**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 106ᵀ, 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 106ᵀ 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-A,pm) 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 *Microlunatus phosphovorus* 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 106ᵀ (= ACM 5119ᵀ).

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

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**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
of some of these cocci is now known. For example, a Gram-positive coccus which can accumulate polyphosphate was identified as the new bacterium Micrococcus lunatus phosphovorus 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 106T (ACM 5119T). The phenotypic, chemotaxonomic and phylogenetic properties indicate that strain Ben 106T 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 106T. Strain Ben 106T (ACM 5119T) was isolated using a Skerman micro-manipulator (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 106T was stored on GS medium in 20% glycerol at -80 °C.

Physiological and biochemical characterization of strain Ben 106T. Substrate utilization patterns for strain Ben 106T 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 (bioMerieux) 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 medium. Whether its metabolism was oxidative or fermentative (O/F) medium (Oxoid).

Chemotaxonomic characterization. The biomass used for chemotaxonomic characterization of Ben 106T 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 x 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 3.5c (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 a-chymotrypsinase 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 spooned onto a sterile glass pipette.
Tessaracoccus bendigoensis gen. nov., sp. nov.

RESULTS

Culture and morphological characteristics

The isolation of strain Ben 106T 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 106T 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-xylone, 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, D/L-glycerol phosphate, UMP, glyceral, uridine and salicin. Strain Ben 106T could not metabolize the following sugars and sugar derivatives: cellobiose, l-fucose, D-psicose, D-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, D-carboxylic acid, adenosine, 2'-deoxyadenosine, AMP, TMP, D-tagatose, methyl α-D-mannoside and sedoheptulosan.

The following acids were metabolized by Ben 106T: acetic acid, D-gluconic acid, α-hydroxybutyric acid, D/L-lactic acid, D/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 106T following Gram staining. Bar, 10 μm. (c) Scanning electron micrograph of strain Ben 106T showing the characteristic tetrad morphology. Bar, 2 μm. (d) Light micrograph of strain Ben 106T stained with the polyphosphate stain of Rees et al. (1992). Bar, 10 μm.
the following acids, amino acids and their derivatives were metabolized by strain Ben 106T: cis-aconitic acid, citric acid, formic acid, d-galacturonic acid lactone, d-galacturonic acid, d-glucosaminic acid, d-glucuronic acid, β-hydroxybutyric acid, α-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, succinic acid, bromosuccinic acid, sebacic acid, succinic acid, sebacic acid, succinic acid, succinic acid, l-aspartic acid, l-glutamic acid, l-aspartic acid, l-glutamic acid, glycyl-L-glutamic acid, l-pyroglutamic acid, γ-aminobutyric acid, urocanic 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 106T, which was not able to metabolize the following amino acids, amines and their derivatives: D-alanine, L-alanine, L-alanylglucine, 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 106T could not metabolize Tween 40, Tween 80, glucuronamidase, alaminamide, lactamidase, 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 106T 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 106T 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 106T was 7.5.

**Phylogenetic characteristics**

Almost the complete 16S rRNA gene was sequenced (1410 bases) for strain Ben 106T, 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. The isolate formed a new branch within the family *Propionibacteriaceae* based on ribosomal 16S rRNA sequence data, indicating the position of *Tessarococcus bendigoensis* (strain Ben 106T) within the radiation of representatives of the high-G+C-containing Gram-positive bacteria.
amur 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 106T contained LL-diaminopimelic acid (LL-Apm) 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 peptidoglycan 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, di-phosphatidylglycerol and three unknown glycolipids, one of these unknown glycolipids being detected in high amounts. The fatty acid profile of Ben 106T was characterized by the predominance of anteiso-12-methyltetradecanoic acid (C15:0) (Table 2) and its G+C content was 74 mol%.

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

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.
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Table 2. Percentage cellular fatty acid composition of Ben 106<sup>T</sup>, Friedmanniella antarctica, Luteococcus japonicus, Microlunatus phosphovorus, Propioniferax innocua and Propionibacterium propionicum

The cellular fatty acids iso- (i) and anteiso- (ai) C<sub>15</sub> and C<sub>17</sub> predominate in Propionibacterium propionicum. C<sub>16:0</sub>, hexadecanoic acid; i-C<sub>15:0</sub>, 13-methyltetradecanoic acid; ai-C<sub>15:0</sub>, 12-methyltetradecanoic acid.

<table>
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<th>Fatty acid</th>
<th>Ben 106&lt;sup&gt;T&lt;/sup&gt;</th>
<th>Friedmanniella antarctica*</th>
<th>Luteococcus japonicus*</th>
<th>Microlunatus phosphovorus*</th>
<th>Propioniferax innocua*</th>
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<td>2.3</td>
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* 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<sup>T</sup> 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 Microlunatus phosphovorus 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<sup>T</sup> shares a similar coccoid morphology with Luteococcus japonicus (Tamura et al., 1994), Friedmanniella antarctica (Schumann et al., 1997) and
Microlunatus phosphovorus (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 106T 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 antarctica (Tamura et al., 1994), Friedmanniella antarctica (Schumann et al., 1997) and Microlunatus phosphovorus (Nakamura et al., 1991, 1995a) (Fig. 2), all of which belong to the family Propionibacteriaceae (Stackebrandt et al., 1997).

