Cloning, sequencing and mutagenesis of the genes for aromatic amine dehydrogenase from *Alcaligenes faecalis* and evolution of amine dehydrogenases

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The nucleotide sequence of the aromatic amine utilization (aau) gene region from *Alcaligenes faecalis* contained nine genes (orf-1, aauBEDA, orf-2, orf-3, orf-4 and hemE) transcribed in the same direction. The aauB and aauA genes encode the periplasmic aromatic amine dehydrogenase (AADH) large and small subunit polypeptides, respectively, and were homologous to mauB and mauA, the genes for the large and small subunits of methylamine dehydrogenase (MADH). aauE and aauD are homologous to mauE and mauD and apparently carry out the same function of transport and folding of the small subunit polypeptide in the periplasm. No analogues of the mauF, mauG, mauL, mauM and mauN genes responsible for biosynthesis of tryptophan tryptophylquinone (the prosthetic group of amine dehydrogenases) were found in the aau cluster. orf-2 was predicted to encode a small periplasmic monohaem c-type cytochrome. No biological function can be assigned to polypeptides encoded by orf-1, orf-3 and orf-4 and mutations in these genes appeared to be lethal. Mutants generated by insertions into mauD were not able to use phenylethylamine, tyramine and tryptamine as a source of carbon and phenylethylamine, 3'-hydroxytyramine (dopamine) and tyramine as a source of nitrogen, indicating that AADH is the only enzyme involved in utilization of primary amines in *A. faecalis*. AADH genes are present in *Alcaligenes xylosoxydans* subsp. *xylosoxydans*, but not in other β- and γ-proteobacteria. Phylogenetic analysis of amine dehydrogenases (MADH and AADH) indicated that AADH and MADH evolutionarily diverged before separation of proteobacteria into existing subclasses.

**Keywords:** aau genes, methylamine dehydrogenase, proteobacteria, methylotrophy

**INTRODUCTION**

Considerable amounts of carbon and nitrogen in the environment are sequestered in the form of amines released there by live organisms or post-mortem (Tarr, 1954). The role of amines in the cycling of these elements is as yet unknown. Being abundant in the environment, amines have an impact on humans. Volatile amines, especially methylated amines, have been implicated in the production in the atmosphere of nitric oxide, an important greenhouse gas (Van Neste *et al*., 1987). A constantly growing source of amines in the environment is from human activity. The degradation of amines in nature therefore plays an important role in sustaining their proper balance in organisms and the environment. Oxidation of primary amines is a widespread process in nature, found in both eukaryotic and prokaryotic organisms and carried out by a number of enzymes including quinoproteins (McIntire & Hartmann, 1992). Quinoproteins degrading amines are unusual in that their prosthetic groups are synthesized from amino acid moieties of their polypeptide chains. There are two...
groups of quinoproteins able to oxidize amines: amine oxidases (AOs) and amine dehydrogenases. AOs are found in both eukaryotes and prokaryotes (McIntire & Hartmann, 1992), whereas amine dehydrogenases have been found only in bacteria. Until recently, methylamine dehydrogenase (MADH) was the only known amine dehydrogenase. MADH enzymes from different sources consist of two small and two large subunits with molecular masses ranging from 13 to 15 kDa and from 40 to 45 kDa, respectively, and in all known cases this enzyme is periplasmic (Eady & Large, 1968; Matsumoto, 1978; Haywood et al., 1982; Kenny & McIntire, 1983; Husain & Davidson, 1987; Kirichkin et al., 1990). The small subunit has a quinone prosthetic group called tryptophan tryptophylquinone (TTQ), which is synthesized from two tryptophans of the MADH small subunit polypeptide (McIntire et al., 1991).

A novel type of amine dehydrogenase, called aromatic amine dehydrogenase (AADH), has a much different substrate specificity from that of MADH (Iwaki et al., 1983). AADH has been found so far only in Alcaligenes faecalis during growth of the bacterium on medium containing phenylethylamine (Nozaki, 1987). AADH resembles MADH in many of its properties (Govindaraj et al., 1994). It has two large and two small subunits with molecular masses of 39 and 18 kDa, respectively. AADH is also localized in the periplasm (Zhu et al., 1999). In addition, the chromogenic group of AADH is associated with the small subunit polypeptide and it has been shown to be a quinone. The visual and Raman spectra of both AADH and MADH are nearly identical. These data suggested that the prosthetic group of AADH is TTQ. Azurin has been shown to act as an in vitro electron acceptor for AADH (Edwards et al., 1995).

