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Cysteine Biosynthesis Pathway in the Archaeon *Methanosarcina barkeri* Encoded by Acquired Bacterial Genes?

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The pathway of cysteine biosynthesis in archaea is still unexplored. Complementation of a cysteine auxotrophic *Escherichia coli* strain NK3 led to the isolation of the *Methanosarcina barkeri cysK* gene [encoding *O*-acetylserine (thiol)-lyase-A], which displays great similarity to bacterial *cysK* genes. Adjacent to *cysK* is an open reading frame orthologous to bacterial *cysE* (serine transacetylase) genes. These two genes could account for cysteine biosynthesis in this archaeon. Analysis of recent genome data revealed the presence of bacteria-like *cysM* genes [encoding *O*-acetylserine (thiol)-lyase-B] in *Pyrococcus* spp., *Sulfolobus solfataricus*, and *Thermoplasma acidophilum*. However, no orthologs for these genes can be found in *Methanococcus jannaschii*, *Methanobacterium thermoautotrophicum*, and *Archaeoglobus fulgidus*, implying the existence of unrecognizable genes for the same function or a different cysteine biosynthesis pathway.

Cysteine is an essential amino acid, unique in its ability to form disulfide linkages and also critical in the catalytic centers of many proteins. In bacteria, cysteine is synthesized from serine by incorporation of sulfide or thiosulfate (Fig. 1A). In the first step, *O*-acetylserine is formed by serine transacetylase, the *cysE* gene product. Cysteine is then produced in a reaction catalyzed by the enzyme *O*-acetylserine (thiol)-lyase-A or *O*-acetylserine (thiol)-lyase-B, encoded by the *cysK* and *cysM* genes, respectively (11). Cysteine biosynthesis in plants is quite similar, although the respective genes have only recently been cloned and only one isozyme of *O*-acetylserine (thiol)-lyase has so far been identified (7). In animals, the transsulfuration pathway derives the sulfur group of cysteine from methionine and the carbon skeleton and amino group from serine (Fig. 1B). Methionine is first converted to homocysteine through the intermediate *S*-adenosylmethionine. Cystathionine β-synthase then combines homocysteine and serine to form cystathionine, which yields cysteine upon the action of cystathionine γ-lyase (6).

The pathway of cysteine biosynthesis in the archaeal domain is at present unknown. Identifiable homologs of the bacterial *cysE*, *cysK*, and *cysM* genes have not been identified in the archaeal genomes of *Methanococcus jannaschii*, *Methanobacterium thermoautotrophicum*, *Archaeoglobus fulgidus*, and *Pyrococcus horikoshii* (3, 9, 10, 15). Similarly, homologs of cystathionine β-synthase and cystathionine γ-lyase are currently found only in the genomic sequence of *Aeropyrum pernix* (8). A single isotopic study in *Halobacterium marismortui* and *Sulfolobus acidocaldarius* showed that the sulfur of protein-bound cysteine may be derived from exogenously supplied methionine (20). Still unresolved, however, is whether the conversion occurs directly as in the animal transsulfuration pathway or by a more indirect route. In any case, a different biosynthetic pathway may also be present. It is known that in some bacteria (e.g., *Deinococcus radiodurans*) asparagine is made from aspartate by a tRNA-dependent amidation reaction (5). This route suggested the possibility that cysteine in archaea may be formed from serine in a reaction that involves thiolation of serine misacylated to $tRNA^{Cys}$, a reaction formally analogous to the synthesis of selenocysteine (4).

To address the question of cysteine biosynthesis in archaea, we attempted to complement a cysteine auxotroph of *Escherichia coli* with a genomic library of *Methanosarcina barkeri*, a mesophilic, autotrophic member of the methylotrophic group of methanogens and the type species of its genus (1). Here we report that bacteria-like *cysE* and *cysK* genes are present in *M. barkeri*.

MATERIALS AND METHODS

Bacterial strains, growth conditions, plasmids, and libraries. *E. coli* NK3 (*cysK cysM*) is a cysteine auxotroph (13). Cells were plated on M9 solid medium (14) supplemented with 20 mg of each L-amino acid except cysteine per liter. Where necessary, plates were supplemented with 22 mg of cysteine and 20 mg of kanamycin per liter. pCBS1 is a pBR322-derived plasmid in which the cloned gene is placed under control of the *E. coli trpS* promoter. A genomic *M. barkeri* (Fusaro) library (18) was kindly provided by Dieter Jahn (Freiburg University, Freiburg, Germany). It had been prepared with *Sau*3A1-digested genomic fragments ligated into the *Bam*HI site of the pBK-CMV vector (Stratagene).

