***Friedmanniella spumicola* sp. nov. and *Friedmanniella capsulata* sp. nov. from activated sludge foam: Gram-positive cocci that grow in aggregates of repeating groups of cocci**

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Two Gram-positive, non-motile, non-spore-forming, strictly aerobic, pigmented cocci, strains Ben 107^T and Ben 108^T, growing in aggregates were isolated from activated sludge samples by micromanipulation. Both possessed the rare type A3 γ peptidoglycan. Major menaquinones of strain Ben 107^T were MK-9(H₄) and MK-7(H₂), and the main cellular fatty acid was 12-methyltetradecanoic acid (ai-C_{15:0}). In strain Ben 108^T, MK-9(H₄), MK-9(H₂) and MK-7(H₄) were the menaquinones and again the main fatty acid was 12-methyltetradecanoic acid (ai-C_{15:0}). Polar lipids in both strains consisted of phosphatidyl inositol, phosphatidyl glycerol and diphosphatidyl glycerol with two other unidentified glycolipids and phospholipids also present in both. These data, together with the 16S rDNA sequence data, suggest that strain Ben 107^T belongs to the genus *Friedmanniella* which presently includes a single recently described species, *Friedmanniella antarctica*. Although the taxonomic status of strain Ben 108^T is far less certain, on the basis of its 16S rRNA sequence it is also adjudged to be best placed in the genus *Friedmanniella*. The chemotaxonomic characteristics and DNA–DNA hybridization data support the view that Ben 107^T and Ben 108^T are novel species of the genus *Friedmanniella*. Hence, it is proposed that strain Ben 107^T (= ACM 5121^T) is named as *Friedmanniella spumicola* sp. nov. and strain Ben 108^T (= ACM 5120^T) as *Friedmanniella capsulata* sp. nov.

Keywords: activated sludge, G-bacteria, Gram-positive cocci, *Friedmanniella*, *Microtholunatus*

INTRODUCTION

Cocci are widely seen in many activated sludge biomass samples, especially those from aerobic/anaerobic enhanced biological nutrient removal plants (Knight *et al.*, 1995) and sequencing batch reactors (SBR) (Bond *et al.*, 1997; Liu *et al.*, 1996; Randall *et al.*, 1994;

Carucci *et al.*, 1994; Cech & Hartman, 1993). The Gram-negative cocci, the so-called 'G' bacteria (Cech & Hartman, 1993) have been isolated and their taxonomy resolved (Maszenan *et al.*, 1997). Four isolates from different parts of the world were all shown to be separate species belonging to a single new genus *Amaricoccus* (Maszenan *et al.*, 1997). *Amaricoccus kaplicensis* has also been named, albeit invalidly, as '*Tetracoccus cechii*' by Blackall *et al.* (1997).

However, Gram-positive cocci are also present in activated sludge and some of these have been cultured and their taxonomy resolved (Maszenan *et al.*, 1999), while others still remain unidentified or uncultured

Abbreviations: A₂pm, diaminopimelic acid; EBPR, enhanced biological phosphorus removal; PolyP, polyphosphate; SBR, sequencing batch reactors; S_{SM}, simple matching coefficients; UPGMA, unweighted pair group with mathematical averages.

The GenBank accession numbers for the 16S rRNA sequences of strains Ben 107^T (= ACM 5121^T) and Ben 108^T (= ACM 5120^T) are AF062535 and AF084529, respectively.

(Katoaka *et al.*, 1996; Matsuo, 1994; Matsuzawa & Mino, 1991). The Gram-positive cocci which have been cultured from activated sludge include *Micro-lunatus phosphovor* (Nakamura *et al.*, 1995) in the family *Propionibacteriaceae*, suborder *Propionibacterineae* (Stackebrandt *et al.*, 1997), and *Micro-sphaera multipartita* (Yoshimi *et al.*, 1996) in the family *Microsphaeraceae*, suborder *Frankineae* (Stackebrandt *et al.*, 1997). Both isolates were obtained in Japan and are slow-growing organisms with G+C values of about 70 mol %. Neither have been reported elsewhere in the world. Chemotaxonically, *M. phosphovor* has type A3 γ' peptidoglycan (Schumann *et al.*, 1997), whereas *M. multipartita* has meso-diaminopimelic acid (meso-A₃pm) in its cell wall (Yoshimi *et al.*, 1996). Recently, we isolated another Gram-positive coccus, *Tessaracoccus bendigoensis*, from a laboratory-scale SBR (Maszenan *et al.*, 1999). Based on its 16S rDNA sequence, it was also shown to be a member of the high-G+C-containing Gram-positive bacteria, suborder *Propionibacterineae* (Stackebrandt *et al.*, 1997). The description of this novel isolate, together with data from descriptions of *M. phosphovor* (Nakamura *et al.*, 1995) and *M. multipartita* (Yoshimi *et al.*, 1996), illustrates the taxonomic diversity among the high-G+C Gram-positive cocci found in activated sludge systems.

Other studies (Katoaka *et al.*, 1996; Bond *et al.*, 1995; Ubukata *et al.*, 1994; Wagner *et al.*, 1994; Liu *et al.*, 1996) would suggest that other Gram-positive cocci are also present in activated sludge systems, although none of the organisms seen in these studies in microscopic examinations of biomass samples has been obtained in pure culture. It has been suggested that cocci play an undesirable role in enhanced biological phosphorus removal (EBPR) plants, accumulating carbohydrate (as glycogen) and not polyphosphate (Cech & Hartman, 1993). As part of our ongoing research into studying these cocci in activated sludge biomass, we successfully isolated two orange cocci arranged in aggregates from activated sludge samples in Australia. This paper describes the taxonomy of these isolates after chemotaxonomic characterization and phylogenetic analysis and places them as novel species of the genus *Friedmanniella* (Schumann *et al.*, 1997).

