**NOTE**

Propionivibrio limicola sp. nov., a fermentative bacterium specialized in the degradation of hydroaromatic compounds, reclassification of Propionibacter pelophilus as Propionivibrio pelophilus comb. nov. and amended description of the genus Propionivibrio

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Strain GolChi1T, a mesophilic, anaerobic bacterium, was isolated with quinic acid (1,3,4,5-tetrahydroxy-cyclohexane-1-carboxylic acid) as the sole source of carbon and energy. Of more than 30 substrates tested, only the hydroaromatic compounds quinic acid and shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) were utilized, yielding acetate and propionate as the only fermentation products. Sugars, alcohols, (di-)carboxylic acids, amino acids and aromatic compounds were not fermented and no external electron acceptors were used. Strain GolChi1T is a Gram-negative, rod-shaped, aerotolerant anaerobe that possesses superoxide dismutase; it does not employ the classical hydroaromatic pathway of aerobic bacteria for the degradation of hydroaromatic compounds (no aromatic intermediates involved). 16S-rRNA-based phylogenetic analyses revealed a common origin of this isolate and Rhodocyclus, Propionibacter and Propionivibrio species. High sequence similarity (> 96%) and phenotypic traits indicated a closer relationship between strain GolChi1T and the type species of the monospecific genera Propionivibrio and Propionibacter but, due to its phenotypic properties, strain GolChi1T could not be assigned conclusively to either of these taxa. We propose (i) the amended description of the genus Propionivibrio, (ii) the reclassification of Propionibacter pelophilus Meijer et al. 1999 as Propionivibrio pelophilus comb. nov. and (iii) designation of Propionivibrio limicola sp. nov., with the type strain GolChi1T (∊ DSM 6832T = ATCC BAA-290T).

Keywords: anaerobic degradation, fermentation, hydroaromatic compounds, quinic acid, shikimic acid

The biosynthesis of aromatic compounds via the shikimic acid pathway involves hydroaromatic compounds as important intermediates (Herbert, 1981). Quinic acid and shikimic acid, which are important precursors of lignin and tannin biosynthesis, are stored in considerable amounts in the vacuoles of many vascular plants (Yoshida et al., 1975). The degradation of hydroaromatic compounds by aerobic bacteria and fungi proceeds oxidatively via the hydroaromatic pathway, involving aromatic intermediates (for references, see Brune & Schink, 1992).

Fermentative degradation of hydroaromatic compounds by anaerobic bacteria has been shown only in the past decade. Several strains have been enriched and isolated from marine and freshwater sediments with quinic acid as the sole source of carbon and energy (Brune & Schink, 1992). Two of these isolates, the marine strain VenChi2T and the freshwater strain GolChi1T, have been characterized morphologically...
and physiologically in detail. Both strains degrade hydroaromatic compounds via novel, fermentative pathways that do not involve aromatic intermediates (Brune & Schink, 1992). The unique phenotypic traits of the two strains, however, did not point to a taxonomic affiliation. Here, we present the results of the phylogenetic analysis of strain GolChi1\textsuperscript{T}, together with additional phenotypic data, and propose the designation of a novel species in the genus Propionivibrio. The results of the phylogenetic analysis of strain VenChi2\textsuperscript{T} have been presented elsewhere (Brune et al., 2002).

**Characterization of strain GolChi1\textsuperscript{T}**

Pure cultures of strain GolChi1\textsuperscript{T}, which was originally isolated from freshwater sediment of a eutrophic pond near Tübingen, Germany, were taken from our laboratory collection. Cells were routinely cultivated in oxygen-free, bicarbonate-buffered mineral medium with 5 mM sodium quinate (1,3,4,5-tetrahydroxy-cyclohexane-1-carboxylic acid, sodium salt) as the sole source of carbon and energy. Details are given in the original description (Brune & Schink, 1992).

Strain GolChi1\textsuperscript{T} is restricted to the fermentation of hydroaromatic substrates. More than 30 different substrates were tested, and only quinic acid and shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) were utilized. Sugars (cellobiose, fructose, glucose, erythrose, lactose, ribose, xyllose), alcohols (meso-erythritol, ethanol, glycerol, mannitol), carboxylic acids (citrate, crotonate, fumarate, glycolate, 2-hydroxybutyrate, 3-hydroxybutyrate, 4-hydroxybutyrate, lactate, malate, 2-oxobutyrate, pyruvate, sorbate, tartrate), amino acids (alanine, aspartate, glycine, threonine) and aromatic compounds (gallate, phloroglucinol, protocatechuic acid, resorcinol, 3,4,5-trimethoxybenzoate, 3,4,5-trimethoxycinnamate) were not fermented (Brune & Schink, 1992). External electron acceptors (amorphous ferric iron, nitrate, oxygen, sulfate, sulfur, thiosulfate) were not reduced with lactate, propionate or quinate as the electron donor.