The genetics of MADH has been extensively studied. Complete or partial sequences are available for mau genes from Methylobacterium extorquens AM1 (Chistoserdov et al., 1990, 1994a), two strains of Paracoccus denitrificans (Van Spanning et al., 1990; Ubbink et al., 1991; Chistoserdov et al., 1992; Hautema et al., 1993; Van der Palen et al., 1995, 1997), Methylophilus methylotrophus W3A1 (Chistoserdov et al., 1994b), Methylobacillus flagellatus KT (Gak et al., 1995, 1997) and ‘Methylomonas’ sp. J (Taguchi et al., 1997). All mau genes are clustered in the order mauFBDACJGLMN (mauC and mauL can be missing in some bacteria). The mauB, mauA and mauC genes encode the large and small subunit polypeptides of MADH and amicyanin, respectively. The products of mauE and mauD are likely to be involved in transport and folding of the small subunit polypeptide in the periplasm (Lidstrom & Chistoserdov, 1993; Van Der Palen et al., 1997), whereas mauF and mauGLM are involved in TTQ biosynthesis (Lidstrom & Chistoserdov, 1993; Chistoserdov et al., 1994a; Van der Palen et al., 1995; Gak et al., 1997). The mauFBDACJGLMN genes are sufficient to synthesize enzymically active MADH (Van der Palen et al., 1995; Graichen et al., 1999).

Nothing is known about the genetics of AADH. Therefore, the goals of this research were to clone aromatic amine utilization (aau) genes and elucidate their organization, to verify whether AADH is the only enzyme present in A. faecalis able to oxidize primary amines and to study the distribution of AADH genes among other proteobacteria.

METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this work are shown in Table 1. All Escherichia coli, Pseudomonas aeruginosa, A. faecalis, Alcaligenes xylosoxydans (A. xylosoxydans subsp. xylosoxydans), Ralstonia eutropha and Bordetella bronchiseptica strains were grown in liquid or on solid Luria–Bertani medium as described by Maniatis et al. (1982). Bordetella pertussis, Neisseria gonorrhoeae and Neisseria mucosa were grown on plates containing media recommended by the ATCC (ATCC Catalogue of Bacteria & Bacteriophages). Methylobacillus methylotrophus W3A1 was grown in liquid OK medium (Owens & Keddie, 1969) supplemented with 0.3% (w/v) methylamine. E. coli, Ps. aeruginosa, R. eutropha, A. faecalis and A. xylosoxydans strains were also grown in the OK minimal medium supplemented with 0.25% (w/v) of a source of carbon (acetate, succinate, phenylethylamine, tyramine, dopamine, tryptamine, histamine, phenylmethylamine). Nitrogen-free OK medium was prepared by substituting sodium-containing salts for ammonia-containing salts in equimolar concentrations. It was supplemented with acetate (0.2%, w/v) as a source of carbon and one of the aromatic amines or methylamine (0.05%, w/v) as a source of nitrogen. During growth and transformation of E. coli strains, appropriate antibiotics were added according to Maniatis et al. (1982), except that the concentration of chloramphenicol for pACYC63 derivatives was 0.01 mg ml⁻¹. A. faecalis is resistant to virtually all known antibiotics and mercury ions, except kanamycin and rifamycin, which were used in concentrations of 4 mg ml⁻¹ and 0.2 mg ml⁻¹, respectively.

AADH assay. Biomass of A. faecalis and A. xylosoxydans was grown in liquid OK medium as described above with phenylethylamine as a source of carbon. Crude extracts were obtained and AADH activity measurements were carried out according to Nozaki (1987). One unit of the AADH activity is defined as the amount of enzyme required to reduce 1 nmol dichlorophenolindophenol min⁻¹. Protein concentrations were measured as described earlier (Whitaker & Granum, 1980).

