Molecular genetics and biochemistry of *N*-acetyltaurine degradation by *Cupriavidus necator* H16

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Cupriavidus necator H16 (DSM 428), whose genome has been sequenced, was found to degrade *N*-acetyltaurine as a sole source of carbon and energy for growth. Utilization of the compound was quantitative. The degradative pathway involved an inducible *N*-acetyltaurine amidohydrolase (NaaS), which catalysed the cleavage of *N*-acetyltaurine to acetate and taurine. The degradation of the latter compound is via an inducible, degradative pathway that involves taurine dehydrogenase [EC 1.4.2.–], sulfoacetaldehyde acetyltransferase [EC 2.3.3.15], phosphotransacetylase [EC 2.4.1.8], a sulfite exporter [TC 9.A.29.2.1] and sulfite dehydrogenase [EC 1.8.2.1]. Induction of the expression of representative gene products, encoded by at least four gene clusters, was confirmed biochemically. The acetate released by NaaS was activated to acetyl-CoA by an inducible acetate-CoA ligase [EC 6.2.1.1]. NaaS was purified to homogeneity; it had a K_m value of 9.4 mM for *N*-acetyltaurine, and it contained tightly bound Zn and Fe atoms. The denatured enzyme has a molecular mass of about 61 kDa (determined by SDS-PAGE) and the native enzyme was apparently monomeric. Peptide-mass fingerprinting identified the locus tag as H16_B0868 in a five-gene cluster, *naaROPST* (H16_B0865–H16_B0869). The cluster presumably encodes a LysR-type transcriptional regulator (NaaR), a membrane protein (NaaO), a solute:sodium symporter-family permease [TC 2.A.21] (NaaP), the metal-dependent amidohydrolase (NaaS) and a putative metallochaperone (COG0523) (NaaT). Reverse-transcription PCR indicated that *naaOPST* were inducibly transcribed.

INTRODUCTION

Taurine (2-aminoethanesulfonate) is widespread in the biosphere, as are many taurine derivatives (Huxtable, 1992). One derivative, *N*-acetyltaurine (Fig. 1a), is found at molar concentrations in the viscid droplets applied by the orb spider to its web (Vollrath *et al.*, 1990) as one aspect of catching prey. Bacteria which degrade *N*-acetyltaurine were easily obtained, and work was concentrated on one isolate, *Delftia acidovorans* NAT (Mayer *et al.*, 2006), in which the cleavage of *N*-acetyltaurine was shown to be catalysed by an uncharacterized amidase, *N*-acetyltaurine amidohydrolase [EC 3.5.1.–], now termed NaaS (Fig. 1). NaaS hydrolyses *N*-acetyltaurine to taurine and acetate (Mayer *et al.*, 2006).

D. acidovorans has been termed pathogenic (Horowitz *et al.*, 1990). The non-pathogenic, genome-sequenced *Cupriavidus necator* H16 also utilizes *N*-acetyltaurine as a sole source of carbon and energy for growth, and expresses

NaaS (J. Mayer & A. M. Cook, unpublished). We chose to work with *C. necator* H16, because we had already elucidated the pathway of taurine degradation and of relevant convergent metabolic pathways in this organism (e.g. Denger *et al.*, 2008; Mayer & Cook, 2009; Weinitschke *et al.*, 2007, 2010a), and taurine is one of the products of NaaS (Fig. 1a). This inducible amidohydrolase was purified, and its gene locus identified in a five-gene cluster presumed to encode the inducible proteins in the pathway to yield taurine and acetate, each of which is degraded via acetyl-CoA.

METHODS

Materials. *N*-Acetyltaurine was synthesized and characterized by melting point [234 °C (Lit.: 233–234 °C)], ¹H- and ¹³C-NMR and MALDI-TOF-MS (Mayer *et al.*, 2006). Commercial chemicals were of the highest purity available and they were purchased from Sigma-Aldrich, Merck or Biomol.

Organisms and growth conditions. *Burkholderia phytofirmans* PsJN (DSM 17436), *Cupriavidus necator* H16 (DSM 428) and *Cupriavidus pinatubonensis* JMP134 (DSM 4058) were obtained from the German Culture Collection (DSMZ, Braunschweig, Germany); *Methylobacterium radiotolerans* JCM 2831 was kindly made available

Abbreviations: DLS, dynamic light scattering; TCDB, Transport Classification Database.

