Lewinella agarilytica sp. nov., a novel marine bacterium of the phylum *Bacteroidetes*, isolated from beach sediment

Soon Dong Lee

Correspondence
Soon Dong Lee
sdlee@cheju.ac.kr

Department of Science Education, Cheju National University, Jeju 690-756, Republic of Korea

A Gram-negative, aerobic, light-orange-coloured, marine bacterium (designated SST-19^T) was isolated from beach sediment in Jeju, Korea, and its taxonomic position was determined by means of a polyphasic approach. In a neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain SST-19^T was positioned within the family 'Saprospiraceae', class *Sphingobacteria*, and formed a consistent cluster with *Lewinella persica* ATCC 23167^T (94.7 % sequence similarity). The levels of 16S rRNA gene sequence similarity between the isolate and recognized members of the family ranged from 84 to 89 %, with *Lewinella cohaerens* ATCC 23123^T, *Lewinella nigricans* ATCC 23147^T, *Haliscomenobacter hydrossis* DSM 1100^T and *Saprospira grandis* ATCC 23119^T showing values of 89.0, 88.4, 86.4 and 84.1 %, respectively. Strain SST-19^T required seawater for growth and its cells were unbranched, flexible rods or filaments with gliding motility. The cellular fatty acids consisted mainly of saturated, unsaturated and iso-branched fatty acids, the major components being C_{16:1ω7c} and/or iso-C_{15:0} 2-OH (24.1 %), C_{18:0} (13.5 %), iso-C_{15:0} (11.1 %) and C_{16:0} (10.9 %). The G + C content of the DNA was found to be 51.3 mol%. On the basis of the results from phenotypic and phylogenetic analyses, the isolate represents a novel species of the genus *Lewinella*, for which the name *Lewinella agarilytica* sp. nov. is proposed. The type strain is SST-19^T (=JBRI 2009^T=KCTC 12774^T=JCM 14216^T).

The genus *Lewinella* was proposed by Sly *et al.* (1998) to encompass the marine species of the genus *Herpetosiphon* (Holt & Lewin, 1968). The genus currently contains three species, *Lewinella cohaerens*, *Lewinella persica* and *Lewinella nigricans*, all of which were isolated from marine environments, including beach sand and brown mud. Despite having similar morphological characteristics (Holt, 1989), the genera *Lewinella* and *Herpetosiphon* were found to belong to different phylogenetic lineages, according to data from 16S rRNA gene sequencing studies (Sly *et al.*, 1998). The genus *Herpetosiphon* currently contains the thermo-tolerant freshwater species *Herpetosiphon aurantiacus* (Holt & Lewin, 1968) and *Herpetosiphon geysericola* (Lewin, 1970). This genus is now recognized as belonging to the phylum *Cloroflexi* (Garrity & Holt, 2001). On the other hand, the genus *Lewinella*, together with the genera *Haliscomenobacter* (van Veen *et al.*, 1973) and *Saprospira*

(Gross, 1911), belongs to the phylum *Bacteroidetes* within the class *Sphingobacteria* (Garrity & Holt, 2001) and forms a distinct clade referred to as the family 'Saprospiraceae'. The aim of this paper was to describe the isolation and identification of a Gram-negative, heterotrophic, gliding bacterium designated strain SST-19^T, and to propose that it represents a novel species of the genus *Lewinella*.

Strain SST-19^T was isolated from a sediment sample collected at Samyang Beach on the coast of Jeju, Republic of Korea. A sediment sample was taken at a depth of 30 cm below the surface and was suspended in sterile distilled water. The procedure and medium used for the isolation of bacteria were as described in previous studies (Lee, 2006a, b, c). The isolate was maintained, at –20 and –80 °C, as a glycerol solution supplemented with 60 % (v/v) sterilized natural seawater and 20 % (v/v) distilled water. For the phenotypic and phylogenetic comparisons, the type strains of the three recognized *Lewinella* species, *L. cohaerens* ATCC 23123^T, *L. nigricans* ATCC 23147^T and *L. persica* ATCC 23167^T (all of which were purchased from the American Type Culture Collection) were grown on marine agar (MA; Difco) at 30 °C.

