

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-E2^T) was isolated from freshwater samples containing microbial mats collected at a lake in Skarvsnes, Antarctica (temporary lake name, Lake Tanago Ike). Strain 107-E2^T 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-E2^T was an oligotroph. In the late stationary phase, strain 107-E2^T 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-C_{15:0}, iso-C_{17:1ω9c} and iso-C_{15:1} at 5. The G + C content of genomic DNA was 66.1 mol%. Analysis of the 16S rRNA gene sequences revealed that strain 107-E2^T belonged to the genus *Lysobacter*, and low DNA–DNA relatedness values with closely related species distinguished strain 107-E2^T from recognized species of the genus *Lysobacter*. The phylogenetic situation and physiological characteristics indicated that strain 107-E2^T 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-E2^T (=JCM 18257^T=ATCC BAA-2438^T).

The Antarctica 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^{–1}) 1.0 g tryptone, 0.5 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-E2^T.

Strain 107-E2^T grew well in 0.25 × LB medium without NaCl (l^{–1}: 2.5 g tryptone, 1.3 g yeast extract), and could even grow in 0.01 × LB medium without NaCl (l^{–1}: 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-E2^T was an oligotroph. In addition, strain 107-E2^T grew in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 107-E2^T is AB694977.

Two supplementary figures and a supplementary table are available with the online version of this paper.

Reasoner's 2A medium (Reasoner & Geldreich, 1985), and inorganic metal salts medium (I^{-1} : 6.0 g Na_2HPO_4 , 3.0 g KH_2PO_4 , 1.0 g NH_4Cl , 0.5 g $NaCl$, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$ 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-E2^T 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-E2^T 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-E2^T could not grow in the media at pH 5.0 or pH 10.0. The range of NaCl concentration allowing growth of strain 107-E2^T 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-E2^T 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-E2^T, 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-(hydroxymethyl)-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-E2^T was an aerobic bacterium, and no growth was observed in 0.25 × LB medium containing 0.5 % Na_2SO_4 , 0.5 % $NaNO_3$, 0.5 % $NaHCO_3$ or 0.02 % $FeCl_3$ under anaerobic conditions with the addition of Na_2S . 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-E2^T 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-E2^T 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 ($0.2\text{--}0.3 \times 1.8\text{--}2.7 \mu\text{m}$) 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-E2^T 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-E2^T were *Lysobacter ginsengisoli* Gsoil 357^T (Jung *et al.*, 2008), *Lysobacter panaciterrae* Gsoil 068^T (Ten *et al.*, 2009) and *Lysobacter brunescens* UASM D^T (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-E2^T, 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-E2^T was located in a clade of *L. brunescens* UASM D^T and *L. panaciterrae* Gsoil 068^T in the genus *Lysobacter* (Fig. 2). Although these results clearly indicated that strain 107-E2^T was a member of the genus *Lysobacter*, strain 107-E2^T 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-E2^T were determined by methods previously described (Fukuda *et al.*, 2012). The chemotaxonomic characteristics of strain 107-E2^T and those of related species are shown in Table 1. The G + C content of the genomic DNA of strain 107-E2^T 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-E2^T 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-E2^T in mid-exponential phase were analysed by the Sherlock Microbial Identification System using version

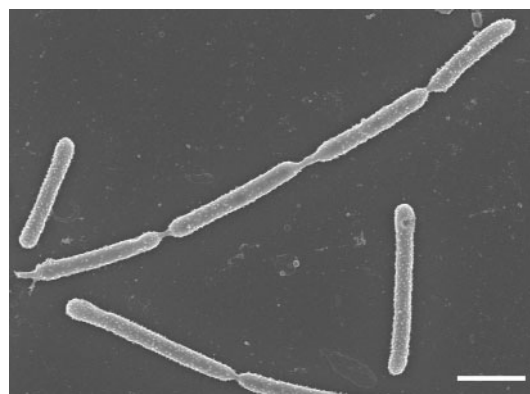


Fig. 1. Scanning electron micrograph of cells of strain 107-E2^T. Bar, 1 μm .

