Lewinella antarctica sp. nov., a marine bacterium isolated from Antarctic seawater

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A marine bacterium, designated IMCC3223^T, was isolated from Antarctic coastal seawater and subjected to a polyphasic taxonomic investigation. Cells of the strain were Gram-negative, short to elongated rods that were orange-coloured but negative for flexirubin-type pigments, obligately aerobic, chemoheterotrophic and devoid of gliding or flagellar motility. On the basis of 16S rRNA gene sequence comparisons, the strain was closely related to members of the genus *Lewinella* (86.3–93.1 %) of the family '*Saprospiraceae*' in the phylum *Bacteroidetes*; the most closely related species was *Lewinella lutea* (93.1 % similarity to the type strain). The DNA G+C content of the strain was 50.3 mol% and the major respiratory quinone was MK-7. The predominant cellular fatty acids were $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH (39.4 %), iso-$C_{15:0}$ (15.9 %), $C_{17:1}\omega7c$ (10.5 %) and $C_{15:1}\omega6c$ (8.9 %). 16S rRNA gene sequence analyses and the phenotypic and chemotaxonomic tests allowed the differentiation of strain IMCC3223^T from all recognized species of the genus *Lewinella*. Therefore, strain IMCC3223^T represents a novel species of the genus *Lewinella*, for which the name *Lewinella antarctica* sp. nov. is proposed. The type strain is IMCC3223^T (=KCCM 42688^T =NBRC 103142^T).

The genus *Lewinella* (Sly *et al.*, 1998) belongs to the family '*Saprospiraceae*' within the phylum *Bacteroidetes* and currently contains the six species *Lewinella cohaerens*, *L. nigricans*, *L. persica*, *L. lutea*, *L. marina* and *L. agarilytica*, which were isolated from marine sources. *L. cohaerens*, *L. nigricans* and *L. persica*, isolated from littoral sediment samples and originally assigned to the genus *Herpetosiphon* by Lewin (1970), were later transferred to the new genus *Lewinella* on the basis of data from 16S rRNA gene sequence analyses (Sly *et al.*, 1998). *L. agarilytica* (Lee, 2007) and *L. lutea* (Khan *et al.*, 2007) were isolated from shallow marine sediment samples, whilst *L. marina* (Khan *et al.*, 2007) was isolated from a sea snail. The members of the genus *Lewinella* are generally characterized as being Gram-negative, aerobic, flexirubin-negative, chemoheterotrophic rods or filaments (up to 25 μm long). In the present study, a Gram-negative, chemoheterotrophic, orange-coloured, non-gliding bacterium, designated strain IMCC3233^T, was isolated from Antarctic seawater and was found to represent a novel species of the genus *Lewinella*.

A seawater sample was collected from the coast of King George Island, Weaver Peninsula, western Antarctica (62° 14′ S 58° 47′ W). Strain IMCC3223^T was isolated using a standard dilution-plating method on an oligotrophic medium, R2A agar (Difco), diluted 1 : 10 (v/v) with aged seawater that had been kept at 8 °C for 2 months. After determination of the optimum growth temperature, cultures of IMCC3223^T were maintained routinely on marine agar 2216 (MA; Difco) at 20 °C.

An almost-complete sequence of the 16S rRNA gene (1452 bp) was obtained for strain IMCC3223^T as described previously (Cho & Giovannoni, 2003). Phylogenetic analyses, including multiple alignment of 16S rRNA gene sequences, determination of sequence similarity and generation of phylogenetic trees, were performed using the ARB package (Ludwig *et al.*, 2004) and PAUP* (Swofford, 2002) as described previously (Cho & Giovannoni, 2006). Sequence comparisons in the ARB database and BLASTN hits from GenBank showed that strain IMCC3223^T was closely related to members of the genus *Lewinella*. This Antarctic isolate was most closely related to *L. lutea* NBRC 102634^T (93.1 %) and shared 86.3–93.1 % 16S rRNA gene sequence similarity with respect to other members of the genus *Lewinella*. To clarify the phylogenetic position of the strain, 1296 unambiguously aligned nucleotide positions were used for the phylogenetic analyses. Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) algorithms. The robustness of the topologies for the maximum-likelihood and neighbour-joining trees was evaluated by means of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC3223^T is EF554367.