METHODS

Isolation and maintenance of bacterial strains. Strain Ben 107^T was isolated from a stable foam on the surface of the aerobic reactor in an activated sludge plant treating mainly wastewater from an orange-juice-processing plant in Mildura, Victoria, Australia by micromanipulation; a Skerman micromanipulator (Skerman *et al.*, 1968) was used to separate cells from filamentous organisms present in the sample. Strain Ben 108^T was obtained from a sample of activated sludge biomass from Hayman Island, Queensland, Australia. Both were successfully micromanipulated (Maszenan *et al.*, 1997) onto plates of standard methods agar (SMA; Difco) supplemented with 1% sterile horse

serum, which were then incubated at 25 °C. Growth was monitored microscopically to check for contamination and, when colonies had developed (usually after 7–10 d), strains Ben 107^T and Ben 108^T were streaked onto fresh plates of R2A agar (Reasoner & Geldreich, 1985). Purity of the micromanipulated cells was checked microscopically and the purified culture was stored at –80 °C in R2A medium in 20% glycerol. The type strain of *Friedmanniella antarctica* (Schumann *et al.*, 1997) was grown on R-medium (Yamada & Komagata, 1972).

Staining procedures and morphology studies. Gram stains were performed according to the modified Hucker method (Hucker, 1921) and confirmed by using 3% KOH (Buck, 1982).

Physiological and biochemical characterization. Enzyme profiles and biochemical characteristics of strains Ben 107^T and Ben 108^T and *F. antarctica* were determined using the API ZYM (bioMérieux) and Microbact 24E (Oxoid) systems according to the manufacturers' instructions. Carbon substrate utilization tests were performed with the BIOLOG GN and GP (Special Diagnostics) systems, or by the methods described by Goodfellow (1971). The results obtained by the two different methods agreed in most cases, although some discrepancies, as indicated in results, were noticed.

Oxidase and catalase activities were determined using standard methods (Smibert & Krieg, 1994). The presence of possible storage granules was detected by microscopic examination of cells after staining according to the method of Rees *et al.* (1992). Cells were grown aerobically in R2A medium incorporating either glucose, acetate or propionate for detection of storage compounds at an initial concentration of 0.15 g l⁻¹. Urease activity, nitrate reduction and motility were determined according to methods previously described (Maszenan *et al.*, 1997).

The physiological and biochemical data for strains Ben 107^T and Ben 108^T and *F. antarctica* together with *T. bendigoensis* and *M. phosphovor* were analysed with numerical taxonomy using the simple matching coefficients (S_{SM}) and the unweighted pair group with mathematical averages (UPGMA) clustering algorithm (Sneath & Sokal, 1973), and the NTSYS-PC software package, version 1.80 (Exeter Software).

Chemical inhibitor tolerance and penicillin inhibition tests. R2A medium was the basal medium used for chemical tolerance tests. Crystal violet (0.001 and 0.0001%), phenol (0.1 and 0.3%), phenyl ethanol (0.03%), sodium azide (0.1 and 0.2%) and NaCl (5 and 7%) were added (final concentrations are shown in parentheses). Cultures were inoculated and any sign of growth was considered positive and taken to mean that the organism was tolerant to that concentration of chemical tested (Goodfellow & Pirouz, 1982). Penicillin sensitivity tests were conducted by placing penicillin disks (Oxoid) at 10 IU concentration on the lawn of R2A plates, prepared by inoculating with swabs soaked in suspensions of cells of strains Ben 107^T or Ben 108^T. Plates were incubated at 25 °C for 2 weeks. If no zone appeared around the disc, then the organism was resistant to penicillin at that concentration.

Culture conditions. The biomass used for phylogenetic studies was obtained by growing strains Ben 107^T and Ben 108^T aerobically in R2A broth or on R2A agar at 25 °C for 2 weeks. *F. antarctica* was grown on R-medium (Yamada &

Komagata, 1972). The biomass for chemotaxonomic analyses was obtained by cultivating strains Ben 107^T and Ben 108^T on R-agar at 22 °C for 2–3 weeks.

Electron microscopy. Cells for TEM studies were prepared by cryofixation and cryosubstitution protocols as described by Lindsay *et al.* (1997), using osmium tetroxide in acetone and embedding the material in epon resin. For chemical fixation studies, cells were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide in the same buffer and embedded in epon, while specimens were prepared for examination by SEM according to the method of Seviour *et al.* (1984).

16S rDNA sequence and phylogenetic analysis. The 16S rDNA from strains Ben 107^T and Ben 108^T was amplified, sequenced and analysed as previously described (Maszenan *et al.*, 1997).

DNA base composition. The DNA for base composition determination was isolated using the method of Maszenan *et al.* (1999) and calculated as previously described (Maszenan *et al.*, 1997).

Chemotaxonomic characterization. Peptidoglycan, menaquinone, cellular fatty acid and polar lipid compositions were all analysed as described by Schumann *et al.* (1997).

DNA–DNA hybridization. DNA–DNA hybridization was carried out according to the method of De Ley *et al.* (1970) with the modification described by Huss *et al.* (1983), using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992).

RESULTS

Culture characteristics

Strain Ben 107^T grew as yellow to pale orange colonies on R2A agar, SMA and R-medium agar, whereas strain Ben 108^T grew as bright orange colonies on all three media. The pigments of both were not water-soluble and are probably carotenoids. Cells of both strains stained unevenly with the Gram stain; the staining reaction depended on the time used for ethanol decolorization (data not shown). Both grew slowly on R2A medium, requiring up to 2 weeks for colonies to appear on agar and turbidity to occur in liquid medium. These organisms are aerobic as no growth occurred below the surface of the stab cultures.

The growth temperature range for Ben 107^T on R2A agar was between 15–37 °C and the optimum temperature was 25 °C. No growth was observed at 5 °C or below and at 45 °C or above. The pH range supporting growth of strain Ben 107^T was between 5.5–8.0, with optimal pH of 7.0–7.5. Strain Ben 107^T did not grow at pH 5.0 or below or at pH 8.5, 9.0 or 9.3, whereas weak growth was observed at pH 5.5. In R2A broth, cells tended to attach to the side of the flask and sediment to the bottom as clumps. With strain Ben 108^T, the growth temperature range was from 20–25 °C, and no growth was observed at 15 °C or below and at 30 °C or above. The pH range supporting

Fig. 1. (a) SEM of strain Ben 107^T showing aggregated cells in repeated layers. Bar, 2 µm. (b) SEM of strain Ben 108^T showing coccoid cells in regular packages associated into aggregates. Bar, 2 µm.

growth of strain Ben 108^T was between 5.5–7.5, with optimal pH of 6.5–7.0. Strain Ben 108^T did not grow at pH 5.0 or below, or at pH 8.0, 8.5, 9.0 or 9.3.

