Nucleotide sequence of the 16S rRNA gene from thermoacidophilic archaea *Sulfolobus acidocaldarius* ATCC33909

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Three species of genus *Sulfolobus: S.acidocaldarius, S.solfataricus* and *S.shibatae* are known. The complete sequences of the 16S rRNA gene from *S.solfataricus* and *S.shibatae* were reported (1, 2). We have sequenced the 16S rRNA gene of *Sulfolobus acidocaldarius* ATCC33909 for the identification of our thermoacidophilic archaeal isolates.

The complete nucleotide sequence of the 16S rRNA gene from *S.acidocaldarius* as well as its 5′ and 3′ flanking regions were determined by the dideoxynucleotide termination method with FITC-labelled primers using HITACHI SQ-3000 DNA sequencer. The 16S rRNA gene encodes 1493 nucleotides and the G+C content is 62.8%.

Total chromosomal DNA from *S.acidocaldarius* ATCC33909 was cleaved with restriction enzymes (*Sac*I, *Xba*I, *Eco*RI, *Xho*I, *Eco*RI−*Xho*I, *Xba*I−*Xho*I, *Sac*I−*Xba*I) and the resulting restriction patterns were analyzed by Southern hybridization using 885 bp of the 16S rDNA fragment amplified by PCR as a probe. A single band was found for each restriction enzymes and combinations mentioned above. This result suggests that the 16S rRNA gene is present in one copy per genome.

To our surprise, the 16S rRNA gene of *S.acidocaldarius* showed 99.9% homology with the corresponding gene of *S.solfataricus* P1 (only one base is different). These results indicate that the P1 strain which was previously identified as *S.solfataricus* should belong to *S.acidocaldarius*. To confirm the above, we have also sequenced 884 bp of 16S rDNA fragment for *S.solfataricus* IF015331 (derived from DSM1616) amplified by PCR. This sequence showed 92.3% homology with the corresponding sequence of *S.acidocaldarius* and P1. This is also supported by Grogan's report (2) which describes the RNA polymerase component pattern of *Sulfolobus* species. Comparison of those four sequences are summarized on the Table 1. *S.shibatae* showed 98.4% homology with the corresponding gene of *S.solfataricus* IF015331 in the region sequenced. These results altogether suggest that *S.shibatae* and *S.solfataricus* are closely related or may be the same species.

Sequences from flanking regions of the 16S rRNA gene, 259 bases upstream and 328 bases downstream, were searched for transcription signals, structural genes or other significant sequences based on the data available for eubacteria and other archaea. The archaeal promoter consensus, TTTATATA (4, 5) was found in 175 nucleotides upstream from the 5′ end of the 16S rRNA coding region. In *T.acidophilum*, stretches of oligo-T preceded by a relatively stable stem and loop structure that may serve as a termination signal was found downstream from the 3′ of the 16S rRNA coding region (3). However 23S rRNA gene starts at about 200 bases downstream from the 3′ end of the *S.acidocaldarius* 16S rRNA gene, and no tRNA gene was found in the spacer region between 16S and 23S sequences. In *E.coli* or halophilic archaea *Halobacterium cutirubrum*, leader sequence before the 16S rRNA coding region can base-pair with sequence lying between 16S and the first spacer tRNA. These regions come together in the precursor rRNA transcript to form long stem structures that serve as site for processing by RNase III (6, 7). A stem structure of 30 bp with a small bulged loop was also found in *S.acidocaldarius*.

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Table 1. Comparison of 16S rRNA genes (%)

	S.acid	*S.sol*-P1	*S.sol*-IFO	*S.shib*
S.acid	−	99.9	92.3*	90.8
S.sol-P1	−	−	92.3*	90.8
S.sol-IFO	−	−	−	98.4*
S.shib	−	−	−	−

*Partial homology around position 500−1400
S.acid, S.acidocaldarius; S.sol-Pi, *S.solfataricus* P1;
S.sol-IFO, *S.solfataricus* IFO15331; *S.shib, S.shibatae*