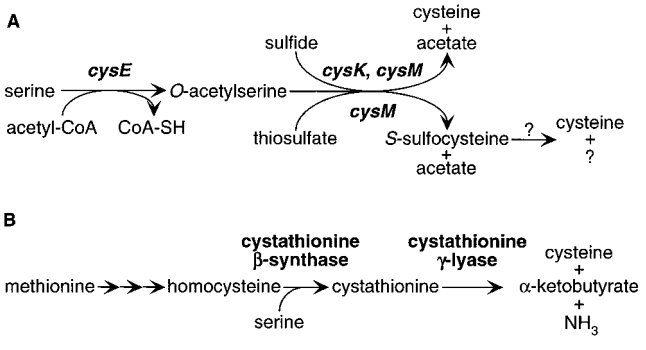

FIG. 1. Known biosynthetic pathways to cysteine. (A) Pathway in enteric bacteria. The question marks indicate a step that is incompletely characterized but in which cysteine may be formed by hydrolysis or by reduction with glutathione, also generating sulfate or sulfite, respectively (19). CoA, coenzyme A. (B) The transsulfuration pathway in animals. The first three reactions involve methyl group transfer via *S*-adenosylmethionine.

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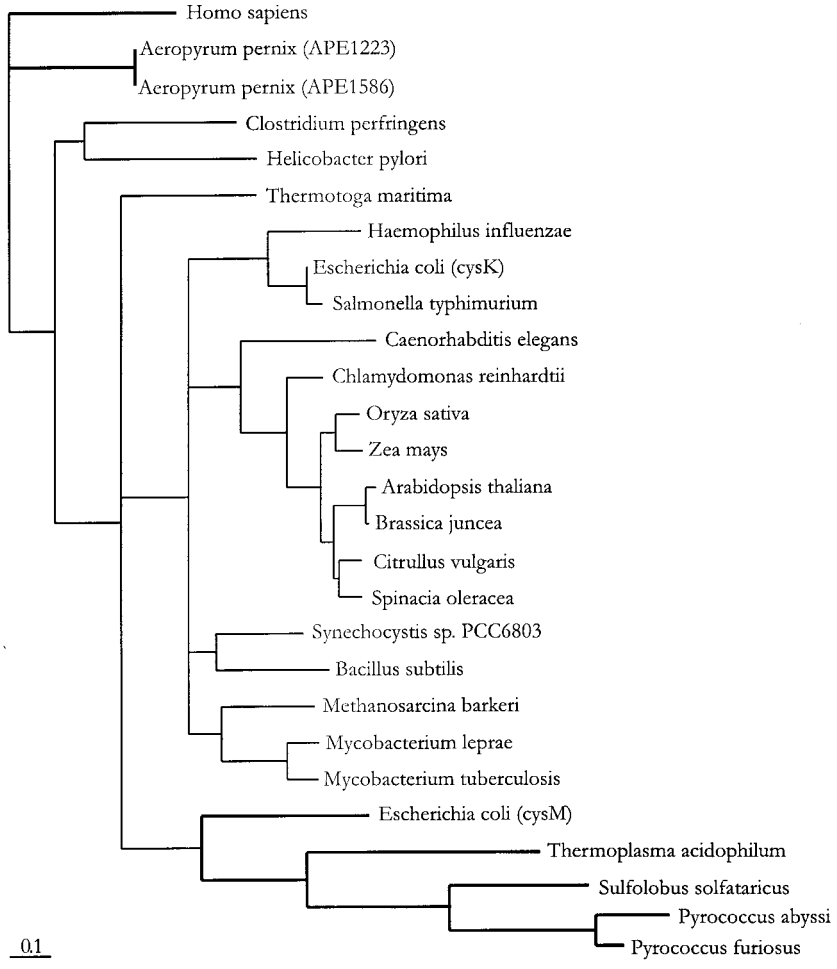

FIG. 2. Unrooted phylogeny of *cysK*-like sequences.

Cloning and sequencing techniques. Strain NK3 was transformed by electroporation in a Bio-Rad gene pulser. The *cysK* sequence in *M. barkeri* was PCR amplified from the complementing clone with *PfuTurbo* DNA polymerase (Stratagene) and primers containing *Nde*I and *Bgl*II restriction sites. To add 3′-deoxyadenosine residues, the reaction was incubated at 72°C for 15 min with *Taq* polymerase (Boehringer Mannheim). The PCR product was gel isolated by using the QIAEX II gel extraction kit (Qiagen) and directly cloned into the pCRII-TOPO vector (Invitrogen). The DNA sequence of the cloned *cysK* PCR product was confirmed prior to subcloning into pCBS1 at the *Nde*I and *Bgl*II restriction sites. Sequencing was performed by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. The GenBank accession number of the *M. barkeri* sequence is AF174138.

