Lysobacter oligotrophicus sp. nov., isolated from an Antarctic freshwater lake in Antarctica

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A Gram-stain-negative, non-spore-forming, rod-shaped, aerobic bacterium (strain 107-E2T) was isolated from freshwater samples containing microbial mats collected at a lake in Skarvsnes, Antarctica (temporary lake name, Lake Tanago Ike). Strain 107-E2T grew between 5 and 25 °C, with an optimum of 23 °C. Moreover, colony formation was observed on agar media even at −5 °C. The pH range for growth was between 6.0 and 9.0, with an optimum of pH 7.0–8.0. The range of NaCl concentration for growth was between 0.0 and 0.5 % (w/v), with an optimum of 0.0 %. No growth was observed in media containing organic compounds at high concentrations, which indicated that strain 107-E2T was an oligotroph. In the late stationary phase, strain 107-E2T produced a dark brown water-soluble pigment. Esterase, amylase and protease production was observed. Antimicrobial-lytic activities for Gram-negative bacteria and yeast were observed. Ubiquinone-8 was the major respiratory quinone. The major fatty acids were iso-C15 : 0, iso-C16 : 0, iso-C17 : 0, C18 : 1ω9c and iso-C15 : 1ω6c at 5. The G+C content of genomic DNA was 66.1 mol%. Analysis of the 16S rRNA gene sequences revealed that strain 107-E2T belonged to the genus Lysobacter, and low DNA–DNA relatedness values with closely related species distinguished strain 107-E2T from recognized species of the genus Lysobacter. The phylogenetic situation and physiological characteristics indicated that strain 107-E2T should be classified as a representative of a novel species of the genus Lysobacter, for which the name Lysobacter oligotrophicus sp. nov. is proposed. The type strain is 107-E2T (=JCM 18257T =ATCC BAA-2438T).

The Antartica is the coldest place on Earth and most of the land surfaces are covered by a thick ice (Bargagli, 2005). However, there are ice-free regions in the vicinity of Syowa Station in East Ongul Island in the Antarctic, and there are many small freshwater lakes (Imura et al., 1999, 2003). These lakes are oligotrophic, and a low diversity of phytoplankton has been reported (Imura et al., 1999). However, microbiological mats with mat- and pillar-like forms, which are composed of filamentous cyanobacteria, diatoms and green algae, have been discovered at the bottom of these lakes (Imura et al., 1999, 2003; Ohtsuka et al., 2006).

Environmental samples were collected by the summer party of the 46th Japanese Antarctic Research Expedition in 2004–2005. Among them, freshwater samples containing microbial mats were collected at the bottom of a freshwater lake in the Skarvsnes region in Antarctica, and a phylogenetically novel bacterium, Rhodoligotrophos appendicifer, was isolated from the sample of Lake Naga-ike (Fukuda et al., 2012). Moreover, the other freshwater samples containing microbial mats collected at the bottom of a lake [temporary name of the lake is Lake Tanago Ike (Imura et al., 2003)] were added onto the modified Luria–Bertani (LB) plate medium containing (l-tryptone, 1.0 g yeast extract, 5 g NaCl and 15 g agar (0.1 × LB plate medium). After incubation under aerobic conditions at 25 °C, the formation of several bacterial colonies was observed on the agar plate. A bacterium forming yellow colonies on the plate was designated strain 107-E2T.

