***Tessaracoccus bendigoensis* gen. nov., sp. nov., a Gram-positive coccus occurring in regular packages or tetrads, isolated from activated sludge biomass**

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An isolate of a Gram-positive bacterium, designated strain Ben 106^T, was obtained in pure culture by micromanipulation of a biomass sample obtained from a laboratory-scale sequencing batch reactor. This isolate grew axenically as cocci or clusters of cocci arranged in regular tetrads and was morphologically similar to the dominant organism observed in the biomass. This morphology resembled that of some Gram-positive and -negative bacteria and the so-called 'G-bacteria' commonly seen in activated sludge samples. Strain Ben 106^T is a non-motile, facultative anaerobe. It is oxidase-negative, catalase-positive and is capable of reducing nitrate. This organism can grow between 20 and 37 °C, with an optimum temperature of 25 °C. The pH range for growth is between 6.0 and 9.0, with an optimum pH of 7.5. The isolate stained positively for intracellular polyphosphate granules. The diagnostic diamino acid of the peptidoglycan is LL-diaminopimelic acid (LL-A₂pm) with a glycine moiety at position 1 of the peptide subunit, which characterizes the presence of a rare peptidoglycan (type A3-γ'). Two menaquinones, MK-9(H₄) and MK-7(H₄), are present and the main cellular fatty acid is 12-methyltetradecanoic acid. The G+C content is 74 mol%. From phenotypic characteristics and 16S rDNA sequence analysis, the isolate differed sufficiently from its closest phylogenetic relatives, namely *Propionibacterium propionicum*, *Propioniferax innocua*, *Friedmanniella antarctica*, *Luteococcus japonicus* and *Micrococcus phosphovor* in the A1 subdivision of the Gram-positive bacteria (i.e. *Firmicutes* with a high G+C content), suborder *Propionibacterineae*, to be placed in a new genus, *Tessaracoccus*, as *Tessaracoccus bendigoensis* gen. nov., sp. nov. The type strain is Ben 106^T (= ACM 5119^T).

Keywords: *Tessaracoccus bendigoensis*, activated sludge, Gram-positive coccus, *Propionibacteriaceae*, G-bacteria

INTRODUCTION

Many early studies on bacterial populations in activated sludge systems have reported the isolation and identification of members of the genus *Micrococcus* (Painter, 1983; Wanner, 1994), a Gram-positive coccus

often arranged distinctively in tetrads. Gram-negative cocci, generically described as 'G-bacteria' (Carrucci *et al.*, 1994; Cech & Hartman, 1993), have also been seen frequently in large numbers in biomass samples from plants around the world. Some of these G-bacteria have been successfully cultured (Cech & Hartman, 1993; Maszenan *et al.*, 1997) and four isolates have recently been characterized and described as belonging to a new genus *Amaricoccus* (Maszenan *et al.*, 1997).

Cocci other than *Amaricoccus* spp. and *Micrococcus* spp. occur in activated sludge systems and the identity

Abbreviations: LL-A₂pm, LL-diaminopimelic acid; PHA, polyhydroxyalkanoate; PolyP, polyphosphate; SBR, sequencing batch reactor.

The GenBank accession number for the 16S rRNA sequence of *Tessaracoccus bendigoensis* strain Ben 106^T is AF038504.

of some of these cocci is now known. For example, a Gram-positive coccus which can accumulate polyphosphate was identified as the new bacterium *Micro-lunatus phosphovorius* in Japan (Nakamura *et al.*, 1991, 1995a, b; Ubukata & Takii, 1994; Ubukata, 1994) and *Microsphaera multipartita* was also obtained from activated sludge biomass from a plant treating carbohydrate-rich wastewater (Takii, 1977a, b; Yoshimi *et al.*, 1996). Neither of these organisms have so far been reported in plants in other parts of the world. Kataoka *et al.* (1996) successfully isolated a *Micrococcus*-like bacterium by culturing activated sludge biomass under starvation conditions prior to isolation, but did not identify the isolate (Kataoka *et al.*, 1996). Gram-positive tetrad cocci are also commonly encountered in biomass from sequencing batch reactors designed for phosphorus removal, but the identities of these organisms remain unknown (Liu *et al.*, 1996; Matsuo, 1994; Matsuzawa & Mino, 1991; Randall, 1994).

The activated sludge system is expected to harbour a wide spectrum of previously undescribed bacteria, so the Gram-positive cocci present may be very diverse and represent novel taxonomic and phylogenetic groups. Until the true diversity of bacteria in activated sludge is better understood and their physiology and ecology are studied, our ability to optimize and control these treatment systems will be limited. As part of our ongoing project to investigate these groups of bacteria, we used micromanipulation (Skerman, 1968) to isolate an organism with cocci in a distinctive tetrad arrangement. This organism was designated strain Ben 106^T (ACM 5119^T). The phenotypic, chemotaxonomic and phylogenetic properties indicate that strain Ben 106^T cannot be assigned to any known genus and should be placed in a new genus, *Tessaracoccus*, as *Tessaracoccus bendigoensis* gen. nov., sp. nov.

METHODS

Isolation and maintenance of strain Ben 106^T. Strain Ben 106^T (ACM 5119^T) was isolated using a Skerman micromanipulator (Skerman, 1968) from activated sludge biomass from a laboratory-scale sequencing batch reactor (SBR) seeded with activated sludge obtained from the Biological Nutrient Removal (BNR) plant in Bendigo, Australia. The SBR (8 l total vol.) was operated under an anaerobic/aerobic cycle regime consisting of 90 min anaerobic, 240 min aerobic periods and a 30 min settle with a subsequent decant period. Four litres of effluent were decanted at the end of each cycle and the mean sludge age was 8 d. The synthetic feed used consisted of (l⁻¹) sodium acetate (100 mg), glucose (400 mg), KH₂PO₄ (57 mg), (NH₄)₂SO₄ (100 mg), NaCl (100 mg), CaCl₂ (50 mg), NaHCO₃ (200 mg) and MgSO₄ (80 mg). The SBR was maintained at 20 °C. Pure cultures were obtained by streaking out colonies arising from micromanipulated cells several times onto fresh GS medium (Williams & Unz, 1985) and incubating plates at 25 °C. Purity was confirmed by microscopic examination of cells from single colonies. Strain Ben 106^T was stored on GS medium in 20 % glycerol at -80 °C.