Strain Ben 106T does not contain the cellular fatty acid 10-methyl-C18:0 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 106T but not in Microlunatus phosphovorus or Propioniferax innocua (Table 2). Although Ben 106T and Microlunatus phosphovorus share a similar type of cell wall peptidoglycan, the presence of 14-methylpentadecenoic acid (iso-C16:1) and 15-methylhexadecenoic acid (iso-C17:1) and the absence of octadecanoic acid (C18:0) in Ben 106T would also support the view that it does not belong to the genus Microlunatus (Table 2). The absence of MK-8(H4) in Ben 106T 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-(9H4), strain Ben 106T 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 106T does not belong to any of these genera.

Based on 16S rDNA sequence data, Ben 106T 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 106T has the nucleotide signature G–T instead of A–T and A instead of G (Table 3). Similarly, Ben 106T differs markedly from members of the family Nocardioidaceae. In addition, Ben 106T 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 106T and Friedmanniella antarctica (Schumann et al., 1997), which also differs...
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**Table 4. 16S rDNA signature nucleotides for Ben 106\textsuperscript{T}, Friedmanniella antarctica and members of the families Propionibacteriaceae and Nocardioidaeae 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.

<table>
<thead>
<tr>
<th>Position*</th>
<th>Propionibacteriaceae</th>
<th>Propionibacteriaceae</th>
<th>Nocardioidaeae</th>
<th>Ben 106\textsuperscript{T} antarctica</th>
<th>Friedmanniella</th>
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<td>603–635</td>
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<tr>
<td>657–735</td>
<td>G-C</td>
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<td>A-T</td>
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<td>G-C</td>
<td>T-A</td>
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<tr>
<td>986–1219</td>
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<td>A-T</td>
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<td>T-A</td>
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<tr>
<td>987–1218</td>
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<td>G-C</td>
<td>T-G</td>
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<tr>
<td>990–1215</td>
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<tr>
<td>1059–1198</td>
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<td>C-G</td>
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</tr>
</tbody>
</table>

* E. coli numbering.

in some of its signature nucleotides (see Tables 3 and 4). It is possible that Ben 106\textsuperscript{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 resolved. Therefore, in spite of different signature nucleotides at some positions, strain Ben 106\textsuperscript{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\textsuperscript{T} cannot be affiliated to any established genus within the family Propionibacteriaceae. Hence we propose that strain Ben 106\textsuperscript{T} should be assigned to a new genus, *Tessaracoccus* gen. nov., with *Tessaracoccus bendigoensis* sp. nov. as type species.

**Description of *Tessaracoccus bendigoensis* sp. nov.**

*Tessaracoccus bendigoensis* (ben.dig.o'eni. 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-xylene, stachyose, palatinose, maltotriose, methyl α-D-glucoside, methyl α-D-galactoside, methyl β-D-galactoside, α-cyclodextrin, β-cyclodextrin, dextrin, amygdalin, arbutin, glycyogen, D-mannitol, methylpyruvate, glucose 1-phosphate, glucose 6-phosphate, DL-glycerol phosphate, UMP, glycerol, uridine and salicin, but not cellubiose, D-fucose, D-psicose, L-rhamnose, D-trehalose, D-melezitose, 3-methylglucose, methyl β-D-glucoside, mannann, 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 sedoheptulose. The following organic acids, amino acids and their derivatives are utilized: acetic acid, D-glucuronic 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, succinic acid, L-aspartic acid, L-glutamic acid, glycyrl-L-aspartic acid, glycyrl-L-glutamic acid, polyphosphate. The organism is oxidase-negative, Gram-positive, non-spore-forming, facultatively aerobic, 0.5–1.1 µm in diameter and arranged in regular tetrams. Cells are non-motile and accumulate polyphosphate. The organism is oxidase-negative, capable of reducing nitrate and catalase-positive. The cell wall contains LL-A,pm with a rare peptidoglycan type, A3-γ'. The major menaquinones are MK-9(H\textsubscript{4}) and MK-7(H\textsubscript{4}). The main fatty acid is anteiso-12-methyltetradecanoic acid and polar lipids are phosphatidylglycerol, phosphatidylglycerol, diphasphatidylglycerol and three unknown glycolipids. The phylogenetic position of the genus is in the high-G+C-content branch of Firmicutes with Microbacterium phosphovorans, 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* gen. nov.**

*Tessaracoccus* (Tes.sa.ra'co.cus. Gr. adj. *tessara* four; Gr. n. *coccos* 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-A,pm with a rare peptidoglycan type, A3-γ'. The major menaquinones are MK-9(H\textsubscript{4}) and MK-7(H\textsubscript{4}). The main fatty acid is anteiso-12-methyltetradecanoic acid and polar lipids are phosphatidylglycerol, phosphatidylglycerol, diphasphatidylglycerol and three unknown glycolipids. The phylogenetic position of the genus is in the high-G+C-content branch of Firmicutes with Microbacterium phosphovorans, Friedmanniella antarctica, Propioniferax innocua and Propionibacterium propionicum as its closest relatives. The G+C content is 74 mol%. Type species is *Tessaracoccus bendigoensis*.
acid, L-pyroglutamic 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-alanlyglycine, 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-phosphorylase, z-galactosidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase. Cells lack trypsin, chymotrypsin, β-glucuronidase, z-fucosidase, lysozene decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and arginine dihydrolase. It does not produce H₂S 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 106T, has been deposited in the Australian Collection of Microorganisms as strain ACM 5119T.

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References


