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Lysobacter spongiicola sp. nov., isolated from a deep-sea sponge

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An aerobic, Gram-negative bacterium, strain KMM 329^T, was isolated from a deep-sea sponge specimen from the Philippine Sea and subjected to a polyphasic taxonomic investigation. Comparative 16S rRNA gene sequence analysis showed that strain KMM 329^T clustered with the species of the genus *Lysobacter*. The highest level of 16S rRNA gene sequence similarity (97.0 %) was found with respect to *Lysobacter concretionis* KCTC 12205^T; lower values (96.4–95.2 %) were obtained with respect to the other recognized *Lysobacter* species. The value for DNA–DNA relatedness between strain KMM 329^T and *L. concretionis* KCTC 12205^T was 47 %. Branched fatty acids 16 : 0 iso, 15 : 0 iso, 11 : 0 iso 3-OH and 17 : 1 iso were found to be predominant. Strain KMM 329^T had a DNA G+C content of 69.0 mol%. On the basis of the phenotypic, chemotaxonomic, DNA–DNA hybridization and phylogenetic data, strain KMM 329^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter spongiicola* sp. nov. is proposed. The type strain is KMM 329^T (=NRIC 0728^T =JCM 14760^T).

The genus *Lysobacter* was initially described by Christensen & Cook (1978) and comprised four species: *Lysobacter enzymogenes*, *L. antibioticus*, *L. brunescens* and *L. gummosus*. Subsequently, seven more species were added to the genus: *Lysobacter concretionis* (Bae *et al.*, 2005), *L. koreensis* (Lee *et al.*, 2006), *L. daejeonensis*, *L. yangpyeongensis* (Weon *et al.*, 2006), *L. niabensis*, *L. niastensis* (Weon *et al.*, 2007) and *L. defluvii* (Yassin *et al.*, 2007). At the time of writing, the natural occurrence of members of the genus *Lysobacter* is known to be restricted to terrestrial habitats. Here we report the polyphasic characterization of a Gram-negative, aerobic bacterium, designated KMM 329^T, isolated from a deep-sea sponge specimen. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain KMM 329^T belonged to the genus *Lysobacter* and could represent a novel species of this genus. Differential phenotypic properties, together with the phylogenetic distinctiveness of KMM 329^T, demonstrated that this strain differed from the recognized *Lysobacter* species. Strain KMM 329^T possessed the proteolytic, haemolytic and bacteriolytic activities that have been reported for *Lysobacter* species (Bae *et al.*, 2005; Yassin *et al.*, 2007). On the basis of the phenotypic and molecular data obtained, strain KMM 329^T represents a novel species of the genus *Lysobacter*.

Strain KMM 329^T was isolated from a sponge specimen (*Pachastrella* sp.), collected in 1991 from the Philippine Sea at a water depth of 750 m, as described previously (Romanenko *et al.*, 2005). The bacterium was grown aerobically on marine agar 2216 (MA) or marine broth, trypticase soy agar, trypticase soy broth or R2A agar (all from Difco) at 25–28 °C, and stored at −80 °C in liquid marine broth supplemented with 30 % (v/v) glycerol. Strain KMM 329^T has been deposited in the Collection of Marine Micro-organisms (Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia). The type strain of *L. concretionis*, KCTC 12205^T, was kindly provided by the Korean Collection for Type Cultures (Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea) for use in this study. The motility of cells grown on an MA slant after half-day cultivation was observed by using oil-immersion phase-contrast microscopy (AX70; Olympus). Flagellation was investigated with a transmission electron microscope

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 329^T is AB299978.

TLCs of the polar lipids of strains KMM 329^T and *L. concretionis* KCTC 12205^T, a maximum-parsimony phylogenetic tree and a table comparing the cellular fatty acid compositions of KMM 329^T and *Lysobacter* species are available as supplementary material with the online version of this paper.