Physiological and biochemical characterization of strain Ben 106^T. Substrate utilization patterns for strain Ben 106^T were

obtained with both GP and GN Biolog systems (Special Diagnostic) as described previously (Maszenan *et al.*, 1997). Enzyme profiles were determined using API ZYM strips (bioMérieux) following the manufacturer's instructions. All biochemical properties were obtained with the Microbact 24E system (Oxoid). Catalase, oxidase, motility tests and growth response to pH and temperature were performed as described by Maszenan *et al.* (1997). Detection of both polyphosphate (PolyP) and polyhydroxyalkanoate (PHA) granules were performed according to the method of Rees *et al.* (1992). Cultures for PolyP and PHA examination were grown on basal GS medium with glucose, acetate or propionate, all at 0.15 g l⁻¹, as carbon sources, at their optimal growth temperature. The possible presence of intracellular PolyP and PHA granules was determined using an Olympus fluorescent microscope (model BH-RFL) fitted with an L-410 exciter filter, L-420 barrier filter and G 580 chromatic beam splitter. The response of the organism to O₂ was determined by stabbing the culture into a GS agar deep. Whether its metabolism was oxidative or fermentative (O/F) was determined with Hugh and Leifson (O/F) medium (Oxoid).

Chemotaxonomic characterization. The biomass used for chemotaxonomic characterization of Ben 106^T was obtained by scraping cells from the surfaces of R-agar plates (Schumann *et al.*, 1997) incubated at 22 °C for 2–3 weeks. Cell wall and lipid components were analysed as described by Schumann *et al.* (1997). Amino acids and peptides in cell wall hydrolysates were analysed by two-dimensional TLC (Schleifer & Kandler, 1972). Cellular fatty acid methyl esters were separated by GC using a non-polar (type OV-1) capillary column (25 m × 0.25 mm i.d.). Polar lipids were separated by two-dimensional TLC on silica gel and identified by spraying with specific reagents and menaquinones were analysed by reversed-phase HPLC (Schumann *et al.*, 1997).

Preparation of specimens for scanning electron microscopy. Samples were prepared for examination by scanning electron microscopy according to the method of Maszenan *et al.* (1997).

16S rDNA sequence and phylogenetic analysis. 16S rDNA amplification and sequencing were carried out as already described by Maszenan *et al.* (1997). Sequences were aligned with genera of the domain *Bacteria* and *Archaea* using the methods of Patel *et al.* (1995). Pairwise evolutionary distances were computed according to the method of Jukes & Cantor (1969). Dendrograms were constructed from evolutionary distances with the neighbour-joining method. Tree topology was examined with 100 bootstrapped data sets. The computer programs used form part of the PHYLIP program version 3.51c (Felsenstein, 1993).

G + C content. The DNA base composition was calculated from the melting temperature determined by the thermal denaturation method of Owen & Lapage, (1976). Cell DNA was isolated using the method of Porteous *et al.* (1994), modified by disrupting the cells with a cell homogenizer (Braun) and incubating them with 50 mg lysozyme ml⁻¹ (Sigma) and 100 U achromopeptidase ml⁻¹ (Sigma) for 60 min at 37 °C before DNA extraction (Porteous *et al.*, 1994). This method was used to overcome difficulties associated with the production of large amounts of extracellular polysaccharide material by this organism. Disrupted cells were incubated with 5 M guanidinium thiocyanate (Promega) for 60 min and the DNA spooled onto a sterile glass pipette.

RESULTS

Culture and morphological characteristics

The isolation of strain Ben 106^T was difficult. A wide range of media routinely used for isolation of bacteria from activated sludge was tested. Only GS medium was successful in supporting the growth of micro-manipulated cells. The organism grew as beige colonies, visible after 2–3 d incubation at 25 °C and shared the distinctive coccoid morphology of cells in tetrads as seen in large numbers in the SBR biomass (Fig. 1). The diameter of the individual coccoid cells ranged from 0.5 to 1.1 µm. Cells could accumulate intracellular PolyP granules aerobically with all three carbon sources examined (Fig. 1d) but PHA granules were not observed.

Physiological and biochemical characteristics

Results obtained with the Biolog GN and GP systems and Microbact 24E system showed that strain Ben 106^T could utilize L-arabinose, D-ribose, D-fructose, D-

galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, sucrose, turanose, D-raffinose, D-xylose, stachyose, palatinose, maltotriose, methyl α-D-glucoside, methyl α-D-galactoside, methyl β-D-galactoside, α-cyclodextrin, β-cyclodextrin, dextrin, amygladin, arbutin, glycogen, D-mannitol, methylpyruvate, glucose 1-phosphate, glucose 6-phosphate, DL-glycerol phosphate, UMP, glycerol, uridine and salicin. Strain Ben 106^T could not metabolize the following sugars and sugar derivatives: cellobiose, L-fucose, D-psicose, L-rhamnose, D-trehalose, D-melezitose, 3-methylglucose, methyl β-D-glucoside, mannan, adonitol, D-arabitol, i-erythritol, *m*-inositol, D-sorbitol, xylitol, monomethylsuccinate, D-galacturonic acid lactone, 2-aminoethanol, 2,3-butanediol, inulin, fructose 6-phosphate, methylsuccinate, adenosine, 2'-deoxyadenosine, AMP, TMP, D-tagatose, methyl α-D-mannoside and sedoheptulosan.

