Transfer of *Blastobacter natatorius* (Sly 1985) to the Genus *Blastomonas* gen. nov. as *Blastomonas natatoria* comb. nov.

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The budding bacterium *Blastobacter natatorius* belongs to the alpha-4 group of the *Proteobacteria* and clusters phylogenetically on a deep branch with *Sphingomonas capsulata*, with which it shares 93.9% 16S rRNA sequence similarity. On phylogenetic, phenotypic, and chemotaxonomic grounds a proposal is made to transfer *B. natatorius* to the genus *Blastomonas* gen. nov. as *Blastomonas natatoria* comb. nov.

The taxonomy of the genus *Blastobacter* Zavarzin 1961 is in a state of confusion. This confusion has arisen due to the lack of a type strain for the type species, *Blastobacter henricii* (14, 25), and to the fact that morphological characteristics, such as budding cell division, which define the genus may not be phylogenetically useful features at the genus level (5) and may group distinctly related species in the genus as currently defined (group 25). Cell division by non-prosthecate budding is confined to the alpha subclass of the *Proteobacteria* but is widely distributed in genera within the subclass.

The genus *Blastobacter* was proposed by Zavarzin (25) to include rosette-forming, budding, rod-shaped or wedge-shaped bacteria which were observed in a filter paper enrichment of reduced iron-containing water from a northern Russian forest brook. Zavarzin was unable to isolate the cells in pure culture, and the description of *Blastobacter henricii* is based on drawings and observations.

Several additional *Blastobacter* species have been validly described since 1961 (8). These include *Blastobacter aggregatus*, *Blastobacter capsulatus*, *Blastobacter denitrificans* (4, 6), and *Blastobacter natatorius* (15, 17). Other taxonomically unvalidated species which have been reported on include "*Blastobacter vicinus"* (7), "*Blastobacter aminooxidans"* (1), and "*Blastobacter novus"* (10).

Several authors have demonstrated that there is a high degree of heterogeneity in the genus *Blastobacter* with respect to phenotype (21), cellular fatty acids and phospholipids (13), and molecular phylogeny (3, 5, 11, 23).

There is a need to revise the taxonomy of the genus *Blastobacter* Henrici 1961, and the phylogenetic evidence of Hugenholtz et al. (5) provides a framework with which to commence the taxonomic revision. An analysis of the 16S rRNA sequences of the validated species *Blastobacter aggregatus*, *Blastobacter capsulatus*, *Blastobacter denitrificans*, and *Blastobacter natatorius* by Hugenholtz et al. (5) showed that the species clustered in three separate groups within the alpha subclass of the *Proteobacteria*. *Blastobacter aggregatus* and *Blastobacter capsulatus* were closely related to each other (96.6% sequence similarity) and to *Agrobacterium tumefaciens* (96.0 to 96.9% sequence similarity) in the alpha-2a group. This confirmed the previous finding of Rothe et al. (11) concerning the close relationship between *Blastobacter aggregatus* and *A. tumefaciens* determined by rRNA oligonucleotide catalog analysis. *Blastobacter denitrificans* grouped with *Rhodosporidomonas palustris* and *Bradyrhizobium japonicum* in the alpha-2b group, a phylogenetic branch distinct from *Blastobacter aggregatus* and *Blastobacter capsulatus*. The relationship of *Blastobacter denitrificans* to *Bradyrhizobium japonicum* confirmed previous observations of Willems and Collins (23) based on 16S rRNA sequence similarities and of Green and Gillis (3) based on rRNA cosins similarities. *Blastobacter natatorius*, on the other hand, was shown by Hugenholtz et al. (5) to be a member of the alpha-4 group.

Hugenholtz et al. (5) recommended that a new type species for the genus *Blastobacter* be selected and described because of the lack of a type strain of the type species. However, given the absence of physiological and phylogenetic information about the type species, *Blastobacter henricii*, it would be difficult to designate one of the other species as the type species of the genus *Blastobacter* with confidence. There is no way of knowing which phylogenetic line the true blastobacters as described by Zavarzin (25) belong to, and such action might cause further taxonomic confusion in the future. Consequently, the genus *Blastobacter* should be reserved at this time for *Blastobacter henricii* in case a culture matching the description can be isolated from the same habitat in the future. New genera need to be described to include the remaining validated species.

