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Reclassification of '*Blastobacter viscosus*' 7d and '*Blastobacter aminooxidans*' 14a as *Xanthobacter viscosus* sp. nov. and *Xanthobacter aminooxidans* sp. nov.

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On the basis of morphological, physiological and genotypic properties '*Blastobacter viscosus*' 7d and '*Blastobacter aminooxidans*' 14a are proposed as new species of the genus *Xanthobacter*, *Xanthobacter viscosus* (type strain 7d^T = VKM B-2253^T = ATCC BAA-298^T) and *Xanthobacter aminooxidans* corrig. (type strain 14a^T = VKM B-2254^T = ATCC BAA-299^T).

The genus *Blastobacter* was proposed by G. Zavarzin for budding bacterial rods observed in a filter paper enrichment of reduced iron-containing water from a forest brook. However, a pure culture was not obtained, and the description of *Blastobacter henricii* was based mainly on morphological observations (Zavarzin, 1961). Subsequently, several *Blastobacter* species were validly described. These include *Blastobacter aggregatus*, *Blastobacter capsulatus*, *Blastobacter denitrificans* (Hirsch & Muller, 1985) and *Blastobacter natatorius* (Sly, 1985; Sly & Hargreaves, 1984). Other species with non-valid names include '*Blastobacter viscosus*' (Loginova & Trotsenko, 1980) and '*Blastobacter aminooxidans*' (Doronina *et al.*, 1984). Several authors have demonstrated a high degree of heterogeneity in the genus *Blastobacter* with respect to their phenotype (Trotsenko *et al.*, 1989; Sitting & Hirsch, 1992) and molecular phylogeny (Rothe *et al.*, 1987; Willems & Collins, 1992; Hugenholtz *et al.*, 1994). Hence, there is a need to revise the taxonomic structure of this genus. An analysis of the 16S rRNA sequences of *Blastobacter aggregatus*, *Blastobacter capsulatus*, *Blastobacter denitrificans* and *Blastobacter natatorius* (Hugenholtz *et al.*, 1994) showed that they were clustered into three separate groups within the α -Proteobacteria. *Blastobacter aggregatus* and *Blastobacter capsulatus* were closely related to each other (96.6% sequence similarity) and to *Agrobacterium tumefaciens* (96.0–96.9% sequence similarity) in the 2a group of the α -Proteobacteria. *Blastobacter denitrificans* grouped with *Rhodopseudomonas palustris* and *Bradyrhizobium japonicum* in the 2b group of the α -Proteobacteria. At first, *Blastobacter natatorius* was transferred to the new genus *Blastomonas* in the 4 group of the α -Proteobacteria as *Blastomonas natatoria* (Sly & Cahill, 1997). More recently, it was

proposed to transfer *Blastomonas natatoria* to the genus *Sphingomonas* (Yabuuchi *et al.*, 1999; Hiraishi *et al.*, 2000).

'*Blastobacter viscosus*' 7d and '*Blastobacter aminooxidans*' 14a were isolated from activated sludge of the Baikal sewage purification system at pulp and papermill (Loginova & Trotsenko, 1980; Doronina *et al.*, 1984). Cells of strains 7d and 14a were rod-shaped to pleomorphic, often bent and occasionally branched, reproduced by budding. Buds were produced polarly and laterally and were ovoid and non-motile. Colonies were yellow pigmented. Some phenotypic properties of strains 7d and 14a were similar to *Xanthobacter* species. Formerly, the Gram reactions of *Xanthobacter* species were described as Gram-positive or Gram-variable (Wiegel & Schlegel, 1984; Wiegel, 1992). However, strains 7d and 14a are Gram-negative bacteria as determined by the ultrastructure of the cell wall, cellular fatty acid composition and quinone system (Trotsenko *et al.*, 1989). Strains 7d and 14a grew autotrophically in an atmosphere of H₂+O₂+CO₂ or with reduced C₁-compounds (methanol or methylated amines) (Loginova *et al.*, 1982; Doronina *et al.*, 1984). By using a polyphasic taxonomic approach we propose that these strains of budding bacteria be reclassified as new species in the genus *Xanthobacter*.