The following acids were metabolized by Ben 106^T: acetic acid, D-gluconic acid, α-hydroxybutyric acid, DL-lactic acid, L-lactic acid and pyruvic acid. None of

Fig. 1. Morphology of Gram-positive cocci obtained from an activated sludge sample. (a) Cells arranged in tetrads in activated sludge biomass from a laboratory-scale SBR, Bendigo, Australia. Bar, 10 µm. (b) Light micrograph of strain Ben 106^T following Gram staining. Bar, 10 µm. (c) Scanning electron micrograph of strain Ben 106^T showing the characteristic tetrad morphology. Bar, 2 µm. (d) Light micrograph of strain Ben 106^T stained with the polyphosphate stain of Rees *et al.* (1992). Bar, 10 µm.

Fig. 2. Phylogenetic dendrogram based on 16S rRNA sequence data, indicating the position of *Tessarococcus bendigoensis* (strain Ben 106^T) within the radiation of representatives of the high-G+C-containing Gram-positive bacteria. All the sequences used in the analysis were obtained from the Ribosomal Database Project, version 5.0 (Maidak et al., 1996). Bootstrap values, expressed as a percentage of 100 replications are shown at the branching points. Only values above 90% were considered significant and therefore reported. Scale bar indicates 10 substitutions per 100 nt.

the following acids, amino acids and their derivatives were metabolized by strain Ben 106^T: *cis*-aconitic acid, citric acid, formic acid, D-galacturonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-pyrroglutamic acid, γ -aminobutyric acid, uroconic acid, D-lactic acid methyl ester, D-malic acid, L-malic acid and *N*-acetyl-L-glutamic acid.

N-Acetyl-D-glucosamine was the only amine derivative metabolized by strain Ben 106^T, which was not able to metabolize the following amino acids, amines and their derivatives: D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, D-serine, L-serine, L-threonine, DL-carnitine, inosine, thymidine, phenylethylamine, putrescine and hydroxy-L-proline. In addition, strain Ben 106^T could not metabolize Tween 40, Tween 80, glucuronamide, alaninamide, lactamide, *N*-acetyl-D-galactosamine and *N*-acetylmannosamine.

The enzyme activities detected by both the API ZYM and Microbact 24E systems were alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase and α -mannosidase. Activities of the following enzymes were not detected:

trypsin, chymotrypsin, β -glucuronidase, α -fucosidase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and arginine dihydrolase.

Strain Ben 106^T did not produce H₂S or indole. It was Voges-Proskauer-positive, produced acetoin, liquefied gelatin and reduced nitrate to nitrite. It was oxidase-negative, catalase-positive and a facultative anaerobe.

Temperature and pH growth ranges

Strain Ben 106^T grew between 20 and 37 °C, with no growth occurring at 5, 15 or 45 °C. The optimal growth temperature was 25 °C. It also grew at a pH range between 6.0 and 9.0, with no growth occurring at pH 5.5 and 9.3. The optimal pH for strain Ben 106^T was 7.5.

Phylogenetic characteristics

Almost the complete 16S rRNA gene was sequenced (1410 bases) for strain Ben 106^T, corresponding to positions 9–1513 of *Escherichia coli* according to the nomenclature of Winker & Woese (1991). Phylogeny based on 16S rRNA analysis indicated that the isolate was a member of Gram-positive *Firmicutes*, with a high G+C content, in the domain *Bacteria*. The isolate formed a new branch within the family *Propionibacteriaceae* emend. Rainey, Ward-Rainey & Stackebrandt (Stackebrandt et al., 1997) and was closest to members of the genera *Propionibacterium* as *Propionibacterium propionicum* (Charfreitag et al., 1988), *Propioniferax* (Pitcher & Collins, 1991; Yokota et al., 1994), *Friedmanniella* (Schumann et al., 1997), *Luteococcus* (Tamura et al., 1994) and *Microthlunatus* (Nak-

Table 1. Comparative phenotypic characters of *Propioniferax innocua*, *Propionibacterium propionicum*, *Luteococcus japonicus*, *Microlunatus phosphovorius*, *Friedmanniella antarctica* and strain Ben 106^T

All isolates are Gram-positive, non-motile and non-spore-forming chemo-organotrophs. +, Positive test result; –, negative test result; v, variable test result; ±, 11–89% of the strains are positive; ND, not determined. Data obtained from (a) Pitcher & Collins (1991) and Yokota *et al.* (1994), (b) Charfreitag *et al.* (1988), Schaal (1986) and Cummins & Moss (1990), (c) Tamura *et al.* (1994), (d) Schumann *et al.* (1997), (e) Nakamura *et al.* (1995) and (f) this study.

