

Reclassification of *Desulfurococcus mobilis* as a synonym of *Desulfurococcus mucosus*, *Desulfurococcus fermentans* and *Desulfurococcus kamchatkensis* as synonyms of *Desulfurococcus amylolyticus*, and emendation of the *D. mucosus* and *D. amylolyticus* species descriptions

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Representatives of the crenarchaeal genus *Desulfurococcus* are strictly anaerobic hyperthermophiles with an organotrophic type of metabolism. Since 1982, five *Desulfurococcus* species names have been validly published: *Desulfurococcus mucosus*, *D. mobilis*, *D. amylolyticus*, *D. fermentans* and *D. kamchatkensis*. Recently, the genomic sequences of all five species became available, promoting the refinement of their taxonomic status. Analysis of full-length high-quality 16S rRNA gene sequences shows that the sequences of *D. mobilis* and *D. mucosus* are 100 % identical and differ by 2.2 % from those of *D. amylolyticus*, *D. fermentans* and *D. kamchatkensis*. The latter three sequences differ from each other by 0.1–0.3 % (99.9 % similarity in the *D. amylolyticus*–*D. kamchatkensis* pair and 99.7 % in the pairs involving *D. fermentans*). *In silico* prediction of DNA–DNA hybridization (DDH) values by comparison of genomes using GGDC 2.0 BLAST + at <http://ggdc.dsmz.de/> produced results that correlated with the 16S rRNA gene sequence similarity values. In the *D. mucosus*–*D. mobilis* and *D. amylolyticus*–*D. kamchatkensis* pairs, the predicted DDH values were 99 and 92 %, respectively, much higher than the recommended 70 % species-delimiting DDH value. Between members of different pairs, these values were no higher than 20 %. For *D. fermentans*, its predicted DDH values were around 70 % with *D. amylolyticus* and *D. kamchatkensis* and no higher than 20 % with *D. mobilis* and *D. mucosus*. These results indicated that *D. mobilis* should be reclassified as a synonym of *D. mucosus*, whereas *D. kamchatkensis* and *D. fermentans* should be reclassified as synonyms of *D. amylolyticus*.

Representatives of the genus *Desulfurococcus* were among the first hyperthermophilic archaea to be isolated and described (Zillig *et al.*, 1982; Zillig & Stetter, 1983). All *Desulfurococcus* species are obligate anaerobes with coccoid cells; they belong to the family *Desulfurococcaceae* and the

order *Desulfuroccales* in the archaeal phylum *Crenarchaeota*. The first two species described, *Desulfurococcus mucosus* and *Desulfurococcus mobilis*, were isolated from Icelandic terrestrial hot springs; they were characterized as organotrophs, capable of utilizing a wide range of proteinaceous substrates by fermentation, facultatively coupled with the reduction of elemental sulfur to hydrogen sulfide (Zillig *et al.*, 1982). Other members of the genus were

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization.

isolated from terrestrial hot springs of the Kamchatka peninsula, Russia. *Desulfurococcus amylolyticus* (Bonch-Osmolovskaya *et al.*, 1988, 2001) was the first described member of this genus able to grow not only on peptides but also on polysaccharides (starch). The latter capacity was even more prominent in another Kamchatka isolate, *Desulfurococcus fermentans*, able to grow on several sugars and polysaccharides including cellulosic substrates (Perevalova *et al.*, 2005). The most recently described Kamchatka isolate, *Desulfurococcus kamchatkensis*, differed phenotypically from the other isolates by its ability to utilize non-hydrolysed structural proteins (alpha-keratin) (Kublanov *et al.*, 2009).

Differentiation between *D. mucosus*, *D. mobilis* (Zillig *et al.*, 1982) and *D. amylolyticus* (Bonch-Osmolovskaya *et al.*, 1988) was based on phenotypic distinctions. In addition, differences in 16S rRNA genes from those of *D. mobilis* (Kjems *et al.*, 1987) and *D. amylolyticus* (Tourova *et al.*, 2000) were then used for the proposal of *D. fermentans* (Perevalova *et al.*, 2005) and *D. kamchatkensis* (Kublanov *et al.*, 2009). However, later it became clear that the quality of some of these sequences was quite low, and the results of their comparison should be treated with caution. At the same time, hybridization with species-specific oligonucleotide probes (Perevalova *et al.*, 2003) indicated that *D. mucosus* and *D. mobilis* probably represented the same species.

