Quadrisphaera granulorum gen. nov., sp. nov., a Gram-positive polyphosphate-accumulating coccus in tetrads or aggregates isolated from aerobic granules

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A Gram-positive bacterium, designated strain AG019T, was isolated by micromanipulation from aerobic granules obtained from a laboratory-scale sequencing batch reactor. This isolate grew axenically as cocci clustered predominantly in tetrads, and was morphologically similar to the dominant organisms observed in the biomass. The morphology also resembled that of the tetrad-forming organisms commonly seen in activated sludge samples. Strain AG019T was found to be an oxidase-negative, catalase-positive, non-motile aerobe that does not reduce nitrate and grows at temperatures between 15 and 40 °C, with an optimum at 37 °C. The pH range for growth was 5–9, with an optimum at pH 7–5. Strain AG019T contained a peptidoglycan with directly cross-linked meso-diaminopimelic acid (type A1c) and lacked mycolic acids. The G+C content of the DNA was 75 mol%. Menaquinone MK-8(H2) was the major isoprenoid quinone. The bacterium stained positively for intracellular polyphosphate granules but not for poly-β-hydroxyalkanoates. It produced capsular material and showed autoaggregation ability. Phenotypic and 16S rRNA gene analyses showed that the bacterium differed sufficiently from its closest phylogenetic relatives, namely members of the suborder Frankineae, which includes the genera Geodermatophilus, Blastococcus, Frankia, Sporichthya, Acidothermus and Microsphaera, that it is proposed that it be placed in a novel genus, Quadrisphaera, as Quadrisphaera granulorum gen. nov., sp. nov. The type strain is AG019T (= ATCC BAA-1104T = DSM 44889T).

The occurrence of coccoid bacteria with a distinctive tetrad morphology first surfaced in activated sludge systems in the early 1990s (Cech & Hartman, 1990). These were Gram-negative cocci and were commonly seen in reactors fed with an acetate/glucose mixture. Since then, tetrad-forming organisms (TFOs) with a similar morphological description have been reported in other activated sludge systems. These organisms belong to several novel genera that span both the Gram-positive and Gram-negative lineages (Maszenan, 2000; Seviour et al., 2000; Tsai & Liu, 2002). TFOs have also been detected during routine microscopic examination of aerobically grown microbial granules cultivated in a laboratory-scale sequencing batch reactor (Dulekgurgen et al., 2003). Aerobic granules are a recent innovation in biological wastewater treatment and are self-immobilized aggregates of bacteria with a strong and compact structure, good settling ability, high biomass retention and an ability to handle high organic loading rates (Moy et al., 2002). The granules are also metabolically versatile, have been cultivated on different biodegradable organic substrates (Jiang et al., 2002, 2004; Moy et al., 2002; Pan et al., 2004; Tay et al., 2001) and are capable of nitrification, denitrification and polyphosphate accumulation (Dulekgurgen et al., 2003; Jang et al., 2003; Lin et al., 2003; Tay et al., 2002; Zeng et al., 2003).

This paper describes the characterization of a Gram-positive TFO, designated strain AG019T, isolated from aerobic granules. The results of phenotypic and phylogenetic...
analyses support the classification of strain AG019T as a novel genus within the family Frankiaceae.

The aerobic granules were cultivated in a laboratory-scale sequencing batch reactor as described previously, with synthetic wastewater containing acetate as the sole carbon source (Moy et al., 2002). Granule samples were harvested 4 weeks after reactor startup, and were disrupted at 2500 r.p.m. for 3 min with a Mini Beadbeater (Biospec Products). Microscopic observations (BX60 apparatus; Olympus) of these granules revealed that the microbial community was dominated by coccolid cells that clustered mostly in tetrads. These coccolid cells were retrieved from the disintegrated biomass using a Skerman micromanipulator (Skerman, 1968) and transferred to GS agar (Williams & Unz, 1985) plates for incubation at 25 °C (Williams & Unz, 1985). Colonies arising from micromanipulated cells were transferred several times to GS agar plates to obtain pure cultures; culture purity was confirmed microscopically by examining cells from single colonies. An axenic culture of strain AG019T was preserved at −80 °C in GS medium (Williams & Unz, 1985) containing 20 % glycerol.

