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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 phylum-level 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; Shooner & 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 20745^T) 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 20745^T is AJ420142.

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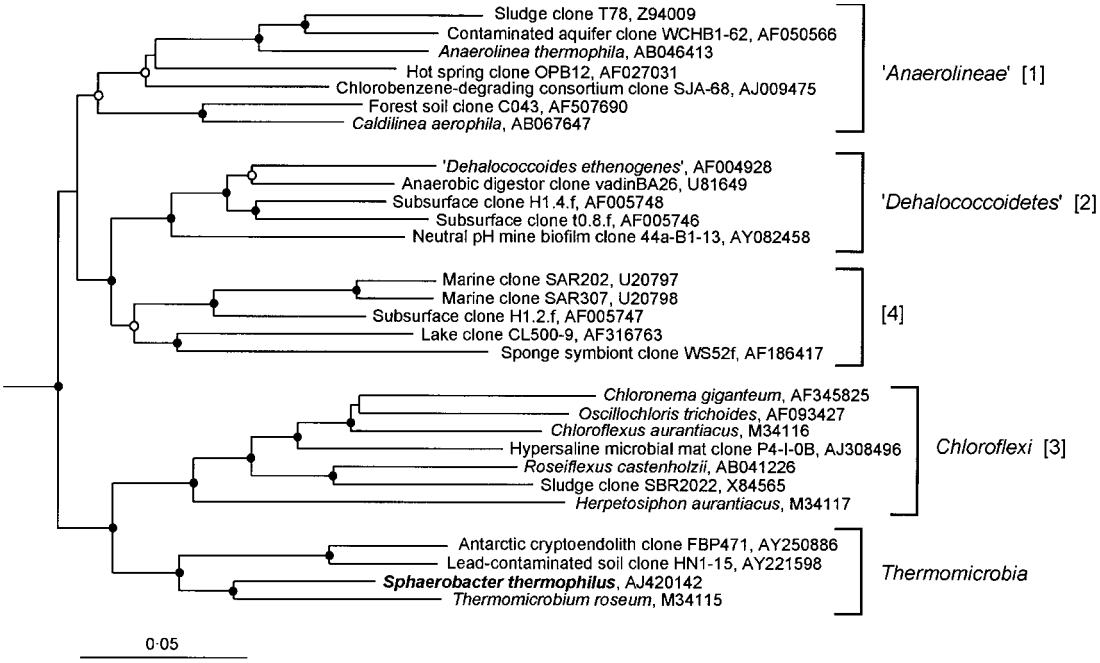

Fig. 1. Evolutionary distance tree of the phylum *Chloroflexi* based on comparative analysis of 16S rRNA gene sequences showing the phylogenetic affiliation of *S. thermophilus* (shown in bold). Branch points supported by evolutionary distance bootstrap resampling and Bayesian posterior probabilities are indicated by filled circles (bootstrap proportion and posterior probability values >90 %) and open circles (both values >75 %). *Escherichia coli* (X80725), *Bacillus subtilis* (D26185) and *Corynebacterium diphtheriae* (X84248) were used as outgroups (not shown). Subdivision (class)-level groupings are indicated by brackets to the right of the figure. Class names follow Garrity & Holt (2001a, b), and informally proposed classes (in quotation marks) are based on representative species names. The numbers in brackets following these names are according to the subdivision numbering in Hugenholtz *et al.* (1998). Bar, 0·05 changes per nucleotide.

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,

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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

The class *Thermomicrobia* subdivides into two orders with validly published names: *Thermomicrobiales* Garrity and Holt 2001 and *Sphaerobacterales* Stackebrandt, Rainey and Ward-Rainey 1997. Pleomorphic, non-motile, non-spore-forming rods. Atypical proteinaceous cell walls.

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