Morphological characteristics

Both organisms grew as clusters of coccoid cells (Fig. 1a, b; Fig. 2a; Fig. 3) where they remained closely associated with each other after cell division as a result of production of extracellular capsular material in large amounts. (Figs 2a). Cells within these clusters were in smaller packages which are delineated by the extracellular material (Figs 2a and 3). The cell clusters of strain Ben 108^T produced a greater amount of a denser extracellular capsule (polysaccharide) than those of strain Ben 107^T. The cell envelope in both strains was Gram-positive, with an easily discerned cytoplasmic membrane and a wall of 20–26 nm in thickness (Fig. 2b). The wall was trilaminar, consisting of two electron-dense layers and an inner more electron-translucent layer (Fig. 2b). Cells of both

Fig. 2. TEM micrographs of thinly sectioned samples of strain Ben 107^T cells. (a) Cluster of cells surrounded by and internally packaged by extracellular capsular material (arrow). Small electron-dense granules (arrowhead) are present in the cells. Bar, 400 nm. (b) Cell envelope showing distinct cell membrane (arrow) and trilaminar cell wall (arrowhead). Bar, 50 nm. (c) Cluster of cells in which some cells display interconnecting bridges (arrow) resulting from incomplete septation. Bar, 400 nm. (d) Cell containing large empty intracellular granules (arrow) which are presumed to be PolyP granules. Bar, 400 nm. (e) Thin section of chemically fixed Ben 107^T showing poorly fixed cells displaying holes (arrow) that result from the falling out of intracellular granules during thin sectioning. Bar, 1 μ m.

strains divided by septation. Frequently, the septation in strain Ben 107^T was incomplete and cells were observed that were well-separated from one another but still connected by a small bridge of cytoplasm (Fig. 2c). Small electron-dense granules were frequently observed in the cytoplasm and were usually more prevalent towards the periphery of the cells (Fig. 2a). The constituents of these granules are unknown. Large intracellular granules were also present in strain Ben

107^T (Fig. 2c), although these appeared to be empty, the contents presumably being lost during processing for electron microscopic observations. These granules may be polyphosphate (PolyP) granules, since this organism stained positively for PolyP when grown aerobically in medium supplemented with glucose, acetate or propionate (data not shown). However, no poly- β -hydroxyalkanoate granules were observed. No large granules were observed in any cells of strain Ben

Fig. 3. TEM micrograph of thinly sectioned cryosubstituted samples of strain Ben 108^T. Cell cluster displaying large amounts of the dense extracellular capsular material which surrounds the cluster and divides it internally into smaller packages of cells. Small electron-dense granules (arrows) are present in the cytoplasm of the cells. Bar, 1 μ m.

108^T. These TEM descriptions are based on material preserved by cryofixation and cryosubstitution, a technique which should produce fewer artefacts than standard chemical fixation techniques, thus giving a more correct picture of the ultrastructure (Graham, 1992). Cells of organisms in the present study processed by chemical fixation appeared quite differently (Fig. 2e): the cytoplasm was much more electron-dense; cells often had an irregular outline; the extracellular capsular matrix was more diffuse and did not delimit the clusters in the same way; the small electron-dense granules and interconnecting bridges of incomplete septation were never seen; and the PolyP granules fell completely out of the section during the thin sectioning process and thus were only observed as holes in cells.

Cells of strain Ben 108^T shared some of the features of strain Ben 107^T. Both strains were also enclosed in extensive capsular material in packets usually of two and four cells (Fig. 3). This capsular material often held them in spherical aggregates. The cell wall organization in strain Ben 108^T was not as clearly resolved as in strain Ben 107^T. However, the wall appeared homogeneous and typically Gram-positive (Fig. 3). Also, no cytoplasmic connections between cells, as seen with strain Ben 107^T, could be detected here. Furthermore, the cells appeared to lack the granules present in strain Ben 107^T.

Strains Ben 107^T and Ben 108^T were both non-motile, lacked flagella and endospores were never observed. Cell stringiness was not seen with 3% KOH treatment, which is consistent with strains Ben 107^T and Ben 108^T both being Gram-positive. The cells were regularly spherical when grown on R2A medium and had

diameters of 0.5–1.4 and 0.6–1.2 μ m for strains Ben 107^T (Fig. 1b) and Ben 108^T, respectively.

Physiological and biochemical characters

These results were obtained using the BIOLOG GN and GP systems. In most cases, when these tests were carried out using the methods of Goodfellow (1971), the same results were obtained. However, some discrepancies were observed. Thus, the utilization of glycerol, maltose, melizitose, sucrose and trehalose for strain Ben 107^T detected with the methods of Goodfellow (1971) did not show up with the BIOLOG system.

The following substrates were utilized by both strains Ben 107^T and Ben 108^T: D-ribose, D-arabitol, sebacic acid, L-arabinose, succinate, D-xylose, xylitol, glycogen, methyl α -D-glucoside, sorbitol, D-fructose, D-cellobiose, mannitol, dextrin, methyl β -D-glucoside, amygdalin, arbutin, methyl α -D-galactoside, palatinose, D-lactic acid methyl ester, pyruvic acid, adenosine, thymidine-5'-monophosphate and quinic acid. However, the following substrates were utilized only by Ben 107^T: i-erythritol, D-saccharic acid, formic acid, D-galactose, turanose, methylpyruvate, α -keto-butyric acid, alaninamide and thymidine.

The following compounds were only utilized by strain Ben 108^T: L-rhamnose, maltose, sucrose, β -cyclodextrin, Tween 40, L-fucose, 2-aminoethanol, 2,3-butanediol, glucose 6-phosphate, maltotriose, sedoheptulosan, stachyose, D-tagatose, lactamide, 2'-deoxyadenosine, α -ketoglutaric acid, propionic acid, succinamic acid, 3-methylglucose, bromosuccinic acid, glucuronamide, D-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-threonine, DL-carnitine, γ -aminobutyric acid, uroconic acid, inosine, D-melizitose, malate and trypsin. However, none of the following compounds were used by either strain: D-mannose, lactose, D-trehalose, D-raffinose, glycerol, m-inositol, α -cyclodextrin, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, gentiobiose, α -D-lactose, D-melibiose, monomethylsuccinate, D-galacturonic acid lactone, D-galacturonic acid, D-gluconic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenyl acetic acid, itaconic acid, putrescine, DL- α -glycerol phosphate, glucose 1-phosphate, mannan, N-acetyl-mannosamine, methyl β -D-galactoside, methyl α -D-mannoside, N-acetyl-L-glutamic acid, adenosine-5'-monophosphate, uridine-5'-monophosphate, fructose 6-phosphate, α -ketovaleric acid, malonic acid, L-proline, L-alanine, L-glutamic acid, glycyl-L-glutamic acid, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, uridine, phenylethylamine, inulin, salicin, lactate, serine, DL-lactic acid, citric acid, acetic acid, *cis*-aconitic acid, N-acetylglucosamine, adenine, L-tyrosine, α -D-glucose and adonitol.