A transmission electron micrograph of cells of strain IMCC3223^T and details of the cellular fatty acid compositions of strain IMCC3223^T and type strains of species of the genus *Lewinella* are available as supplementary material with the online version of this paper.

bootstrap analysis (Felsenstein, 1985) based on 100 and 1000 resamplings of the sequences, respectively. All of the phylogenetic trees generated in this study (Fig. 1) indicated that the Antarctic marine strain belonged to the genus *Lewinella*. Strain IMCC3223^T formed a coherent clade with *L. lutea* NBRC 102634^T within the phylogenetically well-resolved *Lewinella* clade. This phylogenetic inference, together with the level of 16S rRNA gene sequence similarity (Wayne *et al.*, 1987) between strain IMCC3223^T and the other *Lewinella* species (<94 %), suggested that the strain represented a novel species of the genus *Lewinella*.

The following characteristics were investigated using cultures grown on/in MA or marine broth 2216 at 20 °C according to methods described in a previous study (Choi *et al.*, 2007) and standard methods (Smibert & Krieg, 1994): cell morphology and size; colony morphology, size and colour; flagellar and gliding motilities; cellular and flexirubin-type pigments; ranges and optima of temperature, pH and salinity for growth; and oxidase and catalase activities. The MGC anaerobic system and AnaeroPACK Anaero (Mitsubishi Gas Chemical Company) were used to test for anaerobic growth. Cellular pigments were extracted with acetone/methanol (1 : 1, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (Optizen 2120UV; Mechasis). Basic biochemical tests and carbon-source-oxidation tests were performed using API 20NE and API ZYM strips (bioMérieux) and Biolog GN2 MicroPlates, according to the manufacturers' instructions, except that the strips were inoculated with bacterial suspensions in artificial seawater medium (Choo *et al.*, 2007) and incubated at 20 °C for 3 days. Degradation of macromolecules was tested by incubating the Antarctic strain at 20 °C for 4 weeks on MA containing the following: starch (0.2 %, w/v), casein (10 % skimmed milk, w/v), elastin (0.5 %, w/v), chitin (0.5 %, w/v), agar (1.5 %, w/v) and CM-cellulose (0.2 %, w/v). Hydrolysis was indicated by the formation of clear zones around colonies either immediately or after flooding of the plate with the appropriate staining solution (Teather & Wood, 1982). Ten different antimicrobial agents (listed in the species description) were tested using the diffusion plate method (Jorgensen *et al.*, 1999) on MA incubated for 5 days at 20 °C. The DNA G+C content was determined by means of HPLC (Mesbah *et al.*, 1989). The quinone content was analysed, at the Korean Culture Center of Microorganisms (Seoul, Republic of Korea), using reversed-phase HPLC. Cellular fatty acid methyl esters were prepared from cultures grown on MA for 5 days at 20 °C and analysed, at the Korean Culture Center of Microorganisms, using the MIDI Microbial Identification System. For fatty acid analyses, strain IMCC3223^T, *L. cohaerens* ATCC 23123^T, *L. persica* ATCC 23167^T and *L. nigricans* ATCC 23147^T were grown under the same culture conditions.

Phenotypic characteristics of strain IMCC3223^T are listed in Table 1 and in the species description. Cells of strain IMCC3223^T were Gram-negative, orange-coloured, obligately aerobic, chemoheterotrophic, non-motile, short to elongated long rods (see Supplementary Fig. S1, available in IJSEM Online). Strain IMCC3223^T exhibited a number of phenotypic similarities with respect to species of the genus *Lewinella*, including cell morphology, orange-coloured pigments, an NaCl requirement for growth, obligately aerobic growth, the presence of starch and gelatin hydrolysis, MK-7 as the major quinone and the absence of flexirubin-type pigments and gliding motility. These features of strain IMCC3223^T are typical of members of the genus *Lewinella* (Khan *et al.*, 2007). However, several growth characteristics of IMCC3223^T, such as its psychrotolerant nature, its ability to reduce nitrate and its macromolecule-degradation profile, clearly differentiated this strain from the type strains of *Lewinella* species (Table 1). The major fatty acids found in strain IMCC3223^T, i.e. $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH (39.4 %), $C_{15:0}$ (15.9 %), $C_{17:1}\omega7c$ (10.5 %), $C_{15:1}\omega7c$ (8.9 %) and 10-methyl $C_{19:1}$

