

Confusion in the assignments of *Sulfolobus* sequences to *Sulfolobus* species

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Differences between corresponding nucleic acid sequences ascribed to the same *Sulfolobus* species and the identity of sequences ascribed to different *Sulfolobus* species indicated confusion regarding these assignments. Our laboratory has been involved in this confusion and we believe we understand it. This note serves to warn people concerned and to help eliminate the confusion.

The confusion has two roots. First, T.D.Brock, who had isolated and described *Sulfolobus acidocaldarius* (1), distributed samples of the isolate 98/3 both to the Deutsche Sammlung für Mikroorganismen (DSMZ) which keeps it under the designation *Sulfolobus acidocaldarius*, DSM 639, and to T.A.Langworthy from whom colleagues including C.R.Woese, obtained it and used it as a source of RNA and DNA for sequence determination. A 16S rRNA oligonucleotide catalogue (2) and a 5S rRNA sequence (3) were thus assigned to *S.acidocaldarius*.

We obtained two novel *Sulfolobus* isolates, P1, (DSM 1616) and P2, (DSM 1617), from the Pisciarelli hot springs in Italy and found them to differ characteristically from DSM 639, and therefore created a novel species, *S.solfataricus* (4). Among other features, the DNA-dependent RNA polymerase (RNAP) component patterns of DSM 639, (now considered the type strain of *S.acidocaldarius* and DSM 1616, (now considered the type strain of *S.solfataricus*) were characteristically distinct. In this comparison, the strain derived from Brock's isolate 98/3 via T.A.Langworthy, yielded an RNAP exhibiting the same component pattern as that from DSM 1616 (4). Thus, it appears that the same isolate had given rise to two different *Sulfolobus* species. If DSM 639 is *S.acidocaldarius*, the strain obtained from Langworthy must be assigned to *S.solfataricus*. Possibly, the original isolate, which had been purified by dilution, still contained both species and different growth conditions led to their segregation.

The 16S rRNA oligonucleotide catalog ascribed to *S.acidocaldarius* by Woese *et al.* (2) must consequently be that of *S.solfataricus* and, likewise, the 5S rRNA sequence determined by Stahl *et al.* (3) must be that of *S.solfataricus*. Proof for the latter suggestion is furnished by the finding that the sequence of a 5S rRNA from a third acknowledged *S.solfataricus* strain, MT4, isolated by De Rosa *et al.* and originally named Caldariella (5), is identical to that published by Stahl *et al.* (6, 3).

Second, after we had deposited strains P1, (DSM 1616) and P2, (DSM 1617) at the DSM, the P1 culture kept in our laboratory unnoticeably became contaminated by *S.acidocaldarius* and was then distributed to colleagues, including C.R.Woese, as being *S.solfataricus*. When we became aware of inconsistencies

in sequence comparisons, we isolated DNA-dependent RNAP from the contaminant which had overgrown the original strain and found it to exhibit the component pattern characteristic for *S.acidocaldarius*. Consequently, all sequences determined from derived cultures should be ascribed to *S.acidocaldarius*, e.g. the 16S rRNA total sequence assigned to *S.solfataricus* by Olsen *et al.* (7) and the sequence of a 5S rRNA determined by Durovic *et al.* (8), who recognized the erroneous assignment of the strain used as source. Fortunately, DSM 1616 and 1617 now kept by the DSM were confirmed to be uncontaminated strains of *S.solfataricus* by their RNAP component patterns and by restriction fragment patterns of chromosomal DNA.

Two failures in maintaining pure cultures have thus led to reciprocally erroneous sequence assignments. A preliminary analysis of this confusion has been published by Grogan *et al.* (9).

The confusion was also recognized by Kurosawa and Itoh (10), who showed the 16S rRNA sequence determined by Olsen *et al.* (7) to be erroneously assigned to *S.solfataricus* and found 16S rRNA from ATCC 33903 to differ significantly from that of DSM 1616. This was expected because ATCC 33903 is derived from DSM 639, the type strain of *S.acidocaldarius*, and DSM 1616 is the type strain of *S.solfataricus*.

In conclusion, strain DSM 639 and its derivatives, including ATCC 33903, represent *S.acidocaldarius*; strains P1 (DSM 1616), P2 (DSM 1617) (4) and MT4 (5) represent *S.solfataricus*; and DSM 5389 represents *S.shibatae* (9). These strains should be used as references for the assignment of *Sulfolobus* sequences to different species.

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