Proposal to reclassify [Sphingomonas] xenophaga Stolz et al. 2000 and [Sphingomonas] taejonensis Lee et al. 2001 as Sphingobium xenophagum comb. nov. and Sphingopyxis taejonensis comb. nov., respectively

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The sphingomonad group contains bacterial isolates that are quite diverse in terms of their phylogenetic, ecological and physiological properties. Thus, the genus Sphingomonas was divided into four distinct genera, Sphingomonas sensu stricto, Sphingobium, Novosphingobium and Sphingopyxis on the basis of 16S rRNA gene sequence phylogenetic analysis, signature nucleotides, fatty acid profiles and polyamine patterns and this classification is currently widely accepted. In this study, a complete analysis of the 16S rRNA gene sequences of all the members of the group of sphingomonads encompassed in the genera Sphingomonas sensu stricto, Sphingobium, Novosphingobium and Sphingopyxis was inferred by using tree-making algorithms. [Sphingomonas] xenophaga DSM 6838T was found to form a distinct clade with the members of the genus Sphingobium, whereas [Sphingomonas] taejonensis DSM 15583T forms a clade with the members of the genus Sphingopyxis. The respective positions of these strains were also supported by the data for signature nucleotides, 2-hydroxy fatty acid profiles, polyamine patterns and the nitrate reduction properties of the strains. We therefore propose the reclassification of [Sphingomonas] xenophaga and [Sphingomonas] taejonensis as Sphingobium xenophagum comb. nov. (type strain DSM 6838T = CIP 107206T) and Sphingopyxis taejonensis comb. nov. (type strain DSM 15583T = KCTC 2884T = KCCM 41068T), respectively.

The genus Sphingomonas was proposed by Yabuuchi et al. (1990) and defined as a group of Gram-negative, rod-shaped, chemoheterotrophic, strictly aerobic bacteria that possess ubiquinone 10 as the major respiratory quinone, contain glycosphingolipids in their cell envelopes and typically produce yellow-pigmented colonies. The genus is a dynamic group of bacterial isolates with respect to taxonomy and phylogeny, metabolic diversity and the ability to produce extracellular gellan-like polysaccharides (sphinans). Although members of the genus Sphingomonas have created much interest for biotechnological applications in the fields of novel catalysts, bioremediation, fossil fuel desulfurization, novel enzymes and biotin and polysaccharide production (Sutherland, 1999), reorganization of the genus Sphingomonas has also long been a subject of discussion for many taxonomists (Takeuchi et al., 2001; Yabuuchi et al., 2002). Many Gram-negative organisms previously classified as members of the genera Beijerinckia (Khan et al., 1996), Flavobacterium (Yabuuchi et al., 1990) and Pseudomonas (Yrjala et al., 1998), and even Gram-positive organisms from the genus Arthrobacter, have been reclassified as members of the genus Sphingomonas. As a result, the number of species represented by the genus Sphingomonas has increased and this has created quite a diverse group of sphingomonads as far as their ecological, physiological and phylogenetic properties are concerned. Thus, a re-evaluation of the species belonging to the genus Sphingomonas was carried out by Takeuchi et al. (2001). On the basis of phylogenetic analysis, polyamine patterns, fatty acid profiling and nucleotide signature sequences, the genus Sphingomonas was subdivided into four separate genera: Sphingomonas sensu stricto, Sphingobium, Novosphingobium and Sphingopyxis. By and large, this scheme of classification of sphingomonads has been accepted and is in operation (Pal et al., 2005). Currently, the genera Sphingomonas sensu stricto, Sphingobium, Novosphingobium and Sphingopyxis respectively contain 31, 8, 11 and 7 species with validly published names. However, there are still some discrepancies and a few sphingomonads have not yet been re-evaluated in...
the light of the classification proposed by Takeuchi et al. (2001) and are still represented in literature by the genus *Sphingomonas* (Stolz et al., 2000; Fujii et al., 2001; Lee et al., 2001; Yabuuchi et al., 2002). These discrepancies are largely due to the fact that at the time the four new genera were created from the genus *Sphingomonas*, some of the papers that had already been submitted for publication were not available to Takeuchi et al. (2001).

In this paper, we analyse the current status of species of the genus *Sphingomonas* with validly published names on the basis of the information available in the literature on 16S rRNA gene sequences, fatty acid profiling, 16S rRNA signature nucleotides and polyamine patterns. On the basis of our findings, we propose to reclassify [*Sphingomonas* xenophaga](Stolz et al., 2000) and [*Sphingomonas* taejonensis](Lee et al., 2001) as *Sphingobium xenophagum* comb. nov. and *Sphingopyxis taejonensis* comb. nov., respectively, according to the scheme of Takeuchi et al. (2001). The reclassification of *Sphingomonas cloacae* (Fujii et al., 2001) has already been taken up by O. Prakash & R. Lal (unpublished). The reclassification of *Sphingomonas agrestis* has not been undertaken as it is not a species with a validly published name. The taxonomic positioning of the species *Sphingomonas ursincola* (synonym *Blastomonas ursincola*; Hiraishi et al., 2000) and *Sphingomonas suberifaciens* (Yabuuchi et al., 1999) has been debated in the past. These two species have been reported to show some characteristics of the sphingomonads, such as ubiquinone 10, the presence of spermidine and glycosphingolipids (Yabuuchi et al., 2002); however, phylogenetic analysis reveals that the two species do not fall in any cluster of the four sphingomonad genera and form distinct sublines (Fig. 1).

