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Inducible transcription of genes involved in taurine uptake and dissimilation by *Silicibacter pomeroyi* DSS-3^T

Abstract A largely untested hypothesis for the bacterial dissimilation of taurine was explored in *Silicibacter pomeroyi* DSS-3, whose genome has been sequenced. Substrate-specific transcription of candidate genes encoding taurine uptake and dissimilation (*tauABC*, *tpa*, *ald*, *xsc*, *pta*) was found, which corresponded to the induction of Tpa, Ald, Xsc and Pta, that was observed.

Keywords Reverse transcriptase PCR · Taurine dissimilatory pathway · Enzymes of taurine degradation

Introduction

Taurine (2-aminoethanesulfonate), the major organic solute in mammals (on average about 8 mM in the human body), is synthesized in the liver and excreted largely in urine; mammals cannot cleave the C-sulfonate bond (Huxtable 1992; Stipanuk 2004). Corresponding to this ready supply of taurine, many terrestrial and marine bacteria utilize the compound, and two pathways of taurine dissimilation have been hypothesized around the key enzyme, the desulfonative sulfoacetaldehyde acetyltransferase [EC 2.3.3.15] (Xsc) (Fig. 1) (Cook and Denger 2006). *Silicibacter pomeroyi* DSS-3^T, a marine bacterium, was found to grow with taurine as a sole source of carbon and energy, and quantitative utilization was established (González et al. 2003; Denger et al. 2006). A hypothetical degradative pathway was derived from the genome sequence (Fig. 1). It involved an ATP binding-cassette transporter (TauABC) [TC 3.A.1.17.1], a taurine:pyruvate aminotransferase (Tpa) [EC 2.6.1.77], Xsc and phosphate acetyltransferase (Pta) [EC 2.3.1.8] (Moran et al. 2004). The function of putative TauABC, orthologs of the *Eschericha coli* TauABC that was characterized in sulfur assimilation (Eichhorn et al. 2000), has never been tested in a dissimilative pathway, and TauA shares only 23% identity with the characterized protein from *E. coli*. The putative Tpa shares 59 and 33% identity with the established orthologs in *Bilophila wadsworthia* RZATAU and *Rhodococcus opacus* ISO-5, respectively (Laue and Cook 2000a; Denger et al. 2004). The putative Xsc shares 75% identity with the characterized ortholog in *Paracoccus denitrificans* NKNIS (Brüggemann et al. 2004). Pta activity has been observed in some taurine degraders (Cook and Denger 2002; Cook and Denger 2006), but no *pta*-gene has been confirmed to be involved in the degradation of taurine. The need for an alanine dehydrogenase (Ald) [EC 1.4.1.4] in the hypothesis in Fig. 1 is known (Laue and Cook 2000b; Denger et al. 2004), as are roles for a sulfite dehydrogenase (sulfite oxidoreductase, Sor) and exporters of ammonium and sulfate ions, but candidate genes to encode the latter functions are either absent or unknown (Sor) or have not been confirmed (Cook and Denger 2006). Thus, despite the logic of the scheme in Fig. 1, and the utilization of taurine, there is no experimental support for a sometimes tenuous hypothesis (e.g., 23% identity with a confirmed ortholog).

The aim was to test this genome sequence-derived hypothesis, using reverse transcription-PCR and enzyme assays to show that the postulated genes are indeed involved in taurine dissimilation. The structurally related compound cysteate (2-amino-3-sulfopropionate) is utilized as a sole carbon source by *S. pomeroyi* DSS-3 via cysteate sulfo-lyase (SPOA0158) (Denger et al. 2006) and not via Xsc, so we used cysteate-grown cells as one negative control to confirm the specificity of transcription of taurine-induced genes. The other control substrate for growth was acetate.

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Materials and methods

Silicibacter pomeroyi DSS-3^T (González et al. 2003) was grown aerobically at 30°C in a modified *Silicibacter*