The DNA of strains 7d and 14a was extracted by the method of Marmur (1961). DNA–DNA hybridizations were done on nitrocellulose membrane filters (0.22 µm) according to Denhardt (1966) in the following incubation mixture: 0.15 M NaCl and 0.015 M trisodium citrate, pH 7.0, with 30% formamide at 60 °C for 24 h. Deoxy [1',2',5'-³H]CTP and the nick-translation kit N5500 (Amersham International) were used for labelling the DNA probe. 16S rDNAs of strains 7d and 14a were amplified by thermostable DNA-polymerase (USB) via PCR in a mixture containing 1× *Taq*Pol buffer (USB), 0.2 µg chromosomal DNA, 20 pM oligonucleotide

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 7d^T and 14a^T are AF399970 and AF399969, respectively.

primers pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH' (5'-AAGGAAGGTGATCCAG CTCGT-3') (Edwards *et al.*, 1989), 2.5 mM dNTP, 2.5 mM MgCl₂ and 2 U *Taq*. After denaturation at 94 °C for 5 min, the reaction mixture was subjected to 30 thermal cycles (52 °C, 1 min; 72 °C, 1.5 min; 94 °C, 1 min). PCR products were sequenced using the FemtoMol kit (Promega). 16S rDNA was sequenced in both strands by Sanger's method using *Taq* polymerase (Promega) and pA (see above), pC (5'-CTACGGGAGGCAGCAGT-GGG-3'), pE (5'-AAACTCAAAGGAATTGACGG-3'), pD' (5'-GTATTACCGCGGCTGCTG-3'), pF' (5'-ACGAGCT-GACGACAGCCATG-3') and pH' (see above) primers (Edwards *et al.*, 1989). All the procedures were done according to the manufacturer's protocol. All the primers were labelled by T4 polynucleotide kinase (Fermentas, Lithuania) with [γ -³²P]-ATP (Nuklid-Trans, Russia) according to Sambrook *et al.* (1989) manual. Sequencing reactions were performed in a mixture containing 1 × DNaseq buffer (Promega), 0.2 µg amplified 16SrDNAs, 2 pM labelled oligonucleotide primers, ddNTP/dNTP mix (Promega), 2 U sequencing grade *Taq* and 30 rounds of sequencing PCR were carried out (94 °C, 2 min; 42 °C, 15 s; 72 °C, 30 s; 94 °C, 15 s). The determined 16S rDNA sequences were aligned against those of closely related strains by using CLUSTAL version 1.60. Pairwise evolutionary distances were computed with the Jukes–Cantor correction (Felsenstein, 1993) using the DNADIST program and the phylogenetic tree was constructed using the FITCH program in the PHYLIP version 3.5 software package.

Some differential phenetic characteristics of strains 7d and 14a and representatives of the genus *Xanthobacter* are summarized in Table 1.

Table 1. Some differential phenetic characteristics of *Xanthobacter* species

Species: 1, *X. viscosus* 7d^T; 2, *X. aminoxidans* 14a^T; 3, *X. agilis* SA35^T; 4, *X. autotrophicus* 7C^T; 5, *X. flavus* 301^T; 6, *X. tagetidis* TagT2C^T. +, Positive; –, negative.

Characteristic	1*	2†	3‡	4‡	5‡	6§
Pleomorphism	+	+	–	+	+	+
Slime production	+	–	–	+	+	+
Motility	–	–	+	–	–	+
Growth on nutrient broth	+	+	–	+	+	+
Biotin requirement	–	–	–	–	+	–
Growth on:						
H ₂ +CO ₂ at 37 °C	–	–	–	+	+	+
Citrate	–	–	–	+	+	+
Methanol	+	–	+	+	+	+
Methylamine	–	+	–	–	–	With CO ₂
Trimethylamine	–	+	–	–	–	–

*Data from Loginova & Trotsenko (1980).

†Doronina *et al.* (1984).

‡Jenni *et al.* (1987).

§Padden *et al.* (1997).

