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Gene Clusters Involved in Isethionate Degradation by Terrestrial and Marine Bacteria[▽][§]

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Ubiquitous isethionate (2-hydroxyethanesulfonate) is dissimilated by diverse bacteria. Growth of *Cupriavidus necator* H16 with isethionate was observed, as was inducible membrane-bound isethionate dehydrogenase (IseJ) and inducible transcription of the genes predicted to encode IseJ and a transporter (IseU). Biodiversity in isethionate transport genes was observed and investigated by transcription experiments.

The importance of isethionate (2-hydroxyethanesulfonate) was realized when the compound was shown to be the major anion in the squid giant axon (18); it was then found to be a normal component in mammalian tissue (20) and in human urine (15). The source of this isethionate was considered to be the gut flora (11), and a pathway of isethionate formation from taurine (2-aminoethanesulfonate) in bacteria was established (28). The compound is found in large amounts (at about 250 mM) in red algae (for an example, see reference 13) and on orb spiders' webs ($\sim$2 M) (30). Isethionate is widely used in commerce as a counter-ion in the formulation of cationic pharmaceuticals and, in derivatized form, in shampoos and soap replacements (for examples, see references 6 and 29).

We presume that the ubiquity of isethionate explains the widespread bacterial dissimilation of the compound (5, 21). The degradative pathway of isethionate requires an unknown uptake system (see reference 5) and, in *Paracoccus denitrificans* NKNIS, it involves an inducible, 62-kDa, cytochrome *c*-dependent, membrane-bound isethionate dehydrogenase (EC 1.1.2.-), whose product, sulfoacetaldehyde, is desulfonated to acetyl phosphate and sulfite by sulfoacetaldehyde acetyltransferase (Xsc; EC 2.3.3.15) (3, 5, 23, 31). The genes for isethionate dissimilation were unknown (3); only one *xsc* gene was known in strain NKNIS, and it was located in a gene cluster encoding taurine degradation.

Dissimilation of C_2 sulfonates converges at a single Xsc in many organisms (5, 31). Some organisms, however, have several *xsc* paralogues which might represent different complete pathways for different C_2 sulfonates. *Ruegeria pomeroyi* DSS-3 contains the *xsc* gene (SPO3561), which is expressed during growth with taurine (12). Additionally, SPO2360 is annotated as an *xsc* pseudogene (the gene product lacks key amino acids in the active site). The presence of this second *xsc*, coupled

with isethionate utilization (8), led us to hypothesize that the flanking genes could encode isethionate uptake and isethionate dehydrogenase genes (see below). Orthologues of putative isethionate dehydrogenase (IseJ) were found in other organisms, several of them known isethionate degraders, whereas *iseJ* was absent in the genomes of isethionate nonutilizers. The hypothesis was tested in a terrestrial isethionate degrader (*Cupriavidus necator* H16) and a marine isethionate degrader (*R. pomeroyi* DSS-3), where data from growth kinetics, enzymology, and reverse transcription-PCR (RT-PCR) largely confirmed the initial hypothesis.

Gene candidates for isethionate biodegradation. Sequence data were analyzed with Lasergene, SignalP, TMHMM (http://www.cbs.dtu.dk./services/), and PROSITE (http://www.expasy.org/) software. We concentrated on the flanking regions of the *xsc* pseudogene in *R. pomeroyi* DSS-3 (genome accession numbers NC_003911 and NC_006569), especially the five genes in the upstream region. They could encode a dehydrogenase (SPO2359) of the BetA family (glucose-methanol-choline [GMC] oxidoreductases; COG2303), particulate enzymes of 60 to 64 kDa (http://www.brenda-enzymes.info/php/result_flat.php4?ecno=1.1.99.1&organism=), and, divergently transcribed, a tripartite ATP-independent (TRAP) transporter (SPO2358 to SPO2356; TC 2.A.56.-.-) and an IclR-type transcriptional regulator (SPO2355). This allowed the following hypothesis (Fig. 1): that isethionate was transported into the cell via a TRAP transporter, that an orthologue of the membrane-associated, 62-kDa isethionate dehydrogenase found earlier (3) was encoded by the BetA-type gene, and that both moieties were under the control of an IclR-type transcriptional regulator. Degradation of sulfoacetaldehyde would then proceed via Xsc (SPO3561) and Pta (SPO3560) as described elsewhere (Fig. 1) (12).