Strain 107-E2T grew well in 0.25 × LB medium without NaCl (I−: 2.5 g tryptone, 1.3 g yeast extract), and could even grow in 0.01 × LB medium without NaCl (I−: 0.10 g tryptone and 0.050 g yeast extract) at 23 °C. However, no growth was observed in media containing organic compounds at high concentrations at 23 °C (Fig. S1, available in IJSEM Online). These results indicated that strain 107-E2T was an oligotroph. In addition, strain 107-E2T grew in
Reasoner’s 2A medium (Reasoner & Geldreich, 1985), and inorganic metal salts medium (I⁻¹: 6.0 g Na₂HPO₄, 3.0 g KH₂PO₄, 1.0 g NH₄Cl, 0.5 g NaCl, 1 mM MgSO₄, 0.1 mM CaCl₂ and 10 mg thiamine) containing 20 amino acids (I⁻¹: 38 mg alanine, 63 mg arginine hydrochloride, 50 mg asparagine hydrate, 25 mg aspartic acid, 125 mg cysteine hydrochloride hydrate, 25 mg glutamine, 100 mg glutamic acid, 100 mg glycine, 50 mg histidine hydrochloride hydrate, 50 mg isoleucine, 50 mg leucine, 50 mg lysine hydrochloride, 38 mg methionine, 38 mg phenylalanine, 63 mg proline, 38 mg serine, 50 mg threonine, 38 mg tryptophan, 50 mg tyrosine and 25 mg valine), but could not grow in NZCYM medium (Sambrook & Russell, 2001). Strain 107-E2ᵀ grew between 5 and 25 °C in 0.25 × LB liquid medium without NaCl, with optimum growth at 23 °C, but could not grow at 30 °C. When strain 107-E2ᵀ was incubated on the 0.25 × LB plate medium without NaCl for 4 weeks at −2 and −5 °C in a freezing chamber, colony formation was observed. The pH range for growth was between 6.0 and 9.0, with an optimum of between pH 7.0 and 8.0; strain 107-E2ᵀ could not grow in the media at pH 5.0 or pH 10.0. The range of NaCl concentration allowing growth of strain 107-E2ᵀ was 0.0–0.5% (w/v), with an optimum of 0.0%; there was no growth in the media containing 1.0% NaCl. In the late stationary phase, strain 107-E2ᵀ produced a dark brown water-soluble pigment. It is well-known that tyrosinase contributes to melanin formation from tyrosine in various organisms (Claus & Decker, 2006). In the case of strain 107-E2ᵀ, the dark brown pigment was induced on a inorganic metal salts plate medium containing 20 amino acids, and the production of dark brown pigment was not observed on the inorganic metal salts plate medium lacking tyrosine. Furthermore, the production of the pigment was inhibited by addition of 0.3 mM 5-hydroxy-2-(hydroxy-methyl)-4H-pyran-4-one (kojic acid) which is known as a tyrosinase inhibitor (Cabanes et al., 1994; Yoshimoto et al., 1985). These results suggested the water-soluble pigment was melanin. Strain 107-E2ᵀ was an aerobic bacterium, and no growth was observed in 0.25 × LB medium containing 0.5% Na₂SO₄, 0.5% NaNO₃, 0.5% NaHCO₃ or 0.02% FeCl₃ under anaerobic conditions with the addition of Na₂S. 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 107-E2ᵀ was a Gram-stain-negative, catalase-positive, oxidase-positive, non-spore-forming and non-motile bacterium. Further biochemical characteristics were analysed with API 20NE and API ZYM kits (bioMérieux), and antibiotic sensitivity was examined by using ATB VET test (bioMérieux). These analyses were performed at 25 °C according to the manufacturer’s instructions and the results are given in the species description.

The cell morphology of strain 107-E2ᵀ was examined by bright-field microscopy (BX5; Olympus), and rod-shaped cells were observed (data not shown). The ultra-structure of the cells was examined by scanning electron microscopy (S-4700; Hitachi), and rod-shaped cells (0.2–0.3 × 1.8–2.7 μm) in pairs or chains were observed (Fig. 1). Flagella could not be observed by monitoring with a microscope.