The enzyme activities detected with the API ZYM

Table 1. Carbon substrates utilization profiles used to differentiate Ben 107^T, Ben 108^T and *F. antarctica* using the BIOLOG GN and GP Microplate system

+, Utilized; —, not utilized.

Carbon source	Ben 107 ^T	<i>F. antarctica</i>	Ben 108 ^T
i-Erythritol	+	+	—
D-Saccharic acid	+	+	—
Formic acid	+	+	—
Adonitol	+	+	—
L-Rhamnose	—	—	+
Maltose	—	—	+
Sucrose	—	—	+
β -Cyclodextrin	—	—	+
L-Fucose	—	—	+
2-Aminoethanol	—	—	+
2,3-Butanediol	—	—	+
Glucose 6-phosphate	—	—	+
Maltotriose	—	—	+
Sedoheptulosan	—	—	+
Stachyose	—	—	+
D-Tagatose	—	—	+
Lactamide	—	—	+
2'Deoxyadenosine	—	—	+
α -Ketoglutaric acid	—	—	+
Propionic acid	—	—	+
Succinamic acid	—	—	+
Glucuronamide	—	—	+
D-Alanine	—	—	+
L-Alanyl-glycine	—	—	+
L-Asparagine	—	—	+
L-Aspartic acid	—	—	+
Glycyl-L-aspartic acid	—	—	+
L-Histidine	—	—	+
Hydroxy-L-proline	—	—	+
L-Threonine	—	—	+
DL-Carnitine	—	—	+
γ -Aminobutyric acid	—	—	+
Uroconic acid	—	—	+
Inosine	—	—	+
Malate	—	—	+
D-Fructose	+	—	+
D-Galactose	+	—	+
D-Cellobiose	+	—	+
Mannitol	+	—	+
Dextrin	+	—	+
Methyl β -D-glucoside	+	—	+
D-Psicose	+	—	+
Turanose	+	—	+
Methyl-pyruvate	+	—	+
α -Ketobutyric acid	+	—	+
Amygdalin	+	—	+
Arbutin	+	—	+
Methyl α -D-galactoside	+	—	+
3-Methyl glucose	—	+	+
Palatinose	+	—	+
D-Lactic acid methyl ester	+	—	+
Pyruvic acid	+	—	+
Adenosine	+	—	+
Thymidine-5'-monophosphate	+	—	+
Quinic acid	+	—	+
Bromosuccinic acid	—	+	+
Alaninamide	+	—	—
L-Proline	—	+	—
Thymidine	+	—	—

Fig. 4. Dendrogram based on an S_{SM} /UPGMA numerical taxonomy analysis of the phenotypic characteristics of strains Ben 107^T, Ben 108^T, *T. bendigoensis*, *M. phosphovorius* and *F. antarctica*.

system for both strains Ben 107^T and Ben 108^T were as follows: alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase and β -glucosidase. None of the following enzyme activities were detected for either strain Ben 107^T or Ben 108^T: chymotrypsin, β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Trypsin activities were only detected in strain Ben 108^T and not in strain Ben 107^T. Lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase activities were not detected with the Microbact 24E system with either strain Ben 107^T or strain Ben 108^T. Both strains Ben 107^T and Ben 108^T were negative for indole, Voges-Proskauer and threonine deaminase with the Microbact 24E system and did not reduce nitrate to nitrite. Neither gelatin nor casein was hydrolysed. Both strains were catalase- and urease-positive but oxidase-negative. Substrate utilizations and enzymic activities which differed between strain Ben 107^T, strain Ben 108^T and *F. antarctica* are given in Table 1.

Substrate utilization tests in addition to those obtained from BIOLOG GP and GN systems and Microbact 24 were also performed, and the data revealed that strains Ben 107^T and Ben 108^T could not utilize *p*-hydroxybenzoate, benzoate, disodium succinate, Tween 20, 40 and 60, potassium hydrogen tartrate, adenine and DL-tyrosine. Hypoxanthine, elastin and xanthine were not hydrolysed by either strain Ben 107^T or Ben 108^T. Growth of both strains was inhibited by penicillin (10 IU), crystal violet (0.001 and 0.0001 %, w/v), phenol (0.1 and 0.3 %, w/v), phenyl alcohol (0.03 %, w/v), sodium azide (0.1 and 0.2 %, w/v) and NaCl (5 and 7 %, w/v).

Numerical taxonomic analysis performed on all the physiological and biochemical data revealed that strain Ben 107^T and *F. antarctica* formed a tight cluster at an S_{SM} value of 0.82 (Fig. 4) with the UPGMA algorithm [an S_{SM} value of 0.85 is often used to delineate between species (Sneath, 1978)]. Both strain Ben 107^T and *F.*

antarctica linked with *M. phosphovorius* at an S_{SM} value of 0.75 and, in turn, all three strains grouped with *T. bendigoensis* at an S_{SM} value of 0.67, also using the UPGMA algorithm (Fig. 4). However, strain Ben 108^T only linked with all the other organisms examined at a much lower S_{SM} of 0.62 (Fig. 4).

DNA-DNA hybridization and DNA base composition

DNA relatedness, as determined by DNA-DNA hybridization, showed that strain Ben 107^T is only 28 % related to *M. phosphovorius* (JCM 9379^T), 18 % related to *Propioniferax innocua* (DSM 8251), 21 % related to *T. bendigoensis* (ACM 5119^T) and 50 % related to *F. antarctica* (DSM 11053^T). Strain Ben 108^T was only 33 % related to *M. phosphovorius* (JCM 9379^T), 22 % related to *Propioniferax innocua* (DSM 8251), 20 % related to *T. bendigoensis* (ACM 5119^T) and 27 % related to *F. antarctica* (DSM 11053^T). However, strains Ben 107^T and Ben 108^T were only 29 % related to each other. The DNA G+C compositions of strains Ben 107^T and Ben 108^T were 69 and 74 mol %, respectively.

