Ancylobacter polymorphus sp. nov. and Ancylobacter vacuolatus sp. nov.

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The taxonomic positions of ‘Renobacter vacuolatum’ DSM 1277 and ‘Ancylobacter polymorphus’ DSM 2457 were investigated in this study. 16S rRNA gene sequence analysis indicated that both strains belonged to the genus Ancylobacter. DNA–DNA hybridization showed that they differed from Ancylobacter aquaticus DSM 101T and Ancylobacter rudongensis AS 1.1761T. According to molecular and phenotypic characteristics, strain DSM 1277T (=AS 1.2807T) is proposed as the type strain of Ancylobacter vacuolatus sp. nov. At the same time, valid publication of the name Ancylobacter polymorphus sp. nov. is proposed, with the type strain DSM 2457T (=AS 1.2800T = NCIMB 10516T).

According to its curved, small rod shape, Ørskov named the genus Microcylus in 1928 (Ørskov, 1928). However, because of the prior existence of a fungal genus of the same name [Microcylus Saccardo, Sydow & P. Sydow 1904; Sydow & Sydow (1904)], Raj (1983) substituted the name Ancylobacter. Members of Ancylobacter are widespread in nature, particularly in aquatic environments, such as lowland marshes, water reservoirs and wastewater treatment ponds (Raj, 1989). Members of the genus seem to play an important ecological role in oligotrophic methylootrophy (Raj, 1989). Before Ancylobacter rudongensis was described (Xin et al., 2004), there was only one species in the genus, Ancylobacter aquaticus. In addition, various investigators have isolated several closely related bacteria from different countries, for instance ‘Renobacter vacuolatum’ (Nikitin, 1971) and ‘Ancylobacter polymorphus’ (Raj, 1989). However, these species names were neither included in the Approved Lists nor later validly published.

In this study, we studied the physiological, genetic and phylogenetic characteristics of ‘R. vacuolatum’ DSM 1277 and ‘A. polymorphus’ DSM 2457. Phylogenetic analysis showed that strains DSM 1277 and DSM 2457 belonged to the genus Ancylobacter. DNA–DNA hybridization indicated that each of these strains represents a distinct novel species.

A. aquaticus DSM 101T and Ancylobacter spp. DSM 1227 and DSM 2457 were obtained from DSMZ. A. rudongensis AS 1.1761T was obtained from China General Microbiological Culture Collection Center. These strains were incubated at 30°C in Ancylobacter–Spirosoma medium (DSMZ medium 7; DSMZ, 1998). In order to test the requirement for O2, the strains were incubated in Ancylobacter–Spirosoma medium with 0–2% agar. Growth with carbon sources was tested in the basal medium supplemented with vitamins at 0·1% final concentrations of carbon sources. The production of acid and gas from carbohydrates was determined using the method of Hugh & Leifson (1953). Nitrogenase activity was tested as described by Hansson & Phillips (1981). DNase was tested as described by Schreier (1969). Indole production, methyl red and Voges–Proskauer tests, nitrate reduction and denitrification, H2S production, milk reaction, starch hydrolysis, gelatin liquefaction, aesculin hydrolysis, urease, lipase (Tween 80), arginine dihydrolase and phenylalanine deaminase were tested according to the methods of Smibert & Krieg (1981). Genomic DNAs were extracted according to the method of Marmur (1961). The G+C content of the DNA was tested by the thermal denaturation method (Marmur & Doty, 1962). Levels of DNA–DNA hybridization were determined from the initial DNA–DNA liquid reassociation rate as described by De Ley et al. (1970). Renaturation rates were determined in 2 × SSC on a model Lambda Bio 20 UV/Vis spectrophotometer equipped with a temperature program controller (Perkin-Elmer). The optimal renaturation temperature was 79°C.

PCR amplification of the 16S rRNA gene used primers 5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-TACGCTACCTTGTACGACTT-3′. PCR products were sequenced directly using an ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Perkin Elmer) on an ABI PRISM 377XL DNA Sequencer (Applied Biosystems). The
sequences of strains DSM 2457 and DSM 1277 and corresponding sequences retrieved from the GenBank database were aligned using the CLUSTAL X program (version 1.64b; Thompson et al., 1997). Evolutionary distances were calculated using the program within the PHYLIP package (Felsenstein, 1993). The phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987) using the software package TREECON for Windows version 1.3b (Van de Peer & De Wachter, 1994).