(H-7600; Hitachi High-Technologies) using negative staining with 3 % (w/v) phosphotungstic acid. The Gram-staining, oxidase and catalase reactions and the hydrolytic reactions with regard to starch, chitin, DNA and Tweens 80, 40 and 20 were tested according to the standard methods described by Smibert & Krieg (1994). Starch hydrolysis was investigated after 2 days incubation on nutrient agar containing 0.2 % (w/v) soluble starch by flooding the plates with a 1 % (w/v) iodine solution. Hydrolysis of adenine, guanine, tyrosine and xanthine was investigated by observing cleared zones on MA supplemented with these compounds (each at a concentration of 1 %, w/v). The formation of H_2S from thiosulfate was tested using a lead acetate paper strip. The oxidation/fermentation medium of Leifson (1963) was used for testing acid production from carbohydrates, each compound being used at 1 % (w/v). Antibiotic resistance and growth at different temperatures, pHs and NaCl concentrations were studied as described previously (Romanenko *et al.*, 2003, 2005). In addition, biochemical tests were carried out using API 20NE, API ID 32GN, API 50 CH and API ZYM test kits (all from bioMérieux) as described by the manufacturer. Proteolytic activity was assessed in casein- and gelatin-degradation tests as described by Smibert & Krieg (1994). Bacteriolytic activity was determined using plating on agar medium supplemented with autoclaved cells of *Micrococcus luteus* (10^9 cells ml^{-1}). Haemolysis was screened on agar medium containing 5 % (v/v) defibrinated human blood. Clearance zones that formed around bacterial spots on the agar plate after 5 days incubation at 28 °C were considered indicative of the above lytic activities.

For polar lipid and fatty acid analyses, strain KMM 329^T and *L. concretionis* KCTC 12205^T were cultivated on R2A agar at 28 °C for 3 days and lipids extracted using the chloroform/methanol method of Bligh & Dyer (1959). Polar lipids were analysed as described by Vaskovsky & Terekhova (1979). Fatty acid methyl esters were obtained by using acid methanolysis as described by Rowe *et al.* (2000). The resultant fatty acid methyl esters were extracted with hexane and analysed using a GLC-MS gas chromatograph (model 6890; Hewlett Packard) equipped with an HP 5 MS 5 % phenylmethyl siloxane capillary column (30 m × 250 μm × 0.25 μm) and connected to a mass spectrometer (model 5973; Hewlett Packard). The photobiotin-labelled DNA probe microplate method of Ezaki *et al.* (1989) was used to determine the DNA relatedness between strain KMM 329^T and *L. concretionis* KCTC 12205^T. The DNA G+C content was determined as described by Marmur & Doty (1962) and Owen *et al.* (1969). The 16S rRNA gene sequence (1516 bp) of strain KMM 329^T was determined as described by Shida *et al.* (1997). The sequence obtained was compared with 16S rRNA gene sequences retrieved from the GenBank/EMBL/DDBJ databases by using the FASTA program (Pearson & Lipman, 1988). Distances were calculated according to the method of Jukes & Cantor (1969). Phylogenetic trees were constructed by using the maximum-likelihood and maximum-parsimony methods with the PHYLIP package (Felsenstein, 1989) and by using the neighbour-joining method of Saitou & Nei (1987) with the CLUSTAL W program (version 1.8; Thompson *et al.*, 1997).

The marine isolate KMM 329^T was a Gram-negative, aerobic, rod-shaped bacterium that was motile by means of a single polar flagellum (Fig. 1). Cells grown on agar media often appear to be weakly motile or non-motile because of a tendency to lose their flagella easily. It should be noted that members of the genus *Lysobacter* were originally described as non-fruiting, gliding bacteria (Christensen & Cook, 1978); at the time of writing, the genus *Lysobacter* includes both gliding and non-gliding species (Table 1). Weon *et al.* (2006) reported that *L. yangpyeongensis* DSM 17635^T was motile, but the details of the flagellar arrangement were not specified. The present study is the first documented report on the occurrence of flagella among *Lysobacter* species. Physiological, biochemical and chemotaxonomic characteristics of strain KMM 329^T are given in Table 1 and the species description and also in Supplementary Table S1 and Supplementary Fig. S1 (available in IJSEM Online). The fatty acid profile of strain KMM 329^T was characterized by the predominance of branched fatty acids 16 : 0 iso (32.5 %), 15 : 0 iso (23.0 %), 11 : 0 iso 3-OH (15.5 %) and 17 : 1 iso (13.2 %), which corresponds with the profiles reported for recognized species of the genus *Lysobacter* (Bae *et al.*, 2005; Weon *et al.*, 2006, 2007; Yassin *et al.*, 2007). No significant distinctive features were found in the fatty acid profile of strain KMM 329^T in comparison with the profiles of *Lysobacter* species, except for the presence of a somewhat larger amount of 11 : 0 iso 3-OH (15.5 %). Unlike *L. concretionis* KCTC 12205^T and *L. defluvii* DSM 18482^T, strain KMM 329^T did not contain 17 : 0 cyclo. The polar lipid profiles of strains KMM 329^T and *L. concretionis* KCTC 12205^T were similar in terms of their major components, including phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol (Supplementary Fig. S1). In addition, *L.*