Phylogenetic analysis

An almost complete sequence of the 16S rRNA gene for strains Ben 107^T (1469 nt) and Ben 108^T (1465 nt) corresponding to positions 26–1522 of the *Escherichia coli* sequence according to the nomenclature of Winker & Woese (1991) was determined. Phylogeny based on 777 unambiguous bases after 16S rDNA data analysis indicated that both strains Ben 107^T and Ben 108^T are members of the high-G+C Gram-positive bacteria of the class *Firmicutes* in the domain *Bacteria*. The phylogenetic tree (Fig. 5) shows that strains Ben 107^T and Ben 108^T form a coherent cluster together with *F. antarctica* and are related to each other at a similarity level greater than 97.0 %. Strains Ben 107^T, Ben 108^T and *F. antarctica* are only 96.1, 96.9 and 96.4 % similar, respectively, to *M. phosphovorius*, their closest relative when 777 bases were compared. However, when almost complete sequences (1455 unambiguous bases)

Fig. 5. Phylogenetic position of *F. spumicola* (strain Ben 107^T) and *F. capsulata* (strain Ben 108^T) within the suborder *Propionibacterineae* of the high-G + C-content Gram-positive bacteria.

Table 2. Cellular fatty acids compositions of Ben 107^T, Ben 108^T, *Friedmanniella antarctica*, *Tessaracoccus bendigoensis*, *Microlunatus phosphovorus*, *Propioniferax innocua* and *Luteococcus japonicus*

i, Iso; ai, anteiso. Examples of naming fatty acids: C_{15:0}, pentadecanoic acid; i-C_{15:0}, 13-methyltetradecanoic acid; ai-C_{15:0}, 12-methyltetradecanoic acid; C_{15:1}, *cis*-9-pentadecenoic acid.

Strain	Cellular fatty acid composition (%)																					
	C _{13:1}	i- C _{14:0}	C _{14:0}	C _{14:1}	i- C _{15:0}	ai- C _{15:0}	C _{15:0}	i- C _{15:1}	C _{15:1}	i- C _{16:0}	i- C _{16:1}	C _{16:0}	C _{16:1}	C _{17:0}	C _{17:1}	i- C _{17:0}	i- C _{17:1}	ai- C _{17:0}	ai- C _{17:1}	C _{18:0}	C _{18:1}	C _{18:2}
Ben 108 ^T	0	2.5	1.8	0	13.0	58.8	0	0	0	4.4	0	3.7	0	0	0	9.0	0	6.8	0	0	0	0
Ben 107 ^T	0	3.9	3.3	0	13.3	57.7	3.2	0	0	0	0	4.5	0	0	0	0	0	0	0	0	14.1	0
<i>Friedmanniella antarctica</i>	0	2.3	0	0	23.2	54.5	0	0	0	2.4	0	0	2.9	0	0	0	0	0	0	0	0	6.0
<i>Tessaracoccus bendigoensis</i>	2.0	4.1	3.1	2.0	5.0	47.0	0	3.6	6.2	4.7	3.9	5.7	0	0	0	0	8.1	0	0	0	0	0
<i>Microlunatus phosphovorus</i>	0	4.0	2.9	0	22.4	43.5	0	0	0	6.2	0	7.5	0	0	0	0	0	0	0	5.6	0	0
<i>Propioniferax innocua</i>	0	3.2	0	0	20.9	46.5	0	1.2	0	7.3	4.5	0	0	0	0	2.2	1.3	6.7	2.9	0	0	0
<i>Luteococcus japonicus</i>	0	0	1.0	0	0	0	5.6	0	11.7	0	0	4.8	20.5	2.4	48.1	0	0	0	0	0	3.2	0

for strain Ben 107^T and *F. antarctica* were compared, they were found to be 98.5% similar, whereas strain Ben 108^T was 95.9 and 95.8% similar to *F. antarctica* and strain Ben 107^T, respectively, on the basis of 16S rDNA sequence data. All three are most closely related to *M. phosphovorus*, *Luteococcus japonicus*, *T. bendigoensis*, *Propionibacterium propionicus* and *Propioniferax innocua*, organisms which are currently

placed in the suborder *Propionibacterineae* (Stackebrandt *et al.*, 1997).

Chemotaxonomic properties

The cellular fatty acid profile of strain Ben 107^T is characterized by the predominance of: ai-C_{15:0}, 12-methyltetradecanoic acid (57.7 %); C_{18:1}, octa-

Table 3. Comparative phenotypic characters of *Propioniferax innocua*, *Propionibacterium propionicus*, *Luteococcus japonicus*, *Tessaracoccus bendigoensis*, *Microlunatus phosphovor*, *Friedmanniella antarctica*, Ben 107^T and Ben 108^T

Strains: 1, Ben 107^T; 2, Ben 108^T; 3, *Friedmanniella antarctica* (data obtained from Schumann *et al.*, 1997); 4, *Microlunatus phosphovor* (data obtained from Nakamura *et al.*, 1995); 5, *Luteococcus japonicus* (data obtained from Tamura *et al.*, 1994); 6, *Tessaracoccus bendigoensis* (data obtained from Maszenan *et al.*, 1999); 7, *Propionibacterium propionicus* (data obtained from Charfreitag *et al.*, 1988; Schaal, 1986); 8, *Propioniferax innocua* (data obtained from Pitcher & Collins, 1991; Yokota *et al.*, 1994). All isolates are Gram-positive, non-motile, non-spore-forming chemo-organotrophs. v, Variable test result; ND, not determined; d, 11–89% strains are positive.