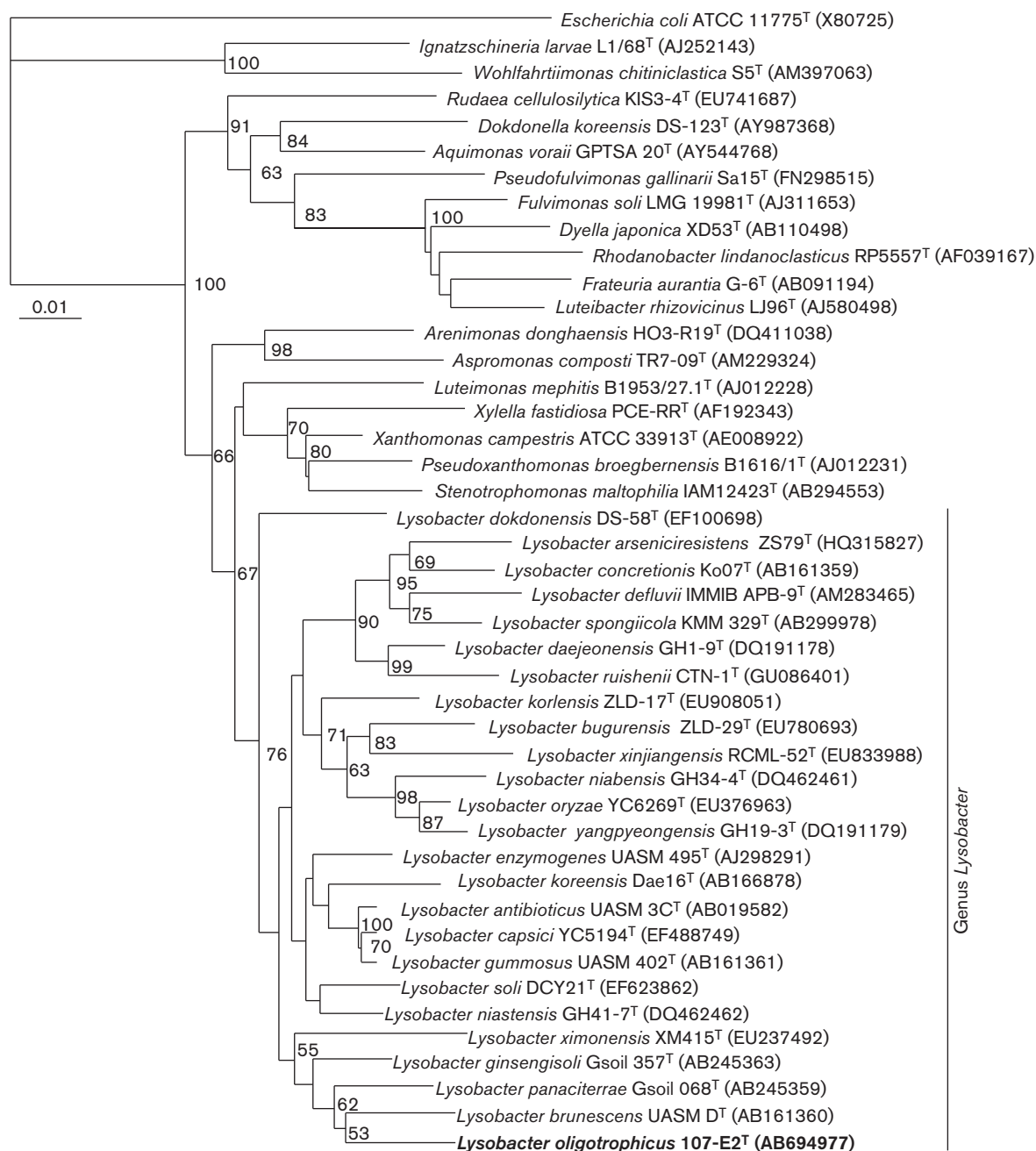


Fig. 2. Phylogenetic tree of strain 107-E2^T and members of the family Xanthomonadaceae based on 16S rRNA gene sequences. This tree was reconstructed using the neighbour-joining method provided by DNA Data Bank of Japan (DDBJ). Bootstrap resampling was performed on 1000 replicates; only values >50% are shown at the branching points. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 1 substitution per 100 nt.

4.10 of the TSBA40 library (MIDI) and GCMS-QP2010 (Shimadzu) equipped with Inert Cap WAX (30 m × 0.25 mm; GL science). The major cellular fatty acids of strain 107-E2^T were iso-C_{15:0} (36.1%), iso-C_{17:1}ω9c (19.7%) and iso-C_{15:1} at 5 (19.0%), and the minor ones were iso-C_{11:0} 3-OH (4.2%), iso-C_{11:0} (4.1%), C_{16:1}ω7c (3.6%), iso-C_{17:0} (2.0%) and C_{16:0} (2.0%). Although

iso-C_{15:0}, iso-C_{16:0} or iso-C_{17:1} is the major cellular fatty acid in most species of the genus *Lysobacter* (Kawamura *et al.*, 2009), the presence of iso-C_{15:1} as a major cellular fatty acid is unique to strain 107-E2^T. The phospholipids pattern on two-dimensional thin-layer chromatography (2D-TLC) revealed the presence of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine in