The putative gene products were compared individually with protein sequences in the NCBI database (http://www.ncbi.nlm.nih.gov/) using the BLAST algorithm. Many orthologues of IseJ were found, and a dendrogram showed four major clusters, two of which presumably represent IseJ in three groups (see Fig. S1 in the supplemental material).

Some 35 organisms, shown in Fig. S1 in the supplemental material, contained at least one additional *ise* gene candidate (see Table S3 in the supplemental material), as well as *xsc*, and these organisms were thus candidates to dissimilate isethio-

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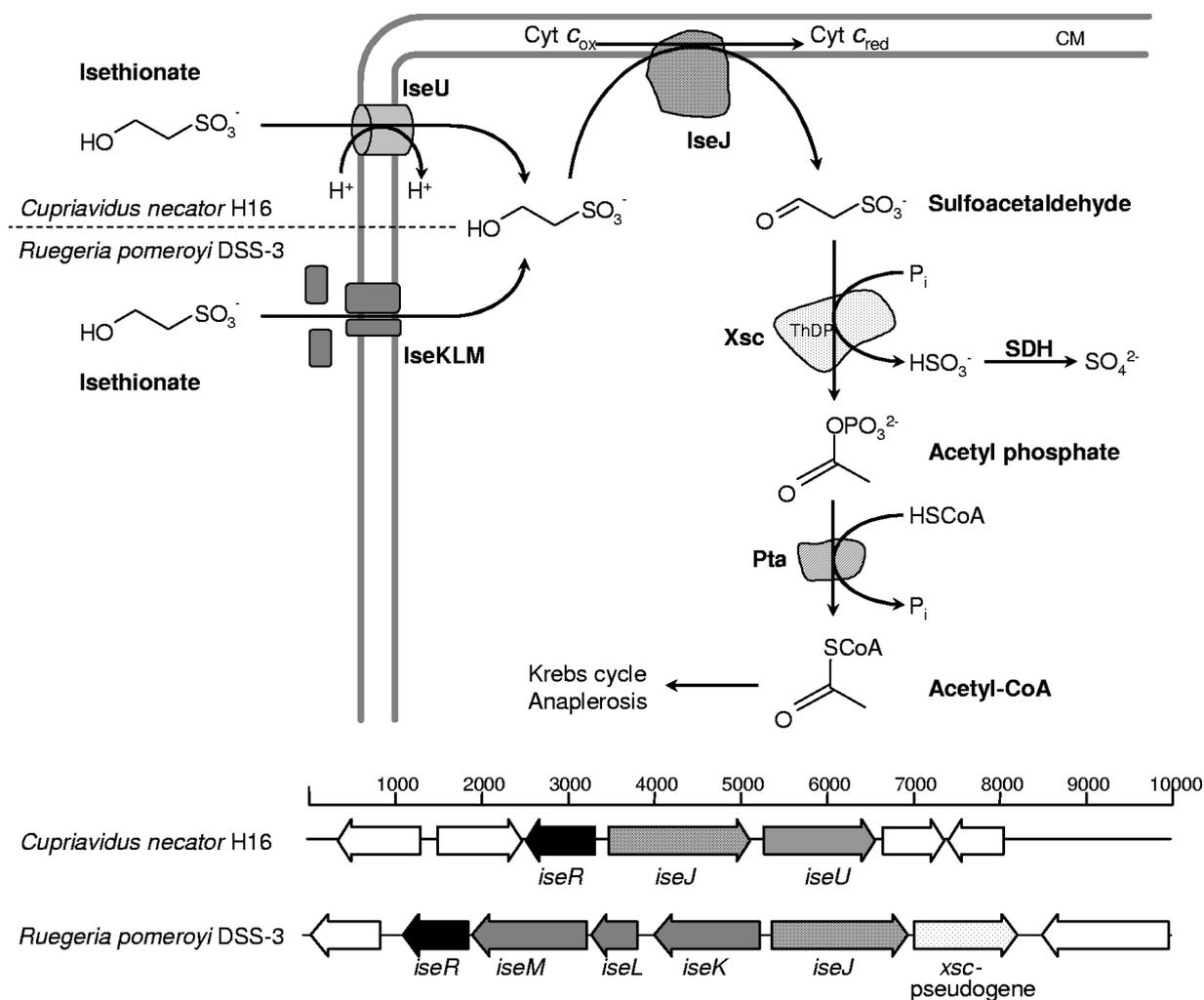‡ Present address: KAUST, Thuwal 23955-6900, Saudi Arabia.