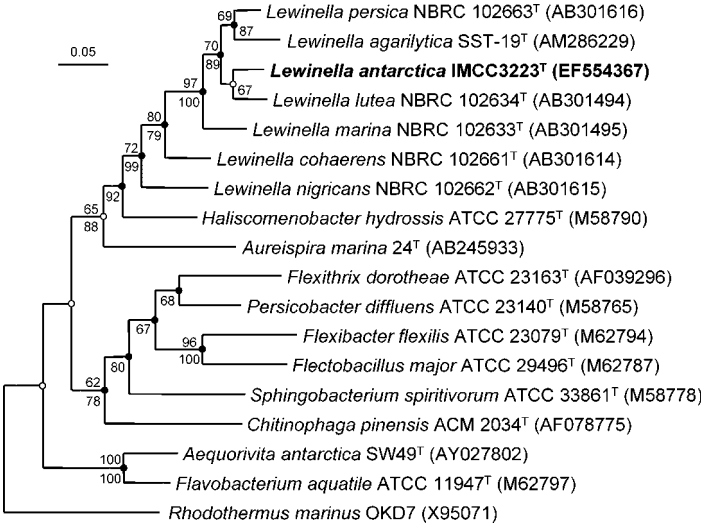

Fig. 1. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain IMCC3223^T and representatives of the phylum *Bacteroidetes*. Bootstrap proportions (>50 %) from both the maximum-likelihood analysis (above nodes) and neighbour-joining analysis (below nodes) are shown. Filled and open circles indicate nodes reproducibly recovered by all three treeing methods and by two treeing methods, respectively. The sequence of *Rhodothermus marinus* OKD7 was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

Table 1. Characteristics that serve to differentiate strain IMCC3223^T from species of the genus *Lewinella*

Strains: 1, strain IMCC3223^T; 2, *L. cohaerens* ATCC 23123^T; 3, *L. nigricans* ATCC 23147^T; 4, *L. persica* ATCC 23167^T; 5, *L. agarilytica* KCTC 12774^T; 6, *L. lutea* NBRC 102634^T; 7, *L. marina* NBRC 102633^T. Data for reference strains were taken from Lee (2007) (*L. agarilytica* KCTC 12774^T) and Khan *et al.* (2007) (other strains). All strains were negative for flexirubin-type pigments, indole production, acid production from glucose, arginine dihydrolase, urea hydrolysis and degradation of chitin. All strains were positive for degradation of starch and gelatin. +, Positive; −, negative; W, weak reaction.

Characteristic	1	2	3	4	5	6	7
Cell shape	Short to elongated rods	Short to elongated rods	Short to elongated rods	Short to elongated rods	Flexible rods or filaments	Short to elongated rods	Short to elongated rods
Colony colour	Orange	Light orange	Black	Dark orange	Light orange	Dark orange	Dull orange
Gliding motility	−	−	−	−	+	−	−
Oxidase	−	+	+	+	+	+	+
Growth at/in:							
4 °C	+	−	−	+	+	−	+
30 °C	−	+	+	+	+	+	+
pH 5.0	+	−	−	−	−	−	−
0.5 % NaCl (w/v)	+	−	−	−	−	−	−
5 % NaCl (w/v)	+	−	−	−	−	−	+
Nitrate reduction	+	−	−	−	−	−	−
β-Galactosidase	W	+	+	+	+	+	+
β-Glucosidase	−	+	+	+	W	+	+
Degradation of:							
Agar	−	−	−	−	+	−	−
Casein	−	+	+	+	+	+	+
CM-cellulose	−	−	−	−	−	+	+
DNA G+C content (mol%)	50.3	45	53	53	51.3	56	61

(4.3 %), also served to distinguish it from members of the genus *Lewinella* (Supplementary Table S1). The low level of 16S rRNA gene sequence similarity (<93.1 %) with respect to species of the genus *Lewinella*, the distinct phylogenetic position (Fig. 1) and the presence of several differentiating phenotypic characteristics (Table 1) demonstrate that strain IMCC3223^T represents a novel species of the genus *Lewinella* (phylum *Bacteroidetes*), for which the name *Lewinella antarctica* sp. nov. is proposed.