Four supplementary tables and two supplementary figures are available with the online version of this paper.

Fig. 1. (a) Hypothetical pathway of *N*-acetyltaurine degradation in *C. necator* H16, (b) the corresponding gene clusters with our annotation of the locus tags and (c) similar *naa* gene clusters in other bacteria. Major pathway genes: TauXY, taurine dehydrogenase; Xsc, sulfoacetaldehyde acetyltransferase; Pta, phosphotransferase; Acs, acetate-CoA ligase. The roles of TauE as a sulfite exporter and TauP as a taurine permease are discussed elsewhere (Weinitschke *et al.*, 2007; Z. Krejčík & A. M. Cook, unpublished), as are AmtB (the ammonium transporter) (H16_A0321 and H16_B1234) and Acs (H16_B0834) (Baldock *et al.*, 2007; Gorzyska *et al.*, 2006).

by S. Vuilleumier, Strasbourg, France; *Paracoccus denitrificans* PD1222 by R. J. M. van Spanning, Amsterdam, The Netherlands; *Rhodopseudomonas palustris* CGA009 by C. S. Harwood, Seattle, USA; and *Roseovarius nubinhibens* ISM and *Ruegeria pomeroyi* DSS-3 by M. A. Moran, Georgia, USA. *D. acidovorans* SPH-1 (DSM 14801) was isolated in our laboratory.

B. phytofirmans PsJN, *C. pinatubonensis* JMP134, *D. acidovorans* SPH-1, *P. denitrificans* PD1222 and *Rh. palustris* CGA009 grew in a phosphate-buffered mineral-salts medium (Thurnheer *et al.*, 1986) with *N*-acetyltaurine (5 mM) as sole added source of carbon. The growth medium for *M. radiotolerans* JCM 2831 was adapted to nitrogen-limited conditions by replacing ammonium chloride with *N*-acetyltaurine (2 mM); the carbon source was succinate (5 mM). *Ru. pomeroyi* DSS-3 and *Ro. nubinhibens* ISM were grown in a marine mineral-salts medium (Denger *et al.*, 2009) with *N*-acetyltaurine (5 mM) as carbon source. *Ro. nubinhibens* ISM grew reproducibly only when the medium was supplemented with 0.05 % yeast extract. All organisms grew aerobically in the dark at 30 °C. Growth experiments were done in 50 ml cultures in 300 ml Erlenmeyer flasks on a shaker: samples were taken at intervals to measure the optical density, to assay protein and to determine the concentrations of sulfate, sulfite and acetate. For enzyme measurements, 100 ml cultures were grown with succinate, *N*-acetyltaurine (5 mM) or taurine (10 mM) as carbon source, and cells were pelleted (30 000 g, 15 min, 4 °C), then washed with 50 mM Tris/HCl buffer, pH 8.0, treated with DNase (0.05 mg DNase I ml⁻¹) and disrupted by three passages through a chilled French pressure cell at 140 MPa. Debris was spun down (30 000 g, 15 min, 4 °C) and discarded. Crude extracts could be used immediately or stored frozen without loss of activity. The membrane/particulate fraction was sedimented by ultracentrifugation (150 000 g, 30 min, 4 °C) and the supernatant fluid was called the soluble fraction. The pellet was washed with 50 mM Tris/HCl buffer, pH 8.0, ultracentrifuged again and resuspended in the same buffer.