Extraction of chromosomal DNA and amplification and sequencing of the 16S rRNA gene were performed as

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain SST-19^T, *Lewinella cohaerens* ATCC 23123^T, *Lewinella nigricans* ATCC 23147^T and *Lewinella persica* ATCC 23167^T are AM286229, AM295254, AM295255 and AM295256, respectively.

Cellular fatty acid compositions of strain SST-19^T and the type strains of recognized species of the genus *Lewinella* are available as a supplementary table with the online version of this paper.

described previously (Lee, 2007). An almost-complete 16S rRNA gene sequence (1415 nt) for strain SST-19^T was determined in this study. A preliminary BLAST search revealed that the organism belonged to the class *Sphingobacteria* within the phylum *Bacteroidetes* (Garrrity & Holt, 2001) and was related to members of the genus *Lewinella*. To check the accuracy of the sequence, the type strains of the three recognized species of the genus *Lewinella* were also subjected to 16S rRNA gene sequencing and then to a preliminary BLAST search. Each of the sequences of the type strains of the three species of the genus *Lewinella* determined in this study (deposited at GenBank/EMBL/DBJ) showed a match with its own previously published sequence (Sly *et al.*, 1998), but a striking difference was observed between each pair of sequences. The levels of sequence dissimilarity were as follows: *L. cohaerens*, 6.1 % (86 out of 1400 nt), *L. nigricans*, 5.5 % (80 out of 1403 nt) and *L. persica*, 2.8 % (40 out of 1414 nt). Most of these differences were found between positions 92 and 242

(nucleotide numbering according to *Escherichia coli* positions; Brosius *et al.*, 1978) in the sequences of *L. cohaerens*, between positions 92 and 193 in those of *L. nigricans* and between positions 326 and 484 in those of *L. persica*.

The 16S rRNA gene sequence of strain SST-19^T was aligned with those of related genera of the class *Sphingobacteria* by using the CLUSTAL_X program (Thompson *et al.*, 1997) and then manually optimized according to the secondary structure of the *E. coli* 16S rRNA gene. Evolutionary distances were calculated by using the model of Jukes & Cantor (1969) and a phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by bootstrap analysis (Felsenstein, 1985) of 1000 replicates. A neighbour-joining tree (Fig. 1) based on an analysis of the 16S rRNA gene sequences showed that strain SST-19^T was related to members of genus *Lewinella* within the family 'Saprospiraceae' and formed a consistent cluster with *L.*

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequence analysis (Saitou & Nei, 1987), showing the position of strain SST-19^T within the radiation of the class *Sphingobacteria*. *Chlorobium limicola* UdG_6037 (AJ299414) was used as the outgroup. Numbers at branches indicate bootstrap percentages (based on 1000 replicates). Bar, 0.1 substitutions per nucleotide position.

persica ATCC 23167^T (94.7 % sequence similarity), with bootstrap support of 100 %. The levels of sequence similarity between the isolate and *L. cohaerens* ATCC 23123^T, *L. nigricans* ATCC 23147^T, *Haliscomenobacter hydrossis* DSM 1100^T and *Saprospira grandis* ATCC 23119^T were 89.0, 88.4, 86.4 and 84.1 %, respectively. On the other hand, the organism showed sequence similarity values of only 77.5–82.0 % with respect to other representatives of the class *Sphingobacteria*.

A cellular fatty acid analysis of strain SST-19^T and the type strains of the three recognized species of the genus *Lewinella* was carried out, using cells grown on MA for 7 days at 30 °C. Fatty acid methyl esters were extracted and analysed according to the instructions of the Sherlock Microbial Identification System (version 6; MIDI). The G+C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989).