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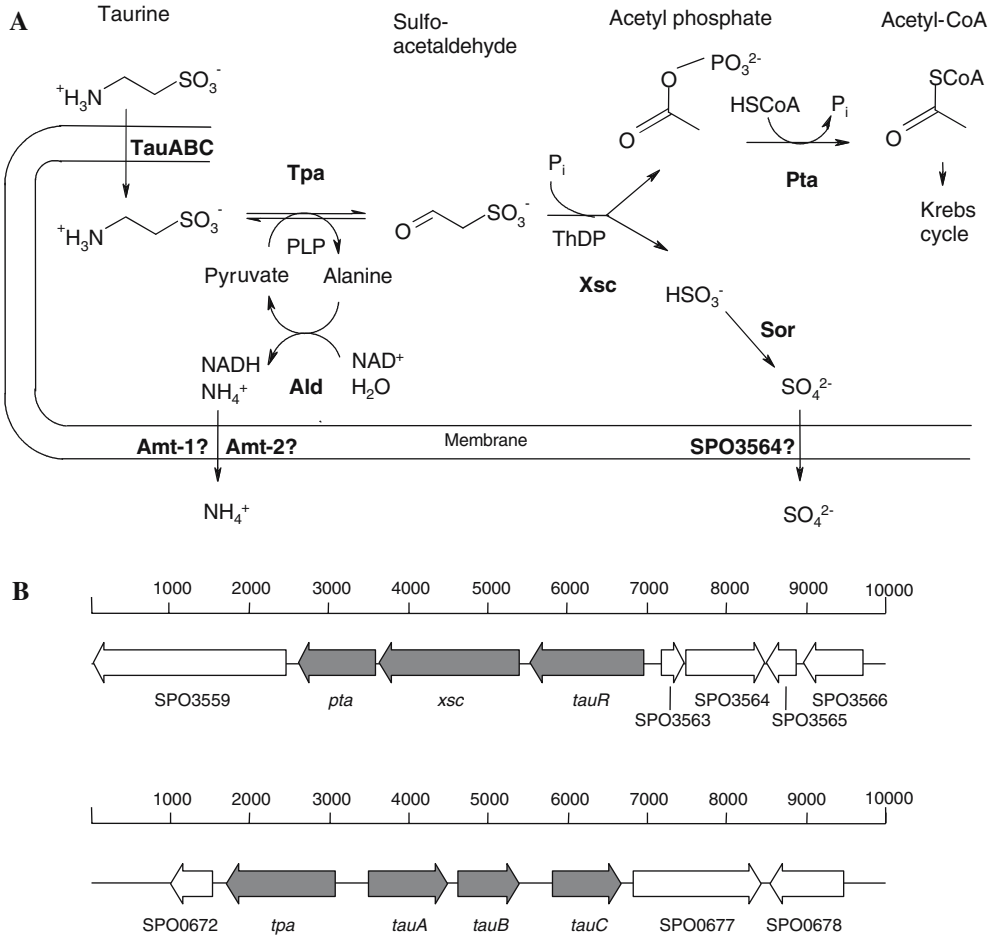

Fig. 1 Hypothetical pathway for taurine degradation in *S. pomeroyi* DSS 3 (adapted from Cook and Denger 2006) (**a**) and the two small gene clusters encoding many of these reactions (**b**). TauABC (taurine ABC transporter), Tpa (taurine:pyruvate aminotransferase), Ald (alanine dehydrogenase), Xsc (sulfoacetaldehyde acetyltransferase), Pta (phosphate acetyltransferase), Sor (sulfite dehydrogenase), SPO3564 (putative sulfate exporter), Amt 1 (AmtB) (ammonia methylammonia transporter 1), Amt 2 (ammonia methylammonia transporter 2)

basal medium (SBM-M) (Denger et al. 2006). The sole added source of carbon and energy was taurine, cysteate or acetate, each at 10 mM. All growth experiments were done in 50-ml cultures in 300-ml Erlenmeyer flasks shaken at 30°C. Samples were taken at intervals to measure optical density at 580 nm. The growth rate (μ) with taurine was 0.12 h^{-1}, with acetate 0.11 h^{-1} and with cysteate 0.07 h^{-1}. Cells for the preparation of total RNA were grown in the required selective medium and harvested by centrifugation in the mid-exponential phase. Total RNA was isolated using the E.Z.N.A bacterial RNA kit (Peqlab Biotechnologie GmbH, Erlangen, Germany) and contaminant DNA was removed with RNase-free DNase (Qiagen, Hilden, Germany). The RNA was tested for presence of contaminant DNA before reverse transcription (RT) by PCR using the primer set SpXscF-SpXscR. The reverse PCR primers (Hermann GbR, Denzlingen, Germany) listed in Table 1 were used for RT with M-MuLV reverse transcriptase (Fermentas GmbH, St. Leon–Rot, Germany). Subsequent PCR reactions were done as described previously (Innis et al. 1990) and amplicons were detected on 1.5% agarose gels according to standard methods (Sambrook et al. 1989). Enzyme assays are cited where required (Table 2).

Results and Discussion

All five enzyme activities (Tpa, Ald, Xsc, Pta and Sor) hypothesized for the utilization of taurine were present in taurine-grown cells and effectively absent in acetate-grown cells (Table 2). Three of these inducible enzymes (Tpa, Xsc and Pta) are also absent in cysteate-grown cells, while a fourth enzyme, sulfite dehydrogenase (Sor), can be anticipated in both sulfite-generating pathways (Table 2). Sor, detected recently (Denger et al. 2006), was confirmed to be independent of cytochrome *c* and to be assayed with ferricyanide as an electron acceptor: no enzyme of this class has been characterized. Further, the data in Table 2 confirm the earlier observation (Denger et al. 2006), that Ald is induced to high levels in cysteate-grown cells.

