

Supplemental material

Gene Clusters Involved in Isethionate Degradation in Terrestrial and Marine Bacteria

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TABLE S1. Organisms and the cultivation media used in this study. FM, fresh water mineral salts medium (18); SBM-M, modified *Silicibacter* basal medium (7); BSWM, modified basal sea water medium (2); DSMZ 133, medium number 133 recommended by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen); LNHM, sterilized seawater medium amended with a vitamin mixture, NH₄Cl and KH₂PO₄ (13).

Organism	Deposition number	Medium
<i>Cupriavidus necator</i> H16 (12)	DSM 428	FM
<i>Roseovarius nubinhibens</i> ISM (9)	DSM 15170 ^T	SBM-M
<i>Roseovarius</i> sp. strain 217 (14)		BSWM
<i>Ruegeria pomeroyi</i> DSS-3 (14, 23)	DSM 15171	SBM-M
<i>Rhodobacterales</i> sp. HTCC2150 (4, 16)		LNHM
<i>Burkholderia xenovorans</i> LB400 (3)	DSM 17367 ^T	FM
<i>Burkholderia phymatum</i> STM815 (19)	DSM 17167 ^T	FM
<i>Burkholderia phytofirmans</i> PsJN (17)	DSM 17436 ^T	FM
<i>Oligotropha carboxidovorans</i> OM5 (11)	DSM 1227 ^T	DSMZ 133
<i>Delftia acidovorans</i> SPH-1 (15)	DSM 12586	FM
<i>Paracoccus denitrificans</i> PD1222 (1)		FM
<i>Paracoccus denitrificans</i> NKNIS (6)	DSM 15418	FM

TABLE S2. Oligonucleotides used in this work. Many oligonucleotides were developed for this project. The 16S-rRNA primers (22), those for *xsc* and *pta* in *C. necator* H16 (21), and those for *xsc* and *pta* in *R. pomeroyi* DSS-3 (10), were described elsewhere.

Organism	Target	Name	Sequence (5' → 3')	
Bacteria	16S	16S-27F	CAG AGT TTG ATC CTG GCT CAG	
		16S-533R	TTA CCG CGG CTG CTG GCA C	
<i>C. necator</i> H16	<i>iseJ</i>	H16iseJF	CAG CGA CGA CGA GAT CCT GCA TT	
		H16iseJR	CGC CGT GCG TCT TCG CGG ATG AAG	
	<i>iseU</i>	H16iseUF	CCG TGA TCG GCG CCA CCA TCG AAT	
		H16iseUR	CCA GGA TGC CGA TGC TGT CAT A	
	<i>xsc</i>	H16xscF	ACC GAC ATC GGC AAC ATC AAC TC	
		H16xscR	GGT TGT AGA AGT CCA CCT GGT TCT	
	<i>pta</i>	H16ptaF	TGG TGT CGA GCT TCT TCC TGA T	
		H16ptaR	GCC GGC TTC CAG GCT GGG AAA C	
<i>R. pomeroyi</i> DSS-3	<i>iseJ</i>	SpIseJF	CTG GCA AAT CGC CTA AGC AAG	
		SpIseJR	GGT TGA ACG GGT AGC CTG CAT TC	
	<i>iseK</i>	SpIseKF	CCT TCA GGA CGC AGT GAT GGA ATC	
		SpIseKR	CTT CAT GTC GGC GGG CAC TTC A	
	<i>iseL</i>	SpIseLF	TCT TCC GGC GCT TTG TCC TGA A	
		SpIseLR	GAG ACC AGG AAC CAC CAT TGC AG	
	<i>iseM</i>	SpIseMF	GGA AAC TGG ACG ATC CTC ATC T	
		SpIseMR	CCA AGG CAG GCA TAG GCG AAA TCC	
	<i>xsc</i>	SpXscF	AAC ATC CCG CGT GAC ATG TGG AC	
		SpXscR	GGC CAG TAT TCC ATG CCA TAA CC	
	<i>pta</i>	SpPtaF	GTC TGA CCA TCC ACG ATC CGG ACA	
		SpPtaR	CGG CCT CGG GCG GAT ACA TCA	
	<i>P. denitrificans</i> NKNIS	<i>xsc-iseJ</i>	PD1222xscF	CCC GTC GAG CGC GTG AAA CCG TC
			PD1222iseJR1	ACC TGC AGG CGC GGC TGG TGT TCA A
<i>iseJ</i>		PD1222iseJF1	ACT GGC AGA CCG ATT GCG TGA AG	
		PD1222iseJR2	GGC TAT TTC CAG ACC ATG CAC AAC	
<i>iseJ-iseU</i>		PD1222iseJF2	CTG CGC CCA GCG GTC GTA ATC CT	
		PD1222iseUR	CTA TAT CGA CGG CGT GTT CTT CAA	
<i>iseU-iseR</i>		PD1222iseUF	GCG GAC GCC CGA TCC TGT CCG AA	
		PD1222iseRR	GCA AGA ACC TCA AGA ATC TGA AGA	

TABLE S3. Bacteria with orthologues of genes presumably able to encode proteins involved in the dissimilation of isethionate, with the general source of the organism and its ability to grow with isethionate as a sole source of carbon and energy for growth.

