

Granulicoccus phenolivorans gen. nov., sp. nov., a Gram-positive, phenol-degrading coccus isolated from phenol-degrading aerobic granules

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A Gram-positive bacterium, designated strain PG-02^T, was isolated by serial dilution from aerobic granules obtained from a laboratory-scale sequencing batch reactor for bioremediation of phenolic wastewater. Strain PG-02^T grew axenically as cocci and is an oxidase-negative and catalase-positive, non-motile facultative anaerobe. It does not reduce nitrate and grows between 15 and 37 °C, with an optimum temperature of 30 °C. The pH range for growth is between 5.0 and 8.5, with an optimum pH of 7.0. Strain PG-02^T contains type A3_γ peptidoglycan (LL-A₂pm←Gly with alanine at position 1 of the peptide subunit). The G + C content of the DNA is 69 mol%. Menaquinone MK-9(H₄) was the major isoprenoid quinone. The polar lipids included diphosphatidylglycerol and phosphatidylglycerol, while 13-methyltetradecanoic acid (i-C_{15:0}) and 1,1-dimethoxy-iso-pentadecane (i-C_{15:0} DMA) were the major components in whole-cell methanolsates. PG-02^T stained positively for intracellular polyphosphate granules but not poly-β-hydroxyalkanoates. It produces capsular material and possesses an autoaggregation capability. Phenotypic and 16S rRNA gene sequence analyses showed that PG-02^T differed from its closest phylogenetic relatives, namely members of the suborder *Propionibacterineae*, which includes the genera *Tessaracoccus*, *Micrococcus*, *Luteococcus*, *Micropruina*, *Propionibacterium*, *Propioniferax*, *Nocardioides*, *Friedmanniella* and *Aeromicrobium*, and that it should be placed in a new genus and species as *Granulicoccus phenolivorans* gen. nov., sp. nov. The type strain of *Granulicoccus phenolivorans* is PG-02^T (= ATCC BAA-1292^T = DSM 17626^T).

Natural phenolic compounds and their derivatives are present in the environment and some enter as intermediates from the biodegradation of natural polymers containing aromatic rings, such as lignins and tannins, aromatic amino acid precursors (Jeong *et al.*, 2003) and xenobiotic compounds (van Schie & Young, 1998). Due to their wide usage in agricultural and industrial processes, phenol and its derivatives are pollutants of environmental concern (Jensen, 1996). Phenol pollution is often associated with pulp mills, coal mines, cooking plants, oil refineries, industrial resin

manufacturing and wood preservation processes and their wastewater (Semple & Cain, 1995; Selvaratnam *et al.*, 1997; Whiteley & Bailey, 2000). In the absence of proper treatment, industrial and agricultural effluents can be an important source of anthropogenic phenol. This, together with the acute toxicity of phenols, has led them to be included as priority pollutants for both the World Health Organization and the US Environmental Protection Agency (Davi & Gnudi, 1999).

The influx of phenol and its derivatives into waste-treatment systems may inhibit microbial activity and result in deterioration of treatment performance and, in extreme cases, complete breakdown of wastewater treatment (Soda *et al.*, 1998; Watanabe *et al.*, 1999). Biodegradation of phenol and phenolics in waste and wastewater is known to be carried out by bacteria of diverse phylogeny, including members from both the *Betaproteobacteria* and *Gammaproteobacteria* (Dapaah & Hill, 1992; Arai *et al.*,

†Deceased 29 July 2005.

Abbreviations: A₂pm, diamino pimelic acid; DMA, dimethyl acetal.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PG-02^T is AY566575.

Details of signature nucleotides within the 16S rRNA gene sequences of strain PG-02^T and related taxa are available as supplementary material in IJSEM Online.

1998; Whiteley & Bailey, 2000). Some of these have been isolated and characterized (Hino *et al.*, 1998; Watanabe *et al.*, 1996, 1998; Whiteley & Bailey, 2000; Rehfuß & Urban, 2005). The ability of bacteria to aggregate is important in the bioremediation of toxic chemicals such as phenol in the activated sludge process, since those capable of aggregation will be retained in the system through biomass recycling and be protected from predatory protozoa (van Limbergen *et al.*, 1998; Farrell & Quilty, 2002). Several factors such as substrate gradients, slow growth rates, stress and predation have been suggested to trigger aggregation, although the precise mechanism is not known (Bossier & Verstraete, 1996).