The 16S rRNA gene sequence of strain 107-E2ᵀ was analysed with similarity search programs (nucleotide BLAST) provided by the National Center for Biotechnology Information (Altschul et al., 1990). The closely related strains to strain 107-E2ᵀ were Lysobacter ginsengisoli Gsoil 357ᵀ (Jung et al., 2008), Lysobacter panaciterrae Gsoil 068ᵀ (Ten et al., 2009) and Lysobacter brunescens UASM DT (Christensen, 2005) in the family Xanthomonadaceae, with 16S rRNA gene sequence similarities of 97.2%, 96.9% and 96.7%, respectively. To examine the phylogenetic position of strain 107-E2ᵀ, a phylogenetic tree with 16S rRNA gene sequences from bacteria belonging to the family Xanthomonadaceae (Fig. 2) was reconstructed by the neighbour-joining method with the CLUSTAL W program (Thompson et al., 1994). Strain 107-E2ᵀ was located in a clade of L. brunescens UASM DT and L. panaciterrae Gsoil 068ᵀ in the genus Lysobacter (Fig. 2). Although these results clearly indicated that strain 107-E2ᵀ was a member of the genus Lysobacter, strain 107-E2ᵀ was located in a branch distinct from known species of the genus Lysobacter.

The G + C content of genomic DNA, respiratory quinone, fatty acids composition and phospholipids pattern of strain 107-E2ᵀ were determined by methods previously described (Fukuda et al., 2012). The chemotaxonomic characteristics of strain 107-E2ᵀ and those of related species are shown in Table 1. The G + C content of the genomic DNA of strain 107-E2ᵀ was 66.1 mol%, which is similar to those of other species of the genus Lysobacter. (Kawamura et al., 2009). The major respiratory quinone of strain 107-E2ᵀ was ubiquinone-8, which is found mostly in members of the order Xanthomonadales (Bae et al., 2005; Saddler & Bradbury, 2005). The cellular fatty acids of strain 107-E2ᵀ in mid-exponential phase were analysed by the Sherlock Microbial Identification System using version

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**Fig. 1.** Scanning electron micrograph of cells of strain 107-E2ᵀ.

Bar, 1 μm.
4.10 of the TSBA40 library (MIDI) and GCMS-QP2010 (Shimazu) equipped with Inert Cap WAX (30 m × 0.25 mm; GL science). The major cellular fatty acids of strain 107-E2T were iso-C₁₅:₀ (36.1 %), iso-C₁₇:₁ω9c (19.7 %) and iso-C₁₅:₁ at 5 (19.0 %), and the minor ones were iso-C₁₁:₀ 3-OH (4.2 %), iso-C₁₁:₀ (4.1 %), C₁₆:₁ω7c (3.6 %), iso-C₁₇:₀ (2.0 %) and C₁₆:₀ (2.0 %). Although iso-C₁₅:₀, iso-C₁₆:₀ or iso-C₁₇:₁ is the major cellular fatty acid in most species of the genus Lysobacter (Kawamura et al., 2009), the presence of iso-C₁₅:₁ as a major cellular fatty acid is unique to strain 107-E2T. The phospholipids pattern on two-dimensional thin-layer chromatography (2D-TLC) revealed the presence of diposphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine in

![Phylogenetic tree of strain 107-E2T and members of the family Xanthomonadaceae based on 16S rRNA gene sequences.](http://ijs.sgmjournals.org)
Table 1. Characteristics that differentiate strain 107-E2T from recognized species of the genus Lysobacter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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</thead>
<tbody>
<tr>
<td>Size (µm)</td>
<td>0.2–0.3 × 1.8–2.7</td>
<td>0.2–0.5 × 7–70</td>
<td>ND</td>
<td>0.7–1.0 × 1.0–4.5</td>
<td>0.5 × 1–3</td>
<td>0.5 × 38.0</td>
<td>0.4 × 4–40</td>
<td>0.4 × 2.0</td>
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<tr>
<td>Colony colour</td>
<td>Y</td>
<td>Y–CH</td>
<td>C</td>
<td>C</td>
<td>Y</td>
<td>Y–DC</td>
<td>P–B</td>
<td>YG</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Catalase/oxidase</td>
<td>+/+</td>
<td>–/–</td>
<td>–/-</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
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<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>β-galactosidase</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+/ND</td>
</tr>
<tr>
<td>NaCl tolerance (%) (optimum)</td>
<td>0–0.5 (0)</td>
<td>&lt;2</td>
<td>0–3</td>
<td>0–2</td>
<td>0–1</td>
<td>0–2 (0)</td>
<td>0–2 (0)</td>
<td>0–2 (0)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>66.1</td>
<td>67.7</td>
<td>67.0</td>
<td>69.3</td>
<td>63.5</td>
<td>69.0</td>
<td>69.2</td>
<td>65.7</td>
</tr>
</tbody>
</table>