Organism (locus tag abbreviation)	<i>iseJ</i>	<i>iseK</i>	<i>iseL</i>	<i>iseM</i>	<i>iseU</i>	<i>iseR</i>	Phylogeny	Habitat	Growth
<i>Ruegeria pomeroyi</i> DSS-3	(SPO) 2359	2358	2357	2356	-	2355	alpha	marine	+
<i>Dinoroseobacter shibae</i> DFL 12	(Dshi_) 0804	0803	0802	0801	-	0800	alpha	marine	n.t. ^a
<i>Jannaschia</i> sp. strain CCS1	(Jann_) 3680	3679	3678	3677	-	3676	alpha	marine	n.t.
<i>Roseobacter denitrificans</i> OCh 114	(RD1_) 4099	4100	4101	4102	-	4103	alpha	marine	n.t.
<i>Roseobacter litoralis</i> OCh 149	(RLO149_) 13345	13340	13335	13330	-	13325	alpha	marine	n.t.
<i>Roseobacter</i> sp. strain CCS2	(RCCS2_) 05824	05819	05814	05809	-	05804	alpha	marine	n.t.
<i>Roseobacter</i> sp. strain MED193	(MED193_) 13178	13183	13188	13193	-	13198	alpha	marine	n.t.
<i>Roseobacter</i> sp. strain SK209-2-6	(RSK20926_) 07202	07207	07212	07217	-	07222	alpha	marine	n.t.
<i>Roseovarius nubinihibens</i> ISM	(ISM_) 12540	12545	12550	12555	-	12560	alpha	marine	+
<i>Roseovarius</i> sp. strain 217	(ROS217_) 05299	05294	05289	05284	-	05279	alpha	marine	+
<i>Roseovarius</i> sp. TM1035	(RTM1035_) 15862	15857	15852	15847	-	15842	alpha	marine	n.t.
<i>Roseovarius</i> sp. Azwk-3b	(RAZWK3B_) 01125	01120	01115	01110	-	01105	alpha	marine	n.t.
<i>Rhodobacteraceae</i> bacterium KLH11	(RKLH11_) 3294 ^b	3142 ^b	3332 ^b	3708 ^b	-	3151 ^b	alpha	marine	n.t.
<i>Octadecabacter antarcticus</i> 307	(OA307_) 3702 ^b	4101 ^b	1989 ^b	2940 ^b	-	2469 ^b	alpha	marine	n.t.
<i>Alphaproteobacterium</i> sp. HTCC2255 ^c	(OM2255_) 05990	-	-	-	-	05985	alpha	marine	n.t.
<i>Rhodobacterales</i> sp. HTCC2150 ^c	(RB2150_) 10786	-	-	-	-	10791	alpha	marine	+
<i>Pelagibacter ubique</i> HTCC1002 ^c	(PU1002) 02111	-	-	-	-	-	alpha	marine	n.t.
<i>Pelagibacter ubique</i> HTCC1062 ^c	(SAR11_) 0858	-	-	-	-	-	alpha	marine	n.t.
<i>Paracoccus denitrificans</i> PD1222	(Pden_) 4278	-	-	-	4279	4280	alpha	terrestrial	+