Transcripts for the candidate genes *tpa*, *ald*, *xsc*, and *pta* were detected in taurine-grown cells but not (or negligibly) in acetate-grown cells (Table 3). These transcripts are in agreement with the enzymic data, and, given the absence of known alternatives, consolidate support for the role of these genes in taurine metabolism.

The anticipated amplicon for the *xsc* gene (SPO3561) was obtained after reverse transcription with primer

Table 1 Genes and primers used in this study

Gene	Gene ID[a]	Name	Sequence (5′ → 3′)	Reference to gene
xsc	SPO3561	SpXscF	AACATCCCGCGTGACATGTGGAC	Brüggemann et al. 2004
		SpXscR	GGCCAGTATTCCATGCCATAACC	
pta	SPO3560	SpPtaF	GTCTGACCATCCACGATCCGGACA	Brüggemann et al. 2004
		SpPtaR	CGGCCTCGGGCGGATACATCA	
	SPO3564	Spo3564F	GCGCCTTTGAAGGACGCGAGAC	Cook and Denger 2006
		Spo3564R	GCAGGAACATCCCGTTGCTGAG	
tpa	SPO0673	SpTpaR	AACTCGGCGCCCGACAGATGA	Cook and Denger 2006
		SpTpaF	TCAGAACGGCAAAGAGCATCT	
tauA	SPO0674	SpTauAF	ACGAGAAGACCGAGCTGGGTA	Cook and Denger 2006
		SpTauAR	CACGGCGTTCTCATAGGTGT	
tauB	SPO0675	SpTauBF	ATTTCAAGGACAAGGCGATCTAC	Cook and Denger 2006
		SpTauBR	CCAAAGGTCACCGTTTTGA	
tauC	SPO0676	SpTauCF	CAGTTCTCGCTGACCGACAAGG	Cook and Denger 2006
		SpTauCR	GGAACAGGGAGAAGCCCAGATG	
ald	SPO0222	SpAldF	GGCATGAGGTCATCATCGAA	Cook and Denger 2006
		SpAldR	GGTCTCATACGCGATGCAGGTCG	
amt 1	SPO2093	SpAmt1F	CGCCTGCCTGATGAGCATTCT	this study
		SpAmt1R	CACACCGGTCTCGCCAAAGATT	
amt 2	SPO3723	SpAmt2F	GCCGAACGAGGAAATCGGCTAT	this study
		SpAmt2R	GAAAGGGCCACAGCTTGATCCG	
16S rRNA		16S 27F	CAGAGTTTGATCCTGGCTCAG	Weisburg et al. 1991
		16S 533R	TTACCGCGGCTGCTGGCAC	

[a]Gene ID according to annotated genome sequence (Moran et al. 2004)

Table 2 Enzyme activities (mkat/kg protein) in crude cell extracts from *Silicibacter pomeroyi* DSS 3 grown with different substrates

Enzyme	Growth of DSS 3 with			Assay in reference
	Taurine	Cysteate	Acetate	
Taurine:pyruvate aminotransferase (Tpa)	16.7	≤ 0.2	≤ 0.2	Laue et al. 1997
L Alanine dehydrogenase (Ald)	9.0	87.6	≤ 0.2	Laue and Cook 2000a
Taurine dehydrogenase (Tdh)	≤ 0.03	≤ 0.03	≤ 0.03	Brüggemann et al. 2004
Sulfoacetaldehyde acetyltransferase (Xsc)	2.2	≤ 0.1	≤ 0.1	Ruff et al. 2003
Phosphate acetyltransferase (Pta)	45.5	≤ 0.1	≤ 0.1	Bergmeyer et al. 1983
Sulfite dehydrogenase (Sor)	3.2	3.8	≤ 0.03	Reichenbecher et al. 1999

SpXscR (Table 3). After reverse transcription with primer SpPtaR (in SPO3562) and cDNA from taurine-grown cells, an amplicon spanning *xsc-pta* was obtained with primers SpXscF and SpPtaR, indicating that *xsc* and *pta* are located on a single transcript. Acetate-grown cells contained only traces of *pta*-transcript, and no induced activity was detected (Table 2).

Table 3 Results of the reverse transcription reactions

Gene	Amplicons found during growth of *S. pomeroyi* DSS 3 with		
	Taurine	Cysteate	Acetate
tauA	+		
tauB	+		
tauC	+		
tpa	+		
ald	+	+	Trace
xsc	+		
pta	+		Trace
amt 1	+	+	+
amt 2			+
spo3564			

The *ald* gene was transcribed under all conditions tested (Table 3), but the transcript in acetate-grown cells was present in trace amounts, and enzyme activity was detected only in taurine- and cysteate-grown cells (see above). The *ald* gene is obviously regulated independently of the *tpa*, *xsc* and *pta* genes, which were not transcribed in cysteate-grown cells (Table 3).