DNA–DNA hybridization. DNA–DNA hybridization and random prime DNA labelling was carried out using the DIG High Prime Labelling and Detection Starter Kit I according to the recommendations of the manufacturer (Boehringer Mannheim). The oligonucleotide AC14 (GGNGCNGAYG-AAYCAAYHAT) was labelled using The Genius System Oligonucleotide Tailing Kit (Boehringer Mannheim). The temperature of hybridization was 6× SS, 0.1% SDS; 1× SSC is 0.15 M sodium chloride and 0.015 M sodium citrate solution, pH 7.0) and washes (0.5× SSC, 0.1% SDS) was 68 °C for homologous DNA–DNA hybridizations (both probe and target DNA from A. faecalis). For heterologous DNA–DNA hybridizations (probe DNA from A. faecalis and target DNA from another bacterium), the temperatures for hybridization and washes were variable from 41 to 56 °C with 3 °C increments. In experiments with the oligonucleotide AC14 as
Table 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
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<tr>
<td>DH5α</td>
<td>F- Δ80d(lacZΔM15 bsdR17 supE44 thi-1 gyrA96 endA1 recA1 relA7 Δ(lacZYA-argF)U169</td>
<td>New England Bio-Labs</td>
</tr>
<tr>
<td>S17-1</td>
<td>F- AproAB thi-1 recA56 RP4-2[TcR::Mu KmR::Tn7 (TpR SmR)] integrated into the chromosome</td>
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<td><em>A. faecalis</em></td>
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<tr>
<td>Rifβ</td>
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<td>Pea1</td>
<td>aauD::KmR rif-l</td>
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<td>ATCC 19695</td>
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<td><strong>Plasmids</strong></td>
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<td>ApR KmR</td>
<td>Pharmacia</td>
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*Where no source or reference is given, the strain or plasmid was derived in this study.

a probe, the temperature of hybridization and washes was 42°C.

**DNA manipulations.** Plasmid isolation, *E. coli* strain transformation, preparative isolation of the DNA fragments from agarose gels, restriction endonuclease digestion, ligation and blunting of ends with Klenow fragment or T4 DNA polymerase were carried out as described by Maniatis et al. (1982). Chromosomal DNA of all bacterial strains was isolated in accordance with the procedure of Marmur (1961). The general strategy of construction and analysis of partial gene libraries was as described by us earlier (Chistoserdov et al., 1990, 1994b). The oligonucleotide AC14 was designed based on the N-terminal sequence of the AADH small subunit polypeptide (GADHII; Govindaraj et al., 1994). A partial clone library was constructed in the vector pRK310, using the fraction of the BclI–BglII digest of the *A. faecalis* chromosome, which hybridized to AC14 (not shown). This fraction had DNA fragments with an approximate size range of 1–6–1–9 kb. These fragments were collected in a well in agarose gels as described by Maniatis et al. (1982) and cloned into pRK310. Clones with positive hybridization to the AC14 probe were identified (approx. 1 per 300 white colonies); plasmids isolated from the positive clones were shown to contain an identical insert of ~1–7 kb in size.

**DNA sequencing.** DNA sequencing was performed by the dideoxy chain termination method on both strands in the SUNY Sequencing Facility on an Applied Biosystems sequencer, model 370A. Plasmid pAYC63 (CmR; Chistoserdov et al., 1992) was used as a vector for subcloning and sequencing in addition to pUC19 (ApR).

**Matings.** Biparental matings were conducted using the *E. coli* strain S17-1 as described previously (Simon, 1984).

**Construction of insertional mau strains.** Insertion mau mutants were constructed by homologous recombination as described previously (Ruvkun & Ausubel, 1981). The KmR cassette from the plasmid pUC4K was used as a selective inactivating marker and the plasmid pAYC61 as a suicide vector (Chistoserdov et al., 1994a).

**Computer analysis of DNA and protein sequences.** Computer analysis was carried out using the GCG (Wisconsin Package version 9.1 of the Genetics Computer Group) and PCGene (Genofit) program packages. DNA and protein sequences were compared to the GenBank and SWISS-PROT databases.
by the BLAST and FASTA programs (Wisconsin Package version 9.1 of the Genetics Computer Group).

