Oceanobacillus locisalsi sp. nov., isolated from a marine solar saltern

Soo-Young Lee,¹ Tae-Kwang Oh,¹ Wonyong Kim² and Jung-Hoon Yoon¹

Correspondence

Jung-Hoon Yoon

jhyoon@kribb.re.kr

¹Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, Republic of Korea

²Department of Microbiology, College of Medicine, Chungang University, 221 Heukseok-dong, Seoul, Republic of Korea

A Gram-stain-variable, motile, moderately halophilic bacterial strain, CHL-21^T, was isolated from a marine solar saltern and its taxonomic position was investigated using a polyphasic approach. Optimal growth of strain CHL-21^T occurred at 30–37 °C, at pH 7.0–7.5 and in the presence of 5–10 % (w/v) NaCl. In phylogenetic trees based on 16S rRNA gene sequences, strain CHL-21^T fell within the cluster comprising members of the genera *Oceanobacillus*, *Ornithinibacillus* and *Paucisalibacillus*. Strain CHL-21^T exhibited 97.1–97.2 % 16S rRNA gene sequence similarity to the type strains of the two subspecies of *Oceanobacillus oncorhynchi* and 92.0–94.7 % 16S rRNA gene sequence similarity to the type strains of other members of the genus *Oceanobacillus* and members of the genera *Ornithinibacillus* and *Paucisalibacillus*. Mean DNA–DNA reassociation values between strain CHL-21^T and the type strains of the two subspecies of *Oceanobacillus oncorhynchi* were 19–21 %. The cell-wall peptidoglycan of strain CHL-21^T was based on meso-diaminopimelic acid, MK-7 was the predominant menaquinone, and anteiso-C_{15:0} and anteiso-C_{17:0} were the major fatty acids. The DNA G+C content was 39.8 mol%. Differential phenotypic properties, including facultatively anaerobic growth and acid production from substrates, together with its phylogenetic and genetic distinctiveness, demonstrated that strain CHL-21^T is distinguishable from recognized *Oceanobacillus* species. On the basis of data presented, strain CHL-21^T represents a novel species within the genus *Oceanobacillus*, for which the name *Oceanobacillus locisalsi* sp. nov. is proposed; the type strain is CHL-21^T (=KCTC 13253^T =CCUG 56608^T).

The genus *Oceanobacillus* was first proposed by Lu *et al.* (2001) with the description of a single species, *Oceanobacillus iheyensis*. Subsequently, four *Oceanobacillus* species with validly published names, *Oceanobacillus oncorhynchi* (Yumoto *et al.*, 2005), *Oceanobacillus chironomi* (Raats & Halpern, 2007), *Oceanobacillus profundus* (Kim *et al.*, 2007) and *Oceanobacillus caeni* (Nam *et al.*, 2008), have been reported; a further species, *Oceanobacillus picturae* (Lee *et al.*, 2006), was described by the reclassification of *Virgibacillus picturae* (Heyrman *et al.*, 2003). In this study, the taxonomic characterization of an *Oceanobacillus*-like bacterium, strain CHL-21^T, which was isolated from a marine solar saltern, is reported.

Sediment samples were collected from a marine solar saltern (35° 41' 59" N 126° 34' 33" E) of the Yellow Sea, Korea, and used for the isolation of bacterial strains. Strain CHL-21^T was isolated by the dilution plating technique at 25 °C on a modified S-G medium (Sehgal & Gibbons,

1960) containing (l⁻¹ distilled water): 100 g NaCl; 20 g MgSO₄·7H₂O; 2 g KCl; 3 g trisodium citrate; 10 g yeast extract; and 7.5 g Casamino acids (pH 7.2). The type strains of the two subspecies of *Oceanobacillus oncorhynchi* and of two other species of the genus *Oceanobacillus* were used as reference strains for DNA–DNA hybridization and/or phenotypic characterization. *Oceanobacillus oncorhynchi* subsp. *oncorhynchi* JCM 12661^T was obtained from the Japan Collection of Microorganisms (JCM), Saitama, Japan. *Oceanobacillus oncorhynchi* subsp. *incaldanensis* DSM 16557^T, *Oceanobacillus chironomi* DSM 18262^T and *Oceanobacillus iheyensis* DSM 14371^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. To investigate morphological, physiological and biochemical characteristics, strain CHL-21^T was routinely cultivated at 30 °C on another modified S-G medium (MSG), which contained (l⁻¹ distilled water): 50 g NaCl; 20 g MgSO₄·7H₂O; 2 g KCl; 3 g trisodium citrate; 10 g yeast extract; and 7.5 g Casamino acids (pH 7.2). Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. Flagellation was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CHL-21^T is EU817570.

determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures. For this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and grids were examined after being air-dried. The Gram reaction was performed by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MSG agar [i.e. MSG medium containing 1.5 % (w/v) agar] and on MSG agar supplemented with 0.1 % (w/v) potassium nitrate, both of which had been prepared anaerobically under a nitrogen atmosphere. Growth at various temperatures (4–50 °C) was measured on MSG agar. The pH range for growth was determined in MSG broth adjusted to various pH (4.5–9.5, at intervals of 0.5) by the addition of HCl or Na₂CO₃. Growth at various NaCl concentrations [0–30 % (w/v), in increments of 1.0 %] was investigated by using liquid media prepared according to the formula of the MSG medium except that NaCl was excluded. Growth in the absence of NaCl was also investigated using trypticase soy broth, in the presence and absence of 0.45 % (w/v) MgCl₂·6H₂O, prepared according to the formula of the Difco medium except that NaCl was excluded. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Nitrate reduction and hydrolysis of aesculin, gelatin, urea and Tweens 20, 40, 60 and 80 were tested as described by Lányi (1987) by using artificial seawater (Bruns *et al.*, 2001) for preparation of media. Hydrolysis of casein, hypoxanthine, starch and xanthine was tested on MSG agar using the substrate concentrations described by Cowan & Steel (1965). Acid production from carbohydrates was determined according to the method of Leifson (1963). Susceptibility to various antibiotics was investigated on MSG plates by using antibiotic discs of the following: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), chloramphenicol (100 µg), kanamycin (30 µg), lincomycin (15 µg), neomycin (30 µg), oleandomycin (15 µg), carbenicillin (100 µg), tetracycline (30 µg), rifampicin (5 µg) and erythromycin (22.5 µg). Enzyme activities were determined by using the API ZYM system (bioMérieux).

Cell biomass for DNA extraction and for analysis of cell-wall and isoprenoid quinones was obtained from cultures grown in MSG broth at 30 °C. Chromosomal DNA was isolated and purified as described by Yoon *et al.* (1996) with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed according to the method described by Yoon *et al.* (2003). The isomer type of the diamino acid in the cell-wall peptidoglycan was analysed using TLC according to the method described by Komagata & Suzuki (1987). Isoprenoid quinones were

extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain CHL-21^T, *Oceanobacillus chironomi* DSM 18262^T, *Oceanobacillus iheyensis* DSM 14371^T, *Oceanobacillus oncorhynchi* subsp. *incaldanensis* DSM 16557^T and *Oceanobacillus oncorhynchi* subsp. *oncorhynchi* JCM 12661^T was harvested after incubation for 3 days at 30 °C on marine agar 2216 (Difco). Fatty acids were extracted and fatty acid methyl esters were prepared by the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined according to the procedure of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the mean of the remaining three values was quoted as the DNA–DNA reassociation value.

Morphological, cultural, physiological and biochemical characteristics of strain CHL-21^T are given in the species description (see below) or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain CHL-21^T determined in this study comprised 1520 nt. A comparative 16S rRNA gene sequence analysis and estimation of phylogenetic relationships showed that strain CHL-21^T was closely related to members of the genera *Oceanobacillus*, *Ornithinibacillus* and *Paucisalibacillus* (Fig. 1). It exhibited 16S rRNA gene sequence similarity values of 97.1–97.2 % to the type strains of the two subspecies of *Oceanobacillus oncorhynchi*, 93.1–94.7 % to the type strains of the other species of the genus *Oceanobacillus*, and 92.0–93.3 % to the type strains of two species of the genus *Ornithinibacillus* and *Paucisalibacillus globulus*. Mean DNA–DNA reassociation values between strain CHL-21^T and the type strains of the subspecies of *Oceanobacillus oncorhynchi* were 19–21 % (19 % for *O. oncorhynchi* subsp. *oncorhynchi* JCM 12661^T; 21 % for *O. oncorhynchi* subsp. *incaldanensis* DSM 16557^T).