Phylogenetic analysis of the 16S rDNA of strains 7d and 14a (Fig. 1) confirmed their location within cluster of the *Xanthobacter* species, as supported by bootstrap values. The 16S rDNA of strain 7d has a high sequence similarity with *Xanthobacter autotrophicus* 7C^T=DSM 432^T (97.8 %) and *Xanthobacter flavus* 301^T=DSM 338^T (95.8 %). Analysis of the 16S rDNA of strain 14a (Fig. 1) showed it also to cluster within the *Xanthobacter* species and is most closely related to *Xanthobacter flavus* 301^T=DSM 338^T (98.5 % sequence similarity), *Xanthobacter tagetidis* Tag T2C^T=DSM 11105^T (96.5 %) and *Xanthobacter agilis* SA35^T=DSM 3770^T (97.2 %).

However, both strains are distinct from each other (22 %) and also from the type strains of known members of the *Xanthobacter* by rather low level of DNA–DNA relatedness (18–32 %) (Table 2). Remarkably, they have very low DNA–DNA similarity with *Blastobacter* species. Based on phenetic characteristics, DNA–DNA hybridization value (not more than 32 %) and 16S rDNA sequence similarities, the strains under study may be regarded as the new *Xanthobacter* species with the names *Xanthobacter viscosus* sp. nov. and *Xanthobacter aminoxidans* sp. nov., respectively. Their formal descriptions are given below.

Fig. 1. Phylogenetic position of *Xanthobacter aminoxidans* strain 14a^T and *Xanthobacter viscosus* strain 7d^T among related micro-organisms of the α -Proteobacteria. Pairwise evolutionary distances were computed by the correction of Jukes–Cantor using programs of PHYLIP (version 3.5) package. 16S rDNA of *Agrobacterium tumefaciens* was used as an outgroup. Bar, 5% evolutionary difference. The numbers at the branch points are bootstrap values from 100 replicates.

Table 2. DNA–DNA hybridization (%) between strains 7d and 14a and representatives of the genera *Xanthobacter* and *Blastobacter*

Strain	7d	14a
<i>Xanthobacter viscosus</i> 7d ^T (=VKM B-2253 ^T)	100	22
<i>Xanthobacter aminoxidans</i> 14a ^T (=VKM B-2254 ^T)	23	100
<i>Xanthobacter autotrophicus</i> DSM 432 ^T	25	21
<i>Xanthobacter flavus</i> DSM 338 ^T	21	32
<i>Xanthobacter agilis</i> DSM 3770 ^T	18	22
<i>Blastobacter aggregatus</i> IFAM 1003 ^T	4	5
<i>Blastobacter denitrificans</i> IFAM 1005 ^T	3	4
<i>Blastobacter capsulatus</i> IFAM 1004 ^T	3	3

Description of *Xanthobacter viscosus* sp. nov.

Xanthobacter viscosus (vis.co'sus. L. masc. adj. *viscosus* sticky).

Gram-negative pleomorphic, often bent and occasionally branched rods, 0.5–0.9 × 1.0–3.2 µm. Non-motile. Reproduction occurs by asymmetric division (budding) of the mother cell to produce a shorter daughter cell. A capsule is formed, but not rosettes. Colonies are yellow, round, convex and of slimy consistency. In liquid medium with methanol or glucose, growth occurs as turbidity, finally with a slimy sediment. An exopolysaccharide consisting of galactose, glucose, rhamnose, xylose and glucuronic acid is formed. The temperature range is 5–34 °C, optimum 28–30 °C, and the pH range is 6.5–8.5, optimum pH 6.8–7.2. Obligately aerobic but potentially, microaerophilic. Catalase- and oxidase-positive. Does not hydrolyse gelatin, starch and cellulose. Urease, phenylalanine deaminase, arginine dihydrolase are not produced. Indole is formed from L-tryptophan. Reduces nitrates to nitrites. Acid but not gas is produced from glucose and fructose. Utilizable carbon sources are methanol, ethanol, propanol, butanol, glycerol, ribose, xylose, glucose, fructose, mannitol, sorbitol, formate, formamide, glyoxylate, pyruvate, butyrate, 2-oxoglutarate, α-ketobutyrate, fumarate, succinate, *cis*-aconitate. Utilizable nitrogen sources are ammonium salts, peptone and amino acids. Fixes N₂ when grown in nitrogen-deficient media. Grows autotrophically in an atmosphere of H₂+O₂+CO₂ and on methanol by the key Calvin-cycle enzymes phosphoribulokinase and ribulosebiphosphate carboxylase. The main ubiquinone is Q-10. The major fatty acid of peptone-yeast-glucose agar-grown cells is C_{18:1} (76.0%). The major phospholipids are phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine. The G+C content of the DNA is 66.3 mol% (T_m). The GenBank accession number for the 16S rRNA gene sequence of strain 7d^T is AF399970. *X. viscosus* strain 7d^T was isolated from activated sludge of a sewage purification system of the Baikal paper mill (Russia). The type strain, 7d^T, is deposited in the All-Russian Collection of Microorganisms as VKM B-2253^T and the American Type Culture Collection as ATCC BAA-298^T.