Phenotypic character	<i>Propioniferax innocua</i> ^a	<i>Propionibacterium propionicum</i> ^b	<i>Luteococcus japonicus</i> ^c	<i>Friedmanniella antarctica</i> ^d	<i>Microlunatus phosphovorius</i> ^e	Strain Ben 106 ^T
O ₂ requirement	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe	Aerobe	Aerobe	Facultative anaerobe
Cell morphology	Pleomorphic rods (0.5–1.2 µm)	Pleomorphic rods (0.2–0.8 µm)	Cocci (0.7–1.0 µm) arranged in pairs and in tetrads	Cocci (0.5–2.2 µm) arranged in packets	Cocci (0.8–2.0 µm) arranged singly and in pairs	Cocci (0.5–1.1 µm) arranged in tetrads
Habitat	Human epidermal surface	Human oral cavity and cervicovaginal secretion	Soil and water	Antarctic sandstone	Sewage treatment plant in Japan	Sewage treatment plant in Australia
Optimum growth temperature (°C)	37	35–37	26–28	22	25–30	25
Growth temperature range (°C)	10–40	30–37	12–38	9–25	5–35	20–37
Optimum growth pH	7.0	ND	ND	6.0–7.2	7.0	7.5
Growth pH range	ND	ND	ND	5.1–8.7	5.0–9.0	5.5–9.3
Presence of metachromatic granules*	+, type of storage granules unknown	ND	ND	+†, polyphosphate	+, polyphosphate	+, polyphosphate
Oxidase	+	ND	+	–	+(weak)	–
Catalase	+	–	+	+†	+	+
Production of indole	–	–	–	–	+	–
Major menaquinone	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄) and MK-7(H ₄)
Diamino acid/murein type	LL-A ₂ pm/A3-γ	LL-A ₂ pm/A3-γ'	LL-A ₂ pm/A3-γ	LL-A ₂ pm/A3-γ'	LL-A ₂ pm/A3-γ'	LL-A ₂ pm/A3-γ'
Polar lipid‡	PE, PG, PL, GL	ND	DPG, PG, PI, GL	PI, PG, DPG, PL	PI, PG, DPG, PL	PI, PG, DPG, PL
Urease	+	–	–	+	+	–
Nitrate reduction	+	+	–	–	+†	+
G + C (mol%)	59–63	63–65	66–68	73	68	74
Carbohydrates utilized:§						
Lactose	–	±	ND	–	–†	+
Raffinose	–	+	+	–	–†	+
Mannitol	–	+	+	–	+†	+
Glycerol	+	±	+	–	–†	+
Arabinose	–	–	+ for D-arabinose – for L-arabinose	+(weak)	+†	+
Inositol	–	±	+	–	+†	–
Sucrose	+	+	+	–	–†	+
Fructose	+	+	+	–	–†	+
Glucose	+	+	+	–	–†	+
Mannose	+	±	+	–	–†	+
Galactose	v	±	+	–	–†	+
Trehalose	+	±	+	–	–†	–
Maltose	+	+	+	–	–†	+
Ribose	ND	±	+	+	+†	+
Xylose	ND	–	–	+(weak)	–†	+
Rhamnose	–	–	–	–	–†	–
Adonitol	–	±	ND	–	–†	–

* *Microlunatus*, *Friedmanniella* and strain Ben 107 contain polyphosphate granules; the granule type for *Propioniferax innocua* has not been determined.

† Data obtained by using the Biolog and Microbact 24E systems (A. M. Maszenan and others, unpublished data).

‡ DPG, diphosphatidylglycerol; GL, unknown glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid.

§ Tested for *Microlunatus phosphovorius* and Ben 106^T under aerobic conditions.

amura *et al.*, 1991, 1995a). Bootstrap analysis demonstrates this relationship was robust with a high confidence value (Fig. 2).

Chemotaxonomic characteristics

The peptidoglycan of strain Ben 106^T contained LL-diaminopimelic acid (LL-A₂pm) and its interpeptide bridge consisted of a single glycine residue. Position 1 of the peptide subunit was occupied by glycine, consistent with the cell wall being of the rare pepti-

doglycan type A3-γ' described by Schleifer & Seidl (1985). The major isoprenoid quinones were MK-9(H₄) and MK-7(H₄) with a peak area ratio of 50:32 (data summarized in Table 1). Its polar lipids consisted of phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and three unknown glycolipids, one of these unknown glycolipids being detected in high amounts. The fatty acid profile of Ben 106^T was characterized by the predominance of anteiso-12-methyltetradecanoic acid (C_{15:0}) (Table 2) and its G + C content was 74 mol %.

Table 2. Percentage cellular fatty acid composition of Ben 106^T, *Friedmanniella antarctica*, *Luteococcus japonicus*, *Micrococcus phosphovor*, *Propioniferax innocua* and *Propionibacterium propionicum*

The cellular fatty acids iso- (i) and anteiso- (ai) C_{15:0} and C_{17:0} predominate in *Propionibacterium propionicum*. C_{16:0}, hexadecanoic acid; i-C_{15:0}, 13-methyltetradecanoic acid; ai-C_{15:0}, 12-methyltetradecanoic acid.

Fatty acid	Ben 106 ^T	<i>Friedmanniella antarctica</i> *	<i>Luteococcus japonicus</i> *	<i>Micrococcus phosphovor</i> *	<i>Propioniferax innocua</i> *
C _{13:1}	2.0	—	—	—	—
i-C _{14:0}	4.1	2.3	—	4.0	3.2
C _{14:0}	3.1	—	1.0	2.9	—
C _{14:1}	2.0	—	—	—	—
i-C _{15:0}	5.0	23.2	—	22.4	20.9
i-C _{15:1}	3.6	—	—	—	—
ai-C _{15:0}	47.0	54.5	—	43.5	46.5
C _{15:0}	—	—	5.6	—	—
C _{15:1}	6.2	—	11.7	—	—
i-C _{16:0}	4.7	2.4	—	6.2	7.3
i-C _{16:1}	3.9	2.9	—	—	4.5
C _{16:0}	5.7	—	4.8	7.5	—
C _{16:1}	3.9	—	20.5	—	—
i-C _{17:0}	—	—	—	—	2.2
ai-C _{17:0}	—	—	—	—	6.7
C _{17:0}	—	—	2.4	—	—
i-C _{17:1}	8.1	—	—	—	1.3
ai-C _{17:1}	—	—	—	—	2.9
C _{17:1}	—	—	48.1	—	—
C _{18:0}	—	—	—	5.6	—
C _{18:1}	—	—	3.2	—	—
C _{18:2}	—	6.0	—	—	—

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

DISCUSSION

Cocci arranged in tetrads have been observed in biomass samples obtained from activated sludge systems from around the world (Carruci *et al.*, 1994; Cech & Hartman, 1993; Jenkins & Tandoi, 1991; Liu *et al.*, 1996; Maszenan *et al.*, 1997; Matsuo, 1994; Matsuzawa & Mino, 1991; Randall, 1994; Ubukata & Takii, 1994; Ubukata, 1994) but only a few such organisms have been isolated in pure culture and characterized. This paper reports a previously undescribed Gram-positive, non-spore-forming, facultatively anaerobic chemo-organotroph with cells in tetrads.