In this paper we propose that *Blastobacter natatorius* be transferred to a new genus as it is phylogenetically (5), phenotypically (15, 21), and chemotaxonomically (13) distinct from the other species of the genus *Blastobacter* as currently defined (15, 21, 25). Hugenholtz et al. (5) found that *Blastobacter natatorius* was most closely related, as determined by 16S rRNA sequence similarity, to *Caulobacter subvibrioides*, *Porphyrobacter neustonensis* (2), and *Erythrobacter longus* (12) in the alpha-4 group of the *Proteobacteria*. We have recently reported (16) that the sequence (accession number M85797) (18) for *C. subvibrioides* used by Hugenholtz et al. (5) and recently by Nohyne et al. (9) in their analyses appears to be the sequence of an organism closely related to *Sphingomonas adhaesiva* and not the sequence of *C. subvibrioides*. Resequencing of the 16S ribosomal DNA of the type strain of *C. subvibrioides* showed that this species clusters with the *Caulobacter* group outside the alpha-4 group and lacks 2-hydroxymyristic acid, which is characteristic of the genus *Sphingomonas* (16). *C. subvibrioides* should no longer be considered a member of the *Sphingomonas* group. Because of this change and the increasing number of genera and species belonging to the alpha-4 group, we undertook a phylogenetic analysis in which we used previously described methods (16); these methods included analyzing the 16S rRNA sequences of a more complete set of species which have become available since the analysis done by Hugenholtz et al. (5) but do not include *C. subvibrioides* to
FIG. 1. Unrooted phylogenetic tree obtained by a neighbor-joining analysis of 16S rRNA sequences, showing the position of Blastobacter natatorius (formerly Blastobacter natatorius) in the alpha-4 group of the Proteobacteria. Scale bar = 10 nucleotide substitutions per 100 nucleotides of 16S rRNA sequences. The analysis included 1,254 nucleotide positions. Bootstrap values from 100 analyses are shown at the branch points. The accession numbers of the sequences used in the analysis are as follows: Blastobacter natatorius ACM 25007T (T = type strain), X73043; Brevundomonas diminuta ATCC 13586T, M50664; Caulobacter bacteroides ATCC 15254T, M83796; Erythrobacter longus Och101T, L11786; Porphyrobacter neutrophilicus ACM 2844T, L11785; Rhizomonas adhaesiva IFO 15211T, D13757; Rhodobacter capsulatus ATCC 11166T, D16426; Rhodobacter sphaeroides ATCC 17023T, X53855; Rhodopseudomonas palustris ATCC 17001T, L11664; Sphingomonas adhaesiva IFO 15099T (= Gifu 11458T), D16146; Sphingomonas capsulata Gifu 11526T (= ATCC 14466T), D16147; Sphingomonas capsulata IFO 15508T, D13723; Sphingomonas paucimobilis IFO 15100T, D13724; Sphingomonas paucimobilis Gifu 2395T (= ATCC 29837T), D16144; Sphingomonas sanguis IFO 15102, D16147; Sphingomonas terrae IFO 15008T, D13723; Sphingomonas yanoikuyae IFO 15102T, D16147; Zymomonas mobilis subsp. mobilis, Zym. mobilis (Rombosal Database Project). The sequence of Agrobacterium tumefaciens IAM 13129T (accession number D12784) was used as the outgroup.

more accurately determine the position of Blastobacter natatorius in the alpha-4 group.

In the phylogenetic tree (Fig. 1) Blastobacter natatorius clusters in a major group containing species of the genus Sphingomonas and Rhizomonas and joins deeply with Sphingomonas capsulata at a level of sequence similarity of 93.9%. Nohynek et al. (9) recently showed that the S. capsulata branch also contains the new species Sphingomonas rosa and Sphingomonas subarctica, which exhibit only 91.9 to 92.4% sequence similarity with Blastobacter natatorius, which continues to be the only representative of a deep branch. The Sphingomonas group is characterized by a number of deeply branching phylogenetic lines which may represent separate genera. The branch which contains Sphingomonas paucimobilis, Sphingomonas paucimobilis, Sphingomonas sanguis, and S. adhaesiva most likely represents the true species of the genus Sphingomonas. Rhizomonas subarctica, on a separate deep branch, was proposed as a member of the new genus Rhizomonas on the basis of 16S rRNA sequence similarity values. It was also suggested by van Bruggen et al. (22) that Sphingomonas yanoikuyae may be a separate species of the genus Rhizomonas, but the deep branch point indicates that this species may be the first member of another new genus. Sphingomonas terrae and Sphingomonas macroglobulida, with a level of sequence similarity of 96.7%, cluster together and are most likely separate species in another new genus. Phenotypic characteristics to describe and differentiate these new genera need to be determined before the new taxa can be proposed formally.

S. capsulata is considered to be representative of a separate genus on the basis of rRNA sequence similarity values and partial sequence similarities (22). It was also suggested by van Bruggen et al. (22) that blastobacter natatorius must also be considered a member of a separate genus.

Members of the major Sphingomonas group, including species of the genera Sphingomonas and Rhizomonas, are characterized by the presence of 2-hydroxymyristic acid (2-OH14:0) in their cellular fatty acids (24). Blastobacter natatorius has been shown by Sittig and Hirsch (13) to contain more than 50% 2-hydroxymyristic acid but no 3-hydroxy fatty acids, which supports its close affinity with the alpha-4 group. These features differentiate Blastobacter natatorius from Blastobacter aggregatus and Blastobacter capsulatus, which do not contain 2-hydroxymyristic acid or other 2-hydroxy fatty acids, but contain 3-hydroxy fatty acids, including levels of 3-OH14:0 (7–18%) (13).