Strain CHL-21^T contained *meso*-diaminopimelic acid as diamino acid in the cell-wall peptidoglycan and MK-7 as the predominant menaquinone. The cellular fatty acid profile of strain CHL-21^T is shown in Table 2, together with those of related species of the genus *Oceanobacillus*. Strain CHL-21^T had large amounts of branched fatty acids; the major components (>10 % of total fatty acids) were anteiso-C_{15:0} and anteiso-C_{17:0}. The fatty acid profile of strain CHL-21^T was similar to those of other species of the genus *Oceanobacillus* in that anteiso-C_{15:0} is the predominant fatty acid, although there were differences in the proportions of some fatty acids, particularly iso-C_{15:0} and

Table 1. Differential phenotypic characteristics of strain CHL-21^T and the type strains of phylogenetically related species of the genus *Oceanobacillus*

Strains: 1, *Oceanobacillus locisalsi* sp. nov. CHL-21^T; 2, *O. chironomi* DSM 18262^T (data from this study and Raats & Halpern, 2007); 3, *O. iheyensis* DSM 14371^T (data from this study and Lu *et al.*, 2001); 4, *O. oncorhynchi* subsp. *incaldanensis* DSM 16557^T (data from this study and Romano *et al.*, 2006); 5, *O. oncorhynchi* subsp. *oncorhynchi* JCM 12661^T (data from this study and Yumoto *et al.*, 2005). All strains are positive for acid production from cellobiose, D-glucose, maltose, D-mannose and D-ribose, but negative for urease (data from this study). +, Positive; –, negative; w, weakly positive reaction.

Characteristic	1	2	3	4	5
Cell size (µm)	0.6–1.0 × 1.3–4.0	0.8–1.0 × 1.3–3.0	0.6–0.8 × 2.5–3.5	0.5–0.8 × 1.2–2.0	0.4–0.6 × 1.1–1.4
Colony colour	Cream–beige	Cream–beige	Cream–white	Beige	White
Spore formation	+	+	+	–	+
Anaerobic growth	+	–	–	–	+
Temperature range (°C) (optimum)	10–45 (30–37)	12–46 (37)	15–42 (30)	10–40 (37)	15–40 (30–36)
NaCl range (%) for growth (optimum)	0–25 (5–10)	0–11 (1–3)	0–21 (3)	5–20 (10)	0–22 (7)
pH range for growth (optimum)	6.0–9.0 (7.0–7.5)	6.5–10.0 (8.5)	6.5–10.0 (7.0–9.5)	6.5–9.5 (9.0)	9–10
Nitrate reduction*	+	+	–	+	+
Hydrolysis of:*					
Gelatin	–	+	+	–	+
Aesculin	+	w	+	+	+
Casein	–	+	+	+	+
Tween 80	+	+	+	w	+
Acid production from:*					
L-Arabinose	+	+	+	+	–
D-Galactose	–	+	–	+	+
D-Fructose	+	–	+	+	+
Lactose	–	+	–	–	–
Melibiose	–	+	–	+	+
Melezitose	+	–	–	+	+
Raffinose	–	w	–	+	+
L-Rhamnose	–	–	w	–	–
Trehalose	+	w	–	+	+
Sucrose	+	–	–	+	+
D-Xylose	+	+	+	+	–
myo-Inositol	+	–	–	+	+
D-Mannitol	+	–	+	+	+
D-Sorbitol	+	–	–	–	+
DNA G + C content (mol%)	39.8	38.1	35.8	40.1	38.5

*Data from this study.

anteiso-C_{17:0} (Table 2). The DNA G + C content of strain CHL-21^T was 39.8 mol%.