Phenotypic character	1	2	3	4	5	6	7	8
O ₂ requirement	Aerobe	Aerobe	Aerobe	Aerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe
Cell morphology	Cocci (0.5–1.4 µm) arranged in packets	Cocci (0.6–1.2 µm) arranged in packets	Cocci (0.5–2.2 µm) arranged in packets	Cocci (0.8–2.0 µm) arranged singly and in pairs	Cocci in pairs and tetrads (0.7–1.0 µm)	Cocci (0.5–1.1 µm) arranged in tetrads	Pleomorphic rods (0.2–0.8 µm)	Pleomorphic rods (0.5–1.2 µm)
Habitat	Sewage treatment plant in Australia	Sewage treatment plant in Australia	Antarctic sandstone	Sewage treatment plant in Japan	Soil and water	Sewage treatment plant in Australia	Human oral cavity and cervicovaginal secretion	Human epidermal surface
Optimum growth temperature (°C)	25	20–25	22	25–30	26–28	25	35–37	37
Growth temperature range (°C)	15–37	20–25	9–25	5–35	12–38	20–37	30–37	10–40
Optimum growth pH	7.0–7.5	6.5–7.0	6.0–7.2	7.0	ND	7.5	ND	7.0
Growth pH range	5.5–8.0	5.5–7.5	5.1–8.7	5.0–9.0	ND	5.5–9.3	ND	ND
Presence of metachromatic granules*	+, PolyP	+, PolyP	+, PolyP	+, PolyP	ND	+, PolyP	ND	+, type of storage granules unknown
Oxidase	–	–	–	+ (weak)	+	–	ND	+
Catalase	+	+	+	+	+	+	–	+
Production of indole	–	–	–	+	–	–	–	–
Production of H ₂ S	+	+	+	ND	–	–	+	–
Major menaquinone	MK-9(H ₄), MK-7(H ₄)	MK-9(H ₄), MK-9(H ₂) and MK-7(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄) and MK-7(H ₄)	MK-9(H ₄)	MK-9(H ₄)
Diamino acid/murein type	LL-A ₂ pm A3γ	LL-A ₂ pm A3γ	LL-A ₂ pm A3γ	LL-A ₂ pm A3γ/§	LL-A ₂ pm A3γ	LL-A ₂ pm A3γ	LL-A ₂ pm A3γ	LL-A ₂ pm A3γ
Polar lipid	PI, PG, DPG, PL, GL	PI, PG, DPG, PL, GL	PI, PG, DPG, PL	PI, PG, DPG, PL	DPG, PG, PI, GL	PI, PG, DPG, PL	ND	PE, PG, PL, GL§
Urease	+	+	+	+	–	–	–	+
Nitrate reduction	–	–	–§	+	–	+	+	+
G + C (mol %)	69	74	73	68	66–68	74	63–65	59–63
Carbohydrates utilized:¶								
Lactose	–	–	–	–†	ND	+	d	–
Raffinose	–	–	–	–†	+	+	+	–
Mannitol	+	–	–	–†	+	+	+	–
Glycerol	–	–	–	–†	+	+	d	+
Arabinose	+	+	+ (weak)	–†	+ for D-arabinose – for L-arabinose	+	–	–
Inositol	–	–	–	–†	+	–	d	–
Sucrose	–	+	–	–†	+	+	+	+
Fructose	+	+	–	–†	+	+	+	+
Glucose	–	–	–	–†	+	+	+	+
Mannose	–	–	–	–†	+	+	d	+
Galactose	+	–	–	–†	+	+	d	V
Trehalose	–	–	–	–†	+	–	d	+
Maltose	–	+	–	–†	+	+	+	+
Ribose	+	+	+	–†	+	+	d	ND
Xylose	+	+	+ (weak)	–†	–	+	–	ND
Rhamnose	–	+	–	–†	–	–	–	–
Adonitol	–	–	–	–†	ND	–	d	–

* Presence of metachromatic granules, *Microlunatus*, *Tessaracoccus*, *Friedmanniella* and strain Ben 107^T contain PolyP granules, the granule type for *Propioniferax innocua* has not been determined.

† Data obtained in this study.

‡ Result obtained by BIOLOG and Microbact 24E (A. M. Maszenan and others, unpublished results).

§ Data obtained from Schumann *et al.* (1997).

|| Abbreviations: PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PL, unidentified phospholipid (s); GL, unidentified glycolipid (s); PI, phosphatidyl inositol and DPG, diphosphatidyl glycerol.

¶ Tested for *Microlunatus phosphovor* and *Tessaracoccus bendigoensis* under aerobic conditions.

decanoic acid (14.1%); and i-C_{15:0}, 13-methyltetradecanoic acid (13.3%). Unbranched fatty acids with 14, 15 and 16 carbon atoms were found in trace amounts (Table 2). Five isoprenoid quinones MK-9(H₄), MK-7(H₂), MK-8(H₂), MK-9(H₂) and MK-8(H₄) with the following ratio of 62:11:7:6:4 were observed. The peptidoglycan of strain Ben 107^T contained LL-A₂pm and its interpeptide bridge had a single glycine residue and position 1 of the peptide subunit is occupied by glycine, properties which are characteristic of the type A3γ' peptidoglycan (Schleifer & Seidl, 1985). The polar lipid profiles of strains Ben 107^T and Ben 108^T are similar as both contained phosphatidyl inositol, phosphatidyl glycerol, diphosphatidyl glycerol and two unidentified glycolipids and phospholipids as shown in Table 3. Strain Ben 108^T also had the type A3γ' peptidoglycan with LL-A₂pm and glycine moiety occupying position 1, but its respiratory menaquinones differed from those of strain Ben 107^T, as it possesses MK-7(H₄) in addition to MK-9(H₄) and MK-9(H₂), in the following ratio of 8:72:10, and lacks MK-8(H₂) and MK-8(H₄). The cellular fatty acid profile of strain Ben 108^T is similar to that of strain Ben 107^T with ai-C_{15:0}, 12-methyltetradecanoic acid (58.8%), as the dominant fatty acid. However, differences were also observed between them as shown in Table 2.

DISCUSSION

Strains Ben 107^T and Ben 108^T, both isolated from activated sludge, and *F. antarctica* are all non-motile, oxidase-negative, orange-pigmented, slow-growing, Gram-positive cocci, appearing as aggregates of cells. These aggregates become layered, probably as cell division occurs in more than one plane, and the cells are embedded in capsular material, which is particularly extensive with strain Ben 108^T. Strain Ben 107^T and *F. antarctica*, the only currently described member of the genus *Friedmanniella* (Schumann *et al.*, 1997), share very similar biochemical and physiological characteristic, despite being isolated from totally different habitats. Strain Ben 108^T differs considerably in its phenotype from these two organisms (Fig. 4).