Additional growths tests on d-glucose (5 mM) and disodium l-malate, disodium fumarate and sodium l-lactate (each 10 mM) performed in medium supplemented with L-phenylalanine, L-tyrosine and L-tryptophan (each 50 \(\mu\)M) were negative. This indicates that the absence of growth on these compounds is not caused by an inability of strain GolChi1\textsuperscript{T} to synthesize aromatic amino acids in the absence of quinate or shikimate as precursors. Other physiological properties of strain GolChi1\textsuperscript{T} have been documented in detail (Brune & Schink, 1992); taxonomically relevant traits are summarized in the species description.

**Phylogenetic analysis**

16S-rRNA-encoding DNA fragments were amplified \textit{in vitro} and sequenced directly as described earlier (Springer et al., 1992; Ludwig et al., 1998). Using the automated tools of the ARB software package (Ludwig & Strunk, 1996), the new 16S rRNA sequences were fitted into an alignment of about 22000 homologous full and partial primary structures available in public databases (Ludwig, 1995). Distance-matrix, maximum-parsimony and maximum-likelihood methods were applied as implemented in the ARB software package. Different datasets were analysed, varying with respect to the sequences of outgroup reference organisms included and alignment positions selected according to their degrees of conservation.

Phylogenetic tree analysis showed that strain GolChi1\textsuperscript{T} represents a phylogenetic subgroup of the \(\beta\)-subclass of the Proteobacteria that comprises Rhodococcus, Propionibacter and Propionivibrio species. Strain GolChi1\textsuperscript{T} shares the highest 16S rRNA sequence similarity (93.3–96.4\%) with species of the genera Ferribacterium (Cummings et al., 1999), Dechloromonas (Achenbach et al., 2001), Rhodococcus (Dewhirst et al., 1990), Propionivibrio (Hippe et al., 1999) and Propionibacterium (Meijer et al., 1999) (Fig. 1). In comprehensive phylogenetic trees, this group is placed in the neighbourhood of Azoarcus, Thauera, Hydrogenophilus and Zoogloea (represented by Azoarcus evansii and Thauera aromatica in Fig. 1; Anders et al., 1995) in the \(\beta\)-subclass of the Proteobacteria.

A closer relationship of strain GolChi1\textsuperscript{T} to Propionibacter pelophilus and Propionivibrio dicarboxylicus (96.4 and 96.0\% sequence similarity, respectively) was supported by all tree analyses performed. Also, the DNA G+C content of strain GolChi1\textsuperscript{T} (61.6 mol\%) is very similar to the values reported for Propionibacter pelophilus (60.8 mol\%); Meijer et al., 1999) and Propionivibrio dicarboxylicus (61 mol\%; Tanaka et al., 1990), whereas it differs slightly from the narrow range of values spanned by members of the genus Rhodococcus (64.8–65.3 mol\%; Trüper & Imhoff, 1992).

**Taxonomic considerations**

In addition to the high 16S rRNA gene sequence similarity, strain GolChi1\textsuperscript{T}, Propionivibrio dicarboxylicus and Propionibacter pelophilus also share a number of phenotypic traits that separate them clearly from their closest phylogenetic relatives. All three strains are chemotrophic organisms with a fermentative metabolism and form propionate and acetate as the major products, which allows their classification as the only members of the \(\beta\)-Proteobacteria that perform a propionic acid fermentation. All three form rod-shaped cells that are motile by a single polar flagellum. Strain GolChi1\textsuperscript{T} and Propionibacter pelophilus are both aerotolerant.