Aerobic granulation, which represents a novel form of cell immobilization without a carrier matrix, was recently used successfully to treat phenolic wastewater at a load that would lead to failure in conventional activated sludge systems (Jiang *et al.*, 2004). It was thought that aggregation of microbial cells into compact granules protected the organisms from possible phenol toxicity (Jiang *et al.*, 2004). In this study, the description of strain PG-02^T, one of several phenol degraders that were isolated from these granules, is presented.

Phenol-degrading aerobic granules were cultivated in a laboratory-scale sequencing batch reactor from activated sludge seed, fed with synthetic wastewater containing phenol as the sole carbon source (Jiang *et al.*, 2004). Granules were harvested 8 weeks after steady-state reactor operation. Granules (2.5 g) were added to 15 ml MP medium, which contained (l^{-1}) 1.0 g $(NH_4)_2SO_4$, 0.2 g $MgCl_2 \cdot 6H_2O$, 0.1 g NaCl, 0.02 g $FeCl_3 \cdot 6H_2O$, 0.01 g $CaCl_2$ and phosphate buffer (1.35 g KH_2PO_4 and 1.65 g K_2HPO_4), with trace elements and vitamins as described by Cote & Gherna (1994). The supernatant was serially diluted with medium from 10^{-1} to 10^{-7} dilutions and 150 μ l aliquots of each dilution was spread onto agar plates containing MP medium solidified with 1.2 % Bacto agar (Difco). Plates were incubated at 25 °C and monitored for 4 weeks for colony growth by examination on a colony counter. Visible colonies were observed after 1 week of incubation. Strain PG-02^T takes 10 days to produce visible colonies on MP agar plates. Culture purity was confirmed by microscopic examination of cells taken from single colonies. An axenic culture of strain PG-02^T was preserved at -80 °C in MP medium containing 20 % glycerol.

PG-02^T is a facultative anaerobe, as growth occurred down the line of inoculation in stab cultures. It grew at 15–37 °C, with optimum growth at 30 °C, and at pH 5.0–8.5, with optimum growth at pH 7.0. Cells stained Gram-positively with the modified Gram-stain method of Hucker (Smibert & Krieg, 1994) and this was confirmed by the absence of stringiness with 3 % KOH treatment (Buck, 1982). No flagella were detected and the motility test confirmed that strain PG-02^T was non-motile (Smibert & Krieg, 1994). Polyphosphate granules were observed by the staining method of Rees *et al.* (1992) in cells grown aerobically with

either glucose, acetate or propionate as the sole carbon source, but polyhydroxyalkanoate granules were not detected when cells were incubated anaerobically. Capsular material was observed with the Indian ink stain.

Physiological and biochemical characteristics of strain PG-02^T are presented in the descriptions of the genus and species. Enzyme profiles and biochemical characteristics of strain PG-02^T were determined using the API ZYM and API 20E systems according to the manufacturer's instructions (bioMérieux). Carbon substrate utilization tests were performed with Biolog GN and GP systems. Cultures were catalase- and urease-positive but oxidase-negative as determined by method of Smibert & Krieg (1994). The genomic G + C content as determined by reversed-phase HPLC (Schumann *et al.*, 1997) was 69 mol%.