FIG. 1. Initial steps of isethionate degradation in *Cupriavidus necator* H16 and *Ruegeria pomeroyi* DSS-3 with the corresponding gene clusters in *C. necator* H16 and *R. pomeroyi* DSS-3. CM, cytoplasmic membrane; IseJ, isethionate dehydrogenase; Xsc, sulfoacetaldehyde acetyltransferase; Pta, phosphate acetyltransferase; SDH, sulfite dehydrogenase; ThDP, thiamine diphosphate. In strain *C. necator* H16, where data are available, SDH is periplasmic (9).

nate. The terrestrial isolates listed in Table S3 in the supplemental material, e.g., *C. necator* H16 (genome accession numbers NC_008313, NC_008314, and NC_005241), were predicted to use a major facilitator superfamily (MFS) isethionate transporter (IseU) instead of the TRAP system (Fig. 1). Our rationale for this is that we predict a higher dilution of isethionate in marine environments than in terrestrial environments. Thus, a facilitator suffices in terrestrial organisms, whereas a powerful pump is needed by marine bacteria, which is consistent with a favored use of TRAP transporters in saline environments (22).

Growth physiology of organisms utilizing isethionate. All organisms (see Table S1 in the supplemental material) except *Rhodobacterales* sp. HTCC2150 were grown aerobically at 30°C in batch culture (media are listed in Table S1 in the supplemental material). Taurine (>99%; Fluka), isethionate (>98%; Fluka), or acetate (>99%; Merck) were sterilized by autoclaving and used as sole added sources of carbon and energy for growth (10 to 20 mM). Growth experiments were done as

described elsewhere (31). Growth was followed turbidimetrically at 580 nm or determined as protein in whole cells (16). Isethionate was determined by ion chromatography (IC) (28). Sulfate was quantified turbidimetrically as an insoluble suspension of $BaSO_4$ (26), and sulfite was quantified as the fuchsin adduct (7).

In growth experiments with *C. necator* H16, ammonium chloride was replaced by ammonium nitrate, which allowed the first determination of isethionate concentration during its degradation by a microorganism (chloride ions interfere during IC detection) (28). Growth with isethionate as the sole source of carbon and energy was rapid ($\mu = 0.35$ h^{-1}) and concomitant with both isethionate utilization and sulfate release, which was quantitative (Fig. 2). The growth yield was 6.6 g protein (mol C)$^{-1}$, confirming quantitative utilization of isethionate (see reference 4). *R. pomeroyi* DSS-3 utilized isethionate ($\mu = 0.06$ h^{-1}) with quantitative recovery of sulfate.

Rhodobacterales sp. HTCC2150 was cultivated at 16°C in sterilized supplemented seawater medium (see Table S1 in the
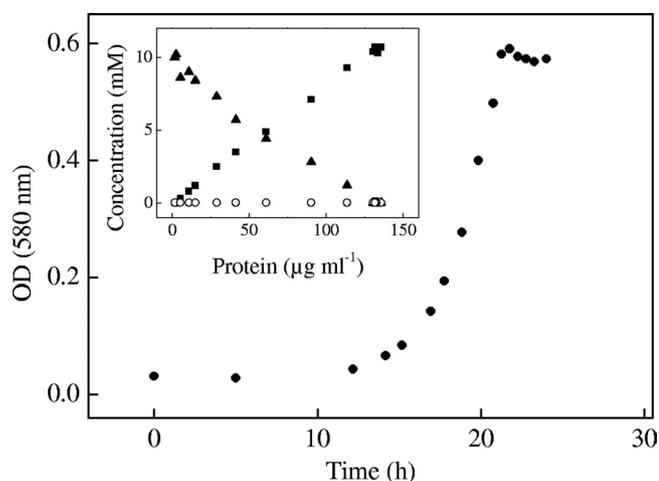
FIG. 2. Growth of *Cupriavidus necator* H16 with 10 mM isethionate as sole source of carbon and energy. Shown are concentrations of substrate and products during growth of *C. necator* H16, plotted as a function of the protein concentration (inlay), isethionate (filled triangles), sulfate (filled squares), and sulfite (open circles). An optical density at 580 nm [OD (580 nm)] of 0.5 corresponded to 113 µg protein ml^{-1}.