Description of *Xanthobacter aminoxidans* sp. nov.

Xanthobacter aminoxidans (am.in.ox'i.dans. N.L. n. *aminum* amine; M.L. v. *oxido* make acid, oxidize; N.L. part. adj. *aminoxidans* amine-oxidizing).

Gram-negative pleomorphic rods, 0.8–1.0 × 1.5–3.5 µm, apparently branching to form V-shaped cells. Non-motile. Reproduction occurs by asymmetric division (budding) of the mother cell to produce a shorter daughter cell. Colonies are yellow, round, convex, bright, non-transparent, smooth-surfaced, with an even edge, homogeneous and of slimy consistency. In liquid medium with methylated amines or glucose the cells do not produce exopolymer. The temperature range is 5–34 °C, optimum 29–32 °C, and the pH range is 6.5–8.5, optimum pH 7.2–7.8. Obligately aerobic, but potentially, microaerophilic. Catalase-, peroxidase- and oxidase-positive. Urease, phenylalanine deaminase and arginine dihydrolase are not produced. Indole is formed from L-tryptophan. Reduces nitrates to nitrites. With glucose medium, acid and gas are not produced. Utilizable carbon sources are monomethylamine, dimethylamine or trimethylamine, ethanol, butanol, mannitol, xylose, glucose, raffinose, glyoxylate, fumarate, malate and lactate. Utilizable nitrogen sources are ammonium salts, nitrates, peptone, methylated amines and some amino acids. Fixes N₂ when grown in nitrogen-deficient media. Grow autotrophically in an atmosphere of H₂+O₂+CO₂ and on methylamines by the key Calvin-cycle enzymes phosphoribulokinase and ribulosebiphosphate carboxylase. Trimethylamine and methylamine are oxidized by the appropriate dehydrogenases and dimethylamine is oxidized by monooxygenase (NADH or NADPH). The major ubiquinone is Q-10. The major fatty acid of peptone-yeast extract-glucose agar-grown cells is C_{18:1} (76.0%). The major phospholipids are phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol. The G+C content of the DNA is 69.1 mol% (T_m). The GenBank accession number for the 16S rRNA gene sequence of strain 14a^T is AF399969. *X. aminoxidans* strain 14a^T was isolated from activated sludge of a sewage-purification system at the Baikal paper mill, Russia. The type strain, 14a^T, is deposited in the All-Russian Collection of Microorganisms as VKM B-2254^T and the American Type Culture Collection as ATCC BAA-299^T.

References

- Denhardt, D. T. (1966). A membrane filter technique for determination of complementary DNA. *Biochem Biophys Res Commun* **23**, 641–646.
- Doronina, N. V., Govorukhina, N. I. & Trotsenko, Y. A. (1984). *Blastobacter aminoxidans*, a new species of bacteria growing autotrophically on methylated amines. *Microbiology* (English translation of *Mikrobiologiya*) **52**, 547–553.
- Edwards, U., Rogall, T., Blöcker, H., Emde, M. D. & Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of

entire genes. Characterization of gene coding for 16S ribosomal RNA. *Nucleic Acids Res* **17**, 7843–7853.

Felsenstein, J. (1993). PHYLIP (phylogeny inference package) version 3.5c. Department of Genetics, University of Washington, Seattle, USA.

Hiraishi, A., Kuraishi, H. & Kawahara, K. (2000). Emendation of the description of *Blastomonas natatoria* (Sly 1985) Sly and Cahill 1997 as an aerobic photosynthetic bacterium and reclassification of *Erythromonas ursincola* Yurkov *et al.* 1997 as *Blastomonas ursincola* comb. nov. *Int J Syst Evol Microbiol* **50**, 1113–1118.

