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SHORT COMMUNICATIONS

Phylogenetic Position of *Desulfurococcus amylolyticus*

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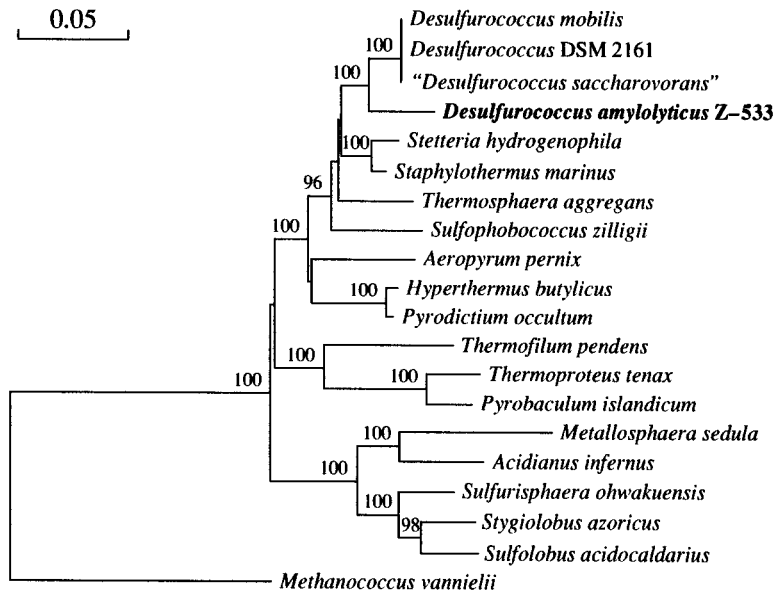
Seven strains of extremely thermophilic, obligately anaerobic cocci were isolated from the terrestrial hot springs of Kamchatka and Kunashir [1]. The temperature optimum for growth of the new isolates was 90–92°C. Investigation of the new isolates revealed their similarity in all major diagnostic features. The best-studied representative of this group, strain Z-533, was described as the type strain of a new species of sulfur-reducing archaea, *Desulfurococcus amylolyticus* [2].

In order to determine the phylogenetic position of this organism and confirm its affiliation to the genus *Desulfurococcus*, the nucleotide sequence of its 16S rRNA gene selectively amplified in vitro with the use of universal primers was analyzed [3]. The amplification was carried out in a reaction buffer composed of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), and 0.001% gelatin. The reaction mixture (100 μl) contained standard concentrations of dNTPs and equimolar concentrations of pA and pH' primers. Thirty amplification cycles with the following temperature profile were performed: DNA denaturation for 30 s at 94°C, annealing of primers for 1 min at 40°C, and elongation for 2 min 30 s at 72°C. After purification on low-melting point agarose, the obtained gene fragment was sequenced on an automatic 373A DNA sequencer using the Ready Reaction Dye Terminator Sequencing Kit with AmpliTag DNA Polymerase (Applied Biosystems). An almost complete 16S rDNA sequence (1405 nucleotides) of strain Z-533 (GenBank accession no. AF 250331), corresponding to positions 28 through 1471 of the *E. coli* numbering, was manually aligned with referent sequences of *Crenarchaeota* type strains. Positions of alignment that had not been sequenced in any of the referent type strains were omitted, and the remaining 1134 nucleotides were used in the analysis. A rooted phylogenetic tree for the archaea under study, with *Methanococcus vannielii* as the outgroup, was constructed and drawn using the TREECON software package [4].



Phylogenetic tree based on 16S rRNA sequence analysis and showing the relationships of strain Z-533 with representatives of the family *Desulfurococcaceae* and other representatives of *Crenarchaeota*. The root was determined by using the sequence of *Methanococcus vannielii* as the outgroup. The scale corresponds to five nucleotide substitutions per 100 nucleotides. The figures show the statistical confidence of the branching order determined by a bootstrap analysis of 100 alternative trees; the values below 95% are not shown.

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The phylogenetic analysis that we carried out showed that strain Z-533 belonged to the family *Desulfurococcaceae* [5] of the order "*Igneococcales*" [6] in the *Crenarchaeota* kingdom (figure), displaying a remote similarity of 92.3–94.7% with the genera *Staphylothermus* [7], *Stetteria* [8], *Sulfophobococcus* [9], and *Thermosphaera* [10]. Strain Z-533 differed from the representatives of these genera in several phenotypic features. Unlike the marine hyperthermophilic *Crenarchaeota* of the genus *Staphylothermus*, it was very sensitive to the presence of NaCl [2]. From the representatives of the genera *Sulfophobococcus* and *Thermosphaera*, strain Z-533 differed in that its growth was stimulated rather than inhibited by sulfur. It differed from *Stetteria* by the inhibitory effect of hydrogen on growth, by its nonobligatory dependence on elemental sulfur, and by the inability to reduce thiosulfate.

The closest relatives of strain Z-533 were the representatives of the genus *Desulfurococcus* (96% 16S rDNA sequence similarity), with which it formed a monophyletic cluster on the phylogenetic tree. A bootstrap analysis showed that strain Z-533 could be affiliated to the genus *Desulfurococcus*a with a 99% confidence. Phenotypically, strain Z-533 is also very close to the genus *Desulfurococcus*, since it is also an organotroph able to ferment peptides and polysaccharides, and this process is stimulated by the presence of elemental sulfur. At present, the genus *Desulfurococcus* comprises two species: *Desulfurococcus mucosus* (the type species of the genus) and *Desulfurococcus mobilis* [5]. It is only the latter species that is represented in the database by two 16S rRNA sequences, and one of them is of the type strain DSM 2161 [11, 12]. The database also includes a 16S rRNA sequence of "*Desulfurococcus saccharovorans*" [6], despite the fact that this organism has not been validated. All three of the sequences present in the database are virtually identical (100% similarity). Although the level of similarity between 16S rDNA sequences of strain Z-533 and representatives of the genus *Desulfurococcus* is fairly high (96%), it is still insufficient to assign the analyzed strain to *D. mobilis* [13]. Based on this evidence, on phenotypic distinctions (the absence of flagella on the cells of strain Z-533 and its capacity to utilize polysaccharides), and a significant difference in the G+C content of the DNA (about 10%), we confirm the affiliation of strain Z-533 with the genus *Desulfurococcus* as a new species under the previously proposed name of *Desulfurococcus amylolyticus*. The new species was described in detail elsewhere [2].

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