However, 16S rDNA sequence data show that strains Ben 107^T and Ben 108^T and *F. antarctica* all cluster at a high (>97%) similarity level, in the high-G+C group of Gram-positive bacteria. These sequence data would suggest that strains Ben 107^T and Ben 108^T are not members of the genera *Propionibacterium* (Schaal *et al.*, 1986; Charfreitag *et al.*, 1988), *Propioniferax* (as *Propioniferax innocua*) (Pitcher & Collins, 1991; Yokota *et al.*, 1994), *Luteococcus* (Tamura *et al.*, 1994) or *Tessaracoccus* (Maszenan *et al.*, 1999), even though they share many chemotaxonomic properties in common with some of them (Table 3). The nearest relative to strains Ben 107^T and Ben 108^T and *F. antarctica* based on 16S rDNA sequence data is *M. phosphovor* clustering at a similarity level only

slightly below 97%, illustrating how closely related these four organisms are to each other. In fact, strain Ben 108^T is closer to *M. phosphovor* than to either Ben 107^T or *F. antarctica* when DNA hybridization values are considered.

Allowing that the separation of *Micrococcus* and *Friedmanniella* into different genera is justifiable, the decision then has to be made as to which of these two genera do Ben 107^T and Ben 108^T best belong. The recognition that *Friedmanniella* should be separated from *Micrococcus* was based on several criteria including their 16S rDNA structures, morphological and physiological characteristic and chemotaxonomic features (Schumann *et al.*, 1997). If only cell arrangement, cell pigmentation and culture growth rate (Schumann *et al.*, 1997) are taken into account, then strains Ben 107^T and Ben 108^T are clearly more closely related to *Friedmanniella* than *Micrococcus*. Furthermore, strains Ben 107^T and Ben 108^T and *F. antarctica* are all oxidase-negative while *M. phosphovor* is oxidase-positive. In fact, strain Ben 107^T is most similar to *Friedmanniella* in all the characteristics described here and there is little doubt that it should be included in this genus. Both strain Ben 107^T and *F. antarctica* also have G-T at position 602-636, while *M. phosphovor*, the only known *Micrococcus* species, has A-T (Table 4).

However, there are enough differences between strain Ben 107^T and *F. antarctica* to indicate that they are different species of the genus *Friedmanniella*. These differences include the additional occurrence of MK-7(H₂), MK-8(H₂) and MK-8(H₄) in strain Ben 107^T, together with two unidentified glycolipids and one unidentified phospholipid, which are not found in *F. antarctica* (Table 3). This view is given additional support by differences in cellular fatty acid compositions (Table 4); strain Ben 107^T contains C_{14:0}, C_{15:0}, C_{16:0} and C_{18:1}, while *F. antarctica* has i-C_{16:0}, C_{16:1} and C_{18:2}. Furthermore, the DNA-DNA hybridization value of <70% homology between them confirms they are not members of a single genomic species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Such a low DNA-DNA homology is unusual, but not unknown in bacteria having high 16S rDNA sequence similarity values (Fox *et al.*, 1992). Hence, we propose that strain Ben 107^T be placed in the genus *Friedmanniella* as a new species, *Friedmanniella spumicola*.

The fate of strain Ben 108^T is more problematic because it seems to fit almost equidistantly between *Friedmanniella* and *Micrococcus* and, although the phylogenetic data support its inclusion as a member of the genus *Friedmanniella*, this claim is harder to justify than with strain Ben 107^T, when the rest of its properties are considered. Large differences in chemotaxonomic properties, and low DNA-DNA hybridization values between strain Ben 108^T and both strain Ben 107^T and *F. antarctica*, confirm that strain Ben 108^T is not a member of either of these two species.

Table 4. 16S rDNA nucleotide signatures for Ben 107^T, Ben 108^T, *Friedmanniella antarctica*, and members of the families *Propionibacteriaceae* and *Nocardioidaceae* within the suborder *Propionibacterineae* (Stackebrandt *et al.*, 1997)

Important differences in the signature nucleotides are highlighted in bold and underlined.

Position*	<i>Propionibacteriaceae</i>	<i>Nocardioidaceae</i>	Ben 107 ^T	Ben 108 ^T	<i>Friedmanniella antarctica</i>	<i>Micrococcus phosphovor</i>	<i>Tessaracoccus bendigoensis</i>
66–103	A–T	G–C	A–T	A–T	A–T	A–T	A–T
328	T	C	T	T	T	T	T
370–391	C–G	G–C	C–G	C–G	C–G	C–G	C–G
407–435	C–G	A–T	C–G	C–G	C–G	C–G	C–G
602–636	A–T	G–T	<u>G–T</u>	A–T	<u>G–T</u>	A–T	G–T
658–748	A–T	T–A	<u>G–A</u>	<u>G–A</u>	<u>G–A</u>	A–T	A–T
686	G	T	G	G	G	G	A
780	A	G	A	A	A	A	A
787	C	A	C	C	C	C	C
819	G	T	G	G	G	G	G
825–875	A–T	G–C	A–T	A–T	A–T	A–T	A–T
1409–1491	A–T	C–G	A–T	A–T	A–T	A–T	A–T

* *E. coli* numbering positions for 16S rDNA base sequences after Winker & Woese (1991).

Thus, the additional presence of MK-7(H₄) together with a cellular fatty acid profile containing i-C_{17:0} and ai-C_{17:0} in strain Ben 108^T separates it from both strains Ben 107^T and *F. antarctica*. Furthermore, the low *S*_{SM} level at which Ben 108^T groups with the other two after numerical taxonomic analysis (Fig. 4) would support this separation. Unfortunately, the signature nucleotide positions do not help resolve its taxonomic position. Sharing G–A at position 658–748 with strain Ben 107^T and *F. antarctica*, where *M. phosphovor* has A–T at these positions, strain Ben 108^T together with *M. phosphovor* has A–T at position 602–636, while strain Ben 107^T and *F. antarctica* both possess G–T (Table 4). No other member of the family *Propionibacteriaceae* shows this pattern.

In summary, it is felt that the taxonomic structures of this group, and the final position of strain Ben 108^T within it, will become clearer only with the isolation of other phylogenetically related strains. The phylogenetic data would, in our opinion, not support the idea that strain Ben 108^T is a new species of *Micrococcus* despite having the highest DNA homology with it or that it belongs to the genus *Tessaracoccus* (Maszenan *et al.*, 1999) despite its phenotypic similarities to both (Fig. 4), or that it should be placed in a new genus. Our view is that strain Ben 108^T is best placed, albeit uneasily, in the genus *Friedmanniella* as a novel species *Friedmanniella capsulata*.