*Y, Yellow; C, cream; CH, chocolate; DC, dark cream; p, pink; b, brown; YG, yellowish-grey; LB, light beige; DY, dark yellow.
†Summed feature 3 contains C₁₆ : ₀7c and/or C₁₅ : ₀ is 2-OH.
Lysobacter oligotrophicus sp. nov.

addition to two unidentified phospholipids and one unidentified lipid (Fig. S2).

Microplate DNA–DNA hybridizations were performed in the presence of 50 % formamide (Ezaki et al., 1989), and the values of DNA–DNA relatedness and standard deviations were calculated on four independent results. The temperature of hybridization was set at 52 °C. When DNA of strain 107-E2T was labelled, the levels of DNA–DNA hybridization to closely related species were lower than 70 %; the DNA–DNA relatedness values were 41 ± 4 % (L. brunescentis UASM D11 and 33 ± 8 % (L. panaciterrae Gsoil 068T) (Table S1). The phylogenetic definition of a species generally would include strains with DNA–DNA relatedness values of approximately 70 % or greater (Wayne et al., 1987), and these results indicated that strain 107-E2T represented a novel species. Meanwhile, the DNA–DNA relatedness values with the closely related species distinguish strain 107-E2T from known species of the genus Lysobacter.

According to these physiological characteristics and the phylogenetic situation, strain 107-E2T represents a novel species of the genus Lysobacter, for which the name, Lysobacter oligotrophicus sp. nov. is proposed.

Description of Lysobacter oligotrophicus sp. nov.

Lysobacter oligotrophicus (o.li.go.tro phi.cus. Gr. adj. oligos little; Gr. adj. trophikos nursing, tending; N.L. masc. adj. oligotrophicus utilizing only a few growth substrates).

Cells are rod-shaped (0.2–0.3 × 1.8–2.7 μm), non-motile and oligotrophic. Growth occurs between −5 and 25 °C (optimum, 23 °C) and between pH 6.0 and 9.0 (optimum, approximately 7.0–8.0). Cells produce black to brown hydrosoluble-pigment in the stationary phase. Catalase and oxidase are produced. Nitrate reduction is not observed. Hydrolyses aesculin, casein, elastin, gelatin, guanine, hippurate, keratine, starch and Tweens 20, 40, 60 and 80, but not carboxymethylcellulose, chitin or xylan. Utilizes adipate, citrate, glconate, and N-acetyl-D-glucosamine as a carbon source, and weak growth is observed in the media containing glucose and malate. Positive for the following enzyme activities as tested with the API ZYM system: cysteine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-Bl-phosphohydrolase and valine arylamidase (weakly positive). Resistant to lincomycin, cotrimoxazole, sulfamethizole and metronidazole, but sensitive to erythromycin, pristinamycin, tylosin, colistin, flumequine, oxolinic acid, nitrofurantoin, fusidic acid, rifampicin, penicillin, amoxicillin, amoxicillin/clavulanic acid, oxacillin, cephalothin, cefoperazone, streptomycin, spectinomycin, kanamycin, gentamicin, apramycin, chloramphenicol, tetracycline and doxycycline. The major respiratory quinone is ubiquinone-8. The major cellular fatty acids are iso-C15:0, iso-C17:0 3–OH and iso-C15:1 ω9c at 5.

The type strain is 107-E2T (=JCM 18257T = ATCC BAA-2438T) which was isolated from a freshwater lake in the Skarvsnes region, Antarctica. The G+C content of the genomic DNA of strain 107-E2T is 66.1 mol%.

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References


