Oceanobacillus profundus sp. nov., isolated from a deep-sea sediment core

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A halotolerant, facultatively alkaliphilic bacterium, designated CL-MP28^T, was isolated from the surface of a sediment core sample collected at a depth of 2247 m in the Ulleung Basin of the East Sea, Korea. Phylogenetic analysis of the 16S rRNA gene sequence of strain CL-MP28^T revealed an affiliation with the genus *Oceanobacillus*. The sequence similarities between the isolate and type strains of members of the genus *Oceanobacillus* were in the range 95.0–96.0 %, indicating that strain CL-MP28^T represented a novel species within the genus. The strain was found to be Gram-positive, rod-shaped and motile by means of peritrichous flagella and was shown to produce ellipsoidal spores. The strain was strictly aerobic and able to grow with NaCl at concentrations in the range 0–14 % (w/v) at pH 7.5. The strain grew at temperatures of 15–42 °C and at pH 6.5–9.5. The major fatty acids were anteiso-C_{15:0} (64.9 %), anteiso-C_{17:0} (11.9 %) and iso-C_{16:0} (7.7 %). The major isoprenoid quinone was MK-7. The DNA G + C content was 40.2 mol%. According to the 16S rRNA gene sequence, DNA–DNA relatedness and physiological data and the fatty acid composition, CL-MP28^T could be assigned to the genus *Oceanobacillus*, but is distinguishable from the recognized species of this genus. Strain CL-MP28^T therefore represents a novel species within the genus *Oceanobacillus*, for which the name *Oceanobacillus profundus* sp. nov. is proposed. The type strain is CL-MP28^T (= KCCM 42318^T = DSM 18246^T).

The genus *Oceanobacillus* was first proposed with the single species *Oceanobacillus iheyensis*, which was isolated from a deep-sea mud sample collected at a depth of 1050 m on the Iheya Ridge of the Nansei Islands and demonstrated an extremely halotolerant and facultatively alkaliphilic phenotype (Lu *et al.*, 2001). The genus description was later emended with the isolation of a halotolerant obligate alkaliphile, *Oceanobacillus oncorhynchi*, isolated from the skin of a rainbow trout (*Oncorhynchus mykiss*) (Yumoto *et al.*, 2005). Recently, [*Virgibacillus*] *picturæ*, isolated from deteriorated mural paintings (Heyrman *et al.* 2003), was reclassified as *Oceanobacillus picturæ* (Lee *et al.*, 2006). Thus, the genus *Oceanobacillus* currently comprises three species, comprising motile, Gram-positive rods that produce ellipsoidal endospores within swollen sporangia (Lee *et al.*, 2006).

In this study, a novel strain, CL-MP28^T, was isolated from the surface of a sediment core sample collected at depth of 2247 m in the Ulleung Basin of the East Sea, Korea. The sediment slurry was spread on a plate containing marine agar 2216 (Difco) and incubated at 20 °C for 2 weeks. Strain CL-MP28^T was isolated on the plate and subsequently purified on marine agar 2216 at 30 °C four times. The strain was maintained at –80 °C both on marine agar 2216 at 4 °C and in marine broth 2216 (Difco) supplemented with 30 % (v/v) glycerol.

The 16S rRNA gene was amplified from a single colony by a PCR with *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified using an AccuPrep PCR purification kit (Bioneer). Sequencing of the 16S rRNA gene was performed with an Applied Biosystems automatic sequencer (ABI3730XL) at MacroGen Corp. (Seoul, Korea). An almost-complete 16S rRNA gene sequence (1354 bp) of strain CL-MP28^T was obtained. This sequence was compared with those available in GenBank by using BLASTN (Altschul *et al.*, 1990) searches. The sequence of strain CL-MP28^T was manually aligned with those of the type strains of the three *Oceanobacillus* species and with those of the type species of other genera in the family *Bacillaceae*, obtained from GenBank and the

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CL-MP28^T is DQ386635.