Table 1. Characteristics that differentiate strain 107-E2^T from recognized species of the genus *Lysobacter*

Taxa: 1, strain 107-E2^T (data from this study); 2, *L. brunescens* UASM D^T (Christensen, 2005; Christensen & Cook, 1978); 3, *L. panaciterrae* Gsoil 068^T (Ten *et al.*, 2009); 4, *L. ginsengisoli* Gsoil 357^T (Jung *et al.*, 2008); 5, *L. ximonensis* XM415^T (Wang *et al.*, 2009); 6, *L. enzymogenes* UASM 495^T (Christensen, 2005; Christensen & Cook, 1978); 7, *L. antibioticus* UASM 3C^T (Christensen, 2005); 8, *L. gummosus* UASM 402^T (Christensen, 2005); 9, *L. capsici* YC5194^T (Park *et al.*, 2008); 10, *L. koreensis* Dae16^T (Lee *et al.*, 2006); 11, *L. niastensis* GH41-7^T (Weon *et al.*, 2007); 12, *L. soli* DCY21^T (Srinivasan *et al.*, 2010); 13, *L. daejeonensis* GH1-9^T (Weon *et al.*, 2006); 14, *L. niabensis* GH34-4^T (Weon *et al.*, 2007); 15, *L. yangpyeongensis* GH19-3^T (Weon *et al.*, 2006); 16, *L. spongiicola* KMM 329^T (Romanenko *et al.*, 2008); 17, *L. concretionis* Ko07^T (Bae *et al.*, 2005). The major quinone in all strains is ubiquinone-8. +, Positive; –, negative; w, weak; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Size (µm)	0.2–0.3 × 1.8–2.7	0.2–0.5 × 7–70	ND	0.7–1.0 × 1.0–4.5	0.5 × 1–3	0.5 × 38.0	0.4 × 4–40	0.4 × 2.0
Colony colour*	Y	Y–CH	C	C	Y	Y–DC	P–B	YG
Motility	–	+ / –	+	+	+	+	+	+
Catalase/oxidase	+ / +	– / +	– / +	+ / +	+ / –	+ / +	+ / +	+ / ND
Nitrate reduction	–	–	–	–	–	–	+	–
β-galactosidase	w	–	–	–	+	+	+	+
Growth temperature (°C) (optimum)	–5–25 (23)	4–50 (30–40)	15–45 (30)	20–30 (25)	10–37	5–40 (25–35)	2–40 (25–33)	10–40 (20)
NaCl tolerance (%) (optimum)	0–0.5 (0)	<2	0–3	0–2	0–1	0–2 (0)	0–2 (0)	0–2 (0)
Major fatty acids	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{15:1}	iso-C _{16:0} , iso-C _{15:0} , iso-C _{17:1} ω9c	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{17:0}	iso-C _{16:0} , iso-C _{17:1} ω9c iso-C _{15:0} , C _{15:0}	iso-C _{16:0} , iso-C _{15:0}	iso-C _{15:0} , iso-C _{16:0} , C _{16:0} , C _{16:0}	iso-C _{15:0} , iso-C _{16:0} , C _{16:0} , iso-C _{11:0} 3-OH	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{11:0} 3-OH
DNA G + C content (mol%)	66.1	67.7	67.0	69.3	63.5	69.0	69.2	65.7

