Phylogenetic Placement of *Sarcina ventriculi* and *Sarcina maxima* within Group I *Clostridium*, a Possible Problem for Future Revision of the Genus *Clostridium*

Request for an Opinion

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The 16S rRNA gene sequences of *Sarcina ventriculi* DSM 286^T (T = type strain) and *Sarcina maxima* DSM 316^T were determined. Phylogenetic analysis revealed that these two species are closely related to each other and belong to group I *Clostridium* (sensu Johnson and Francis). The implications of these phylogenetic findings for future revision of the genus *Clostridium* are discussed.

In 1842 Goodsir described some bundle-shaped organisms observed in the stomach fluid of a patient with digestive tract problems and named these organisms *Sarcina ventriculi* (12). This obligately anaerobic microorganism was first cultured by Beijerinck (1, 2) and has since been isolated from human and animal stomach contents and feces, soil, and the surfaces of cereal seeds (5). After the original description of *S. ventriculi*, many other mostly aerobic, packet-forming cocci were assigned to the genus *Sarcina* as separate species. The morphological criterion of packet formation, however, proved to be unsuitable for genus delineation as studies in the 1960s to 1970s revealed major phenotypic heterogeneity within the genus. Most *Sarcina* species have now been placed in other genera (i.e., the genus *Micrococcus* for some aerobic nonsporing species; the genus *Sporosarcina* for aerobic sporing species; the genus *Methanosarcina* for anaerobic methane fermenters; and the genus *Halococcus* for obligate halophiles [3, 4]), and currently only two species, *S. ventriculi* and *Sarcina maxima*, are retained in the genus *Sarcina* (5). Studies in which Fox et al. (11) used 16S rRNA oligonucleotide cataloging revealed a possible phylogenetic affinity between *S. ventriculi* and some species of the anaerobic spore-forming genus *Clostridium* (*S*_{AB} [see reference 10 for definition] with *Clostridium butyricum* [the type species], *Clostridium scatologenes*, and *Clostridium pasteurianum*, 0.5 to 0.65). Oligonucleotide cataloging has in recent years been largely superseded by complete or partial 16S rRNA (or rRNA gene) sequencing. Complete (or nearly complete) sequences provide far more precision for determining phylogenetic relationships (20). A large number of clostridial 16S rRNA sequences have been published in recent years (15, 17, 18), and these sequences now make it possible to determine more precisely the phylogenetic affinities of the sarcinas. In this paper we describe the 16S rRNA gene sequences of the type strains of *S. ventriculi* and *S. maxima* and the results of a comprehensive phylogenetic analysis of these organisms and the clostridia.

S. ventriculi DSM 286^T (T = type strain) and *S. maxima* DSM 316^T were grown anaerobically in *Sarcina* broth (5 g of

peptone, 5 g of yeast extract, 30 g of glucose, 1 liter of H₂O; pH 6.0) at 37°C, and chromosomal DNAs were prepared as described by Lawson et al. (14). The 16S rRNA genes were amplified by a PCR, purified, and sequenced directly as described previously (19). The sequences which we determined were aligned with sequences of reference organisms obtained from the EMBL data library by using the PILEUP, PRETTY, and UGLY programs of the Genetics Computer Group Sequence Analysis package (7) and a VAX computer. A continuous stretch of 1,343 bases was used in the analysis. Distances were calculated by using the DNADIST program of the Phylogeny Inference package (9), and a bootstrapped phylo-

FIG. 1. Unrooted phylogenetic tree showing the positions of *S. ventriculi* and *S. maxima* within group I *Clostridium*. Bootstrap values greater than 90% are given at the branching points.

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TABLE 1. Similarity values for the 16S rRNA genes of *Sarcina* species and their closest relatives

Taxon	EMBL accession no.	% Similarity with:										
		<i>Sarcina maxima</i> ^a	<i>Sarcina ventriculi</i>	<i>Clostridium acetobutylicum</i>	<i>Clostridium aurantibutyricum</i>	<i>Clostridium beijerinckii</i>	<i>Clostridium botulinum</i> type E	<i>Clostridium butyricum</i>	<i>Clostridium carnis</i>	<i>Clostridium parapertingens</i>	<i>Clostridium parapatrificum</i>	<i>Clostridium perfringens</i>
<i>Sarcina ventriculi</i>	X76649	98.5										
<i>Clostridium acetobutylicum</i>	X68182	92.9	92.7									
<i>Clostridium aurantibutyricum</i>	X68183	92.9	92.3	95.9								
<i>Clostridium beijerinckii</i>	X68179	93.0	93.1	99.2	95.8							
<i>Clostridium botulinum</i> type E	X68170	92.9	92.8	95.8	95.0	96.6						
<i>Clostridium butyricum</i>	X68177	93.3	93.3	97.4	95.2	97.6	96.2					
<i>Clostridium carnis</i>	M59091	94.1	93.3	95.1	95.7	95.3	95.3	95.0				
<i>Clostridium parapertingens</i>	M59102	94.4	94.0	95.3	96.3	95.3	95.1	95.7	96.0			
<i>Clostridium parapatrificum</i>	X73445	93.9	93.8	95.3	97.1	95.9	95.8	96.0	96.4	98.8		
<i>Clostridium perfringens</i>	M59103	93.3	93.3	93.4	92.8	93.6	93.5	93.0	94.2	93.8	93.8	
<i>Clostridium puniceum</i>	X73444	93.4	93.2	97.1	94.5	97.4	96.0	96.9	95.3	95.1	94.4	93.0