Details of the whole-cell fatty acid compositions of strain CL-MP28^T and related *Oceanobacillus* species are available in a supplementary table in IJSEM Online.

Ribosomal Database Project (Cole *et al.*, 2003) databases using known 16S rRNA secondary structure information. Phylogenetic trees were obtained by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of the tree topologies was assessed by using bootstrap analyses based on 1000 replications (for the neighbour-joining and maximum-parsimony methods) or 100 replications (for the maximum-likelihood method). Alignment analyses were carried out using the jPHYDIT program (Jeon *et al.*, 2005), and phylogenetic analysis was carried out using MEGA3 (Kumar *et al.*, 2004) and PAUP* 4.0 (Swofford, 1998). Likelihood parameters were estimated by means of the hierarchical ratio tests in MODELTEST, version 3.04 (Posada & Crandall, 1998). The sequence similarities indicated that the closest relatives of strain CL-MP28^T were *O. iheyensis* JCM 11309^T (95.96 %) and *O. oncorhynchi* R-2^T (95.48 %). Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain CL-MP28^T formed a robust cluster with species of the genus *Oceanobacillus* (Fig. 1). Thus, it is clear that our isolate belongs to the genus *Oceanobacillus*. However, the low levels of similarity (95.0–96.0 %) between the 16S rRNA gene sequence of the novel isolate and those of previously described species of the genus *Oceanobacillus* indicated that strain CL-MP28^T represents a novel species of the genus (Stackebrandt & Goebel, 1994; Rosselló-Mora & Amann, 2001). The relatedness of the genomic DNA was determined by means of dot-blot hybridization. Probe DNA labelling was performed using a nick translation kit (Roche), and hybridization and detection were done using the DIG labelling and detection kit (Roche) according to the manufacturer's instruction. The level of DNA–DNA relatedness between strain CL-MP28^T and *O. iheyensis* KCTC 3954^T was 24.7 %, while that for *O. oncorhynchi* R-2^T was 17.5 %. These values are below the currently accepted limit for DNA relatedness (70 %) for the phylogenetic definition of a species (Stackebrandt & Goebel, 1994) and

therefore provide evidence that our isolate (CL-MP28^T) represents a novel species of the genus *Oceanobacillus*. The DNA G + C content was determined by HPLC analysis (Tamaoka & Komagata, 1984) at the Korean Culture Center of Microorganisms (Seoul, Korea) and was found to be 40.2 mol%.

For the phenotypic and chemotaxonomic analyses, strain CL-MP28^T was routinely cultivated on PYA medium (1 % peptone, 0.5 % yeast extract, 0.1 % K₂HPO₄, 0.02 % MgSO₄·7H₂O, 3 % NaCl, 1.5 % Bacto agar, pH 7.5; Lu *et al.*, 2001). Gram-staining was performed as described by Smibert & Krieg (1994). The cell morphology was examined using phase-contrast microscopy and transmission electron microscopy (EX2; JEOL) with cells grown at 30 °C on a PYA agar plate. Electron microscopy showed that the cells were peritrichously flagellated rods (0.2–0.4 × 0.8–2 µm). Anaerobic growth was checked on PYA using the GasPak anaerobic system (BBL) for 12 days. Hydrolysis of casein, DNA and Tweens 40, 60 and 80 and catalase and oxidase activities were determined according to the protocols described by Barrow & Feltham (1993). Other enzyme activities were also assayed using the API 20NE system (bioMérieux) according to the manufacturer's instruction, except that cell suspensions were prepared using a 3 % (w/v) NaCl solution. Acid production from various carbohydrates was determined by employing the API 50 CH system (bioMérieux) according to the manufacturer's instructions. All suspension media were supplemented with 3 % NaCl (final concentration). For the API 20NE and API 50 CH analyses, *O. iheyensis* KCTC 3954^T was used as a reference strain. The temperature range for growth was determined using PYA broth (the same composition as PYA but without the agar) incubated at various temperatures in the range 5–40 °C (using increments of 5 °C) and at 40–45 °C (using increments of 1 °C). PYA broth and PYA broth containing 100 mM NaHCO₃/Na₂CO₃ were used, respectively, for growth experiments at pH 6.0–8.5 and pH 9.0–10.5. Final pH was adjusted with HCl or NaOH solution. The NaCl concentrations allowing growth of strain CL-MP28^T were

