Molecular genetics and biochemistry of \(N\)-acetyltaurine degradation by \textit{Cupriavidus necator} H16

Karin Denger, Sabine Lehmann and Alasdair M. Cook

Department of Biology, The University, D-78457 Konstanz, Germany

\textit{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], an 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 acetyl-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, \textit{naa}OPST (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 \textit{naa}OPST 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, \textit{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 hydrolys\(es\) \(N\)-acetyltaurine to taurine and acetate (Mayer et al., 2006).

\textit{D. acidovorans} has been termed pathogenic (Horowitz et al., 1990). The non-pathogenic, genome-sequenced \textit{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 \textit{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)], \(^1\)H- and \(^{13}\)C-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. \textit{Burkholderia phytofirmans} PsJN (DSM 17436), \textit{Cupriavidus necator} H16 (DSM 428) and \textit{Cupriavidus pinatubonensis} JMP134 (DSM 4058) were obtained from the German Culture Collection (DSMZ, Braunschweig, Germany); \textit{Methyllobacterium radiotolerans} JCM 2831 was kindly made available.
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. Krejcík & A. M. Cook, unpublished), as are AmtB (the ammonium transporter) (H16_A0321 and H16_B1234) and Acs (H16_B0834) (Baldock et al., 2007; Gorzsinska 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. *Actinobacillus acidovorans* PH1 (DSM 14801) was isolated in our laboratory.

*B. phytofirmans* PsJN, C. pinatubonensis JMP134, *D. acidovorans* PH1, *P. denitrificans* PD1222 and *Rh. palustris* CGA009 grew in a phosphate-buffered mineral-salts medium (Thurnheer et al., 1986) with N-acetyltyramine (5 mM) as sole added source of carbon. The growth medium for *M. radiotolerans* N with precipitate removed by centrifugation (30 000 × g, 15 min, 4 °C) 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), then washed with 50 mM Tris/HCl buffer, pH 8.0, treated with DNase (0.05 mg DNase 1 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-acetyltyramine 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 0.15 M ammonium sulfate. As a third purification step, the rebuffered active fraction with 50 mM Tris/sulfate buffer, pH 8.0, was applied and NaaS eluted at 0 mM ammonium sulfate, sulfite and acetate. As a fourth purification step with 50 mM Tris/sulfate buffer, pH 8.0, including decreasing gradient of ammonium sulfate in 50 mM Tris/sulfate buffer, pH 8.0, was applied and NaaS eluted at 0 mM ammonium sulfate, sulfite and acetate.

**Analytical methods.** Growth was followed as turbidity at 580 nm or quantified as protein in a Lowry-type reaction (Cook & Hütter, 1981). Sulfite 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 hyroxamate (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). Sulfocacetate was quantified by ion chromatography with suppression (Denger et al., 2004). Km 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 (Viscotec, 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.

**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.tcdb.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-acetyltyramine as sole source of carbon and energy for growth. We could not assay for N-acetyltyramine, but the release of sulfate was concomitant with growth and no sulfate was detected in the
medium during growth. The recovery of sulfonate-sulfur as sulfate was about 90%. The growth rate ($\mu$) was 0.3 h$^{-1}$ and the growth yield (for all four carbon atoms) was 4.7 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 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. phytotormans PsIn, 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. nubinhibens 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 mkat (kg protein)$^{-1}$] sufficient to meet the specific degradation rate observed in growing cells [4.5 mkat (kg protein)$^{-1}$]. Furthermore, the activities of enzymes representative for the taurine degradative pathway were found: taurine dehydrogenase (TauXY), sulfoacetaldelyde acetyltransferase (Xsc) and sulfite dehydrogenase (SorAB) (Table 1, Fig. 1a). Extracts of taurine-grown cells also contained taurine dehydrogenase (TauXY), sulfoacetaldelyde 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 tauine from N-acetyltaurine (Fig. 3). The $K_m$ value was 9.4 ± 1.3 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 mol Zn (mol NaaS)$^{-1}$ as was iron at about 0.7 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 [mkat (kg protein)$^{-1}$] of crude extracts of C. necator H16 under different growth conditions