Enzyme purification. The amidohydrolase was purified from 1 l cultures grown with 5 mM *N*-acetyltaurine as carbon source in 5 l Erlenmeyer flasks on a shaker. Harvested cells were treated as described above to give the soluble fraction, which was loaded onto an anion-exchange chromatography column (Mono Q, HR 10/10, Pharmacia) with 50 mM Tris/sulfate buffer, pH 8.0, as eluent at a flow rate of 1.0 ml min⁻¹. A gradient of sodium sulfate up to 0.5 M was applied and active NaaS eluted at about 100 mM sodium sulfate. The active fraction was brought to 1.7 M ammonium sulfate, the precipitate removed by centrifugation (30 000 g, 15 min, 4 °C) and the supernatant fluid was subjected to hydrophobic interaction chromatography on Phenyl Superose HR 10/10 (Pharmacia). A linear decreasing gradient of ammonium sulfate in 50 mM Tris/sulfate buffer, pH 8.0, was applied and NaaS eluted at 0 mM ammonium sulfate. As a third purification step, the rebuffed active fraction with highest activity was loaded onto the anion-exchange chromatography column in 50 mM Tris/sulfate buffer, pH 9.5, and NaaS eluted at about 100 mM sodium sulfate. The active fraction was concentrated using Vivaspinn 500 units (10 kDa cut-off, PES membrane; Sartorius). Gel filtration (Superose 12 HR 10/30, Pharmacia) was used as a fourth purification step with 50 mM Tris/sulfate buffer, pH 8.0, including 150 mM sodium sulfate as the eluent at a flow rate of 0.4 ml min⁻¹. Standard high-molecular-mass proteins (conalbumin, ovalbumin, carbonic anhydrase and RNase A) were used to generate a calibration curve and the molecular mass of native NaaS was estimated by interpolation.

Enzyme assays. Taurine dehydrogenase [EC 1.4.2.-] (TauXY) was assayed spectrophotometrically with beef-heart cytochrome *c* or ferricyanide as the electron acceptor (Brüggemann *et al.*, 2004). Sulfoacetaldehyde acetyltransferase [EC 2.3.3.15] (Xsc) was quantified

by the colorimetric determination of acetyl phosphate (Ruff *et al.*, 2003). Sulfite dehydrogenase [EC 1.8.2.1] (SorAB) was assayed spectrophotometrically with ferricyanide as electron acceptor (Reichenbecher *et al.*, 1999). Activity of *N*-acetyltaurine amidohydrolase was measured discontinuously as the *N*-acetyltaurine-dependent formation of acetate and taurine (Mayer *et al.*, 2006). To examine the effects of metal availability on NaaS function, the metal chelators 2,2'-bipyridyl (2.5 mM) or EDTA (25 mM) were added to purified NaaS in 50 mM Tris/HCl, pH 8.0, and incubated for 15 min at room temperature before adding *N*-acetyltaurine as substrate and assaying as above. Acetate-CoA ligase [EC 6.2.1.1] was assayed discontinuously, following product analysis by HPLC (Baldock *et al.*, 2007).

Analytical methods. Growth was followed as turbidity at 580 nm or quantified as protein in a Lowry-type reaction (Cook & Hütter, 1981). Sulfate was determined turbidimetrically as a suspension of BaSO₄ (Sörbo, 1987). Sulfite was quantified as the fuchsin-derivative as described elsewhere (Denger *et al.*, 2001). Ammonium ion was assayed colorimetrically by the Berthelot reaction (Gesellschaft Deutscher Chemiker, 1996). Acetyl phosphate was determined chemically as iron(III) acetyl hydroxamate (Racker, 1962). Acetate was quantified by GC (Laue *et al.*, 1997). Reversed-phase HPLC was used to quantify taurine and *N*-methyltaurine after derivatization with 2,4-dinitrofluorobenzene (Laue *et al.*, 1997). Sulfoacetate was quantified by ion chromatography with suppression (Denger *et al.*, 2004). *K_m* values were derived by hyperbolic curve fitting. SDS-PAGE and staining were done by standard methods (Laemmli, 1970). Metal analyses by inductively coupled plasma mass spectrometry were done under contract by the Spurenanalytisches Laboratorium Dr Baumann (Pirkensee, Maxhütte-Haidhof, Germany). The molecular mass of native, purified proteins was determined by dynamic light scattering (DLS) on an 802 DLS photometer (Viscotek, Dual Attenuation Technology). The molecular mass was calculated using OmniSIZE 3.0 software. Peptide-mass fingerprinting was done at the Proteomics Centre of the University of Konstanz; trypsin was the protease used.