Sittig and Hirsch (13) in a chemotaxonomic study of the budding and/or hyphal bacteria determined that the major cellular fatty acids of Blastobacter natatorius were n16:1d9 (13.3%), n16:0 (17.2%), and n18:1d11 (61.5%). The major hydroxy fatty acids were 2-OH14:0 (52.2%), 2-OH16:1d11 (16.2%), and 2-OH16:0 (25.7%). It was also shown that the major phospholipids were phosphatidylglycerol, phosphatidyl-

ethanolamine, phosphatidylglycerolphosphatidylethanolamine, and phosphatidylcholine and that Blastobacter natatorius contained ubiquinone Q10.

On the basis of its phenotypic and chemotaxonomic features and its phylogenetic position, we propose that Blastobacter natatorius be transferred to the new genus Blastomonas as Blastomonas natatorius (natatorius = a unit, monad; L.M. fem. n. Blastomonas, a budding monad). Cells are gram negative and rod shaped or wedge shaped with a straight or slightly curved axis. Cells are usually 0.5 to 0.8 by 1 to 3 pm. Older cells may become elongated and reach lengths of 10 pm or more. Some cells may have a swollen or bloated appearance. Cells occur singly or in pairs and may form rosettes. Each rosette-forming cell has a simple mucilaginous holdfast at its nonreproductive pole by which it attaches to other cells or to solid surfaces. No stalks, prosthecae, or other holdfast structures differentiate Blastobacter natatorius from Blastobacter aggregatus and Blastobacter capsulatus, which do not contain 2-hydroxymyristic acid or other 2-hydroxy fatty acids, but contain 3-hydroxy fatty acids, including levels of 3-OH14:0 (7–18%) (13).

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Chemoorganotrophic. Good growth occurs on peptone-

yeast extract agar or peptone-yeast extract-glucose agar. Growth occurs on glucose-ammonium sulfate agar. No vitamin requirement. Colony pigmentation is yellow.

Aerobic. Catalase and oxidase positive. Acid but no gas is produced from glucose. Nitrate is not reduced.

Contains ubiquinone Q10. Contains 2-hydroxymyristic acid as a major component of the cellular fatty acids.
The guanine-plus-cytosine content of genomic DNA is 65 mol% (as determined by the thermal denaturation method). Member of the alpha-4 group of the alpha subclass of the Proteobacteria.

Found in freshwater. The type species is Blastomonas natatoriae.

**Description of Blastomonas natatoriae** (Sly 1985) comb. nov.

The description of *Blastomonas natatoriae* (nat.ato.ta.to'ri.a M.L. fem. adj. natatoria, of a swimming place [pool]) is the same as the genus description with the following additional characteristics described in the effective publication of *Blastobacter natatorius* (15) and chemotaxonomic data from the study of Sittig and Hirsch (13).

Colonies grown on peptone-yeast extract agar for 3 days at 28°C are yellow and round and have entire edges, diameters of 0.5 to 1.0 mm, and high convex elevations. The colony surface is shiny, and the growth is easily emulsified. After further incubation the colonies have a rubbery consistency and may be removed intact from the agar surface by touching with a wire loop. Colonies on Staley PYG medium (19) after 4 days at 28°C are pale pink and 0.5 mm in diameter.

Catalase, oxidase, phosphatase, and DNase are produced. Gelatin and Tween 80 are hydrolyzed, but cellulose, chitin, alginate, starch, tributyrin, casein, and dextran are not hydrolyzed. There is no hemolytic activity. Urease, phenylalanine deaminase, arginine dihydrolase, indole, and H2S are not produced. Nitrate is not reduced. An alkaline reaction occurs in Hugh-Leifson medium. Acid but no gas is produced from fructose, glyc erol, maltose, mannose, melezitose, and sucrose in media containing Andrades indicator.

The following chemotaxonomic information was determined by Sittig and Hirsch (13) for the type strain. Contains ubiquinone Q10. The major cellular fatty acids are n16:1d9, n16:0, and n18:1d11. The major hydroxy fatty acids are 2-OH14:0, 2-OH16:1d11, and 2-OH16:0. The major phospholipids are phosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidyl ethanolamine, and phosphatidylcholine.

The guanine-plus-cytosine content of the DNA of the type strain is 65 mol% (as determined by the thermal denaturation method).

The type strain is strain ACM 2507 (= ATCC 35951 = DSM 3183 = NCIMB 12085), which was isolated from a freshwater swimming pool.

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**REFERENCES**