The cellular fatty acid profile of strain SST-19^T consisted mainly of saturated, unsaturated and branched fatty acids. Small amounts of hydroxy and 10-methyl fatty acids were also detected. The predominant fatty acids were summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 24.1 %), C_{18:0} (13.5 %), iso-C_{15:0} (11.1 %) and C_{16:0} (10.9 %). The cellular fatty acid compositions of strain SST-19^T and the type strains of the three recognized species of the genus *Lewinella* are shown in Supplementary Table S1 (available in IJSEM Online). With regard to the fatty acid compositions, considerable heterogeneity was found between the members of the genus *Lewinella*: *L. cohaerens* ATCC 23123^T and *L. nigricans* ATCC 23147^T contain iso-C_{15:0}, iso-C_{15:1} F, C_{18:0} and C_{16:0} as major components, whereas *L. persica* ATCC 23167^T, like strain SST-19^T, contains summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) as an additional major constituent and has a relatively small amount of iso-C_{15:1} F in comparison with the former group. The DNA G+C content of strain SST-19^T was found to be 51.3 mol%.

The morphology of strain SST-19^T was examined with a transmission electron microscope using cells grown for 3 days on MA at 30 °C. Gliding motility was investigated using the hanging drop method with cells grown in 0.1 × marine broth (Difco) for 24 h at 30 °C (Bernardet *et al.*, 2002). Colony pigmentation was checked visually and recorded after 7 days incubation at 30 °C on MA. The requirement for seawater or artificial sea salts (Sigma) for growth was investigated by using the procedure and media reported previously (Lee, 2007). Strain SST-19^T showed growth on yeast extract-malt extract agar (Shirling & Gottlieb, 1966) and nutrient agar (Difco) supplemented with natural seawater, but not with artificial sea salts. The cells were unbranched, sheathed, flexible rods or filaments (0.4–0.6 × 1.5–5.0 µm) that consisted of three or four cells, each of 1–2 µm in length (Fig. 2). Motility by means of flagella was not observed, but cells were capable of motility by gliding. Strain SST-19^T formed colonies that were light orange in colour and smooth and convex with entire edges.

Fig. 2. Transmission electron micrograph of a cell of strain SST-19^T grown on MA for 3 days at 30 °C. Bar, 0.5 µm.

The colonies reached diameters of 1–2 mm after 7 days incubation.

The physiological and biochemical properties of strain SST-19^T and the type strains of the three recognized species of the genus *Lewinella* were examined using MA as the basal medium and incubation for 7 days at 30 °C, unless indicated otherwise. The results of the morphological, physiological and biochemical tests are given in Table 1 and the species description. Temperature (4–42 °C) and initial pH (pH 4.1–12.1) ranges for growth were determined. Tolerance of NaCl and artificial sea salts (Sigma) for growth were studied on MA using NaCl or sea salts at final concentrations of 1–9 % (w/v) and 0.5–7.0 % (w/v), respectively. Gram stain, catalase and oxidase activities and the degradation of casein, starch and DL-tyrosine were tested as described previously (Lee, 2006c). Hydrolysis of cellulose and chitin was tested on MA supplemented with 0.5 % CM-cellulose or 0.5 % colloidal chitin. Additional degradation tests involving hypoxanthine and xanthine were performed using MA as the basal medium, as described by Gordon *et al.* (1974). The results from the degradation tests were recorded after incubation for 14 days at 30 °C. Tests for other physiological and biochemical characteristics were performed using API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions, but the results for assimilation tests in the API 20NE strips were recorded after incubation for 5 days at 30 °C. The cells were grown on MA for 2 days at 30 °C and suspended in a solution of 1 % (w/v) sea salts before inoculation. Characteristics that serve to differentiate between strain SST-19^T and the type strains of

Table 1. Differential characteristics of strain SST-19^T (*Lewinella agarilytica* sp. nov.) and the type strains of recognized species of the genus *Lewinella*