Strain AG019T possessed the distinctive morphology of TFOs, i.e. cocci arranged in tetrads or clusters (Fig. 1). Strain AG019T was slow-growing and took 7 days to appear as visible colonies on a GS agar plate; it was probably an aerobe, as no growth occurred down the line of inoculation as visible colonies on a GS agar plate; it was probably an aerobe, as no growth occurred down the line of inoculation. The aerobic granules were cultivated in a laboratory-scale sequencing batch reactor as described previously, with synthetic wastewater containing acetate as the sole carbon source, but polyhydroxyalkanoate granules were not detected when cells were grown anaerobically using the dual staining method of Rees et al. (1992). No endospores were detected. Capsular materials were observed with the Indian ink stain (Difco).

A flocculation assay was performed on strain AG019T, as described previously (Malik et al., 2003). Strain AG019T was inoculated into 200 ml GS medium, then incubated at 20 °C with shaking at 100 r.p.m. The optical density (OD) of the mixed suspension was measured at a wavelength of 600 nm as initial OD (OD\text{initial}). The cell suspension was allowed to stand at 20 °C, then the supernatant layer was carefully pipetted at different standing times for OD measurement. The aggregation index was calculated on the basis of the OD value (OD) at a predefined standing time (t), as follows:

\[
\text{Aggregation index (\%)} = \frac{\text{OD}_t - \text{OD}_\text{initial}}{\text{OD}_\text{initial}} \times 100
\]

Flocculation-assay results showed that strain AG019T had high autoaggregation ability. The aggregation index reached 25 % after a standing time of between 150 and 210 min.

The physiological and biochemical characteristics of strain AG019T are presented in the descriptions of the genus and species. Enzyme profiles and biochemical characteristics of strain AG019T were determined using the API ZYM and API 20E systems according to the manufacturer’s instructions (bioMérieux). Carbon-substrate utilization tests were performed with the Biolog GN and GP systems (Special Diagnostics). The cells were catalase-positive but oxidase- and urease-negative, as determined by the method of Smibert & Krieg (1994). The G+C content of the genomic DNA was 75 mol%, as determined by using the reverse HPLC method (Schumann et al., 1997).

The peptidoglycan, menaquinone, cellular fatty acid and polar lipid compositions were analysed as described by Schumann et al. (1997). Strain AG019T possessed type A1:\gamma peptidoglycan with meso-diaminopimelic acid as the diagnostic diamino acid (Schleifer & Kandler, 1972). The cells lacked mycolic acid, but contained four isoprenoid quinones, MK-8(H\text{2}), MK-7(H\text{2}), MK-6(H\text{2}) and MK-9(H\text{2}), in the ratio 88·3:2:1. The polar lipids present included diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol; the cellular fatty acid profiles of strain AG019T were dominated by 12-methyl tetradecanoic acid and hexadecanoic acid (Table 1).

The almost-complete 16S rRNA gene of strain AG019T was amplified and sequenced using methods described previously (Maszenan et al., 1997). A sequence of 1403 nucleotide bases was obtained in both directions, corresponding to positions 20–1471 of Escherichia coli according to the nomenclature of Winker & Woese (1991), and was manually aligned against reference sequences of close relatives by

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**Fig. 1.** Scanning electron micrograph of strain AG019T showing cocci in tetrad and cluster arrangement. Bar, 3 μm.
using the alignment editor in BioEdit (Hall, 1997). Sequence analysis was performed using BLAST (Altschul et al., 1997), CLUSTAL W (Thompson et al., 1994), 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 that contained an unambiguous alignment of 1314 bases of strain AG019T and its closest relatives. A phylogenetic tree was constructed from evolutionary distances by using the FITCH program in PHYLIP (Felsenstein, 1985). Bootstrap confidence values were obtained with 100 resamplings.