Cells of strains DSM 1277 and DSM 2457 were Gram-negative, curved rods, 0.8–1.0 μm in diameter, non-motile, with a capsule and gas vesicles. Colonies were white, convex, round, entire and 0.5–1.0 mm in diameter after 7 days incubation on nutrient agar plates. When they grew in liquid medium, they produced pellicles. They produced poly-β-hydroxybutyrate granules. They could fix free nitrogen while growing on Döbereiner nitrogen-free medium (Döbereiner et al., 1976). Strain DSM 1277 did not grow at 4 or 37 °C, and strain DSM 2457 did not grow at 4 or 50 °C. Optimum growth occurred at 28–30 °C and pH 7.0. Strain DSM 1277 showed no growth at pH 5.5 or 11.0, and strain DSM 2457 showed no growth at pH 4.5 but grew at pH 11.0. Strain DSM 1277 grew on medium containing 0–2.5 % (w/v) NaCl, whereas strain DSM 2457 grew on medium containing 0–3 % (w/v) NaCl. The two strains were positive for oxidase, whereas strain DSM 2457 grew on medium containing 0–3 % (w/v) NaCl. The two strains were positive for oxidase, catalase and urease activities and negative for DNase, phenylalanine deaminase, lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. Lipase (Tween 80) and arginine deaminase. 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growth at 28–30 °C and at pH 7-0. Grows in 0–2.5 % (w/v) NaCl. Oxidase-, catalase- and urease-positive. Negative for DNase, phenylalanine deaminase, lipase (TWEEN 80) and arginine dihydrolase. Does not produce H₂S or indole. Methyl red and Voges–Proskauer tests are negative. Reduces nitrates. Does not grow anaerobically with nitrate. Hydrolyses aesculin and gelatin but not starch. Utilizes the following substrates as carbon and energy sources: maltose, L-arabinose, fructose, glycerol, mannitol, sorbitol, starch, adonitol, ribose, mannose, aesculin, amygdalin, sodium gluconate, ketogluaric acid, alanine, proline, fumaric acid, arginine, sodium acetate, sodium malonate, sodium citrate, sodium succinate, sodium propionate, sodium lactate, sodium malate and methanol. Utilizes the following substrates weakly as carbon and energy sources: cellobiose, galactose, glucose, xylose, methyl D-glucoside, melezitose, sodium formate and salicin. Does not utilize the following substrates as carbon and energy sources: rhamnose, dextrin, lactose, inulin, raffinose, sucrose, trehalose, sorbose, melibiose, dulcitol, inositol, meso-erythritol, sodium tartrate, sodium hippurate and sodium lactate. Forms acid but no gas from mannose, ribose, glycerol, mannitol, inulin, sorbitol, galactose, cellobiose, rhamnose, salicin, glucose, fructose, ribose, galactose, D-arabinose, fructose and lactose. Does not form acid or gas from inositol, raffinose, melezitose, starch, glycerogen, methyl D-glucoside, sucrose, maltose, sorbose, trehalose, dulcitol or meso-erythritol. The DNA G+C content of the type strain is 65.5 mol%.

The type strain is strain DSM 1277T (=AS 1.2807T), isolated from soil in Russia, previously described as ‘Renobacter vacuolatum’ (Nikitin, 1971).

Description of Ancylobacter polymorphus sp. nov.

Ancylobacter polymorphus (pơ.ly.mor’ phus. N.L. masc. adj. polymorphus polymorphic).

Gram-negative, curved rods, 0.8–1.0 μm in diameter, non-motile, with a capsule and gas vesicles. Colonies are white, convex, round, entire and 0.5–1.0 mm in diameter after 7 days incubation on nutrient broth agar plates. Can produce pellicles when growing in liquid medium. Produces poly-β-hydroxybutyrate granules. Fixes free nitrogen while growing on Döbereiner nitrogen-free medium. No growth at 4 or 50 °C or at pH 4–5. Optimum growth at 28–30 °C and at pH 7-0. Grows at pH 11-0. Grows in 0–3 % (w/v) NaCl. Oxidase-, catalase- and urease-positive. Negative for DNase, phenylalanine deaminase, lipase (TWEEN 80) and arginine dihydrolase. Does not produce H₂S or indole. Methyl red and Voges–Proskauer tests are negative. Reduces nitrates. Does not grow anaerobically with nitrate. Hydrolyses aesculin and gelatin but not starch. Utilizes the following substrates as carbon and energy sources: maltose, methyl D-glucoside, adonitol, glycerol, glucose, ribose, inulin, L-arabinose, fructose, galactose, mannitol, dextrin, meso-erythritol, xylose, sorbitol, amygdalin, ketogluaric acid, alanine, proline, fumaric acid, arginine, sodium acetate, sodium malonate, sodium citrate, sodium succinate, sodium propionate, sodium lactate, sodium malate and methanol. Utilizes the following substrates weakly as carbon and energy sources: cellobiose, galactose, glucose, xylose, methyl D-glucoside, melezitose, sodium formate and salicin. Does not utilize the following substrates as carbon and energy sources: rhamnose, dextrin, lactose, inulin, raffinose, sucrose, trehalose, sorbose, melibiose, dulcitol, inositol, meso-erythritol, sodium tartrate, sodium hippurate and sodium lactate. Forms acid but no gas from mannose, ribose, glycerol, mannitol, inulin, sorbitol, galactose, cellobiose, rhamnose, salicin, glucose, fructose, ribose, galactose, D-arabinose, fructose and lactose. Does not form acid or gas from inositol, raffinose, melezitose, starch, glycerogen, methyl D-glucoside, sucrose, maltose, sorbose, trehalose, dulcitol or meso-erythritol. The DNA G+C content of the type strain is 65.5 mol%.

The type strain is strain DSM 2457T (=AS 1.2800T = NCIMB 10516T), isolated from river mud (Maclennan et al., 1974).

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References


Fig. 1. Phylogenetic tree of strains DSM 1277 and DSM 2457 and related taxa based on 16S rRNA gene sequence comparisons using the neighbour-joining method (Saitou & Nei, 1987). Accession numbers of reference sequence are given after the strain names. Numbers represent confidence levels from 1000 replicate bootstrap samplings. Bar, 5 substitutions per 100 nucleotide positions.


