Phylogenetic position of *Chitinophaga pinensis* in the *Flexibacter-Bacteroides-Cytophaga* phylum

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Comparison of the 16S rRNA gene sequence determined for *Chitinophaga pinensis* showed that this species is most closely related to *Flexibacter filiformis* in the *Flexibacter-Bacteroides-Cytophaga* phylum. These two chitinolytic bacteria, which are characterized by transformation into spherical bodies on ageing, belong to a strongly supported lineage that also includes *Cytophaga arvensicola*, *Flavobacterium ferrugineum* and *Flexibacter sancti*. The lineage is distinct from the microcyst-forming species *Sporocytophaga myxococcoides*.

Keywords: *Chitinophaga pinensis*, 16S rRNA, phylogeny, bacterial taxonomy, *Flexibacter-Bacteroides-Cytophaga* phylum

In 1981, Sangkhobol & Skerman described the species *Chitinophaga pinensis* to include five strains of a long, filamentous, chitinolytic, gliding bacterium that transforms on ageing into spherical bodies. The authors likened the coccoid bodies to the microcysts of *Sporocytophaga myxococcoides* (Leadbetter, 1989). Sangkhobol and Skerman noted that the myxospores germinated without any clear evidence of capsule shedding as previously described by Leadbetter (1963) for *Sporocytophaga myxococcoides*, nor did they exhibit the high refractility of *Sporocytophaga myxococcoides* microcysts under phase microscopy. No comparison with other characteristics of *Sporocytophaga myxococcoides* myxospores such as heat and desiccation resistance was made. *Chitinophaga pinensis* was differentiated from *Sporocytophaga myxococcoides* on the bases of cells more than four times the length of *Sporocytophaga myxococcoides*, an ability to hydrolyse chitin but not cellulose, and a G + C content of its genomic DNA of 43–46 mol% compared with 36 mol% for *Sporocytophaga myxococcoides*.

Skerman (1989) later introduced the term microcyst to describe the spherical coccoid bodies but used this term interchangeably with the terms myxospores and resting stage without any clear definitions. Reichenbach (1992) has disputed the formation of microcysts by *Chitinophaga pinensis* and drew attention to the similarities between the morphology of *Chitinophaga pinensis* and *Flexibacter filiformis* (Reichenbach, 1992).

Current knowledge of the phylogenetic relationships of the flexibacteria derived from 16S rRNA catalogues (Paster et al., 1985) shows that *Flexibacter filiformis* ('*Flexibacter elegans*' strain Fx e1) and *Sporocytophaga myxococcoides* are on phylogenetically distinct lines within the *Flexibacter-Bacteroides-Cytophaga* phylum.

To date, no 16S rRNA sequence has been available for *Chitinophaga pinensis* to determine its phylogenetic relationship with the other two species of gliding flexibacteria (*Flx. filiformis* and *Sporocytophaga myxococcoides*) reported to form spherical bodies (myxospores, microcysts or resting stages) on ageing. In this study we have sequenced the 16S rDNA of the *Chitinophaga pinensis* type strain ACM 2034T (= DSM 2588T) and compared this with the available sequences of *Flexibacter filiformis*, *Sporocytophaga myxococcoides* and other reference sequences in the *Flexibacter-Bacteroides-Cytophaga* phylum.

The sequencing of the 16S rDNA of *Chitinophaga pinensis* and the phylogenetic analysis was carried out using methods previously described (Sly et al., 1998). Direct PCR amplification of 16S rDNA was performed and the PCR products were purified using Promega Magic PCR Prep DNA Purification according to the manufacturer's instructions. The PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) was used with the primers 27f,
The dendrogram of relationships (Fig. 1) inferred from a neighbour-joining analysis of the corrected dissimilarity values over a length of 987 nucleotides (restricted by a short 1263 nucleotide sequence for Cytophaga arvensicola) indicated that Chitinophaga pinensis belongs to a strongly supported lineage in the Flexibacter–Bacteroides–Cytophaga phylum. Members of the lineage also include Flavobacterium ferruginium, Cytophaga arvensicola, Flexibacter sancti, and its nearest relative, Flexibacter filiformis. Nakagawa & Yamasato (1993) have suggested previously that F. ferruginium, Flexibacter sancti and Cytophaga arvensicola may constitute a distinct genus on the bases of their phylogenetic relationship determined from 16S rDNA sequence similarities, their strictly respiratory metabolism, MK-7 menaquinone content and a base composition in the range 42.8–48.6 mol%, which is at the high end of the range for the genus Flexibacter (Reichenbach, 1989). Nakagawa & Yamasato (1996) later showed that Flexibacter filiformis is also a member of this phylogenetic lineage, but on the basis of bootstrap values showed that the relationship of Flavobacterium ferruginium to the other three members is not strongly supported. The current study has added weight to the emerging taxonomic importance of this deeply branched and well supported lineage which now also includes Chitinophaga pinensis. This lineage is well separated from the lineage which contains Flexibacter flexilis, the type species of the genus, and supports the transfer of the member species to a new genus. Woese et al. (1990) previously reported the widespread distribution of the Flexibacter species in the FBC phylum and showed that Flexibacter flexilis and Flexibacter sancti belonged to widely separated lineages. However, there appear to be few phenotypic characteristics which unify the members of the phylo-type. As mentioned earlier, Chitinophaga pinensis and
Flexibacter filiformis are quite similar in morphology and share the ability to degrade chitin. Cytophaga arvensicola and Flexibacter sancti on the other hand cannot degrade chitin, have much shorter cells and do not form spherical bodies on ageing. In spite of their superficial phenotypic similarity, the 95.3% sequence similarity between the 16S rRNA genes of Chitinophaga pinensis and Flexibacter filiformis over a length of 1445 nucleotides is indicative of their separate species status (Stackebrandt & Goebel, 1994). The fact that Sporocytophaga myxococcoides belongs to a separate phylogenetic lineage in the Flexibacter–Bacteroides–Cytophaga phylum as previously reported (Paster et al., 1985), indicates that transformation to spherical bodies is not phylogenetically exclusive and may have been an early evolutionary trait or, more likely, that the nature of the spherical body in Chitinophaga pinensis and Flexibacter filiformis is different to that in Sporocytophaga myxococcoides, as previously suggested by Reichenbach (1992).

References


