Polymorphobacter multimanifer gen. nov., sp. nov., a polymorphic bacterium isolated from antarctic white rock

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A Gram-stain-negative, non-spor-forming, aerobic, oligotrophic bacterium (strain 262-7T) was isolated from a crack of white rock collected in the Skallen region of Antarctica. Strain 262-7T grew at temperatures between −4 and 30 °C, with optimal growth at 25 °C. The pH range for growth was between pH 6.0 and 9.0, with optimal growth at approximately pH 7.0. The NaCl concentration range allowing growth was between 0.0 and 1.0 %, with an optimum of 0.5 %. Strain 262-7T showed an unprecedented range of morphological diversity in response to growth conditions. Cells grown in liquid medium were circular or ovoid with smooth surfaces in the lag phase. In the exponential phase, ovoid cells with short projections were observed. Cells in the stationary phase possessed long tentacle-like projections intertwined intricately. By contrast, cells grown on agar plate medium or in liquid media containing organic compounds at low concentration exhibited short- and long-rod-shaped morphology. These projections and morphological variations clearly differ from those of previously described bacteria. Ubiquinone 10 was the major respiratory quinone. The major fatty acids were C₁₇:₁ω₆c (28.2 %), C₁₆:₁ω₇c (22.6 %), C₁₈:₁ω₇c (12.9 %) and C₁₅:₀-2-OH (12.3 %). The G+C content of genomic DNA was 68.0 mol%. Carotenoids were detected from the cells. Comparative analyses of 16S rRNA gene sequences indicated that strain 262-7T belongs to the family Sphingomonadaceae, and that 262-7T should be distinguished from known genera in the family Sphingomonadaceae. According to the phylogenetic position, physiological characteristics and unique morphology variations, strain 262-7T should be classified as a representative of a novel genus in the family Sphingomonadaceae. Here, a novel genus and species with the name Polymorphobacter multimanifer gen. nov., sp. nov. is proposed (type strain 262-7T = JCM 18140T = ATCC BAA-2413T). The novel species was named after its morphological diversity and formation of unique projections.

The climate in Antarctica, with its extreme cold, dryness, strong winds, seasonally strong UV radiation and low concentration of organic materials, makes it difficult for most organisms to sustain life. However, a great diversity of microorganisms has been found in icy environments, such as permafrost, polar oceans, snow, lake ice, sea ice and cryoconite holes (Karr et al., 2006; Prisco, 2007; Priscu et al., 1998; Rothschild & Mancinelli, 2001; Tindall, 2004). In addition, the presence of chasmolithic, hypolithic and endolithic communities have been reported (Cary et al., 2010). The rocks of Antarctica are considered to provide physical stability and to protect microbial communities from UV radiation and dryness. A white rock sample was collected by the summer party of the 46th Japanese Antarctic Research Expedition in 2004–2005 from the Skallen region in Antarctica (Yamada et al., 2011). Interestingly, the interior of the white rock was colourful (green, pink, yellow and...
revealed that strain 262-7 T was a Gram-stain-negative, (doubling time, 5.2 h). When strain 262-7T was grown OD660 reached approximately 0.9 after 70 h of incubation. Analyses of Gram-staining, catalase and oxidase activity, motility and spore formation were performed by methods described previously (Yamada, et al., 2011).