<i>Paracoccus denitrificans</i> NKNIS		+ ^d	-	-	-	+ ^d	+ ^d	alpha	terrestrial	+
<i>Sinorhizobium medicae</i> WSM419	(Smed_)	4865	-	-	-	4866	4864	alpha	terrestrial	n.t.
<i>Oligotropha carboxidovorans</i> OM5	(OCAR_)	6748	-	-	-	6747	6749	alpha	terrestrial	+
<i>Methylobacterium extorquens</i> CM4	(Mchl_)	4211	-	-	-	4212	-	alpha	terrestrial	-
<i>Burkholderia multivorans</i> ATCC 17616	(Bmul_)	4528	-	-	-	4527	4529	beta	terrestrial	n.t.
<i>Burkholderia phymatum</i> STM815	(Bphy_)	6221	-	-	-	6220	6222	beta	terrestrial	+
<i>Burkholderia phytofirmans</i> PsJN	(Bphyt_)	4377	-	-	-	4378	4376	beta	terrestrial	+
<i>Burkholderia xenovorans</i> LB400	(Bxe_)	B0698	-	-	-	B0699	B0697	beta	terrestrial	+
<i>Burkholderia</i> sp. strain 383	(Bcep18194_)	B2005	-	-	-	B2004	B6006	beta	terrestrial	n.t.
<i>Burkholderia multivorans</i> CGD2M	(BURGMUCGD2M_)	5173	-	-	-	-	5174	beta	terrestrial	n.t.
<i>Burkholderia cenocepacia</i> J2315	(BCAM)	1170	-	-	-	1171	1169	beta	terrestrial	n.t.
<i>Cupriavidus necator</i> H16	(H16_)	B1851	-	-	-	B1852	B1850	beta	terrestrial	+
<i>Cupriavidus taiwanensis</i>	(RALTA_)	B1541	-	-	-	B1542	B1540	beta	terrestrial	n.t.
<i>Delftia acidovorans</i> SPH-1	(Daci_)	5947	-	-	-	5948	5946	beta	terrestrial	+
<i>Verminephrobacter eiseniae</i> EF01-2	(Veis_)	4282	-	-	-	4283	-	beta	terrestrial	n.t.
<i>Variovorax paradoxus</i> S110	(Vapar_)	3836	-	-	-	3837	3835	beta	terrestrial	n.t.

^a n.t., not tested. ^b Contiguous genes are represented by these numbers. ^c Neither IseKLM nor IseU is present, but a TerC-like transporter and a possible TRAP transporter can be suggested for these bacteria. ^d Data generated in this paper.