RT-PCR was done as detailed elsewhere (Krejčík *et al.*, 2008) using the primers listed in Supplementary Table S1, available with the online version of this paper. The RNA was tested for residual DNA with primer pair naaS-H16_B0868-f and naaS-H16_B0868-r. Chromosomal DNA from boiled cells of *C. necator* H16 (or *Ru. pomeroyi* DSS-3, as appropriate) was used as a positive control for PCRs. PCR products were visualized on 1.0 % agarose gels.

Bioinformatic analyses. Sequences of chromosome 2 of *C. necator* H16 (accession no. NC_008314) were obtained from the National Center of Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>), whose BLAST (Altschul *et al.*, 1997) and Conserved Domain server was used, as was the BLAST server of the Transport Classification Database (<http://www.tcd.org>), which is coupled to a predictor of transmembrane helices. Orthologue neighbourhood regions were compared with the IMG system of the DOE Joint Genome Institute (<http://img.jgi.doe.gov/cgi-bin/w/main.cgi>) and sequence data up to April 2011 were included. Primers were designed using the subroutine PrimerSelect of the Lasergene software package (DNASTAR).

RESULTS

Growth physiology

C. necator H16 grew exponentially with *N*-acetyltaurine as sole source of carbon and energy for growth. We could not assay for *N*-acetyltaurine, but the release of sulfate was concomitant with growth and no sulfite was detected in the

medium during growth. The recovery of sulfonate-sulfur as sulfate was about 90 %. The growth rate (μ) was 0.3 h^{-1} and the growth yield (for all four carbon atoms) was $4.7 \text{ g protein (mol C)}^{-1}$, a value which indicates a complete mass balance for carbon (Cook, 1987). The specific degradation rate for *N*-acetyltaurine was calculated to be $4.5 \text{ mkat (kg protein)}^{-1}$.

Some 33 bacteria with sequenced genomes were candidates to utilize *N*-acetyltaurine as a sole source of carbon (or of nitrogen) (choice based on biochemical and BLAST data presented below). Eight of these organisms were tested for growth with the compound. Whereas *B. phytofirmans* PsJN, *C. pinatubonensis* JMP134, *D. acidovorans* SPH-1, *P. denitrificans* PD1222 and *Ru. pomeroyi* DSS-3 utilized *N*-acetyltaurine as a sole source of carbon and excreted stoichiometric amounts of sulfate, *Ro. nubinihibens* ISM did neither. *Rh. palustris* CGA009 utilized *N*-acetyltaurine as a sole source of carbon, but with a low yield, which was interpreted to represent utilization of only the acetyl moiety of *N*-acetyltaurine. Not sulfate but sulfoacetate was generated. This organism shows quantitative excretion of sulfoacetate when taurine is utilized as a nitrogen source (Denger *et al.*, 2004); there was, thus, mass balance for the utilization of *N*-acetyltaurine by *Rh. palustris*. *M. radiotolerans* JCM 2831 also utilized the compound as a sole source of nitrogen and also excreted sulfoacetate (Krejčík, 2009).

Enzyme activities in crude extracts and purification of NaaS from *C. necator* H16

Extracts of *N*-acetyltaurine-grown cells contained *N*-acetyltaurine amidohydrolase (NaaS) (Table 1) at a specific activity [$8.4 \text{ mkat (kg protein)}^{-1}$] sufficient to meet the specific degradation rate observed in growing cells [$4.5 \text{ mkat (kg protein)}^{-1}$]. Furthermore, the activities of enzymes representative for the taurine degradative pathway were found: taurine dehydrogenase (TauXY), sulfoacetaldehyde acetyltransferase (Xsc) and sulfite dehydrogenase (SorAB) (Table 1, Fig. 1a). Extracts of taurine-grown cells also contained taurine dehydrogenase (TauXY), sulfoacetaldehyde acetyltransferase (Xsc) and sulfite dehydrogenase (SorAB), but not NaaS (Table 1). NaaS was, thus, inducible. Extracts of succinate-grown cells contained no

activity of enzymes representing taurine degradation (Table 1), which were induced separately from NaaS. The second product from NaaS was acetate (Fig. 1). Acetate-CoA ligase (Acs) was present in extracts of *N*-acetyltaurine-grown cells, whereas it was absent in extracts of taurine-grown cells (Table 1). Acetate-CoA ligase (Acs) was, thus, also inducible. Further metabolism of both products of the NaaS reaction was routed to intermediary metabolism, consistent with the growth yield observed and with its implication of quantitative metabolism of *N*-acetyltaurine.