To our knowledge, this is the first study to use micro-manipulation on aerobic granules for isolating TFOs. Conventional isolation techniques such as serial dilution and the spread-plate method have a low success rate for the isolation of TFOs because these micro-organisms grow slowly, and fast-growing bacterial cells often outgrow them (Maszenan, 2000; Seviour et al., 2000). Previous studies of TFOs in activated sludge systems have resulted in the isolation of several novel genera of the Actinobacteria, some of which are thought to play a major role in biological phosphorus removal in activated sludge plants (Christensson et al., 1998; Seviour et al., 2000). Strain AG019T stained positively for polyphosphate when grown under aerobic conditions using either glucose or acetate as the sole carbon source, but no polyhydroxyalkanoate accumulation was observed under anaerobic conditions with acetate. Flocculation studies also show that strain AG019T can autoaggregate with an aggregation index of 25% after 120 min. The ability both to autoaggregate and synthesize capsular material might confer on strain AG019T a selective advantage over other bacteria in aerobic granular sludge systems in allowing them to overcome cell washout and thus remain in the reactor, and also to avoid predation from bacteria scavengers such as ciliates and protozoa (Cech et al., 1994; Seviour et al., 2000).

Analysis of the 16S rRNA gene from strain AG019T revealed that it is a member of the Gram-positive bacteria in the high-G+C-content group Actinobacteria classis nov. in the domain Bacteria (Stackebrandt et al., 1997). Pairwise comparison of 16S rRNA gene sequences revealed that strain AG019T was 95% similar to Kineococcus-like bacteria and Sporichthya, 93% similar to the type strain of Kineococcus aurantiacus, less than 94% similar to members of the genera Frankia and Blastococcus, and 93% similar to members of the genera Acidothermus, Geodermatophilus and Microsphaera, as shown in Fig. 2. Strain AG019T is related to members of the genus Microsphaera (Yoshimi et al., 1996), which belongs to the suborder Frankineae. However, strain AG019T is different from the other TFOs in the

<table>
<thead>
<tr>
<th>Cellular fatty acid composition</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetradecanoic acid (C14:0)</td>
<td>3.2</td>
</tr>
<tr>
<td>13-Methyl tetradecanoic acid (i-C15:0)</td>
<td>4.0</td>
</tr>
<tr>
<td>12-Methyl tetradecanoic acid (a-C15:0)</td>
<td>56.7</td>
</tr>
<tr>
<td>Pentadecanoic acid (C15:0)</td>
<td>3.6</td>
</tr>
<tr>
<td>14-Methyl pentadecanoic acid (i-C16:0)</td>
<td>4.1</td>
</tr>
<tr>
<td>Hexadecanoic acid (C16:0)</td>
<td>14.9</td>
</tr>
<tr>
<td>15-Methyl hexadecanoic acid (i-C17:0)</td>
<td>2.2</td>
</tr>
<tr>
<td>14-Methyl hexadecanoic acid (a-C17:0)</td>
<td>4.1</td>
</tr>
<tr>
<td>cis-Octadecanoic acid (C18:1)</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Fig. 2. Phylogenetic tree based on analysis of 16S rRNA gene sequences of strain AG019T and representatives of the Actinobacteria. All sequences used in the analysis were obtained from GenBank. Bootstrap values, expressed as a percentage of 100 replications, are shown at the branching points. Bar, 10 substitutions per 100 nt.
**Actinobacteria** group, which includes the genera *Tessaracoccus* (Maszenan et al., 1999a), *Friedmanniella* (Maszenan et al., 1999b) and *Tetrasphaera* (Maszenan et al., 2000).

