDs on IMG are given in parentheses. Percentage homologies with *cbbM* gene of TAG-1 are shown in blue. The tree was rooted with *cbbM* gene of *R. rubrum* F11 and the gene of *G. capsiferiformans* ES-2 is also shown as reference. The tree was created with 1000 bootstrap iteration and the values below 50 are not reported.

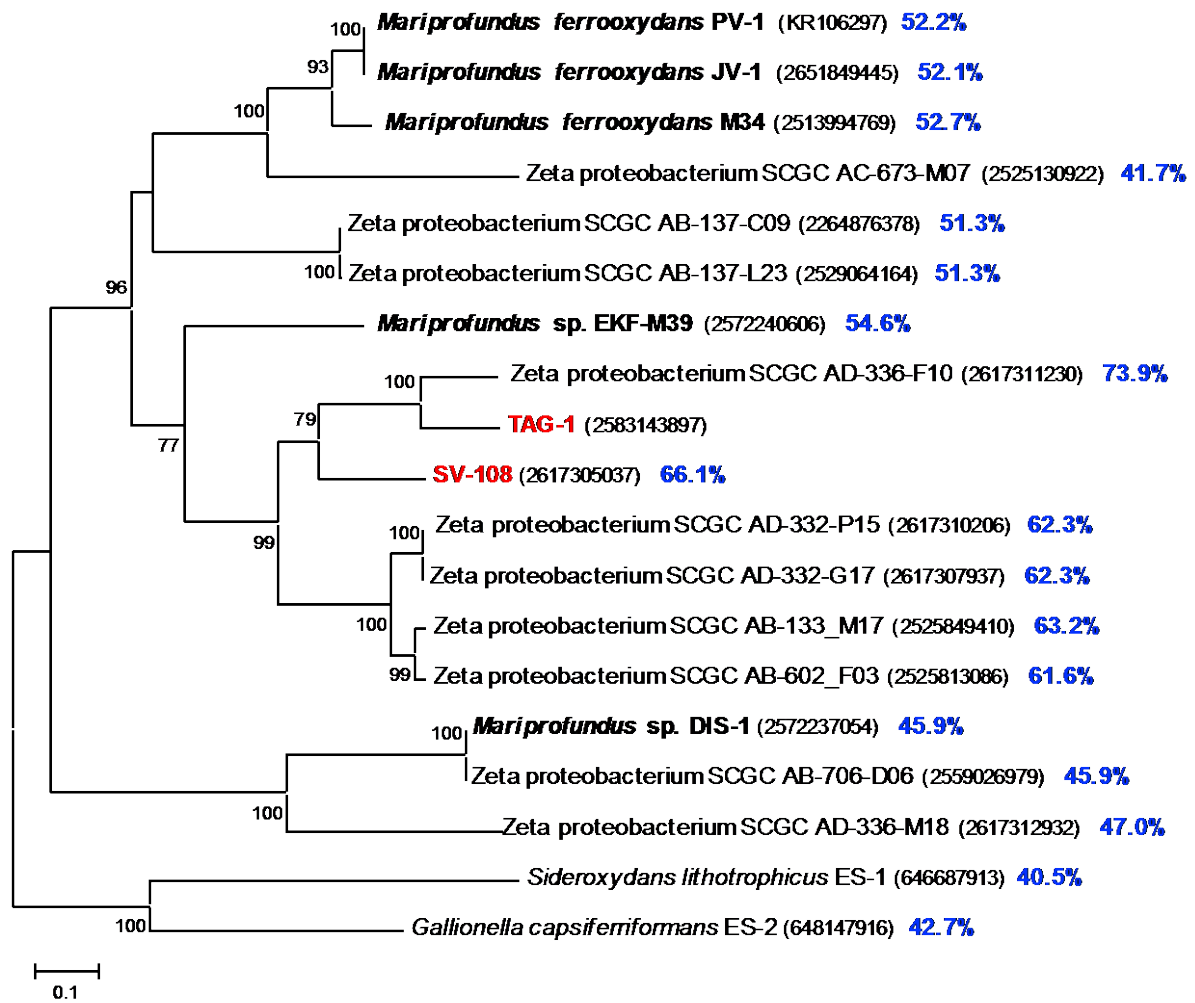


Figure S7. Maximum-likelihood phylogenetic tree and gene synteny of Cyc2 gene homologs of Zetaproteobacteria isolates and SAGs. Genes of *G. capsiferiformans* ES-2 and *S. lithotrophicus* ES-1 are also shown as references. Isolated strains of Zetaproteobacteria are in bold, and GenBank accession no. or Gene IDs on IMG are given in parentheses. Percentage homologies with Cyc2 gene of TAG-1 are shown in blue. The tree was created with 1000 bootstrap iteration and the values below 50 are not reported.

Table S1. Presence of flagellar-formation genes in genomes of Zetaproteobacteria isolates.