Strain 262-7T could grow well in 0.25 × LB medium (l⁻¹: 2.5 g tryptone, 1.3 g yeast extract and 5.0 g NaCl in tap water) under aerobic conditions. However, no growth was observed in 0.75 × LB medium containing (l⁻¹) 7.5 g tryptone and 3.8 g yeast extract. These results indicated that strain 262-7T is an oligotroph. Strain 262-7T could also grow in 0.25 × LB/ASW medium (l⁻¹: 2.5 g tryptone, 1.3 g yeast extract, 5.0 g NaCl, 0.75 g MgCl₂·6H₂O, 1.5 g MgSO₄·7H₂O, 0.25 g (NH₄)₂SO₄, 0.050 g NaHCO₃, 0.075 g CaCl₂·2H₂O, 0.13 g KCl, 0.11 g KH₂PO₄, 0.013 g NaBr, 0.005 g SrCl₂·6H₂O, 0.025 g ferric ammonium citrate) and quarter strength Difco Marine Broth 2216 medium (BD Difco). Strain 262-7T grew at −4–30 °C with an optimum growth temperature of 25 °C in 0.25 × LB medium, while no growth was observed at temperatures above 37 °C. When strain 262-7T grew on 0.25 × LB plate medium containing 20 g agar l⁻¹ at −4 °C in a freezing chamber, colony formation was observed. The pH range for growth was pH 6.0–9.0, with an optimum of approximately pH 7.0. Strain 262-7T was able to grow at NaCl concentrations of 0.0–1.0% (w/v), with an optimum of 0.5% (w/v). When strain 262-7T was cultivated in 0.25 × LB medium under optimal growth conditions, the maximum OD₆₆₀ reached approximately 0.9 after 70 h of incubation (doubling time, 5.2 h). When strain 262-7T was grown on 0.25 × LB plate medium, brown, raised and circular colonies were formed with an entire margin. Strain 262-7T could not grow in 0.25 × LB medium containing 5.0 g l⁻¹ of either Na₂SO₄ or NaNO₃ under anaerobic conditions. Analyses of Gram-staining, catalase and oxidase activity, motility and spore formation were performed by methods described previously (Yamada, et al., 2011). The results revealed that strain 262-7T was a Gram-stain-negative, catalase-positive, oxidase-positive, non-spore-forming and motile bacterium. Further biochemical characteristics were analysed by using API 20NE and API ZYM kits (bioMérieux) and antibiotic sensivities were examined by using an ATB VET kit (bioMérieux); results are given in the species description.

The morphology of the cells cultivated in liquid medium was examined by a bright-field microscope (BX51; Olympus). Cells in the logarithmic and stationary phases of growth were examined, and circular and ovoid cells were observed (data not shown). The ultrastructures of cells were examined by scanning electron microscopy (S-4700; Hitachi), and it became clear that strain 262-7T showed morphology variations depending on growth phase and growth medium. When cells in the lag phase were observed, circular or ovoid cells with smooth surfaces were observed (Fig. 1a). In the exponential phase, ovoid cells with short projections were observed (Fig 1b, c). Cells in the stationary phase possessed long tentacle-like projections which were intertwined intricately (Fig 1d). It has been reported that rod-shaped and coccid cells coexist in the case of Porphyrobacter neustonensis (Hiraishi & Imhoff, 2005). However, the projections and morphology variations seen in strain 262-7T are clearly distinct from those reported in other bacteria, and may represent a response to nutrient deficiency. It has been reported that Prosthecomicrobium hirschii and Ancalomicrobium adetum harbour long projections called prosthecae (Staley, 1968, 1984). In the case of A. adetum, it has been suggested that the long and straight prosthecae help the bacterium to resist ingestion by protists (Bianchi, 1989; Young, 2007). However, the straight projections of A. adetum seem quite different from those of strain 262-7T in appearance. In the case of a stalked bacterium, Caulobacter vibrioides (former name, Caulobacter crescentus), the stalk (prosthecae) affixes the organism to solid surfaces in aqueous environments, and the length of the stalk is regulated by the availability of nutrients (Young, 2007). The cells of strain 262-7T gradually became smaller in response to the progression of the projections (Fig 1c, d). It seemed that the surface area of the cell was expanded by the production of the projections, and the projections of strain 262-7T might be used for nutrient uptake in the oligotrophic environments present in the gaps inside the rock. By contrast, it could be possible that cells were connected by the projections, and the projections might be involved in biofilm formation. A tendency of 262-7T cells to aggregate in the stationary phase was observed.