A four-step purification of NaaS (see Methods) yielded an apparently homogeneous enzyme with a recovery of 1 % and a purification factor of 175 (Fig. 2, Table 2). The enzyme catalysed the release of equimolar acetate and taurine from *N*-acetyltaurine (Fig. 3). The K_m value was $9.4 \pm 1.3 \text{ mM}$. The enzyme could be stored for several weeks at 4°C without loss of activity. The molecular mass of the denatured enzyme was about 61 kDa (Fig. 2), consistent with the value (59.9 kDa) derived from the gene sequence (see below). When separated on a calibrated gel filtration column, the retention time of the native enzyme indicated a molecular mass of 37 kDa. Given the large errors to which this method is prone (le Maire *et al.*, 1996), we interpret the data to support a monomeric enzyme. Analyses by DLS gave values of about 80 kDa for the native protein. Together these data are most compatible with the protein being a monomer.

NaaS has been auto-annotated in genome sequences (see below) as a metal-dependent amidohydrolase, so we tested the effect of chelating agents on NaaS activity. Preincubation of the enzyme with EDTA had no effect on its activity, and only a slight inhibitory effect was detected upon preincubation with bipyridyl. We then analysed the purified enzyme directly for the metals Co, Ni, Zn and Fe. Neither cobalt nor nickel was present at levels above the limit of detection. Zinc was detected in the preparations at about $0.9 \text{ mol Zn (mol NaaS)}^{-1}$ as was iron at about $0.7 \text{ mol Fe (mol NaaS)}^{-1}$. Analogous amides were tested as substrate for NaaS. None of the compounds, *N*-acetylhomotaurine, acetamide and *N*-oleoyl-*N*-methyltaurine, was hydrolysed as judged by the release of acetate or methyltaurine, as appropriate.

Table 1. Specific enzyme activities [$\text{mkat (kg protein)}^{-1}$] of crude extracts of *C. necator* H16 under different growth conditions

Nat, *N*-acetyltaurine; bdl, below detection limit [the limits of detection for NaaS, TauXY, Xsc, SorAB and Acs were 0.2, 0.02, 0.1, 0.02 and $0.5 \text{ mkat (kg protein)}^{-1}$, respectively].

Enzyme	Nat grown	Taurine grown	Succinate grown
<i>N</i> -Acetyltaurine amidohydrolase, NaaS [EC 3.5.1.-]	8.4	bdl	bdl
Taurine dehydrogenase, TauXY [EC 1.4.2.-]	0.3	0.8	bdl
Sulfoacetaldehyde acetyltransferase, Xsc [EC 2.3.3.15]	0.5	1.2	bdl
Sulfite dehydrogenase, SorAB [EC 1.8.2.1]	13.2	17.8	0.3
Acetate-CoA ligase, Acs [EC 6.2.1.1]	4.9	bdl	2.4

Fig. 2. Electropherograms (SDS-PAGE) of denatured proteins from different stages of the purification of NaaS from *C. necator* H16. Lanes: 1, soluble fraction of *N*-acetyltaurine-grown cells; 2, active fraction after first anion-exchange chromatography; 3, active fraction after hydrophobic-interaction chromatography; 4, active fraction after second anion-exchange chromatography; 5, purified enzyme after gel filtration chromatography; 6; molecular mass markers (with masses in kDa).

The locus tag of *naaS* and the *naa* gene cluster(s)