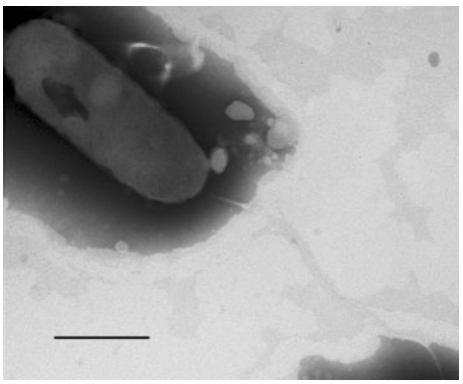

Fig. 1. Transmission electron micrograph of a negatively stained cell of strain KMM 329^T, showing a single polar flagellum. Bar, 0.5 μm.

Table 1. Differential phenotypic characteristics of strain KMM 329^T and species of the genus *Lysobacter*

Strains: 1, KMM 329^T (data from the present study); 2, *L. concretionis* KCTC 12205^T (data from Bae *et al.*, 2005); 3, *L. daejeonensis* GH1-9^T (Weon *et al.*, 2006); 4, *L. defluvii* DSM 18482^T (Yassin *et al.*, 2007); 5, *L. antibioticus* DSM 2044^T; 6, *L. brunescens* DSM 6979^T; 7, *L. enzymogenes* DSM 2043^T; 8, *L. gummosus* DSM 6980^T (data in columns 5–8 from Swings & Christensen, 1989); 9, *L. koreensis* KCTC 12204^T (Lee *et al.*, 2006); 10, *L. niabensis* DSM 18244^T; 11, *L. niastensis* DSM 18481^T (Weon *et al.*, 2007); 12, *L. yangpyeongensis* GH19-3^T (Weon *et al.*, 2006). All strains are positive in API 20NE tests for gelatin hydrolysis and are negative for indole production, glucose fermentation, arginine dihydrolase, urease and for the assimilation of potassium gluconate, capric acid, adipic acid and phenylacetic acid. In API ID 32GN tests, all strains (except for *L. defluvii* DSM 18482^T, for which no data were available) were negative for the assimilation of D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, salicin, L-fucose, capric acid, potassium 2-ketogluconate and 4-hydroxybenzoic acid. +, Positive; −, negative; W, weak reaction; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
DNA G+C content (mol%)	69.0	63.8	61.7	67.1	69.2	67.7	69.0	65.7	68.9	62.5	66.6	67.3
Gliding motility	−	+	−	+	+	+	+	+	ND	−	+	−
Nitrate reduction	−	−	+	−	+	−	−	−	−	−	+	−
β-Galactosidase	−	−	−	−	+	−	+	+	−	−	+	−
Hydrolysis of:												
Aesculin	−	−	+	−	+	+	+	+	−	−	+	−
Starch	−	−	−	−	−	−	−	−	−	+	+	+
Tween 80	+	−	−	−	−	−	−	−	−	ND	ND	−
API 20NE and API ID 32GH tests for assimilation of:												
D-Glucose	−	−	+	−	+	−	+	+	−	−	W	−
D-Mannose	−	−	−	−	+	−	+	+	−	−	−	−
N-Acetylglucosamine	−	−	−	−	+	−	+	+	−	−	+	−
Maltose	−	−	+	−	+	−	+	+	−	−	+	−
Sucrose	−	−	−	ND	−	−	+	+	−	−	−	−
Melibiose	−	−	−	ND	−	−	+	+	−	−	−	−
L-Rhamnose, L-arabinose, D-sorbitol, D-mannitol, 3-hydroxybenzoic acid, propionic acid	−	−	−	−	−	−	−	−	+	−	−	−
Malic acid	−	−	−	−	+	−	+	−	−	−	−	−
Trisodium citrate	−	−	−	+	−	−	−	−	+	−	−	−
Sodium acetate	−	+	+	ND	+	−	W	W	−	−	−	−
Glycogen	−	+	+	ND	+	−	+	+	−	−	−	+
Potassium 5-ketogluconate	+	−	−	ND	−	−	−	−	−	−	−	−
L-Serine	−	−	−	ND	+	−	+	+	+	−	−	−
L-Proline	−	+	−	ND	+	−	+	+	−	−	−	−
L-Histidine	−	−	−	ND	+	−	W	−	−	−	+	−
3-Hydroxybutyric acid	−	+	+	ND	+	−	+	+	−	−	−	+
Valeric acid	−	+	+	ND	+	−	+	W	+	−	−	−
Enzyme activities (API ZYM)*												
α-Galactosidase	−	−	−	−	−	−	+	+	ND	ND	ND	−
α-Glucosidase	−	−	+	−	−	+	+	−	ND	ND	ND	+
β-Glucosidase	−	−	+	−	+	+	+	+	ND	ND	ND	+
N-Acetyl-β-glucosaminidase	−	−	−	−	−	−	−	−	ND	ND	ND	+