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain CL-MP28^T with respect to *Oceanobacillus* species and related taxa. Only bootstrap percentages above 60 % are shown (1000 resamplings) at branching points. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 nucleotide substitutions per site.

determined by using PYA broth supplemented with various NaCl concentrations (0, 1, 2, 3, 5, 7, 8, 10, 13, 15, 18, 20, 21, 22 and 25 %, w/v). All media were sterilized with sterile 0.22 µm pore-size syringe filters (Adventec MFS). Growth at different pHs, NaCl concentrations and temperatures was measured by monitoring changes in OD₆₀₀ over time. All of the experiments were performed under aerobic conditions. The isolate was revealed to be Gram-positive and to produce ellipsoidal spores positioned terminally within swollen sporangia. The acid production from amygdalin demonstrated by strain CL-MP28^T differentiates it from the recognized species of the genus *Oceanobacillus*; furthermore, the species of the genus can be clearly distinguished from each other using a minimal combination of phenotypic

characteristics (β -galactosidase and gelatinase) (Table 1). The morphological, physiological and biochemical characteristics of the isolate are given in Table 1 and in the species description.

Isoprenoid quinones were isolated according to Minnikin *et al.* (1984) and analysed by HPLC as described by Collins (1985). The major isoprenoid quinone in CL-MP28^T is MK-7. The fatty acid methyl esters present in whole cells grown on PYA at 27 °C for 48 h were analysed by gas chromatography according to the instructions of the Microbial Identification System (MIDI) at the Korean Culture Center of Microorganisms. The fatty acid profile of CL-MP28^T was dominated by branched fatty acids: anteiso-C_{15:0} (64.9 %),

Table 1. Differential phenotypic characteristics of strain CL-MP28^T and related *Oceanobacillus* species

Strains: 1, CL-MP28^T (this study); 2, *O. iheyensis* KCTC 3954^T (unless indicated, data from Lu *et al.*, 2001); 3, *O. oncorhynchi* R-2^T (Yumoto *et al.*, 2005); 4, *O. picturae* KCTC 3821^T (Heyrman *et al.*, 2003). +, Positive; –, negative; w, weakly positive; vw, very weakly positive; ND, not determined; All strains are motile, Gram-positive, oxidase- and catalase-positive and produce ellipsoid spores and are negative for indole production. None of the strains produced acid from D-arabinose, L-rhamnose, *myo*-inositol, L-fucose or 5-keto-D-gluconate.

Characteristic	1	2	3	4
Spore position	Terminal	Subterminal or terminal	Subterminal	Terminal
Anaerobic growth	–	–	+	–
Temperature range (°C) (optimum)	15–42 (35)	15–42 (30)	15–40 (30–36)	5–40 (25–35)
NaCl range (%) for growth (optimum)	0–14 (1–3)	0–21 (3)	0–22 (7)	Weak growth without added salt (5–10)
pH range for growth (optimum)	6.5–9.5 (7.5–8.5)	6.5–10 (7.0–9.5)	9–10	7–10*
Urease	–	–	+†	–
Nitrate reductase	+	–	+	+
β -Galactosidase	+	–†	–	+
Hydrolysis of:				
Gelatin	+	+	–	vw
Aesculin	+	+	+†	w
Casein	+	+	–	+
Tween 40	w	+	+	w†
Tween 60	+	+	–	+†
Tween 80	–	+†	–	–†
Acid production from:				
Glycerol	w	+	–	w
Galactose	–	–	–	w
D-Glucose	+	+	+	w
D-Fructose	+	+	+	w
D-Mannose	+	+	+	w
Mannitol	+	w	–	w
N-Acetylglucosamine	+	w	–	w
Amygdalin	+	–	–	–
Maltose	+	+	+	–
D-Melibiose	–	–	+	w
D-Trehalose	+	–	+	w
Glycogen	–	–	–	w
D-Turanose	w	w	–	w
DNA G+C content (mol%)	40.2	36	38.5	40

*Data from Lee *et al.* (2006).