Using the taxonomic scheme of Stackebrandt et al. (1997), it is clear that strain AG019T fits readily within the suborder *Frankineae*, except that A–T is found instead of G–C at nucleotide positions 127–234, and that A–T is present instead of G–C at positions 141–222 (see Table S1 available as supplementary data in IJSEM Online). The most closely related genera show complete concurrence with the scheme of Stackebrandt et al. (1997) for the suborder *Frankineae*. On the other hand, the 16S rRNA structure of strain AG019T also contains several nucleotide pairs that are different from those of members of the families *Frankiaceae*, *Geodermatophilaceae*, *Microsphaeraceae*, *Sporichthyaceae* and *Acidothermaceae* (see Table S2 available as supplementary data in IJSEM Online), lending support to the notion that strain AG019T belongs to a novel genus in the suborder *Frankineae*.

Strain AG019T differs from the cells of the genus *Frankia*, which occur as chains of cocci, while *Geodermatophilus* and *Blastococcus* are pleomorphic and occur as rods and cocci. *Sporichthya* and *Microsphaera*, in the suborder *Frankineae*, are cocci. However, the cell-wall peptidoglycan of *Sporichthya* contains L- diaminopimelic acid and the menaquinones MK-9(H2) and MK-9(H3) (Rainey et al., 1993), while strain AG019T possesses meso-diaminopimelic acid and MK-8(H2) as its dominant menaquinones. Although strain AG019T, members of the genus *Kineococcus* (Yokota et al., 1993; Phillips et al., 2002) and members of the genus *Microsphaera* (Yoshimi et al., 1996) contain meso-diaminopimelic acid in their cell-wall peptidoglycan, strain AG019T has a major menaquinone profile that is different from that of *Microsphaera* and *Kineococcus*. Members of the genera *Microsphaera* and *Kineococcus* possess MK-8(H4) and MK-9(H4), respectively, whereas MK-8(H3) is the predominant quinone in strain AG019T.

Pairwise comparison of 16S RNA gene sequences revealed that strain AG019T was 95% similar to *Kineococcus*-like bacteria and *Sporichthya*, 93% similar to the type strain of *Kineococcus aurantiacus*, less than 94% similar to members of the genera *Frankia* and *Blastococcus*, and 93% similar to members of the genera *Acidothermus*, *Geodermatophilus* and *Microsphaera* (Fig. 2). These results, taken together with the phylogenetic analysis described earlier, show that strain AG019T is different from members of the genera *Frankia*, *Blastococcus*, *Microsphaera*, *Acidothermus*, *Sporichthya* and *Geodermatophilus* (Table 2).

Strain AG019T is only 97.2% similar to its closest phylogenetic relative, strain G10, which originated from Namibia and which had been previously reported to be equidistantly related to *Geodermatophilus obscurus*, *Frankia* and *Acidothermus cellulolyticus* (Eppard et al., 1996). In this study, strains AG019T and G10 form a coherent group. However, on the basis of chemotaxonomic properties such as the presence of a greenish to dark pigment, faint glossy colonies and a reproduction mechanism involving binary fission and budding, as described by Eppard et al. (1996), strain G10 appears to be more closely related to the genus *Geodermatophilus* than to strain AG019T. Thus the phylogenetic evidence and chemotaxonomic properties presented so far support the placement of strain AG019T in a novel genus, for which we propose the name *Quadrisphaera*, with *Quadrisphaera granulorum* as the type species.

**Description of Quadrisphaera gen. nov.**

*Quadrisphaera* (qua dri sphe’ra. L. pref. numer. adj. quadr- four; L. fem. n. sphera a ball, globe, sphere; N.L. fem. n. *Quadrisphaera* fourfold balls, coccus in tetrad).

Gram-positive, non-spore-forming cocci, 1·2–3·0 μm in diameter, occurring in tetrad arrangement, fitting the morphological description of TFOs. MK-8(H2) is the predominant menaquinone. The phylogenetic position is in the family *Frankiaceae*.

The type species is *Quadrisphaera granulorum*.

**Description of Quadrisphaera granulorum sp. nov.**

*Quadrisphaera granulorum* (gra nu lo’rum. L. gen. pl. neut. n. *granulum* from, or of, granules).