Strain CHL-21^T was distinguishable from phylogenetically related species of the genus *Oceanobacillus* by differences in several phenotypic characteristics, as listed in Table 1. The phylogenetic and genetic distinctiveness and differential phenotypic properties of strain CHL-21^T are sufficient to categorize it as a member of a species that is distinct from recognized species of the genus *Oceanobacillus* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, based on the data presented, strain CHL-21^T is considered to represent a novel species of the genus *Oceanobacillus*, for which the name *Oceanobacillus locisalsi* sp. nov. is proposed.

Description of *Oceanobacillus locisalsi* sp. nov.

Oceanobacillus locisalsi (lo.ci.sal'si. L. masc. n. *locus* place; L. adj. *salsus* salted, salt; N.L. gen. n. *locisalsi* of a salt place).

Cells are Gram-stain-variable rods (0.6–1.0 × 1.3–4.0 µm). Motile by means of a single polar flagellum. Central ellipsoidal endospores are observed in swollen sporangia. Colonies are circular, slightly convex, smooth, cream–beige in colour and 0.5–1.0 mm in diameter after incubation for 3 days at 30 °C on MSG agar. Mg²⁺ ions are required for growth. Growth under anaerobic conditions is observed on MSG agar and on MSG agar supplemented with nitrate. Catalase-positive and oxidase-negative. Tweens 20, 40 and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain CHL-21^T, other species of the genus *Oceanobacillus* and members of some other related taxa. Filled circles indicate nodes that were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bootstrap values (expressed as percentages of 1000 replications) >50% are shown at branch points. The outgroup was *Alicyclobacillus acidocaldarius* DSM 446^T (GenBank accession no. X60742). Bar, 0.01 substitutions per nucleotide position.

60 are hydrolysed, but hypoxanthine, xanthine and starch are not. Indole is not produced. Susceptible to penicillin G, ampicillin, cephalothin, novobiocin, tetracycline, rifampicin, chloramphenicol, lincomycin, oleandomycin and carbenicillin, but not to polymyxin B, streptomycin, gentamicin, kanamycin, neomycin or erythromycin. In assays with the API ZYM system, leucine arylamidase, trypsin and α -chymotrypsin are present, but alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, acid phosphatase, α -galactosidase,

β -glucuronidase, α -glucosidase, β -galactosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diamino acid. The predominant menaquinone is MK-7. The major fatty acids (>10% of total fatty acids) are anteiso- $C_{15:0}$ and anteiso- $C_{17:0}$. Other phenotypic characteristics are given in Table 1.

The type strain is CHL-21^T (=KCTC 13253^T =CCUG 56608^T), isolated from a marine solar saltern of the Yellow Sea, Korea. The DNA G+C content of the type strain is 39.8 mol%.

Table 2. Cellular fatty acid composition (%) of strain CHL-21^T and the type strains of phylogenetically related species of the genus *Oceanobacillus*

Strains: 1, *Oceanobacillus locisalsi* sp. nov. CHL-21^T; 2, *O. chironomi* DSM 18262^T; 3, *O. iheyensis* DSM 14371^T; 4, *O. oncorhynchi* subsp. *incaldanensis* DSM 16557^T; 5, *O. oncorhynchi* subsp. *oncorhynchi* JCM 12661^T. Data from this study. tr, Trace amount (<0.5%); –, not detected.

Fatty acid	1	2	3	4	5
Straight-chain					
$C_{14:0}$	tr	tr	0.6	–	–
$C_{15:0}$	–	0.6	tr	–	–
$C_{16:0}$	2.3	0.7	1.6	1.1	0.9
$C_{18:0}$	1.2	–	0.6	–	–
Unsaturated					
$C_{16:1\omega 7c}$ alcohol	–	–	6.1	tr	tr
Branched					
iso- $C_{14:0}$	1.7	19.5	20.3	19.5	14.6
iso- $C_{15:0}$	7.3	1.5	32.5	11.8	8.3
anteiso- $C_{15:0}$	46.2	47.4	20.3	26.3	36.8
iso- $C_{16:0}$	6.7	20.3	12.1	27.4	25.8
iso- $C_{17:0}$	4.0	tr	1.8	4.1	1.8
anteiso- $C_{17:0}$	30.4	9.7	3.0	9.6	11.7

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