Reclassification of *Sphaerobacter thermophilus* from the subclass *Sphaerobacteridae* in the phylum *Actinobacteria* to the class *Thermomicrobia* (emended description) in the phylum *Chloroflexi* (emended description)

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*Sphaerobacter thermophilus* was originally classified as the deepest branching member of the phylum *Actinobacteria* (high-G+C, Gram-positive bacteria) based on 16S rRNA gene comparative analysis. However, the analysis lacked suitable outgroups, and additional 16S rRNA gene sequences indicate that it is most closely related to *Thermomicrobium roseum*, which it also resembles phenotypically. Furthermore, both species are reproducibly affiliated with the phylum *Chloroflexi* (green non-sulfur bacteria), despite *T. roseum* currently being classified in its own phylum, the *Thermomicrobia*. Transfer of *Sphaerobacter* to the class *Thermomicrobia*, and transfer of the class *Thermomicrobia* to the phylum *Chloroflexi*, are proposed. Descriptions for the phylum *Chloroflexi* and the class *Thermomicrobia* are emended to reflect the proposed changes in classification.

*Sphaerobacter thermophilus* is a Gram-positive, non-spore-forming bacterium that was isolated from an aerobic thermophilic sludge (Hensel et al., 1989). Comparative analysis of a partial 16S rRNA sequence obtained by reverse transcriptase sequencing (GenBank/EMBL/DDBJ accession no. X53210) suggested, at the time, that *S. thermophilus* was the most deeply branching member of the phylum *Actinobacteria* (high-G+C, Gram-positive bacteria; Demharter et al., 1989). This phylogenetic assignment has been disseminated through the literature (Carreto et al., 1996; Embley & Stackebrandt, 1994; Monciardini et al., 2003; Rainey & Stackebrandt, 1993; Rappé et al., 1999; Shoenner & Tyagi, 1995; Wiegel, 1998), culminating in a formal classification as a novel suborder of the *Actinobacteria* (Stackebrandt et al., 1997). However, the initial analysis by Demharter et al. (1989) was compromised by insufficient reference sequences (outgroups) and, as more 16S rRNA gene sequences have become available, it is apparent that *S. thermophilus* is not reproducibly affiliated with the *Actinobacteria* and is instead a member of the phylum *Chloroflexi* (green non-sulfur bacteria; Björnsson et al., 2002; Hugenholtz et al., 1998).

We have obtained an almost complete 16S rRNA gene sequence for the type strain of *S. thermophilus* (DSM 20745T) using methods described previously (Rainey et al., 1996) and confirm that its phylogenetic placement is within the *Chloroflexi* (Fig. 1), on the basis of evolutionary distance and Bayesian inference as previously described (Janssen & Hugenholtz, 2003). Fig. 1 shows only the *Chloroflexi* phylum sequences (ingroup) used in the analysis; they were rigorously tested for monophyly using a variety of bacterial outgroup sequences representing multiple phyla (not shown; datasets available on request), as described previously (Dalevi et al., 2001).

The closest relative of *S. thermophilus* is *Thermomicrobium roseum* (Fig. 1), which it resembles phenotypically. Both are thermophilic, obligately aerobic, chemoheterotrophic, catalase-positive, non-motile, non-spore-forming, short irregular-shaped rods with similar DNA G+C content (Demharter et al., 1989; Garrity & Holt, 2001b). The cell membrane of *T. roseum* contains distinctive long-chain diols instead of the familiar glycerol-based lipids (Pond et al., 1986) and these have been postulated to contribute to membrane stabilization at high temperatures (Pond &

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Sphaerobacter thermophilus* DSM 20745T is AJ420142.
Langworthy, 1987). *S. thermophilus* has not been investigated for its cell membrane composition, but given its relationship to *T. roseum* and the fact that it is a thermophile, it may also contain diol-based lipids.

*T. roseum* has long been recognized to be affiliated with the phylum *Chloroflexi* on the basis of 16S rRNA comparative analysis (Woese, 1987), but is currently classified in its own bacterial phylum, *Thermomicrobia* (Garrity & Holt, 2001b). We propose to transfer the class *Thermomicrobia* to the phylum *Chloroflexi* to acknowledge this phylogenetic relationship formally (Fig. 1). In addition to the class *Thermomicrobia*, at least four other major lineages exist in the phylum *Chloroflexi* (Fig. 1), three of which now have cultivated representatives: the well-characterized class *Chloroflexi*, containing the phototrophic genera *Chloroflexus*, *Chloronema*, *Heliothrix* and *Oscillochloris* and the chemoheterotrophic genus *Herpetosiphon* (Garrity & Holt, 2001a); the 'Dehalococcoidetes', containing the tetrachloroethene-respiring coccus *Dehalococcoides ethenogenes* (Maymó-Gatell et al., 1997); and, most recently, the 'Anaerolineae', containing the thermophilic filamentous bacteria *Anaerolinea thermophila* and *Caldilinea aerophila* (Sekiguchi et al., 2003). The 'Anaerolineae' are not monophyletic in all analyses (note node support <90% in Fig. 1) and may need to be subdivided as new sequences come to hand. Detailed comparative studies of *S. thermophilus* and other members of the *Chloroflexi* may reveal unifying chemotaxonomic and ultrastructural features of this interesting phylum. For example, some members of the *Chloroflexi*, including *S. thermophilus*, have unusual cell envelopes rich in protein and lacking significant amounts of peptidoglycan (Demharter et al., 1989; Maymó-Gatell et al., 1997; Merkel et al., 1980).

We conclude, on the basis of 16S rRNA gene sequence comparative analysis, that *S. thermophilus* should be reassigned to the class *Thermomicrobia* in the phylum *Chloroflexi*. We suggest retaining the order and family proposed for *S. thermophilus* (Sphaerobacterales and Sphaerobacteraceae, respectively; Stackebrandt et al., 1997) in the class *Thermomicrobia* until a more rigorous comparison of *S. thermophilus* and *T. roseum* is performed and further representatives of this class are characterized.

Emended description of the phylum *Chloroflexi* 
Garrity and Holt 2001

The phylum *Chloroflexi* is a deep-branching lineage of the *Bacteria*. On the basis of comparative 16S rRNA gene sequence analysis of isolates and environmental clones, the phylum can be divided into at least five major subdivisions,
including the classes Chloroflexi and Thermomicrobia. Mostly Gram-negative, filamentous bacteria with unusual cell envelopes. No peptidoglycan or atypical peptidoglycan present in cell walls. Lipopolysaccharide-containing outer membrane not present in studied representatives.

Emended description of the class Thermomicrobia Garrity and Holt 2001


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References