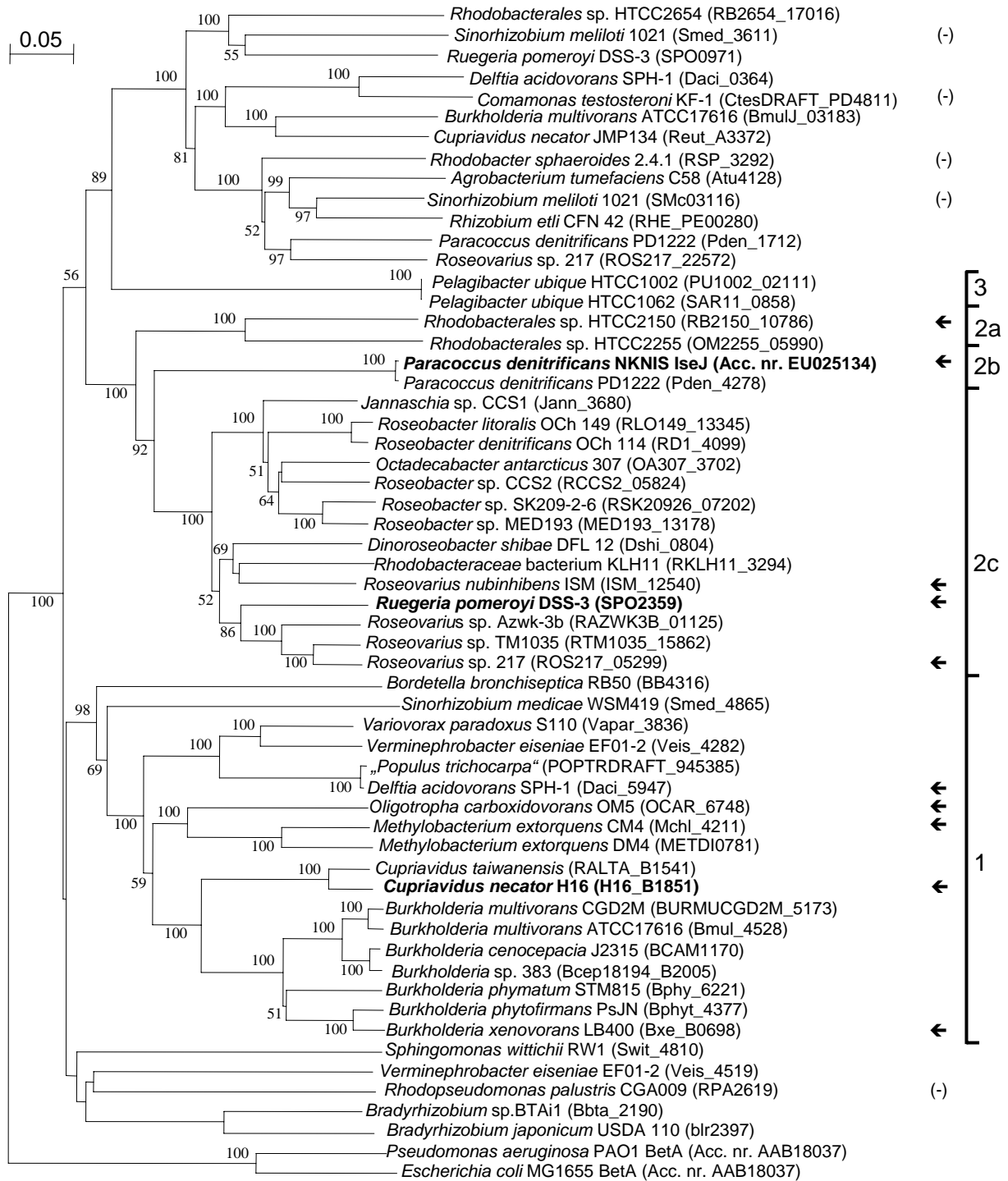


FIG. S1. Phylogenetic tree (generated with NJ plot based on a Clustal X alignment) of orthologues of putative isethionate dehydrogenases (IseJ). The three organisms given in **boldface** were used in this work; the outgroups represent characterized authentic BetA enzymes. Organisms indicated by **bold arrows** utilize isethionate as a source of carbon and energy for growth, those marked by minus sign (-) do not. GenBank accession numbers or locus tags are given in parentheses. The draft genome of *Populus trichocarpa* (poplar tree) apparently encodes IseJ, but the gene does not involve splicing and shares 99 % identity with the *Delftia acidovorans* sequence. We assume that bacterial DNA was sequenced. The choline dehydrogenases BetA of *P. aeruginosa* PAO1 and *E. coli* K12 substrain MG1655 were taken as outgroups.

Group 1 includes the terrestrial betaproteobacteria and is represented by *C. necator* H16, while Group 2c represents the original hypothesis in marine alphaproteobacteria represented by *R. pomeroyi* DSS-3 (see main paper), and Group 2b represents two strains of a terrestrial alphaproteobacterium, with an MFS transporter. Group 2a represents marine alphaproteobacteria, with the TerC-family transporter. The latter is also found in two sequenced genomes of *P. ubique* (Group 3) and in metagenomic sequences from the Sargasso Sea sequencing project (20). Most groups of orthologues of IseJ are distinct and include only potential isethionate utilizers. The exception is in Group 1, which includes *Bordetella bronchiseptica* RB50, an organism lacking the *xsc* gene. We wonder whether this candidate IseJ, if active, might be (i) involved in sulfur assimilation, presumably via a transaminase and taurine dioxygenase (BB3661) (see 8) or (ii) whether *B. bronchiseptica* has an unrecognized Xsc, as in *Bilophila wadsworthia* (5).

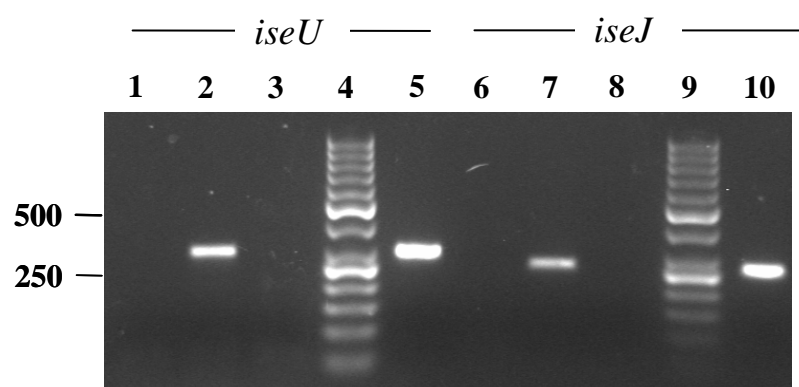


FIG. S2. Transcription of *iseU* and *iseJ* in *Cupriavidus necator* H16 during growth with isethionate. Transcripts from a representative RT-PCR experiment with a predicted length of 293 bp (for primer pair H16iseUF/H16iseUR) or 254 bp (for primer pair H16iseJF/H16iseJR). Lanes 1 and 6, negative control (no DNA); lane 2 and 7, RT-PCR products from RNA of isethionate-grown cells; lane 3 and 8, RT-PCR products from RNA of acetate-grown cells; lanes 4 and 9, 50 bp DNA ladder; lanes 5 and 10, positive control (*C. necator* H16 chromosomal DNA as template).

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