The phylogenetic trees were constructed using two independent algorithms: distance analyses with Jukes–Cantor corrections (programs DNADIST (Jukes–Cantor) and FITCH from the PHYLIP package (version 3.5c, http://evolution.genetics.washington.edu/phylip.html) and parsimony using PAUP (version 3.1.1).

**RESULTS AND DISCUSSION**

**Cloning and nucleotide sequence of the aau gene cluster from *A. faecalis***

A 1.7 kb *BclI* fragment which hybridized with the AC14 probe was cloned as described in Methods. This fragment was completely sequenced and three open reading frames (ORFs) were identified in it. One of these ORFs was truncated and encoded a polypeptide containing the previously determined N-terminal sequence of the AADH small subunit polypeptide (Govindaraj et al., 1994). Two other ORFs encoded polypeptides highly homologous to MauE and MauD and were called *aauE* and *aauD*.

To clone other *aau* genes, additional cloning experiments were carried out. Chromosomal DNA of *A. faecalis* was digested with *EcoRI* and hybridized with the labelled 1.7 kb fragment. Two, 2 kb and 4.6 kb, *EcoRI* fragments were identified by hybridization. Both fragments were excised from agarose gels, cloned and sequenced as described above.

**Computer analysis of the aau gene cluster organization and polypeptides encoded by the mau gene cluster**

ORFs for nine genes (*orf-1, aauBEDA, orf-2, orf-3, orf-4 and hemE*) were found in the two *EcoRI* fragments, all putatively transcribed in the same direction (Fig. 1).

Based on identity with the corresponding genes of the *mau* gene cluster, the second and fifth ORFs were tentatively identified as *aauB* and *aauA* genes. They encode the periplasmic aromatic amine dehydrogenase large (346 aa, 38222 Da) and small (182 aa, 19652 Da) subunit polypeptides, respectively. The identity between AuaA and MauA from *Methylophilus methylotrophus* W3A1 was 60% and the identity between AuaB and MauB from the same bacterium was 31%. The third and fourth genes in this gene cluster encode polypeptides highly similar to the MauE and MauD polypeptides; these polypeptides were therefore denoted AuaE (213 aa, 22327 Da) and AuaD (213 aa, 22917 Da). The identities between MauE (from *Methylophilus methylotrophus* W3A1) and AuaE and MauD (from *Methylophilus methylotrophus* W3A1) and AuaD were 45 and 60%, respectively. AuaB, AuaA and AuaD are putative periplasmic polypeptides similar to their Mau counterparts. A leader sequence of AuaD resembles that of MauD in that it lacks positively charged amino acids in its N-terminus, which are commonly found in all other leader sequences. Both AuaE and MauE are membrane-spanning polypeptides with four predicted hydrophobic helices. AuaA and MauAs are very similar (Fig. 2), particularly around the areas where tryptophans involved in TTQ biosynthesis are located. Overall, all amino acids which are believed to be involved in formation of the active centre and catalysis of TTQ-containing enzymes (Chen et al., 1991) are identical in both AuaA and MauA (Asp32, Asp76, Thr122, Trp57 and Trp108, *Par. denitrificans* numbering). The only exception is Tyr119 in MauA, which is replaced with Phe in AuaA. MauA and AuaA share similarities in their leader polypeptides (Fig. 1): both leader polypeptides are unusual and possess the ‘double-Arg’ motif, which is believed to be necessary to transport into the periplasm polypeptide with covalently attached coenzymes or prosthetic groups (Berks, 1996).