Hirsch, P. & Muller, M. (1985). *Blastobacter aggregatus* sp. nov., *Blastobacter capsulatus* sp. nov. and *Blastobacter denitrificans* sp. nov., new budding bacteria from freshwater habitats. *Syst Appl Microbiol* **6**, 281–286.

Hugenholtz, P., Stackebrandt, E. & Fuerst, J. A. (1994). A phylogenetic analysis of the genus *Blastobacter* with a view to its future reclassification. *Syst Appl Microbiol* **17**, 51–57.

Jenni, B., Aragno, M. & Wiegel, K. W. (1987). Numeric analysis and DNA-DNA hybridization studies on *Xanthobacter* and emendation of *Xanthobacter flavus*. *Syst Appl Microbiol* **9**, 247–253.

Loginova, N. V. & Trotsenko, Y. A. (1980). *Blastobacter viscosus* – a new species of autotrophic bacteria utilizing methanol. *Microbiology* (English translation of *Mikrobiologiya*) **48**, 644–651.

Loginova, N. V., Govorukhina, N. I. & Trotsenko, Y. A. (1982). Autotrophic metabolism of methanol in *Blastobacter viscosus*. *Microbiology* (English translation of *Mikrobiologiya*) **50**, 428–434.

Marmur, J. R. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–214.

Padden, A. N., Rainey, F. A., Kelly, D. P. & Wood, A. P. (1997). *Xanthobacter tagetidis* sp. nov., an organism associated with *Tagetes* species and able to grow on substituted thiophenes. *Int J Syst Bacteriol* **47**, 394–401.

Rothe, B., Fisher, A., Hirsch, P., Sitting, M. & Stackebrandt, E. (1987). The phylogenetic position of the budding bacteria *Blastobacter aggregatus* and *Gemmobacter aquatilis* gen. nov. sp. nov. *Arch Microbiol* **147**, 92–99.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sitting, M. & Hirsch, P. (1992). Chemotaxonomic investigation of budding and hyphal bacteria. *Syst Appl Microbiol* **15**, 209–222.

Sly, L. I. (1985). Emendation of the genus *Blastobacter* Zavarzin 1961 and description of *Blastobacter natatorius* sp. nov. *Int J Syst Bacteriol* **35**, 40–45.

Sly, L. I. & Cahill, M. (1997). Transfer of *Blastobacter natatorius* (Sly 1985) to the genus *Blastomonas* gen. nov. as *Blastomonas natatoria* comb. nov. *Int J Syst Bacteriol* **47**, 566–568.

Sly, L. I. & Hargreaves, M. H. (1984). Two unusual budding bacteria isolated from a swimming pool. *J Appl Bacteriol* **56**, 479–486.

Trotsenko, Y. A., Doronina, N. V. & Hirsch, P. (1989). Genus *Blastobacter*. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 1963–1968. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

Wiegel, J. (1992). The genus *Xanthobacter*, pp. 2365–2383. In *The Prokaryotes*, 2nd edn. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer-Verlag.

Wiegel, J. & Schlegel, H. G. (1984). Genus *Xanthobacter*. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 325–333. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Willems, A. & Collins, M. D. (1992). Evidence for a close genealogical relationship between *Afipia* (the causal organism of cat scratch disease) *Bradyrhizobium japonicum* and *Blastobacter denitrificans*. *FEMS Microbiol Lett* **96**, 241–246.

Yabuuchi, E., Kosako, Y., Naka, T., Suzuki, S. & Yano, I. (1999). Proposal of *Sphingomonas suberifaciens* (van Bruggen, Jochimsen and Brown 1990) comb. nov., *Sphingomonas natatoria* (Sly 1985) comb. nov., *Sphingomonas ursincola* (Yurkov *et al.* 1997) comb. nov., and emendation of the genus *Sphingomonas*. *Microbiol Immunol* **43**, 339–349.

Zavarzin, G. A. (1961). Budding bacteria. *Microbiology* (English translation of *Mikrobiologiya*) **30**, 774–791.