Some Gram-positive bacteria with high G + C contents have been suggested to play a major role in biological phosphorus removal (Bond *et al.*, 1995; Nakamura *et al.*, 1995b; Wagner *et al.*, 1994) but the organisms responsible for phosphate removal in activated sludge have not yet been identified with any conviction (Jenkins & Tandoi, 1991; Knight *et al.*, 1995). Strain Ben 106^T can accumulate PolyP aerobically, while no PHA was observed, but the factors affecting accumulation of these polymers are not known. Its physiology

needs to be studied to determine whether its behaviour fits the empirical models of biological phosphorus removal in activated sludge (e.g. van Loosdrecht *et al.*, 1997) or the model for phosphorus uptake by slow-growing bacteria such as *Micrococcus phosphovor* as proposed by Nakamura *et al.* (1995a).

DNA probes based on 16S rRNA sequence data should be used to identify this organism *in situ* so that its possible role in phosphate removal in activated sludge plants can be better assessed and its true similarity or otherwise to organisms sharing the same microscopic morphology (i.e. cocci in regular packets or in a tetrad) in activated sludge biomass resolved (Matsuo, 1994; Matsuzawa & Mino, 1991). More isolates of this organism should be sought from activated sludge biomass so that their physiological and ecological diversity can be clarified to give a much clearer understanding of the possible role of Gram-positive cocci in activated sludge systems and to clarify the taxonomic uncertainties discussed below.

Strain Ben 106^T shares a similar coccoid morphology with *Luteococcus japonicus* (Tamura *et al.*, 1994), *Friedmanniella antarctica* (Schumann *et al.*, 1997) and

Table 3. Comparison of the signature nucleotides of 16S rDNA for the families *Propionibacteriaceae* and *Nocardioidaceae* (Stackebrandt *et al.*, 1997) with Ben 106^T and *Friedmanniella antarctica*

Differences in the signature nucleotides from the designated signatures described by Stackebrandt *et al.* (1997) to delineate families within the *Actinobacteria* and whose taxonomic implications are discussed in the text are indicated by bold type.

Position*	<i>Propionibacteriaceae</i>	<i>Nocardioidaceae</i>	Ben 106 ^T	<i>Friedmanniella antarctica</i>
66–103	A–T	G–C	A–T	A–T
328	T	C	T	T
370–391	C–G	G–C	C–G	C–G
407–435	C–G	A–T	C–G	C–G
602–636	A–T	G–T	G–T	G–T
658–748	A–T	T–A	A–T	G–A
686	G	T	A	G
780	A	G	A	A
787	C	A	C	C
819	G	T	G	G
825–875	A–T	G–C	A–T	A–T
1409–1491	A–T	C–G	A–T	A–T

* *E. coli* numbering.

Micrococcus phosphovor (Nakamura *et al.*, 1991, 1995a), but not the characteristic hemispherical, lipped, cells of the latter (Nakamura *et al.*, 1995a) nor the pleomorphic rod morphology of *Propionibacterium propionicum* (Schaal, 1986; Charfreitag *et al.*, 1988). On the criterion of 16S rDNA sequence data, strain Ben 106^T is related, but not especially closely, to *Propionibacterium propionicum* (Schaal, 1986; Charfreitag *et al.*, 1988), *Propioniferax innocua* (Pitcher & Collins, 1991; Yokota *et al.*, 1994), *Luteococcus japonicus* (Tamura *et al.*, 1994), *Friedmanniella antarctica* (Schumann *et al.*, 1997) and *Micrococcus phosphovor* (Nakamura *et al.*, 1991, 1995a) (Fig. 2), all of which belong to the family *Propionibacteriaceae* (Stackebrandt *et al.*, 1997).

Strain Ben 106^T does not contain the cellular fatty acid 10-methyl-C_{18:0} or phosphatidylethanolamine, which supports the view that it does not belong to the genus *Aeromicrobium* (Miller *et al.*, 1991; Tamura & Yokota, 1994). Unsaturated fatty acids with 13, 14, 15 and 16 carbon atoms are found in Ben 106^T but not in *Micrococcus phosphovor* or *Propioniferax innocua* (Table 2). Although Ben 106^T and *Micrococcus phosphovor* share a similar type of cell wall peptidoglycan, the presence of 14-methylpentadecenoic acid (iso-C_{16:1}) and 15-methylhexadecenoic acid (iso-C_{17:1}) and the absence of octadecanoic acid (C_{18:0}) in Ben 106^T would also support the view that it does not belong to the genus *Micrococcus* (Table 2). The absence of MK-8(H₄) in Ben 106^T clearly suggests that it does not belong to the genus *Nocardioides* (Collins *et al.*, 1994; Tamura & Yokota, 1994). In addition to the predominant menaquinone MK-9(H₄), strain Ben 106^T contains an additional menaquinone, MK-7(H₄),

in substantial amounts, thus differing from all other genera in the currently recognized family *Propionibacteriaceae*. It also differs from *Propioniferax innocua* as its cell wall peptidoglycan contains glycine at position 1 of the peptide subunit, while *Propioniferax innocua* has L-alanine at that position (Schumann *et al.*, 1997), although Yokota *et al.* (1994) erroneously reported glycine at the same position. These chemotaxonomic data, summarized in Table 1, strongly support the other phenotypic data and phylogenetic data that Ben 106^T does not belong to any of these genera.