**Fig. 1.** Genetic maps of the *mau* gene cluster from *Methylophilus methylotrophus* W3A1 and *Methylobacterium extorquens* AM1 and the *aau* gene cluster from *A. faecalis*. Arrows show direction of transcription. Asterisks denote hairpin structures with free energies of 10 kcal mol$^{-1}$ (42 kJ mol$^{-1}$) or more, some of which were shown to function as transcription terminators in *E. coli*. The *orf-1, orf-2* and *orf-3* ORFs from different bacteria do not encode homologous polypeptides. Open boxes, periplasmic proteins; vertically hatched boxes, cytoplasmic proteins; diagonally hatched boxes, membrane proteins.
The first ORF (orf-1) was truncated. However, 59 amino acids encoded by orf-1 were sufficient to ascertain that Orf-1 is not similar to MauF usually found in this position in mau gene clusters. Moreover, a homology search in the GenBank and SWISS-PROT databases did not reveal any polypeptides homologous to Orf-1. The ORF immediately downstream of auuA (orf-2) was predicted to encode a small periplasmic monohaem c-type cytochrome (166 aa, 18580 Da). This cytochrome c shares relatively high identity (36%) with cytochrome c552 from Thermus thermophilus (Titani et al., 1985). The haem c binding sites of cytochrome c552 and Orf-2 are near-identical. The premature form of Orf-2 does not reveal any polypeptides homologous to Orf-1.

Fig. 2. Alignment of amino acid sequences of MauA or AuuA from: A, Par. denitrificans; B, ‘Thiobacillus versutus’; C, A. faecalis; D, Methyllobacillus flagellatus KT; E, Methyllobacterium methylophilus W3A1; F, Methyllobacterium extorquens AM1; G, Methylophilus methylophilus strain S1. Tryptophans involved in TTQ binding sites of cytochrome c are included. The double Arg motif is underlined. Vertical arrows indicate the signal peptidase cleavage site. Asterisks indicate amino acids that are identical in all sequences; dots indicate conservative substitutions.

Construction of auu mutants and their properties

Our preliminary experiments showed that A. faecalis is able to utilize phenylethylamine, tryptamine and tyramine as both carbon and nitrogen sources. In addition, dopamine may serve as a source of nitrogen (it was possible to select mutants with the frequency 10^{-7} able to utilize dopamine as a source of carbon). Two other amines, histamine and phenylmethylaniline, did not serve as sources of either carbon or nitrogen for A. faecalis.

It is known for methylotrophic bacteria that two different amine-oxidizing systems may reside in the same bacterial cell (Chistoserdov et al., 1994a). To determine a number of amine-oxidizing enzymes, a series of auu mutants was generated. The Km^R cassette from pUC4K was inserted into auuD in such an orientation that aph and auuA were transcribed in the same direction and this construction was introduced into the chromosome of A. faecalis in the suicide vector pAYC61. Km^R revertants appeared on the plates with a high frequency as large colonies (2 x 10^{-7}); however, an equal number of small Km^R colonies appeared as well. Six large and six small colonies were selected, their DNA was isolated and digested with Bgl II and Bcl I and the digest was hybridized with the labelled 1.7 kb fragment and the labelled Km^R cassette. All large colonies did not contain inserts, whereas all six small colonies contained the Km^R cassette. Of the six clones with the Km^R cassette, four contained only the cassette itself, whereas two other clones contained the entire suicide vector.
The growth spectra of the four aauD::KmR mutants and the wild-type strain of *A. faecalis* were studied. All four mutants turned out to be identical and differ from the wild-type in that they could not utilize phenylethylamine, tyramine and tryptamine as sources of carbon. They also lost the ability to utilize phenylethylamine, tyramine and dopamine as sources of nitrogen. Tryptamine still served as a source of nitrogen. It is possible that *A. faecalis* is able to oxidize the indole moiety of tryptamine and, therefore, to use it as a source of nitrogen. Thus, it appears that AADH is the only enzyme involved in utilization of primary amines in *A. faecalis*.

Based on a homology search in GenBank, no biological function can be assigned to polypeptides encoded by orf-1, orf-3 and orf-4. Although orf-2 encodes a c-type cytochrome, its biological function is unclear. The cloned portion of orf-1 is very short and therefore this gene cannot be used for insertional mutagenesis. Therefore, the KmR cassette was inserted into each of the orf-2, orf-3 and orf-4 genes and cloned into the suicide vector pACYC. The resulting suicide plasmids were introduced into *A. faecalis* in a manner similar to that with aauD. Only large colonies were generated in each of the three matings; 400 colonies randomly picked from each mating were re-streaked on plates with mineral medium supplemented with tyramine or acetate. None of the colonies had any detectable phenotype. Finally, 100 randomly picked colonies for each mating were of the colonies had any detectable phenotype. Further experiments suggested that AADH genes are present only in *A. xylosoxydans* and not in other β-proteobacteria (*R. eutropha*, *B. pertussis*, *B. bronchiseptica*, *N. gonorrhoeae*, *N. mucosa*), *E. coli* or *P. aeruginosa*. The presence of AADH activity in crude extracts of *A. xylosoxydans* further confirms this notion.