Nevertheless, strain GolChi1\textsuperscript{T} is clearly separated from the existing species. Propionivibrio dicarboxylicus is a curved rod, utilizes maleate, fumarate and l-malate and decarboxylates succinate to propionate, whereas Propionibacter pelophilus ferments simple organic compounds (sugars, dicarboxylic acids, sugar
alcohols) and reduces nitrate. None of these traits is present in strain GolChi1T. Interestingly, growth tests performed with Propionibacter pelophilus DSM 12018T revealed that this organism is also capable of fermenting quinic acid and shikimic acid, forming acetate and propionate as the major products (data not shown). Nevertheless, since strain GolChi1T can be distinguished clearly from the existing species not only by its unique metabolism but also by phylogenetic distance (Stackebrandt & Goebel, 1994), it should be assigned to a separate species.

The genus Propionivibrio was described by Tanaka et al. (1990) and contains a single species, Propionivibrio dicarboxylicus. The phylogenetic position of Propionivibrio dicarboxylicus (Hippe et al., 1999) was published almost simultaneously with the description of the genus Propionibacter, which contains Propionibacter pelophilus as its only species (Meijer et al., 1999). The small differences in the 16S rRNA gene sequences of these species and their similar fermentation patterns have prompted Hansen (2002) to suggest the inclusion of Propionibacter pelophilus, and propose to allocate all three species to the genus Propionivibrio.

Amended description of Propionivibrio Tanaka et al. 1990 emend.

Gram-negative rods (straight or curved). Do not form spores. May be motile by means of a single polar flagellum. Multiply by binary fission. Chemo-organotrophic metabolism. Substrates are fermented to propionate and acetate as major products. Strictly anaerobic to aerotolerant. Some species may use external electron acceptors. Based on their 16S rRNA sequences, members of this genus form a monophyletic group within the β-subclass of the Proteobacteria. The type species is Propionivibrio dicarboxylicus Tanaka et al. 1990.

Description of Propionivibrio limicola sp. nov.

Propionivibrio limicola (li.mi.co.la. L. n. limus mud; L. v. colere to inhabit; N.L. adj. limicola living in mud).

Cells are straight, slender rods, 0.6–0.7 μm wide and 1.5–2.5 μm long. Cells are motile (polar montrichously flagellated), Gram-negative, oxidase-negative, catalase-negative, superoxide-dismutase-positive. No spores are formed. Chemo-organotrophic, fermentative metabolism; external electron acceptors are not used. Contains no cytochromes. Quinic acid and shikimic acid are the only substrates, which are fermented to acetate, propionate and CO₂ as the only products. No growth with sugars (cellobiose, fructose, glucose, erythrose, lactose, ribose, xylose), alcohols (meso-erythritol, ethanol, glycerol, mannitol), car-
boxylic acids (citrate, crotonate, fumarate, glycolate, 2-hydroxybutyrate, 3-hydroxybutyrate, 4-hydroxybutyrate, lactate, malate, 2-oxobutyrate, pyruvate, sorbate, tartrate), amino acids (alanine, aspartate, glycine, threonine) or aromatic compounds (gallate, phloroglucinol, protocatechuate, resorcinol, 3,4,5-trimethoxybenzoate, 3,4,5-trimethoxyacinnamate). External electron acceptors (amorphous ferrous iron, nitrate, oxygen, sulfate, sulfur, thiosulfate) are not used. Aerotolerant; growth occurs in non-reduced media when incubated under air without agitation. pH range for growth is 6.0–8.0, with an optimum around pH 7.0–7.5. Temperature optimum is 37 °C; no growth at 45 °C. Optimal growth in freshwater medium (identical growth rates with quinic acid and shikimic acid: μ = 0.22 h⁻¹). Growth is inhibited completely in brackish medium with 10 g NaCl and 14 g MgCl₂·6H₂O.

DNA base ratio: 61.6 ± 0.2 mol% G+C. Habitat: anoxic freshwater sediment. Type strain: GolChi1T (= DSM 6832T = ATCC BAA-290T).

Description of Propionivibrio pelophilus comb. nov.

Basonym: Propionibacter pelophilus Meijer et al. 1999. The genus Propionibacter was described by Meijer et al. (1999) to harbour the newly described species Propionibacter pelophilus. Following the suggestion of Hansen (2002), we propose to reclassify Propionibacter pelophilus as Propionivibrio pelophilus comb. nov. as per emendation of the description of Propionivibrio. By this transfer, the genus Propionibacter loses its only species and becomes void. The traits ‘nitrāte reduced to nitrite’ and ‘utilizes N₂ as nitrogen source’, formerly included in the description of Propionibacter (Meijer et al., 1999), are added to the description of Propionivibrio pelophilus.

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References