The first sequenced complete genome of a *Desulfurococcus* representative was that of *D. kamchatkensis* (Ravin *et al.*, 2009), followed by the complete genomes of *D. mucosus* (Wirth *et al.*, 2011) and *D. fermentans* (Susanti *et al.*, 2012). At present, complete or draft (*D. mobilis* and *D. amylolyticus*) genome sequences of the type strains of all five species are available in NCBI GenBank. The genome size of desulfurococci varies from 1 198 142 bp in *D. mobilis* to 1 384 116 bp in *D. fermentans*. The genome DNA G+C values are about 45 mol% in *D. amylolyticus*, *D. kamchatkensis* and *D. fermentans* and about 53 mol% in *D. mucosus* and *D. mobilis*. The availability of all five genomes provides an opportunity to refine the taxonomic structure of the genus *Desulfurococcus* using *in silico* DNA–DNA hybridization (DDH).

Analysis of full-length high-quality 16S rRNA gene sequences, which had been available (*D. mobilis*, Kjems *et al.*, 1987) or became available from genome assemblies of the type strains of other *Desulfurococcus* species, shows that the sequences of *D. mobilis* and *D. mucosus* are 100 % identical and differ by 2.2 % from those of *D. amylolyticus*, *D. fermentans* and *D. kamchatkensis*. The latter three sequences differ from each other by 0.1–0.3 % (99.9 % similarity in the *D. amylolyticus*–*D. kamchatkensis* pair and 99.7 % in the pairs involving *D. fermentans*).

For the *in silico* hybridization, we used the genome sequences of the type strains of *Desulfurococcus* species from NCBI GenBank. *In silico* prediction of DDH values was performed using GGDC 2.0 BLAST+ at the DSMZ GGDC site (Meier-Kolthoff *et al.*, 2013) and produced results that correlated

with the 16S rRNA gene sequence similarity values. DDH values predicted by the recommended formula 2 are presented in Table 1. In the *D. mucosus*–*D. mobilis* and *D. amylolyticus*–*D. kamchatkensis* pairs, these values were 99 and 92 %, respectively, much higher than the recommended 70 % species-delimiting DDH value (Tindall *et al.*, 2010). The predicted DDH values between members of different pairs were no higher than 20 %. For *D. fermentans*, its predicted DDH values were around 70 % with *D. amylolyticus* and *D. kamchatkensis*, and no higher than 20 % with *D. mobilis* and *D. mucosus*. We also calculated average nucleotide identity (ANI) values between the genomes by using the ANI calculator (Goris *et al.*, 2007; <http://enve-omics.emory.edu/ani/>) with default parameters. In the *D. mucosus*–*D. mobilis* and *D. amylolyticus*–*D. kamchatkensis* pairs, these values were 99.9 and 99.2 %, respectively, showing affiliation of the members of each pair with the same species. The ANI values in the *D. amylolyticus*–*D. fermentans* and *D. kamchatkensis*–*D. fermentans* pairs were 96.1 and 96.7 %, respectively, the recommended species-delimiting value being 95 % (Goris *et al.*, 2007).

Comparison of proteins encoded by the genomes was performed using the SEED (Overbeek *et al.*, 2005) sequence-based comparison tool. The obtained comparison tables were processed using a simple in-house program that calculated the following overall results of the comparison of the proteomes by SEED. The deduced proteomes contained from 1272 (*D. mobilis*) to 1475 (*D. fermentans*) proteins. In total, 1045 proteins were encoded in the genomes of all five desulfurococci (*Desulfurococcus* core protein-encoding genes at the current state of knowledge), 404 proteins were encoded in only one of the five genomes (from 37 in *D. mucosus* to 120 in *D. fermentans*) and 475 proteins were encoded in two to four genomes. Thus, in total, the *Desulfurococcus* panproteome includes, at the present state of knowledge, 1924 proteins.

Average amino acid identity values of orthologues were 99.5 % in the *D. mucosus*–*D. mobilis* pair (proteins without orthologues in the counterpart deduced proteome comprised 13 and 8 %, respectively) and 98.9 % in the *D. amylolyticus*–*D. kamchatkensis* pair (8 and 10 % of proteins had no orthologues in the counterpart proteome). Average amino acid identity values of orthologues between members of different pairs were about 70 %, with the share of proteins without orthologues in counterpart proteomes being about 15 %. For *D. fermentans*, average amino acid identity values of its proteome with those of *D. amylolyticus* and *D. kamchatkensis* were about 96 %, with 12–14 % of proteins without orthologues in the proteomes. With the proteomes of *D. mucosus* and *D. mobilis*, average amino acid identities were about 70 %, with the share of proteins without orthologues in counterpart proteomes being about 15 %.

Thus, it is evident from the complete 16S rRNA gene sequence similarity values, ANI values and predicted DDH values, as well as from comparisons of the deduced proteomes, that all validly described representatives of the

Table 1. *In silico* genome hybridization of five *Desulfurococcus* representatives that had validly published species names

Predictions of *in vitro* DDH and, in parentheses, ANI values. ND, Not determined. Reciprocal comparisons were performed only for pairs involving *D. fermentans*.