Transcripts of the candidate genes (*tauABC*) encoding a taurine transport system in *S. pomeroyi* DSS-3 were present during growth with taurine, but not during growth with cysteate or acetate (Table 3, Fig. 2). This is direct evidence that an ATP binding-cassette transporter is involved in the uptake of taurine during taurine dissimilation.

The gene of SPO3564, a potential permease with some sequence identity to presumptive sulfate exporters (Cook and Denger 2006), was not transcribed under the conditions tested (Table 3). The hypothesis of Cook and Denger (2006), that this might be the sulfate exporter, is apparently wrong.

Four genes for ammonia-methylammonia transporters (Amt) were identified on the chromosome (Moran et al. 2004). Based on a comparison with the known Amt

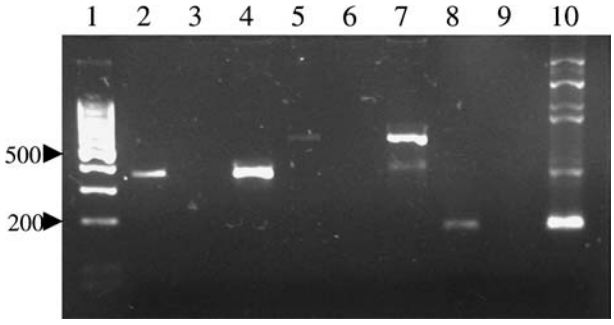

Fig. 2 Transcription of *tauABC* in taurine and acetate grown cells. Lane 1; Marker 100 bp: RT PCRs with primers; lanes 2 4 SpTauAF/SpTauAR, lanes 5 7 SpTauBF/SpTauBR, lanes 8 10, SpTauCF/SpTauCR. Templates for reverse transcriptions: lanes 2/5/8, RNA from taurine grown cells of *S. pomeroyi* DSS 3; lanes 3/6/9, RNA from acetate grown cells of *S. pomeroyi* DSS 3. Lanes 4/7/10: positive controls using chromosomal DNA of *S. pomeroyi* DSS 3

proteins (Thomas et al. 2000), two of these were selected for RT analysis, *amt*-1 ((*amtB*) = *spo*2093) and *amt*-2 (= *spo*3723). Whereas *amt*-1 was transcribed constitutively, *amt*-2 was transcribed in acetate-grown cells only (Table 2). This would suggest that Amt-1, which belongs to the ammonia transporting and sensory proteins [TC 2.A.49.1.1], could be involved in ammonium homeostasis, while Amt-2 belongs to the high affinity ammonium/methylammonium transporters [TC 2.A.49.2.3], and could be part of an uptake system for ammonia during growth with a substrate, which does not contain combined nitrogen. This suggestion needs to be tested experimentally.

Cook and Denger (2006) hypothesized a second pathway for taurine dissimilation, which involves taurine dehydrogenase [EC 1.4.2.-], that is presumably encoded by *tauXY* (Brüggemann et al. 2004; Weinitschke et al. 2006). No enzyme assay for taurine dehydrogenase showed activity (Table 2). This is in agreement with the absence of *tauXY* genes in the genome of *S. pomeroyi* DSS-3.

Conclusion

Although several taurine dissimilatory enzymes have been assayed routinely (Laue and Cook 2000a; Ruff et al. 2003; Brüggemann et al. 2004), direct proof that a candidate gene was transcribed was often lacking. The present RT-PCR studies show clearly that the genes (*tauABC*, *tpa*, *ald*, *xsc*, *pta*) that were proposed to play a role in taurine degradation (Moran et al. 2004; Cook and Denger 2006) were induced during growth with taurine. The enzyme assays confirmed the activity of the gene products to be present in taurine-grown cells. We thus have evidence for the genes encoding the complete taurine catabolic pathway including uptake and dissimilation, and possibly for export of ammonia, whereas sulfite oxidation and excretion of excess anions in *S. pomeroyi* DSS-3 are still undefined.

The levels of sequence identity between the candidate genes in *S. pomeroyi* DSS-3 and orthologs with known function (Ruff et al. 2003; Brüggemann et al. 2004) were sufficient to assign their function in taurine dissimilation correctly. Our experience with newly available genome sequences (Ruff et al. 2003; Brüggemann et al. 2004; this study, T.H.M. Smits and A.M. Cook, unpublished results) shows that orthologs with high levels of identity to known genes for taurine dissimilation may fairly be presumed to encode enzymes or transporters of the predicted function. This fact is still largely ignored by the automated programs to annotate genome sequences.

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