Peptidoglycan, menaquinone and polar lipid compositions were analysed as described by Schumann *et al.* (1997). Fatty acids were extracted and analysed following the instructions of the Microbial Identification System operating manual (MIDI, 1999) and as described by Kämpfer & Kroppenstedt (1996). Strain PG-02^T possessed a type A3 γ peptidoglycan (LL-A₂pm←Gly with alanine at position 1 of the peptide subunit; type A41.1 according to <http://www.dsmz.de/species/murein.htm>). The peptidoglycan amino acids were alanine/glycine/glutamic acid/LL-diaminopimelic acid (LL-A₂pm) in a molar ratio of 1.5 : 0.8 : 1.0 : 1.0, as determined by gas chromatography (MacKenzie, 1987). Cells contained two isoprenoid quinones, MK-9(H₄) and MK-8(H₄), with a composition ratio of 42 : 1. Polar lipids included diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids and three minor phospholipids, and 13-methyltetradecanoic acid (i-C_{15:0}) and 1,1-dimethoxy-iso-pentadecane (i-C_{15:0} DMA) were the two major components of whole-cell methanolsates, respectively contributing 50.5 and 37.4 % to the total. Traces of 12-methyltetradecanoic acid (ai-C_{15:0}), C_{12:0} DMA and 9,10-cyclo C_{19:0} DMA were also detected (Table 1). 1,1-Dimethoxy-iso-pentadecane (i-C_{15:0} DMA) was identified on the basis of its relative retention times on polar (Varian VF-23ms; 0.25 mm × 30 m) and non-polar (5 % phenyl methyl silicone, 0.2 mm × 25 m) gas chromatography columns, and its presence was confirmed by GC-MS using a non-polar OV-1 column (0.15 mm × 25 m), which revealed fragment ions at *m/z* 75 and 241. When these were compared with the mass spectrum of i-C_{16:0} DMA, which generated a fragment at *m/z* 255 (Männistö *et al.*, 2000), the expected mass difference of -14 was observed.

The nearly full-length 16S rRNA gene of strain PG-02^T was amplified and sequenced using methods described previously (Maszenan *et al.*, 1997). A 16S rRNA gene sequence of 1320 nucleotides was obtained in both directions, corresponding to positions 20–1471 of the *Escherichia coli* sequence according to the nomenclature of Winker & Woese (1991). This sequence was aligned manually against sequences of its close relatives using the alignment editor in BioEdit (Hall, 1997). Sequence analysis was performed

Table 1. Composition of the whole-cell methanolsate of strain PG-02^T

Fatty acid	Content (%)
Tetradecanoic acid (C _{14:0})	0.10
Pentadecanoic acid (C _{15:0})	0.06
Hexadecanoic acid (C _{16:0})	0.26
11-Methyl dodecanoic acid (i-C _{13:0})	0.45
12-Methyl tridecanoic acid (i-C _{14:0})	0.23
13-Methyl tetradecanoic acid (i-C _{15:0})	50.52
14-Methyl pentadecanoic acid (i-C _{16:0})	0.19
15-Methyl hexadecanoic acid (i-C _{17:0})	0.35
12-Methyl tetradecanoic acid (ai-C _{15:0})	1.80
14-Methyl hexadecanoic acid (ai-C _{17:0})	0.22
1,1-Dimethoxy dodecane (C _{12:0} DMA)	5.26
1,1-Dimethoxy tetradecane (C _{14:0} DMA)	0.15
1,1-Dimethoxy hexadecane (C _{16:0} DMA)	0.18
1,1-Dimethoxy iso-pentadecane (i-C _{15:0} DMA)	37.42
1,1-Dimethoxy anteiso-pentadecane (ai-C _{15:0} DMA)	0.31
1,1-Dimethoxy 9,10-methylene-nonadecane (9,10-cyclo C _{19:0} DMA)	2.09

with BLAST (Altschul *et al.*, 1997) and CLUSTAL W (Thompson *et al.*, 1994) and SIMILARITY_RANK and SUGGEST_TREE in the Ribosomal Database Project, version 8.0 (Maidak *et al.*, 1997). Distance analysis was performed on a final dataset of an unambiguous alignment of 1162 bases of strain PG-02^T and its closest relatives. A phylogenetic tree was constructed from evolutionary distances using the FITCH program in PHYLIP (Felsenstein, 1985). Bootstrap confidence values were obtained with 1000 resamplings.

The sequence data reveal that PG-02^T is a member of the *Actinobacteria* in the domain *Bacteria* (Stackebrandt *et al.*, 1997). Pairwise comparison of the 16S rRNA gene sequence revealed that strain PG-02^T was 95% similar to *Propioniferax innocua* ATCC 49929^T (Pitcher & Collins, 1991; Yokota *et al.*, 1994) and strains of *Microlunatus phosphovorius* (Nakamura *et al.*, 1995), less than 95% similar to the type strains of *Luteococcus japonicus* and *L. sanguinus* (Tamura *et al.*, 1994) and *Friedmanniella antarctica*, *F. capsulata* and *F. spumicola* (Schumann *et al.*, 1997; Maszenan *et al.*, 1999b), less than 93% similar to the type strains of *Tessaracoccus bendigoensis* (Maszenan *et al.*, 1999a), *Propionibacterium propionicum*, *Propionibacterium avidum*, *Propionibacterium microaerophilum*, *Propionibacterium jensenii*, *Propionibacterium freudenreichii* subsp. *shermanii*, *Propionibacterium australiense* and *Propionibacterium lymphophilum* (Charfreitag *et al.*, 1988) and strains of *Nocardiodes fulvus*, *N. luteus*, *N. albus*, *N. jensenii*, *N. dubius* and *N. kribbensis* (Collins *et al.*, 1994; Tamura & Yokota, 1994) and less than 92% similar to *Micropruina glycofenica* Lg2^T (Shintani *et al.*, 2000), as shown in Fig. 1.