When cells cultivated on 0.25 × LB agar medium were analysed, long-rod-shaped cells (more than 10 μm in length) were observed among short-rod-shaped cells (Fig 1e). To distinguish live cells from dead cells, nucleic acids...
of strain 262-7T were stained with SYTO9 dye, which stains cells with intact and damaged membranes, and with propidium iodide (PI) dye, which only stains cells with damaged membranes, and the stained cells were examined by fluorescence microscopy (Fig. S1, available in the online Supplementary Material). As a result, nucleic acids of most of the long-rod-shaped cells were stained only by SYTO9 dye, suggesting that the long-rod-shaped cells were alive. A similar observation was reported for Caulobacter vibrioides, and the elongated cells displayed increased resistance to stress (Wortinger et al., 1998). Moreover, when strain 262-7T was cultivated in media containing organic compounds at low concentrations [0.1 × LB medium (1 L⁻¹: 1.0 g tryptone, 0.5 g yeast extract and 5.0 g NaCl in tap water) or 0.01 × LB medium (1 L⁻¹: 0.1 g tryptone, 0.05 g yeast extract and 5.0 g NaCl in tap water)], the existence of rod-shaped cells (approx. 3–10 μm in length) was observed (data not shown). Strain 262-7T was isolated from oligotrophic environment, and, therefore, strain 262-7T may be a rod-shaped bacterium in the natural environment. The rod-shaped cell formation of strain 262-7T may be of advantage for adaptation to oligotrophic environments or for resistance to environmental changes. Although flagella could not be observed by scanning electron microscopy, a flagellum was observed by atomic force microscopy (OLS3500; Olympus) (Fig. 1f).

When the 16S rRNA gene sequence of strain 262-7T was analyzed with similarity search programs (nucleotide BLAST) provided by the National Center for Biotechnology Information (Altschul et al., 1990), related known strains were Sandarakinorhabdus limnophila so42T (Gich & Overmann, 2006) and Sphingomonas fennica K101T (Wittich et al., 2007) in the family Sphingomonadaceae, with 16S rRNA gene sequence similarities of 95.0 and 94.6%, respectively. To examine the phylogenetic position of strain 262-7T, a phylogenetic tree based on 16S rRNA gene sequences from bacteria belonging to the order Sphingomonadales (Fig. 2) was reconstructed with the CLUSTAL W program (Thompson et al., 1994). The order Sphingomonadales contains two families, Sphingomonadaceae and Erythrobacteraceae (Lee et al., 2005). Although some pleomorphic genera belong to the family Erythrobacteraceae (Hiraishi & Imhoff, 2005), strain 262-7T was located in the clade of the family Sphingomonadaceae. In addition, strain 262-7T was located in a branch distinct to the previously recognized genera belonging to the family Sphingomonadaceae, although strain 262-7T was located in a clade with Sandarakinorhabdus limnophila and Sandaracinobacter sibiricus. This result indicated that strain 262-7T is relatively closely related to the genera Sandarakinorhabdus and Sandaracinobacter, and suggested that strain 262-7T is a phylogenetically novel bacterium in the family Sphingomonadaceae.

The G+C content of the genomic DNA, respiratory quinone, fatty acids and polar lipids pattern were analysed by previously described methods with slight modification (Fukuda et al., 2012; Yamada, et al., 2011). The fatty acids composition was analysed by the Sherlock Microbial Identification System using version 6.0 of the TSBA6 library. The alkaline-stable lipids were extracted by the method of Matsuyama et al. (2008). The chemotaxonomic characteristics of strain 262-7T and those of the genera in the family Sphingomonadaceae are shown in Table 1. The G+C content of the genomic DNA was 68.0 mol%, which was in the same range as those of members of the genera Sandaracinobacter and Sphingomonas, and is slightly higher than that of members of the other genera in the family Sphingomonadaceae. The major respiratory quinone of strain 262-7T was ubiquinone 10, which is the most

![Fig. 2. Phylogenetic tree of strain 262-7T and members of the order Sphingomonadales based on 16S rRNA gene sequences. This tree was reconstructed using the neighbour-joining method provided by DNA Data Bank of Japan (http://clustalw.ddbj.nig.ac.jp/index.php?lang=en). Bootstrap resampling was performed 1000 times and only values observed in more than 50% of the replicas are shown at branching points. The 16S rRNA gene sequence of Escherichia coli ATCC 11775T was used as an outgroup. Circles at nodes indicate nodes recovered with bootstrap values >50% (filled circles) or >70% (open circles) in a maximum-likelihood tree reconstructed using the Dnaml program in the PhyLP package (Felsenstein, 2009). Bar, 2 substitutions per 100 nucleotides.](http://clustalw.ddbj.nig.ac.jp/index.php?lang=en)
Table 1. Characteristics that differentiate strain 262-7\textsuperscript{T} from the genera in the family Sphingomonadaceae