Results obtained with the Biolog GN and GP systems and the API 20E system showed that strain AG019T has the following characteristics (in addition to those described in the genus description). Utilizes α-cyclodextrin, α-DL-glycerol phosphate, Tween 40, arbutin, glucose 1-phosphate, Tween 80, glucose 6-phosphate, adonitol, L-arabinose, D-arabitol, glucuronamide, cellobiose, D-psicose, D-mannitol, D-melezitose, D-melibiose, L-serine, methyl β-D-glucoside, psicose, D-xyllose, methyl pyruvate, pyruvate, 2-aminoethanol, mono-methyl succinate, glycerol, L-serine, turanose and glycerol. Strain AG019T cannot metabolize the following: β-cyclodextrin, dextrin, glycogen, inulin, mannann, N-acetyl D-galactosamine, N-acetylgalactosamine, N-acetylmannosamine, amygdalin, D-arabitol, cellobiose, L-erythritol, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-glucuronic acid, α-D-glucose, m-inositol, α-D-lactulose, α-D-lactose, maltose, D-mannitol, D-mannose, D-melezitose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, palatinose, D-raffinose, L-rhamnose, salicin, sedoheptulose, D-sorbitol, stachyose, sucrose, D-trehalose, xylitol, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylactic acid, α-ketoglutaric acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, itaconic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, lactamide, D-lactic acid methyl ester, D-malic acid, L-malic acid, propionic acid, succinic acid, succinic...
Table 2. Comparative phenotypic characteristics of strain AG019<sup>T</sup> and members of the suborder Frankineae

All isolates are Gram-positive. Symbols: +, positive test result; −, negative test result; +/-, variable test result; ND, not determined.

<table>
<thead>
<tr>
<th>Phenotypic characteristic</th>
<th><em>Frankia</em>&lt;sup&gt;*&lt;/sup&gt;</th>
<th><em>Geodermatophilus</em>&lt;sup&gt;*&lt;/sup&gt;</th>
<th><em>Blastococcus</em>&lt;sup&gt;*&lt;/sup&gt;</th>
<th><em>Microsphaera</em>&lt;sup&gt;†&lt;/sup&gt;</th>
<th><em>Sporichthya</em>&lt;sup&gt;‡&lt;/sup&gt;</th>
<th><em>Acidothermus</em>&lt;sup&gt;§&lt;/sup&gt;</th>
<th>Strain AG019&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O&lt;sub&gt;2&lt;/sub&gt; requirement</strong></td>
<td>Aerobic to microaerophilic</td>
<td>Aerobic</td>
<td>Aerobic to microaerophilic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td><strong>Cell morphology</strong></td>
<td>Actinomycetes producing septate and filamentous mycelium</td>
<td>Motile rods, irregularly shaped aggregates of coccolid cells</td>
<td>Motile rods, irregularly shaped aggregates of coccolid cells</td>
<td>Cocci</td>
<td>Aggregate of coccolid cells in tetrad arrangements</td>
<td>Slender rods and floccules</td>
<td>Aggregates of coccolid cells in tetrad arrangements</td>
</tr>
<tr>
<td><strong>Motility</strong>&lt;sup&gt;</td>
<td></td>
<td>&lt;/sup&gt;</td>
<td>−, non-motile</td>
<td>+, motile</td>
<td>−, non-motile</td>
<td>−, non-motile</td>
<td>+/−</td>
</tr>
<tr>
<td><strong>Habitat</strong></td>
<td>Soil, plant roots</td>
<td>Desert soil, sea</td>
<td>Sea</td>
<td>Sugar waste activated sludge</td>
<td>Soil</td>
<td>Sugar waste activated sludge</td>
<td>Soil</td>
</tr>
<tr>
<td><strong>Optimum growth temperature (°C)</strong></td>
<td>20–36</td>
<td>26</td>
<td>ND</td>
<td>25</td>
<td>20–30</td>
<td>50–60</td>
<td>37</td>
</tr>
<tr>
<td><strong>Optimum growth pH</strong></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7-0</td>
<td>ND</td>
<td>5-0</td>
<td>7-5</td>
</tr>
<tr>
<td><strong>Growth temperature range (°C)</strong></td>
<td>10–37</td>
<td>10–37</td>
<td>ND</td>
<td>10–35</td>
<td>15–37</td>
<td>37-70</td>
<td>15–40</td>
</tr>
<tr>
<td><strong>Growth pH range</strong></td>
<td>6-4–6-8</td>
<td>ND</td>
<td>ND</td>
<td>5-0–9-0</td>
<td>ND</td>
<td>3-5–7-0</td>
<td>5-0–9-0</td>
</tr>
<tr>
<td><strong>Oxidase</strong></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Cell-wall type</strong></td>
<td>meso-Diaminopimelic acid (type III);&lt;sup&gt;¶&lt;/sup&gt; lacks mycolic acid</td>
<td>meso-Diaminopimelic acid (type III); lacks mycolic acid</td>
<td>ND</td>
<td>meso-Diaminopimelic acid</td>
<td>LL-Diaminopimelic acid</td>
<td>meso-Diaminopimelic acid</td>
<td>meso-Diaminopimelic acid</td>
</tr>
<tr>
<td><strong>Major menaquinone</strong></td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>ND</td>
<td>MK-8(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;), MK-9(H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>ND</td>
<td>MK-8(H&lt;sub&gt;2&lt;/sub&gt;)</td>
</tr>
<tr>
<td><strong>Polar lipid(s)</strong>&lt;sup&gt;#&lt;/sup&gt;</td>
<td>PIM, PI, DPG</td>
<td>PII</td>
<td>ND</td>
<td>PI, PG, DPG</td>
<td>ND</td>
<td>DPG, PG, PI</td>
<td></td>
</tr>
<tr>
<td><strong>Nitrate reduction</strong></td>
<td>ND</td>
<td>+/−</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>66–71</td>
<td>73–75</td>
<td>70–75</td>
<td>68</td>
<td>70–71</td>
<td>61</td>
<td>75</td>
</tr>
</tbody>
</table>