Using the taxonomic scheme of Stackebrandt *et al.* (1997), it is clear that strain PG-02^T fits readily within the suborder

Propionibacterineae. All of the closest related genera show complete concurrence with the 16S rRNA signature nucleotides of the taxonomic scheme of Stackebrandt *et al.* (1997) for members of the suborder *Propionibacterineae* with the exception of *Micropruina glycofenica*, which has A–T instead of G–C at nucleotide positions 987 : 1218, and *T. bendigoensis* and *Microlunatus* sp. Y-73, which respectively have T–A and A–G instead of A–T at positions 671 : 735 (see Supplementary Table S1 available in IJSEM Online). Further analysis of the 16S rRNA structure of strain PG-02^T also concurred with the signature scheme for the family *Propionibacteriaceae*. However, *T. bendigoensis*, *F. antarctica*, *Friedmanniella* sp. Ellin171 and *F. spumicola* have G–T instead of A–T at positions 602 : 636. At positions 658 : 748, instead of A–T, *Micropruina glycofenica* has A–A, members of the genus *Friedmanniella* have G–A and *Microlunatus* sp. Y-73 has G–T (Supplementary Table S2). On the basis of these signature nucleotides, strain PG-02^T may belong to a novel genus in the family *Propionibacteriaceae*. However, the scheme of Stackebrandt *et al.* (1997) will need modification when more actinobacterial isolates within the suborder *Propionibacterineae* and family *Propionibacteriaceae* are characterized.

Strain PG-02^T differs morphologically from members of the genera *Luteococcus*, *Friedmanniella* and *Tessaracoccus*, which occur predominantly as cocci in pairs and tetrads. Even though members of *Micropruina* and *Microlunatus* share a similar morphology with strain PG-02^T in that they all occur as single cocci or cocci in pairs, members of both genera are aerobic, while strain PG-02^T is facultatively anaerobic based on growth observed down the stab culture. Furthermore, strain PG-02^T differs from *Micropruina glycofenica* as it stores polyphosphate instead of intracellular glycogen and does not contain *meso*-A₂pm in its peptidoglycan. Distinguishing characteristics of strain PG-02^T are detailed in Table 2.

Pairwise comparison of 16S rRNA gene sequences revealed that the sequence of strain PG-02^T was 95% similar to those of its closest phylogenetic relatives, *Microlunatus phosphovorius* and *Propioniferax innocua*. However, strain PG-02^T is different from *Microlunatus phosphovorius* in that it contains the isoprenoid quinone MK-8(H₄) and its polar lipids lack phosphatidylinositol. Strain PG-02^T is also different from *Propioniferax innocua* as the latter exhibits the characteristic pleomorphic rod morphology, and strain PG-02^T cells lack the polar lipid phosphatidylethanolamine (Table 2). One unusual chemotaxonomic characteristic of strain PG-02^T that differentiates it from members of the genera *Luteococcus*, *Friedmanniella*, *Tessaracoccus*, *Propioniferax*, *Micropruina* and *Microlunatus* is the presence of i-C_{15:0} DMA. 1,1-Dimethyl acetals have been detected in actinobacterial facultative anaerobes such as *Propionibacterium freudenreichii* and *Propionibacterium jensenii* within the family *Propionibacteriaceae* (Kämpfer *et al.*, 2000) and also in the aerobic psychrophiles of the genera *Frigoribacterium* and *Subtercola* in the family *Microbacteriaceae* (Kämpfer