	PV-1	JV-1	M34	EKF-M39	DIS-1	TAG-1	SV-108
Flagellar biosynthetic protein FlhA	+	+	+	+	+	+	+
Flagellar biosynthetic protein FlhB	+	+	+	+	+	+	+
Flagellar transcriptional activator FlhC							
Flagellar transcriptional activator FlhD							
Flagellar hook-basal body complex protein FliE	+	+	+	+	+	+	
flagellar M-ring protein FliF	+	+	+	+	+	+	+
Flagellar motor switch protein FliG	+	+	+	+	+	+	+
Flagellar assembly protein FliH		+				+	
Flagellar protein FliJ						+	
Flagellar hook-length control protein FliK		+	+	+		+	
Flagellar motor switch protein FliM	+	+					
Flagellar motor switch protein FliN	+	+	+	+	+	+	+
Flagellar protein FliO		+				+	
Flagellar biosynthetic protein FliP	+	+	+	+	+	+	+
Flagellar biosynthetic protein FliQ	+	+	+	+	+	+	+
Flagellar biosynthetic protein FliR	+	+	+	+	+	+	+
Flagellar protein FliS	+	+	+	+		+	
Flagellar protein FliT							
Flagellar basal body P-ring formation protein FlgA		+	+	+		+	
Flagellar basal-body rod protein FlgB	+	+	+	+	+	+	+
Flagellar basal-body rod protein FlgC	+	+	+	+	+	+	+
Flagellar basal-body rod modification protein FlgD	+	+	+	+	+	+	+
Flagellar hook protein FlgE	+	+	+	+	+	+	+
Flagellar basal-body rod protein FlgF					+	+	+
Flagellar basal-body rod protein FlgG	+	+	+	+	+	+	+
Flagellar L-ring protein precursor FlgH	+	+	+	+	+	+	+
Flagellar P-ring protein precursor FlgI	+	+	+	+	+	+	+
Flagellar hook-associated protein 1 FlgK	+	+	+	+	+	+	+
Flagellar hook associated protein 3 FlgL		+					
Flagellar hook-associated protein 2	+	+	+	+		+	
Flagellum-specific ATP synthase	+		+	+	+	+	+
Flagellin		+	+			+	+

Table S2. Presence of chemotaxis genes in genomes of Zetaproteobacteria isolates.

	PV-1	JV-1	M34	EKF-M39	DIS-1	TAG-1	SV-108
Chemotaxis protein MotA	+	+	+	+	+	+	+
Chemotaxis protein MotB	+	+	+	+	+	+	+
Methyl-accepting chemotaxis protein	+	+	+	+	+	+	
Methyl-accepting chemotaxis protein I, serine sensor receptor				+			+
Methyl-accepting chemotaxis protein II, aspartate sensor receptor							
Methyl-accepting chemotaxis protein III, ribose and galactose sensor receptor							
Methyl-accepting chemotaxis protein IV, peptide sensor receptor							
Aerotaxis sensor receptor protein						+	+
Two-component system, chemotaxis family, sensor kinase CheA	+	+	+	+	+	+	+
Two-component system, chemotaxis family, response regulator CheB				+	+	+	+
Two-component system, chemotaxis family, CheB/CheR fusion protein			+	+			
Chemotaxis protein CheC							
Chemotaxis protein CheD				+			+
Chemotaxis protein methyltransferase CheR			+	+	+	+	+
Two-component system, chemotaxis family, response regulator CheV							
Purine-binding chemotaxis protein CheW	+	+	+	+		+	+
Chemotaxis protein CheX							
Two-component system chemotaxis family, response regulator CheY				+	+	+	+
Chemotaxis protein CheZ							
Methyl-accepting chemotaxis protein Hemerythrin	+	+		+		+	

Table S3. Summary of growth studies of TAG-1 and SV-108 at different temperatures and initial pH values.

	Temperature (°C)						Initial pH				
	5	10	20	25	30	37	5.5	6.0	6.5	7.0	7.5
TAG-1	+/-	+	++	++	+/-	-	+	++	++	++	+
SV-108	+/-	++	++	+	+/-	-	-	+	++	++	+

Table S4. Statistics for genomes of TAG-1 and SV-108

Strain	Taxon ID	Total bases	Total genes	Predicted protein coding genes	RNA genes	rRNA genes	tRNA genes	GC (%)
TAG-1	2582580733	2163552	2230	2184	46	6	37	42.68
SV-108	2617270712	2142334	2244	2208	36	2	31	43.02

Table S5. Presence of nitrogen-related genes in genomes of Zetaproteobacteria isolates.

	PV-1	JV-1	M34	EKF-M39	DIS-1	TAG-1	SV-108
Assimilatory nitrate reductase				+	+	+	+
Nitrite reductase (NADH)				+	+	+	+
Nitrite reductase (NO-forming)				+			
Nitrate/nitrite transporters				+	+	+	+
Nitric oxide reductase						+	+
Nitrous oxide reductase							
Nitrilase	+	+	+	+	+	+	+
Nitrogenase ABC-type			+	+			
Nitrate/sulfonate/bicarbonate transport system			+	+	+	+	+
Ammonium transporter	+	+	+	+	+	+	+
Nitrogen regulatory protein P-II	+	+	+	+	+	+	+

Table S6. Abundance of genes for ROS protection in genomes of Zetaproteobacteria isolates.

	PV-1	JV-1	M34	EKF-M39	DIS-1	TAG-1	SV-108
peroxiredoxin	5	5	6	6	5	4	5
catalase	0	0	0	0	2	0	0
catalase-peroxidase	0	0	0	0	2	1	0
cytochrome c peroxidase	2	2	2	4	3	2	2
glutathione peroxidase	0	0	0	0	1	0	0
Fe-dependent peroxidase	1	1	1	1	1	0	0
superoxide dismutase (SOD)	1	1	1	1	1	0	0
Total	9	9	10	12	15	7	7