A peptide-mass fingerprint of the purified NaaS from *C. necator*, with 42% coverage of the deduced sequence, showed it to be encoded by the gene H16_B0868. This is part of a predicted five-gene cluster (H16_B0865–H16_B0869), which was largely conserved in 11 other bacterial genomes (Table 3), and we termed the genes *naaROPST* (Fig. 1b). We hypothesized that the LysR-type transcriptional regulator (H16_B0865) encoded by *naaR* is responsible for regulating the expression of the inducible NaaS and other proteins needed to convert extracellular *N*-acetyltaurine to intracellular acetate and taurine. NaaO (H16_B0866) is a short (68 amino acids), hypothetical protein predicted to have a leader peptide and one transmembrane helix in the mature protein, which, thus, may have a role in transport. The sodium:solute symporter (SSS; [TC 2.A.21.–]) (Faham *et al.*, 2008; Reizer *et al.*, 1994) family permease (H16_B0867), NaaP, was presumed to transport *N*-acetyltaurine into the cell. NaaS is known to be the amidohydrolase (H16_B0868),

whose automatic genome annotation as ‘metal-dependent’ is apparently correct (see above). NaaT (COG0523) may be a Zn-related metallochaperone (e.g. Haas *et al.*, 2009; Kuchar & Hausinger, 2004); certainly the overlapping *naaST* gene pair is found in all 33 gene clusters in which *naaS* is found (Table 3, Supplementary Tables S2, S3 and S4). Furthermore, a dendrogram of all NaaT orthologues and other selected COG0523 members showed that NaaT clusters with established Zn-related metallochaperones (Supplementary Fig. S1).

A somewhat different cluster of *naa* genes is found in 14 genomes (Supplementary Table S2), with one overlap to Table 3, *D. acidovorans* SPH-1, an *N*-acetyltaurine-utilizer which occurs in both tables. The representative organism here is *Ru. pomeroyi* DSS-3. In Table S2, *naaOP* is replaced by *naaABB'CC'*, which presumably encode an ATP-binding cassette (ABC) transporter [TC 3.A.1.5.–]. An analogous cluster is found in other organisms (Supplementary Table S3), which could encode a TRAP-transporter. The candidate organism from Supplementary Table S3 that we tested (*Ro. nubinhibens* ISM) did not grow with *N*-acetyltaurine: but *naaR* is missing, replaced by another amidase gene, so we presume that the cluster is corrupted. Finally, the *naaST* gene pair is found in pathogens such as *Bordetella bronchiseptica* beside a gene encoding a periplasmic binding protein (Supplementary Table S4). There is often no recognizable regulator gene in the organisms in Supplementary Tables S2, S3 and S4.

Transcriptional analyses of genes in the *naa* cluster in *C. necator* H16 and *Ru. pomeroyi* DSS-3

RT-PCR studies with *C. necator* H16 showed that no transcript of *naaS* was detected in succinate-grown cells, whereas a clearly visible signal was observed in *N*-acetyltaurine-grown cells (Fig. 4). Furthermore, three other genes (*naaO*, *naaP* and *naaT*) in the cluster (Fig. 1b) are equally strongly transcribed (Fig. 4).

Ru. pomeroyi DSS-3 grows with *N*-acetyltaurine (see above), and the genome contains *naaST* (Fig. 5) neighbouring candidate genes for a five-component ABC transporter (*NaaABB'CC'*). RT-PCR data indicated that *naaST* were, indeed, inducibly transcribed, with a low level of constitutive transcription (Supplementary Fig. S2). All

Table 2. Purification table of *N*-acetyltaurine amidohydrolase of *C. necator* H16

Purification step	Total protein (mg)	Total activity (nkat)	Recovery (%)	Specific activity [mkat (kg protein) ⁻¹]	Purification (-fold)
Crude extract	85.5	684	100	8	1
Soluble fraction	63.2	619	91	10	1.2
1st anion exchange	8.9	427	62	48	6
Hydrophobic interaction	0.4	233	34	614	77
2nd anion exchange	0.1	116	17	970	121
Gel filtration	0.01	8	1	1400	175

Fig. 3. Reaction products from *N*-acetyltaurine amidohydrolase, NaaS. The purified enzyme (0.01 mg in the 1 ml assay) was used. Key: taurine (▲) and acetate (□) concentrations with active NaaS; negative control, taurine (△) and acetate (■) concentrations without protein in the assay.

five candidates for the ABC transporter were transcribed inducibly, with a (usually) low level of constitutive transcription, representatives of which are shown in Fig. 5.