Phylogenetic methods. The phylogenetic tree was assembled with the maximum likelihood method (20,000 puzzling steps) implemented in the program PUZZLE 4.0 (16). Sequences were aligned with the CLUSTAL X (1.8) program (17). The unpublished *Thermoplasma acidophilum* sequence was determined by A. Ruepp (Max-Planck-Institut für Biochemie, Martinsried, Germany).

RESULTS AND DISCUSSION

A *cysK*-like *M. barkeri* gene complements a cysteine auxotrophic mutant of *E. coli*. We obtained a plasmid by complementing the cysteine auxotrophic *E. coli* strain NK3 (13) with a genomic library of *M. barkeri* and selecting for growth on minimal medium lacking cysteine. The ability of the isolated plasmid to confer cysteine prototrophy was confirmed upon retransformation of NK3. Sequencing of this DNA revealed open reading frames (ORFs) with high similarity to bacterial *cysK* and *cysE* genes, in addition to a *nifS*-like gene. As shown in Fig. 1, the bacterial *cysE* and *cysK* genes encode the enzymes for biosynthesis of cysteine from serine and sulfide. The *M. barkeri cysK* sequence was PCR amplified and cloned into pCBS1, allowing constitutive expression of the gene from the *E. coli trpS* promoter. The pCBS1-*cysK* construct conferred cysteine prototrophy on the NK3 strain as judged by growth on minimal medium lacking cysteine. Thus, the *M. barkeri cysK* gene was active in vivo in complementing the *E. coli* cysteine-deficient strain.

Characterization of the *cysE* and *cysK* genes of *M. barkeri*. The *cysE* and *cysK* genes of *M. barkeri* are adjacent, separated by 260 nucleotides. This is reminiscent of some of the bacteria, such as *Mycobacterium tuberculosis* and *Thermotoga maritima*, where these genes are in a similar arrangement. The *cysE* and *cysK* sequences of *M. barkeri* have 48.2 and 49.7 mol% G+C contents, respectively, slightly above the 39 to 44 mol% G+C content of the *M. barkeri* species as determined by buoyant density (2).

The *M. barkeri cysK* gene product was highly similar to *O*-acetylserine (thiol)-lyase-A sequences from bacteria and plants (62% identity with *M. tuberculosis*, 60% identity with *Synechocystis* sp., 58% identity with *E. coli*, 57% identity with *Bacillus subtilis*, and 56% identity with *Chlamydomonas reinhardtii*). The sequence of the two *O*-acetylserine (thiol)-lyases (A and B) are clearly closely related (43% identity for the *E. coli* enzymes), and there is also similarity between these enzymes and cystathionine β-synthase from the animal pathway (Fig. 1).

A database search also revealed *cysK*-related genes in other archaea. The *A. pernix* genome (8) contains two ORFs (APE1223 and APE1586) with some similarity (in TblastN searches) to bacterial *cysK*. However, they also show similarity to cystathionine β-synthase. In the phylogenetic analysis (Fig. 2), these ORFs do not group with the bacterial genes. Rather, they cluster with the human cystathionine β-synthase gene and therefore may have a related activity. Furthermore, the genomes of *T. acidophilum* (A. Ruepp and W. Baumeister, unpublished results), *Pyrococcus abyssi*, *Pyrococcus furiosus*, and *Sulfolobus solfataricus* contain orthologs to the bacterial *cysM*-encoded *O*-acetylserine (thiol)-lyase-B. Thus, these organisms may use the bacterial cysteine biosynthesis pathway, if *cysE* orthologs were also present. A search of these genomes did not reveal an obvious ORF; therefore, biochemical analysis will be required. Likewise, the methanogens *M. jannaschii* and *M. thermoautotrophicum* do not contain recognizable *cysE*- and *cysK*-related ORFs, which still leaves open the question of whether a different pathway of cysteine formation exists.

We generated a phylogenetic tree of *cysK*- and *cysM*-related sequences to gain some insight into the relationship of these genes (Fig. 2). The *M. barkeri* gene is related to bacterial *cysK* orthologs and may have been transferred from bacteria. A wide-host-range plasmid from *Synechococcus* sp. containing *cysE*- and *cysK*-like genes was also capable of complementing the respective mutations in *E. coli* (12), as was the gene for *O*-acetylserine (thiol)-lyases from watermelon (*Citrullus vulgaris* [13]). Ignoring thermophilic differences, it is conceivable that complementation of NK3 could also be achieved with the archaeal sequences which group with *cysM* from *E. coli* (Fig. 2). However, this has yet to be demonstrated. Likewise, the possible functions of the *cysK*-like sequences from *A. pernix* (Fig. 2) are intriguing. Finally, while *M. barkeri* may contain a typical bacterial cysteine-biosynthetic pathway, cysteine formation remains unexplained in archaea lacking homologous sequences of the well-described cysteine biosynthetic pathways.

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