^a The EMBL accession number of *S. maxima* is X76650.

genetic tree (Fig. 1) was produced by using the NEIGHBOR, DNABOOT, and CONSENSE programs of the same package. The 16S rRNA gene sequences which we determined were deposited in the EMBL data library under accession numbers X76649 and X76650.

The partial 16S rRNA gene sequences of *S. ventriculi* and *S. maxima* consisted of 1,461 and 1,399 nucleotides, respectively, and exhibited 98.5% similarity (corresponding to 20 nucleotide differences). Sequence similarity calculations revealed that *S. ventriculi* and *S. maxima* were highly related to group I *Clostridium* of Johnson and Francis (13) (approximate 16S rRNA sequence similarity range, 89 to 94%). The similarity values obtained with the closest relatives of both *Sarcina* species, as calculated by using the GAP program of the Genetics Computer Group package, are shown in Table 1. Significantly lower levels of sequence relatedness (<85%) were obtained with other clostridial lineages (data not shown). The results of distance matrix analyses confirmed these affinities, with the two *Sarcina* species clustering well within the confines of this major *Clostridium* group (Fig. 1).

The genus *Clostridium*, as presently constituted, contains more than 100 species and is phylogenetically extremely heterogeneous. 16S rRNA sequencing has revealed that members of the genus *Clostridium* belong to several major lines of descent, some of which almost certainly represent new or distinct genera (15, 17, 18). Almost one-half of all currently described clostridial species are members of a single clade, referred to as group I *Clostridium* of Johnson and Francis (13). The genus *Clostridium* is clearly in need of extensive taxonomic revision, and there is a growing view (6, 15) that group I *Clostridium*, which includes the type species, *C. butyricum*, could form the nucleus of a redefined genus. Restricting the genus *Clostridium* to the group I organisms would result in a genealogically coherent, albeit deep, genus and would do much to resolve the phylogenetic absurdity of the present system. Placement of *S. ventriculi*, the type species of the genus *Sarcina*, in group I *Clostridium*, however, presents a serious nomenclatural problem for any such future proposed generic emendation. The genus *Sarcina* (Goodsir) was proposed in

1842 (12) and as such has nomenclatural priority over the genus *Clostridium* (Prazmowski), which was described in 1880 (16). There is no question that nomenclatural rules are necessary and should be followed in most cases. However, strict adherence to nomenclatural priority would in this case result in the loss of the name *Clostridium*, with all *Clostridium* species becoming *Sarcina* species! Such a change would, in our opinion, be quite unacceptable to the scientific community, as the name *Clostridium* is widely used and the genus contains several important human and animal pathogens (e.g., *Clostridium botulinum*, *Clostridium perfringens*, and *Clostridium tetani*). Abandoning the name *Clostridium* also makes no phenotypic sense and would cause unnecessary confusion as clostridial species lack the typical parcel shape implied by the name *Sarcina*.

rRNA (or gene) sequencing is revolutionizing microbial systematics and provides an immensely powerful method for elucidating phylogenetic relationships. It is important that taxonomists capitalize on this revolution and do not shirk from constructing more phylogenetically meaningful classification schemes. As the results of rRNA sequence analysis fundamentally challenge many currently accepted classifications (including our perception of which phenotypic traits are good indicators of relatedness), a more pragmatic interpretation of the rules of priority may become necessary in order to minimize nomenclatural confusion. We believe that a more phylogenetically based classification of the clostridia is an achievable goal for the next few years, with the group I organisms forming the basis of a redefined genus *Clostridium*. From a purely practical point of view, we feel that if this occurs, the rule of priority should be set aside and *Sarcina* species should be included in an emended genus *Clostridium*. We therefore request an opinion from the Judicial Commission as to whether it would be an acceptable option to conserve the generic name *Clostridium* Prazmowski 1880 (type species, *C. butyricum* Prazmowski 1880).

After the submission of the manuscript for this paper, the rRNA gene sequence of *S. ventriculi* GIFU 7886 was published by Ezaki et al. (8). This sequence exhibits a level of similarity

of 95.9% (corresponding to 54 base differences when 1,332 positions were compared) with the sequence of the *S. ventriculi* type strain. Ezaki et al. (8) included only one member of *Clostridium* group I (*C. perfringens* ATCC 13124^T) and therefore did not describe the precise genealogical placement of *S. ventriculi* within this major group.

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