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Nat grown</th>
<th>Taurine grown</th>
<th>Succinate grown</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyltaurine amidohydrolase, NaaS [EC 3.5.1.-]</td>
<td>8.4</td>
<td>bdl</td>
<td>bdl</td>
</tr>
<tr>
<td>Taurine dehydrogenase, TauXY [EC 1.4.2.-]</td>
<td>0.3</td>
<td>0.8</td>
<td>bdl</td>
</tr>
<tr>
<td>Sulfoacetaldelyde acetyltransferase, Xsc [EC 2.3.3.15]</td>
<td>0.5</td>
<td>1.2</td>
<td>bdl</td>
</tr>
<tr>
<td>Sulfite dehydrogenase, SorAB [EC 1.8.2.1]</td>
<td>13.2</td>
<td>17.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Acetate–CoA ligase, Acs [EC 6.2.1.1]</td>
<td>4.9</td>
<td>bdl</td>
<td>2.4</td>
</tr>
</tbody>
</table>
showed it to be encoded by the gene H16 _B0868. This is necator
A peptide-mass fingerprint of the purified NaaS from C. necator
markers (with masses in kDa).
enzyme after gel filtration chromatography; 6; molecular mass
fraction after second anion-exchange chromatography; 5, purified
fraction after hydrophobic-interaction chromatography; 4, active
2, active fraction after first anion-exchange chromatography; 3, active
symporter (SSS; may have a role in transport. The sodium : solute
transmembrane helix in the mature protein, which, thus,
protein predicted to have a leader peptide and one
H16. Lanes: 1, soluble fraction of NaaS from C. necator
et al. (Faham et al., 2008; Reizer et al., 1994) family permease (H16 _B0867), NaaP,
acetyltaurine to intracellular acetate and taurine. NaaO
was presumed to transport NaaS and other proteins needed to convert extracellular
-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. nubinhibiens 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.

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

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

![Image](https://via.placeholder.png)

**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.

<p>| Table 3. Clusters of <em>naa</em> genes similar to that found in <em>C. necator</em> H16 |
|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Organism</strong></th>
<th><strong>Tag</strong></th>
<th><strong>naaR</strong></th>
<th><strong>naaO</strong></th>
<th><strong>naaP</strong></th>
<th><strong>naaS</strong></th>
<th><strong>naaT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cupriavidus necator</em> H16</td>
<td>H16_</td>
<td>B0865</td>
<td>B0866</td>
<td>B0867</td>
<td>B0868</td>
<td>B0869</td>
</tr>
<tr>
<td><em>Cupriavidus pinatubonensis</em> JMP134</td>
<td>Reut_</td>
<td>B5754</td>
<td>B5755</td>
<td>B5756</td>
<td>B5757</td>
<td>B5758</td>
</tr>
<tr>
<td><em>Brevibacterium michellei</em> ATCC 94030</td>
<td>HMPREF0183_</td>
<td>0126</td>
<td>0125</td>
<td>0128</td>
<td>0127</td>
<td></td>
</tr>
<tr>
<td><em>Burkholderia cenocepacia</em> J2315</td>
<td>BCAS</td>
<td>0092</td>
<td>0094</td>
<td>0095</td>
<td>0096</td>
<td>0097</td>
</tr>
<tr>
<td><em>Burkholderia multivorans</em> ATCC 17616†</td>
<td>Bmul_</td>
<td>6055</td>
<td>6054</td>
<td>6053</td>
<td>6052</td>
<td>6051</td>
</tr>
<tr>
<td><em>Burkholderia multivorans</em> CGD1</td>
<td>BURMUCGD1_</td>
<td>6339</td>
<td>6338</td>
<td>6337</td>
<td>6336</td>
<td>6335</td>
</tr>
<tr>
<td><em>Burkholderia multivorans</em> CGD2</td>
<td>BURMUCGD2_</td>
<td>6579</td>
<td>6578</td>
<td>6577</td>
<td>6576</td>
<td>6575</td>
</tr>
<tr>
<td><em>Burkholderia multivorans</em> CGD2M</td>
<td>BURMUCGD2M_</td>
<td>6569</td>
<td>6568</td>
<td>6567</td>
<td>6566</td>
<td>6565</td>
</tr>
<tr>
<td><em>Burkholderia pyhtofirmans</em> PsJN</td>
<td>Bphyt_</td>
<td>4357</td>
<td>4361</td>
<td>4360</td>
<td>4359</td>
<td>4358</td>
</tr>
<tr>
<td><em>Burkholderia</em> sp. strain 383</td>
<td>Bcep18194_</td>
<td>C7632</td>
<td>C7629</td>
<td>C7628</td>
<td>C7627</td>
<td>C7626</td>
</tr>
<tr>
<td><em>Burkholderia</em> sp. strain TJ149</td>
<td>B1M_</td>
<td>27391†</td>
<td>27396</td>
<td>27401</td>
<td>27406</td>
<td>27411†</td>
</tr>
</tbody>
</table>

*A* 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.
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. 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).

---

**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 macrellneri 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 xylosidans C54 (Fig. 5).

ACKNOWLEDGEMENTS

We are grateful to many organizations for making their genome sequence data generally available, but especially to GenoMik and TIGR, for the complete genome sequences of C. necator H16 and R. pomeroiyi DSS-3, respectively. We thank W. Welte and B. Philipp, University of Konstanz, for access to the DLS machine and software, and permission to use his ‘L2’ laboratory, respectively. This work was supported by the University of Konstanz and by the Deutsche Forschungsgemeinschaft (DFG).

REFERENCES


Edited by: G. H. Thomas