†Data from this study: *O. oncorhynchi* R-2^T was tested with API 20NE, using a suspension medium supplemented with 7 % NaCl (at pH 10).

anteiso-C_{17:0} (11.9%), iso-C_{16:0} (7.7%), iso-C_{14:0} (5.6%) and iso-C_{15:0} (3.8%) (see Supplementary Table S1 available in IJSEM Online). The relative proportions of the predominant fatty acids in CL-MP28^T were different from those of *O. iheyensis* KCTC 3954^T and *O. oncorhynchi* R-2^T, but were relatively similar to those of *O. picturae* KCTC 3821^T. However, the absence of summed feature 7 (C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t), the relatively low optimal NaCl concentration for growth and the lack of growth at temperatures below 15 °C of strain CL-MP28^T differentiate it from *O. picturae* KCTC 3821^T.

Some phenotypic characteristics of strain CL-MP28^T, e.g. acid production from amygdalin and a relatively low optimal NaCl concentration for growth, clearly distinguish the novel isolate from previously described species of the genus *Oceanobacillus*. Furthermore, the DNA–DNA relatedness data supported our suggestion that strain CL-MP28^T should be classified within a novel species within the genus *Oceanobacillus* (Stackebrandt & Goebel, 1994). In conclusion, phylogenetic analyses based on 16S rRNA gene sequences, DNA–DNA relatedness and physiological and chemotaxonomic features suggest that strain CL-MP28^T represents a novel species of the genus *Oceanobacillus*, for which the name *Oceanobacillus profundus* sp. nov. is proposed.

Description of *Oceanobacillus profundus* sp. nov.

Oceanobacillus profundus (pro.fun'dus. L. masc. adj. *profundus* from the deep).

Cells are Gram-positive, peritrichously flagellated, straight rods that are approximately 0.2–0.4 × 0.8–2 µm in size and produce ellipsoidal spores terminally positioned within swollen sporangia. Colonies are circular and creamy white in colour. Obligately aerobic or facultatively alkaliphilic. Grows at 15–42 °C (optimum 35 °C) and pH 6.5–9.5 (optimum pH 7.5–8.5). Catalase- and oxidase-positive. The NaCl range for growth is 0–14% (w/v) at pH 7.5 (optimum 1–3%). Nitrate reductase, β-galactosidase (ONPG) and DNase activities are present and hydrolysis of gelatin, aesculin, casein and Tweens 40 and 60 is demonstrated. Indole production, urease and hydrolysis of Tween 80 are absent. Acids are produced from glycerol, D-xylose, D-glucose, D-fructose, D-mannose, mannitol, N-acetylglucosamine, amygdalin, maltose, lactose, D-trehalose and D-turanose, but not from D-arabinose, galactose, L-rhamnose, myo-inositol, D-melibiose, glycogen or L-fucose. The DNA G + C content is 40.2 mol%. The major fatty acids are anteiso-C_{15:0} (64.9%), anteiso-C_{17:0} (11.9%), iso-C_{16:0} (7.7%), iso-C_{14:0} (5.6%), iso-C_{15:0} (3.8%) and C_{16:0} (2.5%). The major isoprenoid quinone is MK-7.

The type strain, CL-MP28^T (=KCCM 42318^T=DSM 18246^T), was isolated from the surface of a sediment core sample collected at a depth of 2247 m in the Ulleung Basin of the East Sea, Korea.

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