*API ZYM test results for *L. defluvii* DSM 18482^T, *L. antibioticus* DSM 2044^T, *L. brunescens* DSM 6979^T, *L. enzymogenes* DSM 2043^T and *L. gummosus* DSM 6980^T were obtained from the study of Yassin *et al.* (2007).

concretionis KCTC 12205^T contained minor amounts of an unknown lipid and an unknown glycolipid. The DNA G+C content of strain KMM 329^T was 69.0 mol%, which is within the range of values (61.7–69.2 mol%) reported for recognized *Lysobacter* species. This value is close to those determined for *L. enzymogenes*, *L. antibioticus* and *L. koreensis* strains (69.0, 69.2 and 68.9 mol%, respectively) but is significantly higher than the values (61.7–63.8 mol%) determined for *L. concretionis*, *L. daejeonensis* and *L. niabensis* (Table 1).

A phylogenetic analysis based on 16S rRNA gene sequences placed strain KMM 329^T within the genus *Lysobacter*, adjacent to *L. concretionis* KCTC 12205^T (Bae *et al.*, 2005) (Fig. 2). The same relationship was confirmed by 16S rRNA gene sequence dendrograms based on different treeing algorithms (Supplementary Fig. S2). Isolate KMM 329^T showed a 16S rRNA gene sequence similarity of 97.0 % with respect to *L. concretionis* KCTC 12205^T and showed 96.4 % similarity or less with respect to other recognized *Lysobacter* species. According to Stackebrandt & Goebel

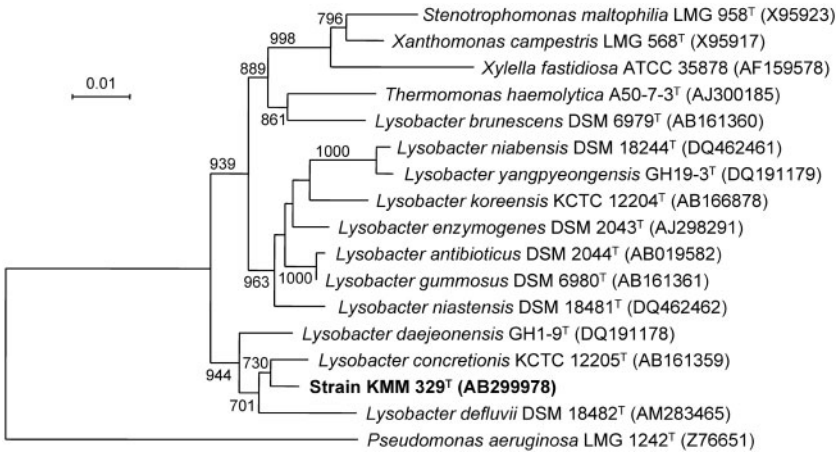

Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between isolate KMM 329^T and species of the genus *Lysobacter*. Numbers at nodes indicate bootstrap values >700 from 1000 resamplings. Bar, 0.01 substitutions per nucleotide position.

(1994), a 16S rRNA gene sequence similarity value of 97.0 % is the threshold for bacterial species differentiation. The relatively low 16S rRNA gene sequence similarities (97.0–95.2 %) obtained for strain KMM 329^T clearly demonstrated that the novel isolate could not belong to any of the recognized *Lysobacter* species. The DNA–DNA relatedness between strain KMM 329^T and *L. concretionis* KCTC 12205^T was determined because they shared a 16S rRNA gene sequence similarity of 97.0 %. The value obtained was 47 %, which is significantly below the value of 70 % proposed by Wayne *et al.* (1987) for species discrimination. On the basis of phenotypic and chemotaxonomic characteristics, 16S rRNA gene sequence similarities and the level of DNA–DNA relatedness, strain KMM 329^T could be considered to represent a separate species of the genus *Lysobacter*.