Based on 16S rDNA sequence data, Ben 106^T is only 95.9% similar to its closest relative *Propionibacterium propionicum*. A comparison of its 16S rDNA signature nucleotides with members of the family *Propionibacteriaceae* reveals many similarities but also several differences to those suggested by Stackebrandt *et al.* (1997) for circumscribing members of this family. These are summarized in Tables 3 and 4. For example, at positions 602–636 and 686 (*E. coli* numbering system) Ben 106^T has the nucleotide signature G–T instead of A–T and A instead of G (Table 3). Similarly, Ben 106^T differs markedly from members of the family *Nocardioidaceae*. In addition, Ben 106^T also has a different nucleotide signature at position 671–735 to that considered diagnostic for the suborder *Propionibacterineae* (i.e. A–T), possessing T–A instead (Table 4).

It is possible that the description of the family *Propionibacteriaceae* within the suborder *Propionibacterineae* (Stackebrandt *et al.*, 1997) needs to be amended to include Ben 106^T and *Friedmanniella antarctica* (Schumann *et al.*, 1997), which also differs

Table 4. 16S rDNA signature nucleotides for Ben 106^T, *Friedmanniella antarctica* and members of the families *Propionibacteriaceae* and *Nocardiodaceae* within the suborder *Propionibacterineae*

Differences in the signature nucleotides from the designated signatures described by Stackebrandt *et al.* (1997) to delineate families within the *Actinobacteria* and whose taxonomic implications are discussed in the text are indicated by bold type.

Position*	<i>Propionibacterineae</i>	<i>Propionibacteriaceae</i>	<i>Nocardiodaceae</i>	Ben 106^T <i>antarctica</i>	<i>Friedmanniella</i>
127–234	A–T	A–T	A–T	A–T	A–T
603–635	A–T	A–T	A–T	A–T	A–T
657–735	G–C	G–C	G–C	G–C	G–C
671–735	A–T	A–T	A–T	T–A	A–T
986–1219	T–A	T–A	T–A	T–A	T–A
987–1218	G–C	G–C	G–C	G–C	G–C
990–1215	T–G	T–G	T–G	T–G	T–G
1059–1198	C–G	C–G	C–G	C–G	C–G

* *E. coli* numbering.

in some of its signature nucleotides (see Tables 3 and 4). It is possible that Ben 106^T and *Friedmanniella* may represent members of a new family within an amended suborder *Propionibacterineae*, but more isolates of these need to be characterized before this can be resolved. Therefore, in spite of different signature nucleotides at some positions, strain Ben 106^T is best placed currently in the family *Propionibacteriaceae* emend. Rainey, Ward-Rainey & Stackebrandt 1997 (Stackebrandt *et al.*, 1997) because of similarities in most of its phenotypic characteristics to members of this family.

However, our phylogenetic and chemotaxonomic data reveal that strain Ben 106^T cannot be affiliated to any established genus within the family *Propionibacteriaceae*. Hence we propose that strain Ben 106^T should be assigned to a new genus, *Tessaracoccus* gen. nov., with *Tessaracoccus bendigoensis* sp. nov. as type species.

Description of *Tessaracoccus* gen. nov.

Tessaracoccus (Tes.sa.ra'coc.cus. Gr. adj. *tessara* four; Gr. n. *coccus* grain; L. n. *Tessaracoccus* four round cells).

Gram-positive, non-spore-forming, facultatively anaerobic cocci, 0.5–1.1 µm in diameter and arranged in regular tetrads. Cells are non-motile and accumulate polyphosphate. The organism is oxidase-negative, capable of reducing nitrate and catalase-positive. The cell wall contains LL-A₂pm with a rare peptidoglycan type, A3-γ'. The major menaquinones are MK-9(H₄) and MK-7(H₄). The main fatty acid is anteiso-12-methyltetradecanoic acid and polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and three unknown glycolipids. The phylogenetic position of the genus is in the high-G + C-content branch of *Firmicutes* with *Microtholus phosphovor*, *Friedmanniella antarctica*, *Propioniferax innocua* and *Propionibacterium propionicum* as its

closest relatives. The G + C content is 74 mol %. Type species is *Tessaracoccus bendigoensis*.

Description of *Tessaracoccus bendigoensis* sp. nov.

Tessaracoccus bendigoensis (ben.di.go'en. sis. M. L. adj. *bendigoensis* referring to Bendigo, the place of origin of the isolate).

The following sugars and sugar derivatives are utilized: L-arabinose, D-ribose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, sucrose, turanose, D-raffinose, D-xylose, stachyose, palatinose, maltotriose, methyl α-D-glucoside, methyl α-D-galactoside, methyl β-D-galactoside, α-cyclodextrin, β-cyclodextrin, dextrin, amygladin, arbutin, glycogen, D-mannitol, methylpyruvate, glucose 1-phosphate, glucose 6-phosphate, DL-glycerol phosphate, UMP, glycerol, uridine and salicin, but not cellobiose, L-fucose, D-psicose, L-rhamnose, D-trehalose, D-melezitose, 3-methylglucose, methyl β-D-glucoside, mannan, adonitol, D-arabitol, i-erythritol, *m*-inositol, D-sorbitol, xylitol, monomethylsuccinate, D-galacturonic acid lactone, 2-aminoethanol, 2,3-butanediol, inulin, fructose 6-phosphate, methylsuccinate, adenosine, 2'-deoxyadenosine, AMP, TMP, D-tagatose, methyl α-D-mannoside or sedoheptulosan. The following organic acids, amino acids and their derivatives are utilized: acetic acid, D-gluconic acid, α-hydroxybutyric acid, DL-lactic acid, L-lactic acid and pyruvic acid, but not *cis*-aconitic acid, citric acid, formic acid, D-galacturonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic

acid, L-pyrogutamic acid, γ -aminobutyric acid, uroconic acid, D-lactic acid methyl ester, D-malic acid, L-malic acid and N-acetyl-L-glutamic acid. The following substrates are metabolized: N-acetyl-D-glucosamine and uridine. The following substrates are not metabolized: Tween 40, Tween 80, N-acetyl-D-galactosamine, N-acetylmannosamine, glucuronamide, alaninamide, lactamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, D-serine, L-serine, L-threonine, DL-carnitine, inosine, thymidine, phenylethylamine, putrescine and hydroxy-L-proline. It possesses the following enzymes: alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase and α -mannosidase. Cells lack trypsin, chymotrypsin, β -glucuronidase, α -fucosidase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and arginine dihydrolase. It does not produce H_2S and indole. It is Voges-Proskauer-positive and can produce acetoin. It can liquefy gelatin. The natural habitat is activated sludge. The temperature range for growth is between 20 and 37 °C; pH range for growth is between 6.0 and 9.0. Optimal growth is at 25 °C and at pH 7.5. The type strain, Ben 106^T, has been deposited in the Australian Collection of Microorganisms as strain ACM 5119^T.

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REFERENCES

- Bond, P. L., Hugenholtz, P., Kellar, J. & Blackall, L. L. (1995). Bacterial community structures of phosphate-removing and non-phosphate-removing activated sludges from sequencing batch reactors. *Appl Environ Microbiol* **61**, 1910–1916.
- Carucci, A., Majone, M., Ramadori, R. & Rossetti, S. (1994). Dynamics of phosphorus and organic substrates in anaerobic and aerobic phases of a sequencing batch reactor. *Water Sci Technol* **30**, 237–246.
- Cech, J. S. & Hartman, P. (1993). Competition between polyphosphate and polysaccharide accumulating bacteria in enhanced biological phosphate removal systems. *Water Res* **27**, 1219–1225.
- Charfreitag, O., Collins, M. D. & Stackebrandt, E. (1988). Reclassification of *Arachnia propionica* as *Propionibacterium propionicus* comb. nov. *Int J Syst Bacteriol* **38**, 354–357.
- Collins, M. D., Cockcroft, S. & Wallbanks, S. (1994). Phylogenetic analysis of a new LL-diaminopimelic acid-containing coryneform bacterium from herbage, *Nocardioides plantarum* sp. nov. *Int J Syst Bacteriol* **44**, 523–526.
- Cummins, C. S. & Moss, C. W. (1990). Fatty acid composition of *Propionibacterium propionicus* (*Arachnia propionica*). *Int J Syst Bacteriol* **40**, 307–308.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5.1. Seattle: Department of Genetics, University of Washington.
- Jenkins, D. & Tandoi, V. (1991). The applied microbiology of enhanced biological phosphate removal – accomplishments and needs. *Water Res* **25**, 1471–1478.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kataoka, N., Tokiwa, T., Tanaka, Y., Takeda, K. & Suzuki, T. (1996). Enrichment culture and isolation of slow growing bacteria. *Appl Microbiol Biotechnol* **45**, 771–777.
- Knight, G. C., Seviour, E. M., Seviour, R. J., Soddell, J. A., Lindrea, K. C., Strachan, W., DeGrey, B. & Bayly, R. C. (1995). Development of the microbial community of a full scale nutrient removal activated sludge plant during start up. *Water Res* **29**, 2085–2093.
- Liu, W.-T., Mino, T., Nakamura, K. & Matsuo, T. (1996). Glycogen accumulating populations and its anaerobic substrates uptake in anaerobic-aerobic activated sludge without biological phosphorus removal. *Water Res* **30**, 75–82.
- van Loosdrecht, M. C. M., Hooijmans, C. M., Brdjanovic, D. & Heijnen, J. J. (1997). Biological phosphate removal processes. *Appl Microbiol Biotechnol* **48**, 289–296.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996). The Ribosomal Database Project (RDP). *Nucleic Acids Res* **24**, 82–85.
- Maszenan, A. M., Seviour, R. J., Patel, B. K. C., Rees, G. N. & McDougall, B. M. (1997). *Amaricoccus* gen. nov., a gram-negative coccus occurring in regular packages or tetrads isolated from activated sludge biomass. Description of four species belonging to the genus. *Int J Syst Bacteriol* **47**, 727–734.
- Matsuo, Y. (1994). Effect of the anaerobic solid retention time on enhanced biological phosphorus removal. *Water Sci Technol* **30**, 193–202.
- Matsuzawa, Y. & Mino, T. (1991). Role of glycogen as an intracellular carbon reserve of activated sludge in the competitive growth of filamentous and non filamentous bacteria. *Water Sci Technol* **23**, 899–905.
- Miller, E. R., Woese, C. R. & Brenner, S. (1991). Description of the erythromycin-producing bacterium *Arthrobacter* sp. strain NRRL B-3381 as *Aeromicrobium erythreum* gen. nov., sp. nov. *Int J Syst Bacteriol* **41**, 363–368.
- Nakamura, K., Masuda, K. & Mikami, E. (1991). Isolation of a new type of polyphosphate accumulating bacterium and its phosphate removal characteristics. *J Ferment Technol* **71**, 258–263.
- Nakamura, K., Hiraishi, A., Yoshimi, Y., Kawaharasaki, M., Masuda, K. & Kamagata, Y. (1995a). *Microtholunatus phosphovorius* gen. nov., sp. nov., a new gram-positive polyphosphate-accumulating bacterium isolated from activated sludge. *Int J Syst Bacteriol* **45**, 17–22.
- Nakamura, K., Ishikawa, S. & Kawaharasaki, M. (1995b). Phosphate uptake and release activity in immobilized polyphosphate accumulating bacterium *Microtholunatus phosphovorius* strain NM-1. *J Ferment Technol* **80**, 377–382.
- Owen, R. J. & Lapage, S. P. (1976). The thermal denaturation of partly purified bacterial deoxyribonucleic acid and its taxonomic applications. *J Appl Bacteriol* **41**, 335–340.