	<i>D. mucosus</i> DSM 2162 ^T	<i>D. mucosus</i> DSM 2161 (former <i>D. mobilis</i>)	<i>D. amylolyticus</i> Z-533 ^T	<i>D. amylolyticus</i> Z-1312 (former <i>D. fermentans</i>)	<i>D. amylolyticus</i> 1221n (former <i>D. kamchatkensis</i>)
<i>D. mucosus</i> DSM 2162 ^T	100.0 ± 0.0 % (100 %)	98.6 ± 0.6 % (99.9 %) ND (100.0 %)	18.0 ± 2.3 % (79.9 %) 17.7 ± 2.2 % (78.4 %)	17.3 ± 2.2 % (80.1 %) 17.7 ± 2.2 % (78.4 %)	18.0 ± 2.3 % (79.7 %) 18.3 ± 2.3 % (78.6 %)
<i>D. mucosus</i> DSM 2161 (former <i>D. mobilis</i>)					
<i>D. amylolyticus</i> Z-533 ^T		ND (100.0 %) 69.1 ± 2.9 % (96.1 %)		69.1 ± 2.9 % (96.1 %) 100.0 ± 0.0 % (100.0 %)	92.3 ± 1.8 % (99.2 %) 69.5 ± 2.9 % (96.7 %)
<i>D. amylolyticus</i> Z-1312 (former <i>D. fermentans</i>)					
<i>D. amylolyticus</i> 1221n (former <i>D. kamchatkensis</i>)			69.5 ± 2.9 % (96.7 %)	100.0 ± 0.0 % (100 %)	

genus *Desulfurococcus* belong to one of two species, either to *D. mucosus* or to *D. amylolyticus*. Thus, *D. mobilis* should be reclassified as a synonym of *D. mucosus*, whereas *D. kamchatkensis* and *D. fermentans* should be reclassified as synonyms of *D. amylolyticus*.

To gain additional information on the taxonomic structure of the genus *Desulfurococcus*, we isolated 21 new strains belonging to this genus from hot springs of Kamchatka, Kuril Islands, Azores and Iceland on media with peptides or polysaccharides as the growth substrates and elemental sulfur as the electron acceptor. 16S rRNA gene sequence analysis revealed 99–100 % similarity with the type strains of either *D. amylolyticus* or *D. mucosus*. Most strains belonged to *D. amylolyticus*, and only one strain of *D. mucosus* was isolated from an Iceland hot spring sample. This might indicate that the distribution of *D. mucosus* is limited to Icelandic thermal habitats, while that of *D. amylolyticus* has a much wider geography. This conclusion is supported by BLASTN searches in the NCBI nr/nt database.

Note also that, at the time of writing, only representatives of *D. amylolyticus* were found to be able to grow on polysaccharides, while strains of *D. mucosus* grow only on peptides.

Emended description of *Desulfurococcus mucosus* Zillig and Stetter, 1983

The description is based mainly on that of Zillig *et al.* (1982). Cells are cocci, obligately anaerobic and hyperthermophilic. Cells of some strains are non-flagellated and embedded in a slimy matrix. Cells of other strains are peritrichously flagellated and not embedded in matrix. Cell envelope is flexible, and composed of subunits. Growth is optimal at pH 5.5–6.0 and 85 °C. Utilize proteins (casein and casein hydrolysate) and peptides (yeast extract, bacto-tryptone). Elemental sulfur stimulates growth. The DNA G+C content of the type strain is 53 mol%. Isolated from Iceland hot springs. The type strain is DSM 2162^T (=ATCC 35584^T=JCM 9187^T).

Emended description of *Desulfurococcus amylolyticus* Bonch-Osmolovskaya *et al.*, 2001

Cells are cocci, obligately anaerobic and hyperthermophilic. Can be flagellated. Growth is optimal at pH 6.0–6.5 and 80–92 °C. Utilize sugars (glucose, sucrose, fructose, lactose, ribose), polysaccharides (starch, dextran, pectin, cellulose), amino acids (alanine, phenylalanine, serine, tyrosine, ornithine), proteins (casein, casein hydrolysate, keratins, albumin, gelatin) and peptides (yeast extract, tryptone, peptone, beef extract). Elemental sulfur stimulates growth of most strains. The products of fermentation are acetate and molecular hydrogen; hydrogen sulfide is formed in the presence of sulfur. The DNA G+C content of the type strain is 45 mol%. The type strain is Z-533^T (=DSM 3822^T=VKM Z-533^T).

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