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

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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 distantly related species in the genus as currently defined (21, 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 viscosus" (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 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 Rhodospseudomonas 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 cistron 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 M83797) (18) for C. subvibrioides used by Hugenholtz et al. (5) and recently by Nohynek 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
more accurately determine the position of \textit{Blastobacter natatorius} in the alpha-4 group. In the phylogenetic tree (Fig. 1) \textit{Blastobacter natatorius} clusters in a major group containing species of the genus \textit{Sphingomonas} and \textit{Rhizomonas} and joins deeply with \textit{Sphingomonas capsulata} at a level of sequence similarity of 93.9%. Nohynek et al. (9) recently showed that the 3. \textit{capsulata} branch also contains the new species \textit{Sphingomonas rosa} and \textit{Sphingomonas subarctica}, which exhibit only 91.9 to 92.4% sequence similarity with \textit{Blastobacter natatorius}, which continues to be the only representative of a deep branch. The \textit{Sphingomonas} group is characterized by a number of deeply branching phylogenetic lines which may represent separate genera. The branch which contains \textit{Sphingomonas paucimobilis}, \textit{Sphingomonas parapaucimobilis}, and \textit{S. adhaesiva} most likely represents the true species of the genus \textit{Sphingomonas}. \textit{Rhizomonas subarctica}, on a separate deep branch, was proposed as a member of the new genus \textit{Rhizomonas} on the basis of 16S rRNA cistron and sequence similarity values (22). It was also suggested by van Bruggen et al. (22) that \textit{Sphingomonas yanoikuyae} may be a separate species of the genus \textit{Rhizomonas}, but the deep branch point indicates that this species may be the first member of another new genus. \textit{Sphingomonas terrae} and \textit{Sphingomonas macrogoltabidus}, 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.

\textit{S. capsulata} is considered to be representative of a separate genus on the basis of rRNA cistron similarity values and partial sequence similarities (22). At a low level of 16S rRNA similarity between \textit{Blastobacter natatorius} and \textit{S. capsulata} and the distinctive morphological features, \textit{Blastobacter natatorius} must also be considered a member of a separate genus.

Members of the major \textit{Sphingomonas} group, including species of the genera \textit{Sphingomonas} and \textit{Rhizomonas}, are characterized by the presence of 2-hydroxymyristic acid (2-OH14:0) in their cellular fatty acids (21). \textit{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 \textit{Blastobacter natatorius} from \textit{Blastobacter aggregatus} and \textit{Blastobacter capsulata}, which do not contain 2-hydroxymyristic acid or other 2-hydroxy fatty acids, but contain 3-hydroxy fatty acids, including high levels of 3-OH14:0 (81%) (13).

Sittig and Hirsch (13) in a chemotaxonomic study of the budding and/or hyphal bacteria determined that the major cellular fatty acids of \textit{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 phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl ethanolamine, phosphatidylcholine and that \textit{Blastobacter natatorius} contained ubiquinone Q10.

On the basis of its phenotypic and chemotaxonomic features and its phylogenetic position, we propose that \textit{Blastobacter natatorius} be transferred to the new genus \textit{Blastomonas} as \textit{Blastomonas natatorius} comb. nov.

Description of \textit{Blastomonas} \textit{gen. nov.} \textit{Blastomonas} (Blasto-'mo'nas. Gr. \textit{n. blastos}, bud shoot; Gr. \textit{n. monas}, a unit, monad; M-L. fem. n. \textit{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 \textmu m. Older cells may become elongated and reach lengths of 10 \textmu m 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 base. The free bud cells are motile with a single polar flagellum. The budding and/or hyphal cells are usually 0.5 to 1 pm. The free bud cells are motile with a single polar flagellum. The budding and/or hyphal bacteria determined that the major cellular fatty acids of \textit{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 phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl ethanolamine, phosphatidylcholine and that \textit{Blastobacter natatorius} contained ubiquinone Q10.

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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 natatoria.

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

The description of *Blastomonas natatoria* (na.ta.to’ri.a, M.L. fem. adj. natatoria, of a swimming pool [pool]) is the same as the genus description with the following additional characteristics described in the effective publication of *Blastobacter natatorium* (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, glycerol, glucose, maltose, mannose, melezitose, and sucrose in media containing Andrades indicator.

2-OHn16:ldl17 and 2-OHn16:O. The major phospholipids are and n18:ldll. The major hydroxy fatty acids are 2-OHn14:07 none QlO. The major cellular fatty acids are n16:ld9, n16:0, dimethylethanolamine, and phosphatidylcholine.

The guanine-plus-cytosine content of genomic DNA 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**