The almost complete 16S rRNA gene sequences for [*Sphingomonas* xenophaga](DSM 6383T) (1438 nt) (Stolz et al., 2000) and [*Sphingomonas* taejonensis](DSM 15583T) (1443 nt) (Lee et al., 2001), as well as the sequence of *Rhodanobacter lindanilasticus* RP 5557T (Nalin et al., 1999), were retrieved from the GenBank database at NCBI (http://www.ncbi.nlm.nih.gov/). The 16S rRNA gene sequences of [*Sphingomonas* xenophaga](DSM 6383T) and [*Sphingomonas* taejonensis](DSM 15583T) were aligned with the 16S rRNA gene sequences of representative sphingomonads that encompass the four distinct sphingomonad genera by using CLUSTAL_X (version 1.8b; Thompson et al., 1997). Gaps at the 5’ and 3’ ends of the alignment were omitted from further analysis. Evolutionary distance matrices were calculated using the method of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1993).
A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). The stability of the relationships was assessed by bootstrap analysis of 1000 datasets with SEQBOOT and CONSENSE programs in the PHYLIP package (Fig. 1). An identical phylogenetic tree was obtained by the maximum-parsimony method (data not shown). On the basis of the 16S rRNA gene sequence analysis, strains [Sphingomonas] xenophaga DSM 6383T and [Sphingomonas] taejonensis DSM 15583T formed distinct clades within the evolutionary radiations of the genera Sphingobium and Sphingopyxis, respectively, and the positions were supported by high levels of bootstrap confidence. The 16S rRNA gene sequence placed [Sphingomonas] xenophaga DSM 6383T in the neighbourhood of Sphingobium yanoikuyae GIFU 9882T (Yabuuchi et al., 1990). The highest 16S rRNA gene sequence similarities, of 96.5 and 94.8%, were found between [Sphingomonas] xenophaga DSM 6383T and Sphingobium amiense JCM 11777T (Ushiba et al., 2003) and Sphingobium chungbukense KCTC 2953T (Kim et al., 2000; Pal et al., 2005), respectively. In the case of [Sphingomonas] taejonensis DSM 15583T (Lee et al., 2001), the highest gene sequence similarities, of 98.7 and 98.2%, were with Sphingopyxis chilensis DSM 14889T (Godoy et al., 2003) and Sphingopyxis alaskensis DSM 13593T (Vancanneyt et al., 2001), respectively. Additionally, the 16S rRNA gene sequences of [Sphingomonas] xenophaga DSM 6383T (Stolz et al., 2000) and [Sphingomonas] taejonensis DSM 15583T (Lee et al., 2001) contained the signature nucleotides specific for the genera Sphingobium cluster II and Sphingopyxis cluster IV (Takeuchi et al., 2001), respectively, confirming that the strains should be reclassified in the genera Sphingobium and Sphingopyxis, respectively.

The chemical properties of strains [Sphingomonas] xenophaga DSM 6383T (Stolz et al., 2000) and [Sphingomonas] taejonensis DSM 15583T (Lee et al., 2001), nitrate reduction, polyamine pattern and fatty acid profiling, also indicate that the strains show a strong associations with members of the genera Sphingobium and Sphingopyxis, respectively. [Sphingomonas] xenophaga DSM 6383T has been reported to contain 2-hydroxyoxymyristic acid (2-OH 14:0) as the dominant hydroxylated fatty acid (Stolz et al., 2000), whereas [Sphingomonas] taejonensis DSM 15583T contains 2-OH 14:0, 2-OH 15:0 and 2-OH 16:0 (Lee et al., 2001). These fatty acids are characteristic for the genera Sphingobium and Sphingopyxis, respectively. For [Sphingomonas] xenophaga DSM 6383T, the major polyamine has been reported to be spermidine (Stolz et al., 2000). This clearly distinguishes [Sphingomonas] xenophaga DSM 6383T from members of the genus Sphingomonas sensu stricto, as they contain homospermidine as the major polyamine (Takeuchi et al., 2001).

Table 1. Differential phenotypic characteristics of [Sphingomonas] xenophaga DSM 6383T (Stolz et al., 2000) and [Sphingomonas] taejonensis DSM 15583T (Lee et al., 2001) from the representatives of the genus Sphingobium and Sphingopyxis

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Data are taken from earlier studies. Strains: 1, [Sphingomonas] xenophaga DSM 6383T (data from Stolz et al., 2000); 2, Sphingobium amiense JCM 11777T (Ushiba et al., 2003); 3, Sphingobium chungbukense KCTC 2953T (Kim et al., 2000); 4, Sphingobium yanoikuyae GIFU 9882T (Yabuuchi et al., 1990); 5, [Sphingomonas] taejonensis DSM 15583T (Lee et al., 2001); 6, Sphingopyxis alaskensis DSM 13593T (Vancanneyt et al., 2001); 7, Sphingobium chilensis DSM 14889T (Godoy et al., 2003); 8, Sphingopyxis macrogoltabida IFO 15033T (Takeuchi et al., 1993). ++, Positive or present; –, negative or absent; NA, not available.
Description of Sphingopyxis taejonensis (Lee et al. 2001) comb. nov.

Basonym: Sphingomonas taejonensis Lee et al. 2001.

Sphingopyxis taejonensis (tae.jon.′en′sis. N.L. fem. adj. taejonensis referring to Taejon, Korea, the geographical origin of the type strain).

The description is identical to the description given for Sphingomonas taejonensis by Lee et al. (2001). The type strain is JSS54T (= DSM 15583T = KCTC 2884T = KCCM 41068T).

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References