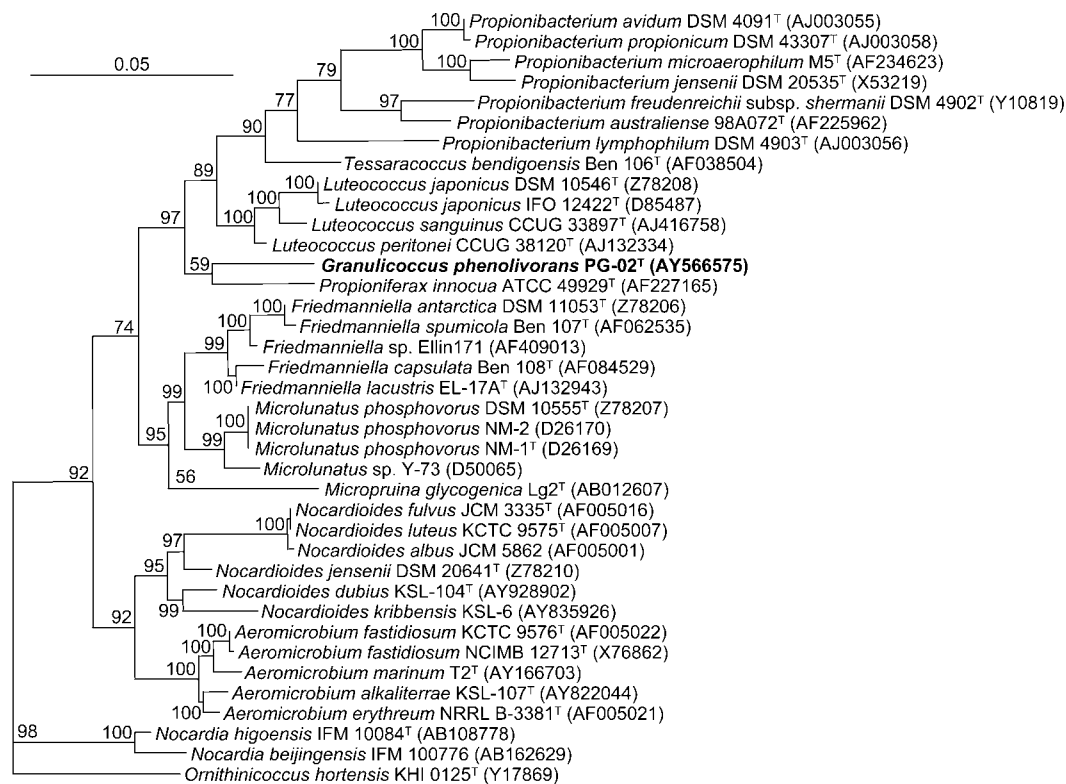


Fig. 1. Phylogenetic tree based on analysis of 16S rRNA gene sequences of strain PG-02^T and representatives of the Actinobacteria, constructed from evolutionary distances using the FITCH program. All sequences used in the analysis were obtained from GenBank. Bootstrap values from 100 replications are shown at branching points. Bar, 5 substitutions per 100 nucleotides.

et al., 2000; Männistö *et al.*, 2000). However, in PG-02^T, i-C_{15:0} DMA made up 37.4% of the total cellular fatty acids, a much higher level than has been detected in other organisms. An increase in the i-C_{15:0} DMA concentration was noted in *Subtercola boreus* and *Subtercola frigoramans* when the growth temperature was lowered from 25 to 4 °C (Männistö *et al.*, 2000). 1,1-Dimethyl acetals are derived from methanolysis of plasmalogens (alk-1'-enyl glyceryl ethers), which are found in the obligate anaerobes *Eubacterium lentum* and *Megasphaera elsdenii* and obligately anaerobic members of the *Clostridia* and the genera *Fusobacterium*, *Propionibacterium*, *Subtercola* and *Frigoribacterium* (Jantzen & Hofstad, 1981; Johnston & Goldfine, 1994; Kaufman *et al.*, 1990; Verhulst *et al.*, 1987; Kämpfer *et al.*, 2000; Männistö *et al.*, 2000). Kaufman *et al.* (1990) suggested that 1,1-dimethyl acetal composition may affect cell membrane fluidity in *Megasphaera elsdenii*.