Description of *Lewinella antarctica* sp. nov.

Lewinella antarctica (ant.arc′ti.ca. L. fem. adj. *antarctica* southern and, by extension, of the Antarctic, where the type strain was isolated).

Gram-negative, chemoheterotrophic, obligately aerobic, psychrotolerant, oxidase-negative and catalase-positive. Devoid of flagellar and gliding motility. Cells are short to elongated rods, 0.9–1.4 μm wide and 2.4–21.2 μm long. Colonies grown on MA for 5 days at 20 °C are circular, pulvinate, entire, butyrous, orange-coloured and 0.2–0.6 mm in diameter. The temperature range for growth is 3–25 °C, with optimum growth at 20 °C. Growth occurs at pH 5.0–10.0 and 0.5–5.0 % NaCl, optimally at pH 6 and 3.0 % NaCl. Flexirubin-type pigments are not produced. Absorption spectra show peaks for cellular pigments at 472 nm (major peak) and 503 nm. Starch and elastin are degraded, but agar, casein, CM-cellulose and chitin are not degraded. In API 20NE strips, nitrate reduction and hydrolysis of aesculin and gelatin are positive, β-galactosidase activity (substrate *p*-nitrophenyl β-D-galactopyranoside) is weakly positive and arginine dihydrolase, acid production from glucose, indole production and urea hydrolysis are negative. Positive results are obtained (in the API ZYM system) for acid phosphatase, alkaline phosphatase, leucine arylamidase, trypsin and valine arylamidase (weakly positive), but negative results are obtained for α-fucosidase, α-chymotrypsin, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase (substrate 2-naphthyl β-D-galactopyranoside), β-glucosidase, β-glucuronidase, cystine arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), *N*-acetyl-β-glucosaminidase and naphthol-AS-BI-phosphohydrolase. The following carbon substrates are oxidized (Biolog GN2 MicroPlates): α-cyclodextrin, glycogen, cellobiose, i-erythritol, D-galactose, α-D-glucose, α-D-lactose, maltose, D-mannitol, melibiose, D-psicose, L-rhamnose, D-sorbitol, sucrose, trehalose, pyruvic acid methyl ester, acetic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketovaleric acid, succinic acid, succinamic acid, L-alaninamide, L-alanine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-serine, DL-carnitine, γ-aminobutyric acid, thymidine, phenylethylamine, 2-aminoethanol and α-D-glucose 1-phosphate. None of the other carbon substrates in Biolog GN2 MicroPlates is oxidized. Susceptible to ampicillin (10 μg), chloramphenicol

col (25 μg), erythromycin (15 μg), rifampicin (50 μg), streptomycin (10 μg), tetracycline (30 μg) and vancomycin (30 μg), but resistant to gentamicin (10 μg), kanamycin (30 μg) and penicillin G (10 μg, weakly). In cultures grown on MA for 5 days at 20 °C, the cellular fatty acids are $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH, iso-$C_{15:0}$, $C_{17:1}\omega7c$, $C_{15:1}\omega6c$, 10-methyl $C_{19:0}$, iso-$C_{15:1}$, iso-$C_{17:0}$ 3-OH, iso-$C_{16:1}$, iso-$C_{17:1}\omega9c$, $C_{16:0}$, $C_{15:0}\omega8c$ and iso-$C_{15:0}$ 3-OH. The major respiratory quinone is MK-7. The DNA G+C content of the type strain is 50.3 mol%.

The type strain, IMCC3223^T (=KCCM 42688^T =NBRC 103142^T), was isolated from surface seawater from Maxwell Bay, King George Island, western Antarctica.

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