- Painter, H. A. (1983). Metabolism and physiology of aerobic bacteria and fungi. In *Ecological Aspects of Used-Water Treatment: Vol. 2, Biological Activities and Treatment Processes*, pp. 11–25. Edited by C. R. Curds & H. A. Hawks. London: Academic Press.
- Patel, B. K. C., Andrews, K. T., Ollivier, B., Mah, R. A. & Garcia, J. L. (1995). Re-evaluating the classification of *Halobacteroides* and *Haloanaerobacter* species based on sequence comparisons of the 16S ribosomal RNA gene. *FEMS Microbiol Lett* **134**, 115–119.
- Pitcher, D. G. & Collins, M. D. (1991). Phylogenetic analysis of some LL-diaminopimelic acid-containing coryneform bacteria from human skin: description of *Propionibacterium innocuum* sp. nov. *FEMS Microbiol Lett* **84**, 295–300.
- Porteous, L. A., Armstrong, J. L., Seidler, R. J. & Watrud, L. S. (1994). An effective method to extract DNA from environment samples for polymerase chain reaction amplification and DNA fingerprint analysis. *Curr Microbiol* **29**, 301–307.
- Randall, A. A. (1994). *The effect of substrate chemistry on enhanced biological phosphorus removal, intracellular phosphate form and location and the resulting population structure of sequencing batch reactors receiving synthetic wastewater*. PhD thesis, Auburn University, AL, USA.
- Rees, G. N., Vasiliadis, G., May, J. W. & Bayly, R. C. (1992). Differentiation of polyphosphate and poly- β -hydroxybutyrate granules in an *Acinetobacter* sp. isolated from activated sludge. *FEMS Microbiol Lett* **94**, 171–174.
- Schaal, K. P. (1986). Genus *Arachnia* Pine and Georg 1969, 269^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1332–1342. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 406–477.
- Schleifer, K. H. & Seidl, P. H. (1985). Chemical composition and structure of murein. In *Chemical Methods in Bacterial Systematics*, pp. 201–219. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Schumann, P., Prauser, H., Rainey, F. A., Stackebrandt, E. & Hirsch, P. (1997). *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. *Int J Syst Bacteriol* **47**, 278–283.
- Skerman, V. B. D. (1968). A new type of micromanipulator and microforge. *J Gen Microbiol* **54**, 287–297.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Takii, S. (1977a). Accumulation of reserve polysaccharide in activated sludge treating carbohydrate wastes. *Water Res* **11**, 79–83.
- Takii, S. (1977b). Bacterial characteristic of activated sludges treating carbohydrate waste. *Water Res* **11**, 85–89.
- Tamura, T. & Yokota, A. (1994). Transfer of *Nocardioides fastidiosa* Collins and Stackebrandt 1989 to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov. *Int J Syst Bacteriol* **44**, 608–611.
- Tamura, T., Takeuchi, M. & Yokota, A. (1994). *Luteococcus japonicus* gen. nov., sp. nov., a new gram-positive coccus with LL-diaminopimelic acid in the cell wall. *Int J Syst Bacteriol* **44**, 348–356.
- Ubukata, Y. (1994). Some physiological characteristics of a phosphate removing bacterium isolated from anaerobic/aerobic activated sludge. *Water Sci Technol* **30**, 229–235.
- Ubukata, Y. & Takii, S. (1994). Induction method of excess phosphate accumulation for phosphate removing bacteria isolated from anaerobic/aerobic activated sludge. *Water Sci Technol* **30**, 221–227.
- Wagner, M., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wedi, D. & Schleifer, K.-H. (1994). Development of rRNA-targeted oligonucleotide probe specific for the genus *Acinetobacter* and its application for *in situ* monitoring in activated sludge. *Appl Environ Microbiol* **60**, 792–800.
- Wanner, J. (1994). *Activated Sludge Bulking and Foaming Control*. Lancaster: Technomic Publishing Company.
- Williams, T. M. & Unz, R. F. (1985). Isolation and characterization of filamentous bacteria present in bulking activated sludge. *Appl Microbiol Biotechnol* **22**, 273–282.
- Winker, S. & Woese, C. R. (1991). A definition of the domain Archaea, Bacteria and Eucarya in terms of small ribosomal RNA characteristics. *Syst Appl Microbiol* **14**, 305–310.
- Yokota, A., Tamura, T., Takeuchi, M., Weiss, N. & Stackebrandt, E. (1994). Transfer of *Propionibacterium innocuum* Pitcher and Collins 1991 to *Propionifera* gen. nov. as *Propionifera innocua* comb. nov. *Int J Syst Bacteriol* **44**, 579–582.
- Yoshimi, Y., Hiraishi, A. & Nakamura, K. (1996). Isolation and characterization of *Microsphaera multipartita* gen. nov., sp. nov., a polysaccharide-accumulating gram-positive bacterium from activated sludge. *Int J Syst Bacteriol* **46**, 519–525.