Based on the presence of i-C_{15:0} DMA in unusually large amounts, together with the other chemotaxonomic properties, phenotypic features and the 16S rRNA gene sequence data presented here, we propose that strain PG-02^T should be classified in a novel genus and species as *Granulicoccus phenolivorans* gen. nov., sp. nov. within the family *Propionibacteriaceae*.

Description of *Granulicoccus* gen. nov.

Granulicoccus [Gra.nu.li.coc'cus. L. neut. n. *granulum* a small grain; L. masc. n. *coccus* a berry; N.L. masc. n. *Granulicoccus* a coccus from (sludge) granules].

Gram-positive, non-spore-forming cocci, 0.3–1.4 µm in diameter (Fig. 2). Contain type A3γ peptidoglycan (LL-A₂pm←Gly with alanine at position 1 of the peptide subunit). Menaquinone MK-9(H₄) is the major isoprenoid quinone. Polar lipids include diphosphatidylglycerol and phosphatidylglycerol. 13-Methyltetradecanoic acid and 1,1-dimethoxy-iso-pentadecane are the major components in whole-cell methanolysates. The genus is a member of the family *Propionibacteriaceae*. The type species is *Granulicoccus phenolivorans*.

Description of *Granulicoccus phenolivorans* sp. nov.

Granulicoccus phenolivorans (phe.no.li.vo'rans. N.L. neut. n. *phenolum* phenol; L. part. adj. *vorans* devouring, consuming; N.L. part. adj. *phenolivorans* consuming phenol).

In addition to the characteristics described for the genus, results obtained with the Biolog GN and GP systems and

Table 2. Comparative phenotypic characters of strain PG-02^T and related members of the suborder *Propionibacterineae*

Data for reference taxa were obtained from Charfreitag *et al.* (1988) (*Propionibacterium*), Tamura *et al.* (1994) (*Luteococcus*), Schumann *et al.* (1997) and Maszenan *et al.* (1999b) (*Friedmanniella*), Nakamura *et al.* (1995) (*Micrococcus*), Maszenan *et al.* (1999a) (*Tessaracoccus*), Shintani *et al.* (2000) (*Micropruina*) and Pitcher & Collins (1991) and Yokota *et al.* (1994) (*Propioniferax*). All isolates are Gram-positive. +, Positive; -, negative; ND, no data available.

Character	<i>Propionibacterium</i>	<i>Luteococcus</i>	<i>Friedmanniella</i>	<i>Micrococcus</i>	<i>Tessaracoccus</i>	<i>Micropruina</i>	<i>Propioniferax</i>	Strain PG-02 ^T
O ₂ requirement	Facultative anaerobes	Facultative anaerobes	Aerobes	Aerobes	Facultative anaerobes	Aerobic chemoorganotrophs	Facultative anaerobes	Facultative anaerobe
Cell morphology (size, in µm)	Pleomorphic rods (0.2–0.8)	Cocci, in pairs and tetrads (0.7–1.0)	Cocci, in packets (0.5–2.2)	Cocci, single and in pairs (0.8–2.0)	Cocci, in tetrads (0.5–1.1)	Cocci, single or pairs and in packets (0.5–2.2)	Pleomorphic rods (0.5–1.2)	Cocci, single and in pairs (0.3–1.4)
Isolation source(s)	Human oral cavity, cervicovaginal secretion	Soil, water	Antarctic sandstone, sewage treatment plant	Sewage treatment plant	Sewage treatment plant	Laboratory sequencing batch reactor	Human epidermal surface	Phenol-degrading aerobic granules
Growth temperature (°C)								
Optimum	35–37	26–28	20–25	25–30	25	30	37	30
Range	30–37	12–38	9–37	5–35	20–37	20–35	10–40	15–37
pH for growth								
Optimum	ND	ND	6.0–7.5	7.0	7.5	7.0	7.0	7.0
Range	ND	ND	5.1–8.7	5.0–9.0	5.5–9.3	6.0–8.0	ND	5.0–8.5
Presence of metachromatic granules*	ND	ND	+, PolyP	+, PolyP	+, PolyP	+, G	+, NK	+, PolyP
Oxidase	ND	+	–	+ (weak)	–	+	+	–
Catalase	–	+	+	+	+	+	+	+
Production of indole	–	–	–	+	–	ND	–	–
Production of H ₂ S	+	–	+	ND	–	ND	–	–
Major menaquinone(s)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄), MK-9(H ₂), MK-7(H ₄), MK-7(H ₂)	MK-9(H ₄)	MK-9(H ₄), MK-7(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄), MK-8(H ₄)
A ₂ pm/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'	meso-A ₂ pm	LL-A ₂ pm/A3-γ	LL-A ₂ pm/A3-γ
Polar lipids†	ND	PG, DPG, PI, GL	PG, DPG, PI, PL, GL‡	PG, DPG, PI, PL	PG, DPG, PI, PL	ND	PE, PG, PL, GL	PG, DPG
Urease	–	–	+	+	–	ND	+	+
Nitrate reduction	+	–	–	+	+	+	+	–
DNA G + C content (mol%)	63–65	66–68	69–74	68	74	71	59–63	69