Taxa: 1, Strain 262-7\textsuperscript{T}; 2, Sandarakinorhabdus (data from Gich & Overmann, 2006); 3, Sandaracinobacter, (Yurkov, 2005a); 4, Sphingomonas (Takeuchi, et al., 2001; Yabuuchi & Kosako, 2005b); 5, Sphingobium (Takeuchi, et al., 2001; Yabuuchi & Kosako, 2005b); 6, Novosphingobium (Takeuchi, et al., 2001; Yabuuchi & Kosako, 2005b); 7, Sphingopyxis (Takeuchi, et al., 2001; Yabuuchi & Kosako, 2005b); 8, Parasphingopyxis (Uchida et al., 2012); 9, Blastomonas (Sly & Hugenholtz, 2005); 10, Sphingomicrobium (Kämpfer et al., 2012); 11, Sphingosinicella (Maruyama et al., 2006); 12, Stakelama (Chen et al., 2010); 13, Zymomonas (Sprenger & Swings, 2005). +, Positive; −, negative or absent; + or −, different reaction in different species; ND, no data; Q, ubiquinone.

<table>
<thead>
<tr>
<th>Characteristics</th>
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<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
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<tbody>
<tr>
<td>Pigmentation (colony colour)</td>
<td>Brown</td>
<td>Orange–red</td>
<td>Yellow–orange</td>
<td>Orange–brown, yellow, white or colourless</td>
<td>Yellow or whitish-brown</td>
<td>Yellow or whitish-brown</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td></td>
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<tr>
<td>Cell morphology</td>
<td>Ovoid to rod (Pleomorphic)</td>
<td>Rod</td>
<td>Long rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Ovoid or rod</td>
<td>Irregular rod</td>
<td>Rod</td>
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<td>Motility</td>
<td>+ / +</td>
<td>− / +</td>
<td>− / +</td>
<td>+ / −</td>
<td>+ or −</td>
<td>+ or −</td>
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<td>ND</td>
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<tr>
<td>Major fatty acids</td>
<td>C_{17:1\omega6c}, C_{16:1\omega7c}, C_{18:1\omega7c}</td>
<td>C_{16:1\omega7c}, C_{17:1}, C_{17:0}</td>
<td>C_{18:1}, C_{16:0}</td>
<td>C_{18:1}, C_{16:0}</td>
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<tr>
<td>Major hydroxyl fatty acids</td>
<td>C_{15:0} 2-OH</td>
<td>iso-C_{14:0} 2-OH</td>
<td>nd</td>
<td>C_{14:0} 2-OH, C_{15:0} 2-OH</td>
<td>C_{14:0} 2-OH</td>
<td>C_{14:0} 2-OH, C_{15:0} 2-OH</td>
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<td>C_{14:0} 2-OH, C_{15:0} 2-OH</td>
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<tr>
<td>Major quinone</td>
<td>Q-10</td>
<td>Q-10</td>
<td>Q-9, Q-10</td>
<td>Q-10</td>
<td>Q-10</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>68.0</td>
<td>64.3</td>
<td>68.5</td>
<td>62–68</td>
<td>59–67</td>
<td>62–67</td>
<td>63.0–65.0</td>
<td>60.1</td>
<td>65</td>
<td>63.4</td>
<td>63.6–63.7</td>
<td>66 ± 0.5</td>
<td>47.5–49.5</td>
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frequently identified respiratory quinone in bacteria belonging to the family Sphingomonadaceae (Yabuuchi & Kosako, 2005a). To analyse the composition of cellular fatty acids, cells of strain 262-7T were cultivated in 0.25× LB medium at 25°C until the late exponential phase of growth. The major cellular fatty acids of strain 262-7T were C17:1ω6c (28.2%), C16:1ω7c (22.6%), C18:1ω7c (12.9%) and C15:0 2-OH (12.3%); C14:0 2-OH (7.1%), C16:0 (3.1%) and C15:1ω6c (3.1%) were detected as minor fatty acids. Trace amounts of iso-C16:0 3-OH (0.9%) were detected. The major nonpolar and 2-hydroxy fatty acids of strain 262-7T were different from those of the related genus Sandarakinorhabdus (Table 1), although fatty acid compositions of species of the genus Sphingomonas are similar to those of strain 262-7T (Takeuchi et al., 2001). The lipids pattern on two-dimensional TLC revealed the presence of phosphatidylglycerol, phosphatidylethanolamine, sphingoglycolipid, an unknown phospholipid and two unknown glycolipids (Fig. S2 and Table S1). The lipids pattern of strain 262-7T was different from the related genera Sphingomonas and genera in the family Sphingomonadaceae (Yabuuchi & Overmann, 2001). The lipids pattern of strain 262-7T was different from the related genera Sphingomonas (Yabuuchi et al., 2003; Takeuchi et al., 2001). Although fatty acid compositions of the closely related species Sandarakinorhabdus limnophila (Gich & Overmann, 2006), pigmentation analysis was performed using cells in the mid–late exponential phase by the modified method described by Saga et al. (2005). The spectra of pigments extracted from cells showed three peaks at 428, 452 and 600 nm derived from bacteriochlorophyll a were observed, contrary to Sandarakinobacter sibiricus and genera in the family Erythrobacteraceae (Yurkov et al., 1997; Yurkov, 2005b).