DISCUSSION

It is clear that *N*-acetyltaurine is utilized quantitatively as a sole source of carbon and energy by *C. necator* H16, with mass balances for carbon and the sulfonate moiety, as observed previously in *D. acidovorans* NAT (Mayer *et al.*, 2008). The gene cluster in *C. necator* H16 is a paradigm for

12 organisms (Table 3), representatives of which also grow with *N*-acetyltaurine, namely *C. pinatubonensis*, *B. phytofirmans* and *D. acidovorans* SPH-1. There is a second paradigm, *Ru. pomeroyi* DSS-3 (Table S2), for 14 organisms, including *P. denitrificans*, which also utilized all the carbon from *N*-acetyltaurine. A variant of this paradigm involves the release of sulfoacetate from taurine, discovered in *Rh. palustris* CGA009 (Denger *et al.*, 2004) and observed in *M. radiotolerans* (Krejčík, 2009). This pathway is active when *Rh. palustris* CGA009 (and presumably also *Rh. palustris* strains HaA2, DX-1 and TIE-1; Supplementary Table S2) utilizes *N*-acetyltaurine as a source of nitrogen. *Roseovarius* sp. strain 217 also utilizes *N*-acetyltaurine (Baldock *et al.*, 2007), but this organism is not found in Table 3, or Supplementary Tables S2, S3 or S4, so we assume that there is an alternative pathway (K. Denger, unpublished). Thus, as for the degradation of other widespread sulfonates (e.g. Weinitschke *et al.*, 2010b), there is considerable diversity in the pathways which have evolved.

N-acetyltaurine has been reported from only one source, the webs of orb spiders (Vollrath *et al.*, 1990). The known and presumed degradative organisms in Table 3 are all terrestrial, which is unsurprising. Supplementary Table S2, however, contains both marine organisms (*Ru. pomeroyi*, *Roseobacter* sp. and *Rhodobacterales* bacterium HTCC2150) and organisms which can grow in the marine environment (*Rh. palustris* strains). We thus postulate a currently unknown marine source of *N*-acetyltaurine.

The first specific interaction of *N*-acetyltaurine with induced cells of *C. necator* H16 is deduced to be binding to the SSS-family membrane transporter, NaaP (possibly NaaOP). The TCDB website mentions single-component SSS transporters [TC 2.A.21] in bacteria, archaea and animals, but not bipartite SSS transporters (represented by

Table 3. Clusters of *naa* genes similar to that found in *C. necator* H16

Organism	Tag	<i>naaR</i>	<i>naaO</i>	<i>naaP</i>	<i>naaS</i>	<i>naaT</i>
<i>Cupriavidus necator</i> H16	H16_	B0865	B0866	B0867	B0868	B0869
<i>Cupriavidus pinatubonensis</i> JMP134	Reut_	B5754	B5755	B5756	B5757	B5758
<i>Delftia acidovorans</i> SPH-1*	Daci_	1982	1983	1984	1985	1986
<i>Brevibacterium mcbrellneri</i> ATCC 94030	HMPREF0183_		0126	0125	0128	0127
<i>Burkholderia cenocepacia</i> J2315	BCAS	0092	0094	0095	0096	0097
<i>Burkholderia multivorans</i> ATCC 17616†	Bmul_	6055	6054	6053	6052	6051
<i>Burkholderia multivorans</i> CGD1	BURMUCGD1_	6339	6338	6337	6336	6335
<i>Burkholderia multivorans</i> CGD2	BURMUCGD2_	6579	6578	6577	6576	6575
<i>Burkholderia multivorans</i> CGD2M	BURMUCGD2M_	6569	6568	6567	6566	6565
<i>Burkholderia phytofirmans</i> PsJN	Bphyt_	4357	4361	4360	4359	4358
<i>Burkholderia</i> sp. strain 383	Bcep18194_	C7632	C7629	C7628	C7627	C7626
<i>Burkholderia</i> sp. strain TJ149	B1M_	27391‡	27396	27401	27406	27411‡

*A very similar set of sequence data are attributed to *Populus trichocarpa*, but we suspect this to represent the presence of *D. acidovorans* on the leaves of the plant during sampling.
†This organism has been sequenced by two organizations, information from only one of which is shown in the table.
‡The first and last genes in this cluster are only partially sequenced.