Strains: 1, SST-19^T; 2, *L. cohaerens* ATCC 23123^T; 3, *L. nigricans* ATCC 23147^T; 4, *L. persica* ATCC 23167^T. Data were taken from Lewin (1970), Holt (1989) and this study. All were positive or weakly positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and *N*-acetyl- β -glucosamidase, but negative for lipase (C14), α -chymotrypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase (API ZYM). With the API 20NE strip, aesculin degradation, gelatin hydrolysis and β -galactosidase gave positive results for all strains, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease were negative for all strains. All assimilated D-glucose, D-mannose, *N*-acetyl-D-glucosamine and maltose, but not D-arabinose, D-mannitol, gluconate, caprate or phenylacetate. All showed growth at an initial pH of 7.1–8.1 and a temperature of 25–30 °C, and tolerated 1 % NaCl on MA. Degradation of cellulose, chitin, hypoxanthine and xanthine was not observed in any of the strains. All required seawater for growth. +, Positive; –, negative; w, weak.

Characteristic	1	2	3	4
Cell size (μ m)	0.4–0.6 $\times$ 1.5–5.0	0.7 $\times$ 60–150	0.7 $\times$ 30–150	0.5 $\times$ 5–50
Pigment of colony	Light orange	Light orange	Yellow	Orange
Growth on MA at/in:				
pH 5.1	+	–	–	–
pH 10.1	+	–	–	–
4 °C	+	–	–	–
10 °C	+	–	–	+
37 °C	+	–	+	–
3 % NaCl	–	–	+	–
Degradation of:				
Agar	+	–	–	–
Casein	+	–	+	–
Starch	+	–	–	–
DL-Tyrosine	+	–	+	–
API ZYM				
Esterase lipase (C8)	–	–	–	w
Trypsin	w	–	w	w
Naphthol-AS-BI-phosphohydrolase	–	–	+	–
α -Glucosidase	w	–	–	w
β -Glucosidase	w	–	–	+
Assimilation of (API 20NE):				
Adipate	–	–	w	–
Malate	+	+	+	–
Citrate	w	+	+	–
DNA G + C content (mol%)	51.3	44.9	53.1	52.6
Source of isolation	Beach sediment	Beach sand	Beach sand	Brown mud

recognized species of the genus *Lewinella* are given in Table 1.

On the basis of the phenotypic and molecular genetic data presented here, strain SST-19^T represents a novel species of the genus *Lewinella*, for which the name *Lewinella agarilytica* sp. nov. is proposed.

Description of *Lewinella agarilytica* sp. nov.

Lewinella agarilytica (a.ga.ri.ly'ti.ca. Malayan n. *agar* agar, gelling polysaccharides from seaweed; N.L. n. *agarum* agar; N.L. adj. *lyticus* -a -um from Gr. adj. *lutikos* dissolving; N.L. fem. adj. *agarilytica* agar-dissolving).

Aerobic, Gram-negative, catalase-positive, oxidase-positive and heterotrophic. Cells are unbranched, sheathed, flexible rods or filaments (0.4–0.6 $\times$ 1.5–5.0 μ m) that contain 3–4

individual cells that are each 1–2 μ m in length. Long filaments (10–150 μ m) are not observed. Gliding motility is observed. Colonies are opaque, convex, circular and light orange in colour. Temperature range for growth is 4–37 °C, with optimum growth at 30 °C. No growth is observed at 42 °C. Growth occurs at pH 5.1–10.1, with optimum growth occurring under alkaline conditions (pH 8.1–9.1). Seawater is required for growth. Growth occurs on MA containing 1 % NaCl or 0.5–1 % artificial sea salts. Agar, casein and starch are hydrolysed. DL-Tyrosine is decomposed. Positive or weakly positive for assimilation of malate and citrate. Adipate is not assimilated. In API ZYM tests, trypsin, α -glucosidase and β -glucosidase activities are weakly positive. Negative for esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase activities. Major cellular fatty acids are C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH (24.1 %), C_{18:0} (13.5 %), iso-C_{15:0} (11.1 %) and C_{16:0}

(10.9%). The DNA G+C content of the type strain is 51.3 mol%.

The type strain, SST-19^T (=JBRI 2009^T=KCTC 12774^T=JCM 14216^T), was isolated from beach sediment on the coast of Jeju Island, Republic of Korea.

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