The phylogenetic distinctness of strain KMM 329^T was supported by its phenotypic characteristics. The differential phenotypic features of strain KMM 329^T and recognized *Lysobacter* species are displayed in Table 1. Isolate KMM 329^T could be distinguished from all of the *Lysobacter* species from its ability to assimilate potassium 5-ketogluconate and its ability to hydrolyse Tween 80. Strain KMM 329^T was similar to *L. niabensis* (Weon *et al.*, 2007) in terms of its carbon-assimilation pattern, but differed in terms of its ability to grow in 5 or 6 % NaCl and its inability to hydrolyse starch. Strain KMM 329^T differed from its close relative *L. concretionis* KCTC 12205^T by its ability to grow at 40–41 °C and in the presence of 5–6 % NaCl, its ability to hydrolyse Tweens 80, 40 and 20 and in its negative reactions for the assimilation of acetate, glycogen, L-proline, 3-hydroxybutyric acid and valeric acid. On the basis of the results obtained, strain KMM 329^T represents a novel species of the genus *Lysobacter*, for which the name *Lysobacter spongiicola* sp. nov. is proposed.

Description of *Lysobacter spongiicola* sp. nov.

Lysobacter spongiicola [spon.gi.i.co'la. L. n. gen. *spongia* sponge; L. suff. *-cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *spongiicola* sponge inhabitant].

Gram-negative, aerobic, oxidase- and catalase-positive rod-shaped bacteria (0.5–0.6 μm wide and 1.3–1.5 μm long), motile by means of single polar flagella. Cells grown on agar media can be observed as non-motile because of their tendency to lose flagella easily. On R2A agar and trypticase soy agar, colonies are yellowish in colour, transparent, smooth and shiny, have regular edges and are 3–4 mm in diameter; on MA or media supplemented with NaCl or natural seawater, colonies are yellow-brownish or brown-reddish in colour. Grows at 5–41 °C, with an optimum at 25–28 °C, but does not grow at 4 or 42 °C. Sodium ions are not required for growth. Growth is observed in 0–6 % (w/v) NaCl. The pH range for growth is pH 5.5–9.5, with an optimum at pH 6.5–8.5. H_2S, ornithine decarboxylase, lysine decarboxylase and phenylalanine deaminase are not produced. Starch, chitin, DNA, adenine, guanine and xanthine are not hydrolysed. Positive for hydrolysis of casein, gelatin, Tweens 80, 40 and 20 and tyrosine. Positive for haemolysis. Acid is not produced from D-glucose, arabinose, mannose, rhamnose, galactose, maltose, fructose, lactose, D-xylose, inositol, mannitol or glycerol. Results from other phenotypic and biochemical tests are listed in Table 1. In addition, in API 50 CH tests, positive results were obtained for the utilization of potassium 5-ketogluconate and D-arabitol and negative results were obtained for the utilization of glycerol, erythritol, D-arabinose, ribose, D- and L-xylose, D-adonitol, methyl β-D-xyloside, galactose, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, mannitol, D-sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, D-lactose, melezitose, trehalose, inulin, raffinose, starch, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- and L-fucose, L-arabitol, gluconate and potassium 2-ketogluconate. In the API ID 32GN test, the assimilation of potassium 5-ketogluconate was weakly positive. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase,

N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Susceptible to the following antibiotics (μg per disc, unless indicated otherwise): erythromycin (15), carbenicillin (100), oleandomycin (15), streptomycin (30), chloramphenicol (30), ampicillin (10), kanamycin (30), neomycin (30), polymyxin B (300 U), ofloxacin (5), rifampicin (5), tetracycline (30), vancomycin (30), nalidixic acid (30) and cephazolin (30). Resistant to the following antibiotics (μg per disc, unless indicated otherwise): benzylpenicillin (10 U), oxacillin (10), lincomycin (15), gentamicin (10) and cephalexin (30). Contains the following fatty acids: 16 : 0 iso (32.5 %), 15 : 0 iso (23.0 %), 11 : 0 iso 3-OH (15.5 %), 17 : 1 iso (13.2 %), 11 : 0 iso (9.5 %), 14 : 0 iso (3.3 %) and 17 : 0 iso (2.8 %). On R2A agar, the following phospholipids are present: phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The DNA G+C content of the type strain is 69.0 mol% (thermal denaturation method).

The type strain, KMM 329^T (=NRIC 0728^T =JCM 14760^T), was isolated from a sponge specimen (*Pachastrella* sp.) collected from the Philippine Sea at a water depth of 750 m.

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