Fig. 4. RT-PCR data to demonstrate inducible transcription of four *naa* genes. Three genes were analysed individually (*naaPST*), whereas the small gene (*naaO*) was detected as a co-transcript with *naaP*. The numbers on the left are fragment sizes (bp). The number adjacent to each gene(s) shows the anticipated length of the gene fragment. Lanes: M, length markers; N, RT-PCR with RNA extracted from *N*-acetyltaurine-grown cells; S, RT-PCR with RNA extracted from succinate-grown cells; +, PCR control with chromosomal DNA; –, negative control (no template).

NaaOP). A BLAST search with NaaO, however, reveals several poor orthologues (e.g. BMQ_2311, Bxe_B1296, Gdia_0759, RER_26730, ROP_33450 in bacteria and TVN0147 in an archaeon) encoded adjacent to candidate SSS-encoding genes of unknown function, so bipartite SSS transporters seem possible.

The second specific interaction of *N*-acetyltaurine is with NaaS, whose two metabolic products enter independent,

inducible metabolic pathways (Table 1). The Naa pathway is, thus, very short, but in a well-controlled, complex metabolic situation (Fig. 1).

NaaS hydrolyses a carbon–nitrogen bond that is not a peptide bond, so it can be allocated to group EC 3.5. in the Enzyme Nomenclature Commission's listing. The substrate is a linear amide, so the enzyme belongs to EC 3.5.1.–. The systematic name, *N*-acetyltaurine amidohydrolase, can be

Fig. 5. Clusters of *naa* genes similar to those found in *Ru. pomeroyi* DSS-3. *Rh. palustris* DX-1 is representative of four gene sequences of this organism (Supplementary Table S2).

simplified to 'acetyltaurine amidase' for the accepted name. The entry could contain 'Comments', namely 'Contains tightly bound Zn and Fe'. The tightness of this binding is indicated by the ineffectiveness of e.g. EDTA to chelate the metals. This, in turn, indicates that the metal(s) does not diffuse into the protein, but that active insertion is involved, presumably by a metallochaperone (e.g. Kuchar & Hausinger, 2004). Another amidase (Nawaz *et al.*, 1996) is one of the few in which the metals (here Co and Fe) have been determined directly.

The reaction catalysed by NaaS (Fig. 1, Fig. 3) might look like a facile hydrolysis, but the chemical inertness of the substrate, which prevented any simple chemical derivatization of the amido-nitrogen or the amido oxo-group, indicates the effectiveness of the catalyst in lowering the activation energy for the reaction. We presume that the metal(s) is one key to the catalysis, as seen in another amidase (Nawaz *et al.*, 1996).

The fourth gene product, NaaT, belongs to the large COG0523 family of metallochaperones, 'a striking example of systematic, homology-based mis-annotation' (Haas *et al.*, 2009). We nonetheless used a dendrogram (Supplementary Fig. S1) to indicate that (i) NaaT clusters with representative Zn-associated metallochaperones, and (ii) NaaT is separate from Fe-associated and presumably Co-associated chaperones. This grouping with Zn-related metallochaperones is consistent with the Zn in NaaS. No experiments were done with NaaT, but the GTPase domain at the N terminus of the protein could energize any manipulation of NaaS and the potential His-containing metal-binding sites in the C-terminus could provide the Zn.

We noted two major paradigms for clusters of *naa* genes (Fig. 1c and Fig. 5) and two minor paradigms (Supplementary Tables S3 and S4), which will not be discussed further. The core of the first paradigm, *naaOPST*, is constant in the betaproteobacteria (Table 3), where the position or orientation of the regulator varies. In the Gram-positive *Brevibacterium mcbrellneri* the core is slightly different, *naaSTOP*, and the regulation is unknown (Fig. 1c). The second paradigm is found in about three subgroups (Fig. 5). Some four alphaproteobacteria encode NaaST on one strand and the transporter on the other strand, upstream of *naaST*, whereas other alphaproteobacteria encode the transporter on the same strand as *naaST*, downstream thereof (Fig. 5). The third subgroup, in betaproteobacteria, encodes the transporter upstream of *naaST* and from the same strand. To add to the biodiversity, an alternative to NaaCC', NaaC'', is deduced to be encoded in *Achromobacter xylosoxidans* C54 (Fig. 5).

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