NOTE

***Propionimicrobium* gen. nov., a new genus to accommodate *Propionibacterium lymphophilum* (Torrey 1916) Johnson and Cummins 1972, 1057^{AL} as *Propionimicrobium lymphophilum* comb. nov.**

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Based upon significant differences in chemotaxonomic properties, i.e., amino acid composition of peptidoglycan, fatty acids and base composition of DNA, and supported by the phylogenetic position of the 16S rDNA sequence the species *Propionibacterium lymphophilum* was reclassified as *Propionimicrobium lymphophilum* comb. nov.

Keywords: *Propionibacterium*, *Propionimicrobium* gen. nov., *Propionimicrobium lymphophilum* comb. nov.

Originally described as '*Bacillus lymphophilus*' (Torrey 1916), '*Corynebacterium lymphophilum*' (Torrey 1916) Ebersson 1918 and '*Mycobacterium lymphophilum*' (Torrey 1916) Krasil'nikov 1949, strain VIP 0202 was included in a taxonomic study on coryneforms and propionibacteria by Johnson & Cummins (1972). Based upon low DNA reassociation with members of *Corynebacterium*, anaerobic growth and the formation of propionic acid, this strain was tentatively classified as *Propionibacterium lymphophilum*. Skerman *et al.* (1980) included *Propionibacterium lymphophilum* in the Approved Lists of Bacterial Names, referring to the publication of Johnson & Cummins (1972) and to a description by Holdeman *et al.* (1977). The *Anaerobe Laboratory Manual* of the Virginia Polytechnic Institute and State University, Blacksburg, VA, USA (VIP), however, only lists physiological and morphological properties but gives no formal description. This was followed in 1986 (Cummins & Johnson, 1986) by the coverage of the genus *Propionibacterium* in *Bergey's Manual of Systematic Bacteriology*. Both Holdeman *et al.* (1977) and Skerman *et al.* (1980) indicated strain VIP 7625B^T (= ATCC 27520^T) as the type strain, but neither Johnson & Cummins (1972), Holdeman *et al.* (1977) nor Cummins & Johnson (1986) refer to VIP 7625B^T but to VIP 0202 as the

strain investigated. According to the Technical Services of ATCC in August 2001, strain ATCC 27520^T was originally received as strain VIP 7625B^T.

In order to determine whether strain VIP7625B^T (= ATCC 27520^T = DSM 4309^T) is a subculture of VIP 0202, we reinvestigated two of the salient characters of strain DSM 4309^T, originally obtained for strain VIP 0202, i.e. base composition of DNA and cell wall composition. Johnson & Cummins (1972) found lysine instead of diaminopimelic acid (A₂pm) as the dicarboxylic amino acid of the peptidoglycan. A₂pm is the diagnostic amino acid of *Propionibacterium* species other than *Propionibacterium lymphophilum*. Using the methods of Schleifer & Kandler (1972), we confirm the presence of lysine and demonstrate the peptidoglycan type to be A4 α (Lys-Asp), in which aspartic acid forms the interpeptide bridge. We also confirm the rather low G+C content of the DNA. Using the method of Mesbah *et al.* (1989), the DSMZ culture has a DNA base composition of 56 mol % G+C, which is slightly higher than the 53–54 mol % reported for VIP 0202. Investigation of the principal isoprenoid quinone (Collins *et al.*, 1977) revealed MK-9(H₄), which is present in all members of the family *Propionibacteriaceae*. Major fatty acids, determined according to the methods of Miller & Berger (1984), are C_{18:1} ω 9c (30 %), anteiso-C_{15:0} (29 %) and C_{16:0} (15 %); smaller components (> 2 to < 5 %) are C_{14:0}, i-C_{15:0}, ai-C_{17:0} and C_{18:1} ω 7c. This composition differs significantly from those reported for *Propionibacterium* strains, in

Fig. 1. Distance matrix tree (De Soete, 1983), displaying the phylogenetic position of *Propionibacterium lymphophilum* DSM 4903^T, described as *Propionimicrobium lymphophilum* in this study, within the radiation of some members of the family *Propionibacteriaceae*. Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 200 bootstrapped trees (only values of 80% and above are shown). Bar, 2 nucleotide changes per 100 nucleotides.

which either branched fatty acids (Moss *et al.*, 1969) or ω -cyclohexane (Kusano *et al.*, 1997) dominate.

The description of this species is based on two strains, VIP 0202 and VIP 0383, which show 75% DNA similarity to one another. Two additional strains shared 100% DNA similarity with strain VIP 0202 and also show cross-agglutination and precipitation with it (cited by Cummins & Johnson, 1972). In their coverage of the species *Propionibacterium lymphophilum* in *Bergey's Manual of Systematic Bacteriology* (1986), Cummins & Johnson state 'In view of this [referring to chemotaxonomic properties] and the small number of strains, the status and taxonomic position of this organism must remain somewhat provisional until more strains are collected and examined'. A combination of chemotaxonomic distinctness and phylogenetic position, based upon comparative sequence analysis of 16S rDNA, has been the main basis for the description of novel genera in the past decade. *Propionibacterium lymphophilum* DSM 4309^T shares less than 91.8% sequence similarity with the other *Propionibacterium* species (Dasen *et al.*, 1998) which themselves show more than 93% similarity among each other. Depending upon the algorithm used, analysis of the 16S rDNA indicates *Propionibacterium lymphophilum* to form either the deepest branch of the genus (Koussémon *et al.*, 2001; NEIGHBOR program [Felsenstein, 1993]), or to branch even among the other genera of *Propionibacteriaceae* (this study, based upon maximum-likelihood [Felsenstein, 1993] or distance matrix analyses [De Soete, 1983], Fig. 1).