supplemental material) because the organism is not cultivable in defined media. Isethionate (2, 5, 10, and 20 µM) or glucose (1 and 10 µM) was added, and the increase in cell densities and final cell yields were determined by flow cytometry on a Guava EasyCyte cell counter (Guava Technologies, Hayward, CA) (27). Growth was carbon limited as shown by comparing counts of cells in outgrown medium which had contained 0.0, 1.0, and 10.0 µM glucose; replicate flasks without the addition of glucose yielded 1.1×10^6 cells ml^{-1}, while additions of glucose resulted in a linear increase of about 5.5×10^4 cells (nmol glucose-carbon)$^{-1}$. Growth with isethionate (2 to 20 µM) resulted in a linear increase in cell yield of about 1.3×10^5 cells (nmol isethionate-carbon)$^{-1}$, showing that the organism utilized isethionate and growth was proportional to the substrate concentration.

Enzyme activities involved in isethionate degradation in *C. necator* H16. Preparation of cell extracts and separation of membrane and soluble fractions were done as described elsewhere (3), and protein content was assayed by protein-dye binding (2). IseJ was assayed spectrophotometrically as the isethionate-dependent reduction of beef heart cytochrome *c* at 550 nm. The reaction mixture contained 50 µmol potassium phosphate buffer, pH 7.2 (with 5 mM MgCl$_2$), 12.5 µmol isethionate, 50 nmol cytochrome *c*, and 0.1 to 0.5 mg protein. The reaction was linear for at least 2 min. Xsc was assayed by the colorimetric determination of acetyl phosphate (23).

Cell extracts of isethionate-grown cells showed activities of both IseJ (0.3 mkat kg^{-1}) and Xsc (2.8 mkat kg^{-1}), whereas extracts of acetate-grown cells did not. After separation of soluble and membrane fractions, Xsc activity (3.4 mkat kg^{-1}) was found solely in the soluble fraction, whereas IseJ (0.1 mkat kg^{-1}) was active in the membrane fraction, as predicted (3). An inducible membrane protein of 60 to 62 kDa was detected in the membrane fraction by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). These enzyme

activities confirmed the inducible presence of the predicted pathway (Fig. 1) (3, 17, 19).

RT-PCR of *ise* gene candidates. Total RNA was isolated using the E.Z.N.A. bacterial kit (Omega Bio-Tek, Doraville, GA). Primers (see Table S2 in the supplemental material) were synthesized by Microsynth (Balgach, Switzerland), and reverse transcription and PCR were performed as described elsewhere (14, 31) using materials from Fermentas GmbH (St Leon-Rot, Germany). PCR products were visualized on 1.5% agarose gels (25). The hypothesis that gene candidates in *C. necator* H16 would encode uptake (*iseU*) and oxidation (*iseJ*) of isethionate was tested by RT-PCR. No transcript was detected in acetate-grown cells, whereas the transcript of each gene (monocistronic transcription) was detected in isethionate-grown cells (see Fig. S2 in the supplemental material). The gene candidate *iseJKLM* of *R. pomeroyi* DSS-3 was hypothesized to encode a TRAP transporter and IseJ (Fig. 1). No transcript was detected in acetate-grown cells, whereas a transcript of each gene was detected in isethionate-grown cells (not shown). We interpret these data as evidence that IseJ and IseKLM in *R. pomeroyi* DSS-3, or IseJ and IseU in *C. necator* H16, are involved in isethionate utilization.