According to its phylogenetic position, strain 262-7T is most closely related to the genera Sandarakinorhabdus and Sandarakinobacter, and is clearly different from other genera in the family Sphingomonadaceae. Fatty acids composition and production of oxidase/catalase are different from the related genera Sandarakinorhabdus and Sandarakinobacter (Table 1). Above all, unique morphology variations and appearance of strain 262-7T were observed. Therefore, strain 262-7T should be classified as a representative of a novel species and genus in the family Sphingomonadaceae, for which the name Polymorphobacter multimanifer gen. nov., sp. nov. is proposed.

**Description of Polymorphobacter multimanifer sp. nov.**

**Polymorphobacter multimanifer** [mul.ti.ma’ni.fer. L. adj. multus many; L. n. manus hand; L. suff. -fer, (from L. v. fero) bearing, carrying, producing; N.L. masc. adj. n. multimanifer many hands bearer, referring to tentacle-like projections].

Colonies are brown, raised, circular and entire. Cells are circular or ovoid with smooth surfaces in the lag phase of growth. In the exponential growth phase, ovoid cells with short projections are observed. Cells in the stationary phase possess long tentacle-like projections intertwined intricately. By contrast, cells on 0.25× LB agar plate medium exhibit short- and long-rod-shaped morphology. Growth occurs between –4 and 30°C (optimum, 25°C), between pH 6.0 and 9.0 (optimum, approximately pH 7.0), and with between 0.0 and 1.0% NaCl (optimum, 0.5%). Cells can utilize maltose and malate as a carbon sources, and weak growth is observed in media containing glucose and mannose. Cannot utilize adipate, arabinose, caprate, citrate, gluconate, mannitol and phenylacetate. Production of acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase is observed. Activities of arginine dihydrolase N-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, β-galactosidase, gelatinase, α-glucosidase, β-glucuronidase, lipase (C4), α-mannosidase and urease are not observed. H2S and indole are not produced. Cells are resistant to amoxicillin, amoxicillin/clavulanic acid, cefoperazone, cefalotin, colistin, cotrimoxazole, erythromycin, lincomycin, metronidazole, oxacillin, penicillin, sulfamethizole and tetracyclins. Cells are sensitive to apramycin, chloramphenicol, doxycycline, enrofloxacin, flumequine, fusidic acid, gentamicin, kanamycin, nitrofurantoin, oxolinic acid, pristinamycin, rifampicin, spectinomycin, streptomycin and tetracycline.

The type strain is 262-7T (=JCM 18140T = ATCC BAA-2413T) and was isolated from a crack of white rock in the Skallen region of Antarctica.

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