Although the availability of more than a single strain is the basis for unravelling the metabolic potential of a species, hardly more than two strains have been included in the description of recent species of the

family *Propionibacteriaceae* (Stackebrandt *et al.*, 1997), e.g. *Propionibacterium cyclohexanicum* (Kusano *et al.*, 1997) and *Propionibacterium microaerophilum* (Koussémon *et al.*, 2001), *Micrococcus phosphovorans* (Nakamura *et al.*, 1995), the two species of *Luteococcus* (Tamura *et al.*, 1994; Collins *et al.*, 2000), *Tessaracoccus bendigoensis* (Maszenan *et al.*, 1999b), *Micropruina glycogenica* (Shintani *et al.*, 2000) as well as the four species of *Friedmanniella* (Lawson *et al.*, 2000; Schumann *et al.*, 1997; Maszenan *et al.*, 1999a).

We thus conclude, on the basis of distinct phylogenetic position in combination with the significant differences in peptidoglycan composition in which *Propionibacterium lymphophilum* differs from the other members of *Propionibacterium*, that a new genus is warranted to accommodate *Propionibacterium lymphophilum*. We therefore propose the generic name *Propionimicrobium* gen. nov. and the species *Propionimicrobium lymphophilum* comb. nov. for this organism. Physiologically this species can be differentiated from members of *Propionibacterium* by a combination of results from aesculin hydrolysis, indole production and nitrate reduction, as well as by a combination of acid production from L-sorbose, adonitol, erythritol, maltose and ribose (Kusano *et al.*, 1997; Koussémon *et al.*, 2001).

Description of *Propionimicrobium* gen. nov.

Propionimicrobium (Pro.pi.on.i.mi.cro'bi.um. N.L. n. *acidum propionicum* propionic acid; Gr. adj. *micro* small; Gr. masc. n. *bios* life. N.L. neut. n. *Propionimicrobium* propionic acid-producing microbe).

The description is based on data of Holdeman *et al.* (1977), Cummins & Johnson (1986), 16S rDNA data of Dasen *et al.* (1998) and our own data. Pleomorphic rods, 0.5–0.8 μ m in diameter $\times$ 1–2.5 μ m in length, often diphtheroid or club-shaped. Cells may be coccoid. Cells may occur singly, in pairs or short chains, in V or Y configurations or in clumps. Gram-positive, non-motile, non-sporeforming chemo-organotrophs. Anaerobic. Fermentation end products include propionic acid, acetic acid, succinic acid, iso-valeric acid and lesser amounts of formic acid. One out of two strains is catalase-positive. The major menaquinone type is MK-9(H₄). The peptidoglycan contains lysine and aspartic acid (Lys-Asp type). Major fatty acids are C_{18:1} ω 9c, anteiso-C_{15:0} and C_{16:0}. The G + C content of the DNA is 53–54 mol% (*T_m*) or 56 mol% (HPLC of digested DNA). Based upon 16S rDNA analysis a member of the family *Propionibacteriaceae*. Type species: *Propionimicrobium lymphophilum* (Torrey 1919) Johnson & Cummins 1972.

Description of *Propionimicrobium lymphophilum* (Torrey 1919) Johnson and Cummins 1972 comb. nov.

Propionimicrobium lymphophilum (lym.pho'phi.lum. L. fem. n. *lympha* clear water, lymph; Gr. adj. *philos* loving; N.L. adj. *lymphophilum* lymph-loving).

The description is taken from Cummins & Johnson (1986) based on the characterization of four strains (Holdeman *et al.*, 1977; Johnson & Cummins, 1972). Surface colonies on horse blood in 4 days are punctiform to 0.5 mm, circular, entire, convex to pulvinate, white, glistening and smooth. Glucose broth cultures (24 h) are turbid, becoming clear, with a ropy sediment and terminal pH of 5.4–5.7. Anaerobic, producing no growth on agar surface incubated aerobically but growth develops in deep broth incubated aerobically. Acid production from adonitol, erythritol, fructose, glucose, maltose, ribose as well as starch and inositol (reaction positive in 40–90 % of strains). No acid from amygdalin, arabinose, cellobiose, dulcitol, aesculin, galactose, glycerol, glycogen, inulin, lactose, mannitol, mannose, melezitose, raffinose, rhamnose, salicin, sorbitol or sorbose. Nitrate reduction is positive for some strains. Aesculin and gelatin not hydrolysed, indole and acetoin not produced, haemolysis negative. Production of gas and growth are weak in 20 % bile. Cell walls contain alanine, glutamic acid, lysine and aspartic acid. Glucose, galactose and mannose are principal cell-wall sugars. Major fatty acids are C_{18:1}ω9c (30 %), anteiso-C_{15:0} (29 %) and C_{16:0} (15 %), smaller amounts (> 2 to < 5 %) are C_{14:0}, i-C_{15:0}, ai-C_{17:0} and C_{18:1}ω7c. Isolated from urinary tract infections and mesenteric ganglion of a monkey. Strains originally described by Torrey (1916) were from lymph nodes of patients suffering from Hodgkin's disease. Type strain: ATCC 27520^T = DSM 4309^T.

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