Currently, all of the genera mentioned above are in the family *Propionibacteriaceae* which, together with the family *Nocardioidaceae*, is in the suborder *Propionibacterineae* (Stackebrandt *et al.*, 1997). This classification was proposed solely on the presence of signature nucleotides in the 16S rDNA (see Table 4) and often families were based on a single characterized isolate (e.g. *Microsphaeraceae*). Strains Ben 107^T, Ben 108^T and *F. antarctica* have nucleotide signatures which differ from those proposed by Stackebrandt *et al.* (1997) for delineating the family *Propionibacteriaceae*, although not those suggested as diagnostic for the suborder *Propionibacterineae*. Strain

Ben 107^T and *F. antarctica* alone have G–A instead of A–T at positions 658–748. However, as mentioned earlier, strain Ben 108^T has A–T at position 602–636, which is also shared by *Micrococcus* species and members of the currently recognized family *Propionibacteriaceae* as proposed by Stackebrandt *et al.* (1997), but which is not found in either strain Ben 107^T, *F. antarctica* or *T. bendigoensis*. It seems probable that as more strains of these high-G + C Gram-positive bacteria are isolated and characterized, more discrepancies with their nucleotide signatures will become apparent and the scheme of Stackebrandt *et al.* (1997) will require substantial modification. Until more strains of the genus *Friedmanniella* are isolated and the diversity of their 16S rDNA nucleotide composition better understood, it might be argued that they could be removed from the currently described family *Propionibacteriaceae*, despite their very high phenotypic similarities to the other genera currently within this family. However, as they all share high levels of phylogenetic similarity, it could also be argued that they should all stay with the other genera discussed above, within the same family. Other genomic fingerprinting methods need to be applied in order to resolve their intergeneric relationships.

Finally, the ecological diversity of members of the genus *Friedmanniella* could be considerable since both strains Ben 107^T and Ben 108^T were isolated from activated sludge foam while *F. antarctica* originated from Antarctic sandstone. Interestingly, *F. antarctica* is reported not to grow in a laboratory below 6 °C (Schumann *et al.*, 1997), an unexpected finding if its true natural habitat is extreme cold. Some Gram-positive bacteria with high-G + C content have been implicated to play a major role in biological phosphorus removal (Bond *et al.*, 1995; Wagner *et al.*, 1994). The recent description of *Tessaracoccus bendigoensis* (Maszenan *et al.*, 1999), which like *F. spumicola* and *F. capsulata* was shown to accumulate PolyP aerobically, would increase the known complexity of organisms which might be responsible for

biological phosphorus removal in activated sludge. The isolation of strains Ben 107^T and Ben 108^T from activated sludge and their ability to accumulate PolyP might indicate that they may also play an important role in EBPR activated sludge systems.

Description of *Friedmanniella spumicola* sp. nov.

Friedmanniella spumicola (spu.mi'co.la. L. fem. n. *spuma* foam; L. suffix *-cola* inhabitant of; M.L. masc./fem. N. *spumicola* inhabitant of foam).

Cells adhere to one another and form aggregates of four and eight. Growth in liquid and solid media is slow and poor requiring up to 2 weeks. Colony colour when grown on solid and liquid media is dark yellow to pale orange. The temperature range for growth is 15–37 °C with an optimum temperature of 25 °C. The pH range over which growth occurs is 5.5–8.0, with an optimum pH of 7.0–7.5. Cells store PolyP granules aerobically. It can utilize the following substrates: i-erythritol, D-saccharic acid, formic acid, D-galactose, turanose, methylpyruvate, α -ketobutyric acid, alaninamide and thymidine. The following substrates were not utilized: L-rhamnose, maltose, sucrose, β -cyclodextrin, L-fucose, 2-aminoethanol, 2,3-butanediol, glucose 6-phosphate, maltotriose, sedoheptulosan, stachyose, D-tagatose, lactamide, 2'-deoxyadenosine, α -ketoglutaric acid, propionic acid, succinamic acid, glucuronamide, D-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-threonine, DL-carnitine, γ -aminobutyric acid, uroconic acid, inosine, D-melezitose, malate, 3-methylglucose, bromosuccinic acid and trypsin. It is also characterized by the presence of C_{18:1} (octadecenoic acid; 14.1%) and i-C_{15:0} (13-methyltetradecanoic acid; 13.3%). Unbranched fatty acids with 14, 15 and 16 carbons were found in trace amounts. Its major menaquinones are MK-9(H₄) and MK-7(H₂), with MK-9(H₂), MK-8(H₂), MK-8(H₄), present in trace amounts. It possesses G-A and G-T at positions 658–748 and 602–636, respectively. The G+C content is about 69 mol%. Mycolic acids are absent. The cell diameter is 0.5–1.4 μ m. It originates from activated sludge. The type strain, strain Ben 107^T, has been deposited in the Australian Collection of Microorganisms, as strain ACM 5121^T.

Description of *Friedmanniella capsulata* sp. nov.

Friedmanniella capsulata (cap.su.la'ta. L. fem. adj. *capsulata* capsulated).

This species is characterized by a bright orange colour when grown in both solid and liquid media. It also can store polyphosphate aerobically. The optimum growth temperature is 20–25 °C. The pH range for growth is 5.5–7.5, with an optimal growth pH of 6.5–7.0. It utilizes L-rhamnose, maltose, sucrose, β -cyclodextrin, L-fucose, 2-aminoethanol, 2,3-butanediol, glucose 6-phosphate, maltotriose, sedoheptulosan, stachyose, D-tagatose, lactamide, 2'-deoxyadenosine, α -ketoglutaric

acid, propionic acid, succinamic acid, 3-methylglucose, bromosuccinic acid, glucuronamide, D-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-threonine, DL-carnitine, γ -aminobutyric acid, uroconic acid, inosine, D-melezitose, malate and trypsin. The following substrates are not utilized: i-erythritol, D-saccharic acid, formic acid, adonitol, D-galactose, D-psicose, turanose, methyl-pyruvate, α -ketobutyric acid, alaninamide and thymidine. Cell diameter is 0.6–1.2 μ m. Cellular fatty acids are characterized by the presence of i-C_{16:0} (14-methylpentadecanoic acid), i-C_{17:0} (15-methylhexadecanoic acid) and ai-C_{17:0} (14-methylhexadecanoic acid). It also contains MK-9(H₄) and MK-9(H₂) and is characterized by the presence of MK-7(H₄). The G+C content is 74 mol%. Mycolic acids are absent. It originates from activated sludge. The type strain, strain Ben 108^T, has been deposited in the Australian Collection of Microorganisms, as strain ACM 5120^T.

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