*G, Glycogen; PolyP, polyphosphate; NK, type of granules not known.

†DPG, Diphosphatidylglycerol; GL, unidentified glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid.

‡Present in *F. spumicola* and *F. capsulata*.

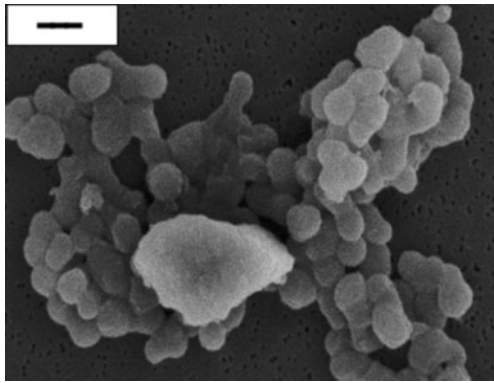


Fig. 2. Scanning electron micrograph of strain PG-02^T showing cocci in pairs and as single cells. Bar, 1 μ m.

API 20E system show that the type strain can utilize the following components: Tweens 40 and 80, L-arabinose, α -D-glucose, α -D-lactose, lactulose, maltose, maltotriose, D-mannose, D-melezitose, D-melibiose, methyl α -D-galactoside, methyl β -D-galactoside, 3-methyl glucose, methyl α -D-glucoside, methyl β -D-glucoside, psicose, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, stachyose, sucrose, D-tagatose, D-trehalose, turanose, D-xylose, *myo*-inositol, D-mannitol, D-sorbitol, xylitol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, adenosine, AMP, TMP, UMP and fructose 6-phosphate. Acids and their derivatives utilized by the type strain include methyl pyruvate, monomethyl succinate, acetic acid, citric acid, D-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, α -, β - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, lactamide, D-lactic acid methyl ester, L- and DL-lactic acid, D- and L-malic acid, propionic acid, pyruvic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, *N*-acetylglutamic acid, L-glutamic acid, glycyl L-glutamic acid and L-pyrroglutamic acid. The type strain can consume amino compounds including glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-phenylalanine, L-proline, L-serine, inosine, uridine, thymidine and putrescine. Gentiobiose is utilized weakly. α -Cyclodextrin, β -cyclodextrin, dextrin, glycogen, inulin, mannan, amygdalin, adonitol, D-arabitol, arbutin, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, 2-aminoethanol, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetylmannosamine, phenyl ethylamine, deoxyadenosine, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, D-serine, L-threonine, DL-carnitine, D-galacturonic acid, formic acid, D-glucosaminic acid, malonic acid, L-aspartic acid, γ -aminobutyric acid and urocanic acid are not utilized. Enzyme activities detected by both the API ZYM and API 20E systems are alkaline phosphatase, esterase, lipase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase and β -glucosidase. Activities of the

following enzymes are not detected with API ZYM: acid phosphatase, esterase lipase, cystine arylamidase, trypsin, chymotrypsin, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Activities of β -galactosidase, urease and gelatinase are detected with API 20E. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are not detected. H₂S and indole are not produced. Voges-Proskauer-negative and does not produce acetoin or reduce nitrate to nitrite. Catalase-positive and oxidase-negative. The genomic G+C content of the type strain is 69 mol%.

The type strain, PG-02^T (= ATCC BAA-1292^T = DSM 17626^T), was isolated from phenol-degrading aerobic granules.

Acknowledgements

We thank Professor Dr Hans Trüper for his assistance in naming the organism.

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