**Distribution of aau genes among other proteobacteria**

*A. faecalis* is the only bacterium known so far to produce AADH. On the other hand, MADH is found in at least three different subclasses of proteobacteria. To ascertain the distribution of the AADH enzymic system among other proteobacteria, we carried out a series of DNA–DNA hybridization experiments.

We selected a number of β-proteobacteria closely related to *A. faecalis*: *A. xylosoxydans*, two Bordetella species and two *Neisseria* species. *R. eutropha* is also a β-proteobacterium but it is more evolutionarily distant from *A. faecalis* (Busse & Auling, 1992). Two γ-proteobacteria used in this study were *E. coli* and *P. aeruginosa*. *E. coli* served as a negative control since it is known that its genome does not contain genes for AADH. *Methylphilus methylotrophus* W3A1 was used as a positive control.

The growth spectra of *A. xylosoxydans*, *R. eutropha* and *P. aeruginosa* on aromatic amines (both *Bordetella* and *Neisseria* spp. are fastidious and would not grow in mineral medium) were studied. *A. xylosoxydans* showed a growth pattern identical to that of *A. faecalis*. Neither *A. faecalis* nor *A. xylosoxydans* showed methylothrophic or methazotrophic growth. *P. aeruginosa* used only histamine as a source of carbon and dopamine and histamine as sources of nitrogen. *R. eutropha* did not use any amine as either carbon or nitrogen sources.

AADH activity with phenylethylamine as a substrate was measured in crude extracts from both *A. faecalis* and *A. xylosoxydans* and was comparable: 95 and 87 units (mg protein)−1, respectively. The same extract did not show any detectable activity with methyamine as a substrate.

**Phylogeny of amine dehydrogenases**

In addition to AADH genes, sequences for MADH genes from at least six micro-organisms are known. To ascertain the evolution of amine dehydrogenases, amino acid sequences of MADH small subunit polypeptides from *Par. denitrificans*, ‘Thiobacillus versutus’ [presently reassigned to *Paracoccus versus* (Katayama et al., 1995)], *Methyllobacterium extorquens* AM1, *Methylphilus methylotrophus* W3A1, *Methylphaga thalassica* S1, *Methyllobacillus flagellatus* KT and *Methylomonas* sp. J and the AADH small subunit polypeptide from *A. faecalis* were aligned. The alignment was used to construct phylogenetic trees using distance and parsimony methods. The resulting trees were identical, therefore only the parsimony tree is shown here (Fig. 3). It is not surprising that all MADHs are more related to each other than to AADH. However, if we look at the same tree based on 16S rRNA phylogeny, the picture will be different. *Methylphilus methylotrophus* W3A1, *Methylbacillus flagellatus* KT and *A. faecalis* belong to the same β-subclass of the Proteobacteria. *Methylphaga thalassica* S1 belongs to the γ-subclass and is more closely related to these three than to *Par. denitrificans* or *Methyllobacterium extorquens* AM1 (γ-subclass). The overall mauA branching pattern for these methylotrophs is similar to...
Conclusions

Four genes involved in aromatic amine utilization by A. faecalis were cloned (aauBEDA). They share a high similarity with mauBEDA genes and appear to be functionally identical. However, no analogues of the mauF, mauG, mauL, mauM and mauN genes responsible for biosynthesis of TTQ (the prosthetic group of amine dehydrogenases) were found in the aau cluster. According to DNA–DNA hybridization experiments, AADH genes are found so far only in the genus Alcaligenes and not in other β- and γ-proteobacteria. Phylogenetic analysis of amine dehydrogenases (MADH and AADH) indicated that AADH and MADH evolutionarily diverged before separation of proteobacteria into the existing subclasses.

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REFERENCES


for the large subunit of methylamine dehydrogenase from *Thiobacillus versutus*. J Bacteriol 175, 6254–6259.


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