Characteristic	9	10	11	12	13	14	15	16	17
Size (µm)	0.3–0.5 × 2.0–20	0.5–0.8 × 1.5–2.0	0.5–0.6 × 2.0–4.0	0.2–0.5 × 0.6–0.9	0.4–0.6 × 3.0–4.0	0.5 × 2.0–5.0	0.4–0.6 × 3.0–4.0	0.5–0.6 × 1.3–1.5	0.7 × 1.0–13.5
Colony colour*	Y–C	Y	LB	Y	DY	Y	Y	Y	Y
Motility	+	ND	+	+	–	–	+	–	+
Catalase/oxidase	+ / +	+ / –	+ / +	+ / +	w / +	+ / +	+ / –	+ / +	+ / +
Nitrate reduction	ND	–	+	+	+	–	–	–	–
β-galactosidase	–	–	+	–	–	–	–	–	–
Growth temperature (°C) (optimum)	15–37 (28)	(30)	10–40 (28)	4–42 (30)	10–37	5–37 (28)	15–40	5–41 (25–28)	(25–30)
NaCl tolerance (%) (optimum)	0–2	0–1	0–1	ND	0–3 (0)	0–1	0–0.5	0–6	ND
Major fatty acids	iso-C _{15:0} , summed feature 3†, C _{16:0} , iso-C _{17:1} ω9c	iso-C _{16:0} , iso-C _{15:0} , iso-C _{17:1} ω9c	iso-C _{16:0} , iso-C _{15:0} , iso-C _{17:1} ω9c	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{17:0}	iso-C _{16:0} , iso-C _{15:0} , iso-C _{14:0}	iso-C _{16:0} , iso-C _{15:0} , iso-C _{17:1} ω9c	iso-C _{16:0} , iso-C _{15:0} , C _{16:1} ω7c alcohol	iso-C _{16:0} , iso-C _{15:0} , iso-C _{11:0} 3-OH, iso-C _{17:1}	iso-C _{15:0} , iso-C _{16:0} , iso-C _{17:1} ω9c
DNA G + C content (mol%)	65.4	68.9	66.6	65.4	61.7	62.5	67.3	69.0	63.8

*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 C_{16:1}ω7c and/or C_{15:0} iso 2-OH.

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-E2^T 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. brunescens* UASM D^T) and 33 ± 8 % (*L. panaciterrae* Gsoil 068^T) (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-E2^T represented a novel species. Meanwhile, the DNA–DNA relatedness values between *L. brunescens* UASM D^T and *L. panaciterrae* Gsoil 068^T were approximately 70 % (Table S1). Although the separation of these two species is based on the low 16S rRNA gene sequence similarity (96.1 %), the high DNA–DNA relatedness values revealed that the two species are closely related to each other (Ten *et al.*, 2009).

Members of the genus *Lysobacter* degrade chitin and often other polysaccharides, and infrequently degrade agar (Christensen, 2005). In addition, members of the genus *Lysobacter* are strongly proteolytic and characteristically lyse a variety of micro-organisms, as well as nematodes. Strain 107-E2^T could hydrolyse aesculin, casein, elastin, gelatin, guanine, hippurate, keratin, starch and Tweens 20, 40, 60 and 80. However, strain 107-E2^T could not hydrolyse carboxymethylcellulose, chitin or xylan. These results indicated that strain 107-E2^T produced esterase, amylase and protease, but not cellulase, chitinase or xylanase. To evaluate an antimicrobial-lytic action, strain 107-E2^T was cultivated on 0.25 × LB plate media without NaCl containing autoclaved cells of Gram-negative bacteria (*Escherichia coli* DH5α *Lysobacter enzymogenes* 495^T, and *Rhodoligotrophos appendicifer* 120-1^T), Gram-positive bacteria (*Bacillus megaterium* 1060^T), yeast (*Saccharomyces cerevisiae* A.C. van Wijk^T or algae *Chlorella vulgaris* NIES-641) for 90 h at 25 °C. Strain 107-E2^T showed halo formation around colonies on the medium containing cells of Gram-negative bacteria and *S. cerevisiae*, but *B. megaterium* and *C. vulgaris* could not be lysed. In general, members of the genus *Lysobacter* do not attack Gram-negative bacteria as vigorously as they do Gram-positive species, and this feature may be for self-protection (Christensen, 2005). However, strain 107-E2^T lysed the Gram-negative species more efficiently than it lysed other micro-organisms. Indeed, the autolysis of strain 107-E2^T was observed in the stationary phase.

Strain 107-E2^T prefers oligotrophic conditions, and the lower salinity tolerance and growth of strain 107-E2^T below freezing point is more unusual than those of related strains (Table 1). In addition, the low DNA–DNA relatedness values with the closely related species distinguish strain 107-E2^T from known species of the genus *Lysobacter*.

According to these physiological characteristics and the phylogenetic situation, strain 107-E2^T 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, gluconate, 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: cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase (weakly positive). Resistant to lincomycin, cotrimoxazole, sulfamethizole and metronidazole, but sensitive to erythromycin, pristnamycin, tylosin, colistin, flumequine, oxolinic acid, enrofloxacin, 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-C_{15:0}, iso-C_{17:1ω9c} and iso-C_{15:1} at 5.

The type strain is 107-E2^T (=JCM 18257^T=ATCC BAA-2438^T) which was isolated from a freshwater lake in the Skarvsnes region, Antarctica. The G+C content of the genomic DNA of strain 107-E2^T is 66.1 mol%.

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