*Normand et al. (1996).
†Yoshimi et al. (1996).
‡Rainey et al. (1993), Tamura et al. (1999).
§Mohagheghi et al. (1986).
||Note: *Sporichthya brevicatena* spores exhibit motility when suspended in sterile distilled water.
¶Type III cell walls contain *meso*-diaminopimelic acid, which contains glutamic acid, alanine, glucosamine and muramic acid.
#Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PII, phosphatidylethanolamine and/or phosphatidylmethanolamine; PIM, phosphatidylinositol mannosides.
acid, N-acetylglutamic acid, bromosuccinic acid, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, glycoll-1-glutamic acid, L-propylglutamic acid, putrescine, 2,3-butanediol, glycoll-1-asparatic acid, L-histidine, hydroxyl-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-propylglutamic acid, D-serine, L-threonine, DL-carntline, γ-aminobutyric acid, adenosine, 2'-deoxyadenosine, inosine, thymidine, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, uridine 5'-monophosphate, phenyl ethlyamine or putrescine. The following acid and acid derivatives are utilized by strain AG019T: glucuronic acid, α-ketobutyric acid, α-ketovaleric acid, DL-lactic acid, L-aspartic acid, L-glutamic acid, uroconic acid and pyruvic acid. The enzyme activities detected by both API ZYM and API 20E are as follows: esterase, esterase lipase, leucine arylamidase, valine arylamidase, naphthol-AS-Bl phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase. Activities of the following enzymes are not detected by API ZYM: alkaline phosphatase, lipase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Activities of the following enzymes are not detected by API 20E: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase. Strain AG019T does not produce H2S or indole. It is Voges-Proskauer-negative, does not produce acetoin and does not reduce nitrate to nitrite. It is Voges-Proskauer-negative, does not produce acetoin and does not reduce nitrate to nitrite. It is catalase-positive but oxidase-negative. The genomic G+C content is 75 mol%.

The type strain, AG019T (= ATCC BAA-1104T = DSM 44889T), was isolated from aerobic granules.

Acknowledgements

We thank Professor H. Truper for his assistance in naming the organism.

References