Data from genome sequencing projects. The gene cluster identified in *C. necator* H16 (*iseRJU*) (sometimes *iseRUJ*) was found in 16 other terrestrial isolates whose genomes have been sequenced (see Table S3 in the supplemental material). This allowed us to predict utilization of isethionate by these organisms, which we confirmed by growth experiments with seven organisms that were available to us (Table S3) (1, 3, 8, 21). In some organisms, e.g., *P. denitrificans* PD1222, *Sinorhizobium medicae* WSM419, and *Dinoroseobacter shibae* DFL 12, the *ise* genes are contiguous with the *xsc* and *pta* genes, presumably encoding a complete and independent isethionate degradative pathway.

The gene cluster identified in *R. pomeroyi* DSS-3, *iseJKLMR*, was found in 13 other marine genome-sequenced organisms (see Table S3 in the supplemental material), which allowed us to predict isethionate dissimilation in those isolates. The hypothesis was confirmed by growth experiments with two strains available to us (Table S3). We regard this as confirmation of the function of IseJKLM.

Two more marine isolates (*Alphaproteobacterium* sp. HTCC2255 and *Rhodobacterales* sp. HTCC2150) (see Table S3 in the supplemental material) contain *iseRJ* (as well as *xsc* and *pta*), but neither *iseKLM* nor *iseU*. *Rhodobacterales* sp. HTCC2150 did, however, utilize isethionate (see above). A TerC family (TC 9.A.30.-.-) hypothetical protein is encoded adjacent to *iseJ*, with a 1-bp overlap. This protein, with six predicted transmembrane helices, may represent a third type of isethionate transporter (RB2150_10781), possibly involving the neighboring genes (RB2150_10776 to RB2150_10771). These three genes are conserved upstream of *iseJ* in *Alphaproteobacterium* sp. HTCC2255 and in two sequences from a metagenome project (24).

Brüggemann et al. (3) predicted the presence of a regulator of isethionate dissimilation. We now presume that IseR, an IclR-type protein, fulfils this function, because it is almost always colocalized with the genes encoding the degradative pathway (Fig. 1; see Table S3 in the supplemental material). The biodiversity of the isethionate pathway, with at least three

types of transporters, concurs with the widespread occurrence of isethionate in the environment.

Sequence prediction in *P. denitrificans* NKNIS. The biochemical background to the present paper is the presence of an inducible, 62-kDa, membrane-bound protein in *P. denitrificans* NKNIS, assumed to be IseJ (3). The genome of a different strain of *P. denitrificans*, PD1222, has now been sequenced (NC_010355), and its nine-gene taurine cluster is essentially identical to the corresponding sequence in strain NKNIS (not shown). We predicted that the *ise* gene cluster in strain NKNIS would also show high similarity to that in strain PD1222. We amplified and sequenced overlapping fragments of the cluster in NKNIS. Chromosomal DNA was isolated as described elsewhere (10), and the PCR primers listed in Table S2 in the supplemental material were used to amplify the fragments. PCR products were purified with the E.Z.N.A. Cycle-Pure kit (Omega Bio-Tek). Sequencing was done at MWG-Biotech AG (Ebersberg, Germany). We confirmed our hypothesis by finding a 3.5-kb fragment which shared 99% identity with the corresponding region (*iseRUJ-xsc*) in strain PD1222. Thus, the isethionate-positive phenotype of *P. denitrificans* NKNIS was confirmed to be accompanied by the *iseRUJ-xsc* genotype.

The power of prediction—that the presence of *iseJ* indicates isethionate utilization in an organism on the one hand, and that membrane-bound isethionate dehydrogenase indicates the presence of *iseJ* in the organism's genome on the other hand—is surely sufficient to establish that *iseJ* encodes isethionate dehydrogenase (EC 1.1.2.-). Nevertheless, in the future, it will hopefully be possible to purify IseJ and identify any cofactors. As an alternative, one could construct a deletion mutant defective in *iseJ*, but these experiments were unsuccessful, despite being able to delete other genes in the same organism (S. Weinitschke, unpublished data).

Nucleotide sequence accession number. The gene sequence reported in this paper has been deposited in the GenBank database (accession no. EU025134).

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