Bacillus litoralis sp. nov., isolated from a tidal flat of the Yellow Sea in Korea

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A Gram-variable, motile, endospore-forming, slightly halophilic bacterial strain, designated SW-211^T, was isolated from a tidal flat of the Yellow Sea in Korea, and was characterized taxonomically by using a polyphasic approach. The organism grew optimally at 37 °C and in the presence of 2–3 % NaCl. Comparative 16S rRNA gene sequence analysis showed that strain SW-211^T forms a distinct phylogenetic lineage within the radiation of the cluster comprising *Bacillus* species. Strain SW-211^T had cell-wall peptidoglycan based on meso-diaminopimelic acid. The predominant menaquinone was MK-7 and the major fatty acids were anteiso-C_{15:0} (34.8 %), iso-C_{15:0} (15.6 %), iso-C_{16:0} (12.5 %) and iso-C_{14:0} (10.0 %). The DNA G + C content was 35.2 mol%. Strain SW-211^T exhibited levels of 16S rRNA gene sequence similarity of <96.2 % with respect to the type strains of recognized *Bacillus* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain SW-211^T (=KCTC 3898^T=DSM 16303^T) was classified in the genus *Bacillus* as the type strain of a novel species, for which the name *Bacillus litoralis* sp. nov. is proposed.

Tidal sediments have been utilized as excellent sources for isolating novel and useful micro-organisms (Yi *et al.*, 2003; Yoon *et al.*, 2003a). Some new genera or species have recently been isolated from tidal sediments in Korea (Yi *et al.*, 2003; Yoon *et al.*, 2003a, b, 2004a, b). In the course of screening novel micro-organisms from a tidal flat near Daepo beach in the Yellow Sea in Korea, many moderately halophilic or halotolerant bacteria have been isolated and characterized taxonomically. Of these isolates, a rod-shaped, endospore-forming bacterial strain, SW-211^T, is described here. Its exact taxonomic position was determined by using a polyphasic approach that included phenotypic properties and phylogenetic analysis based on 16S rRNA gene sequences.

Strain SW-211^T was isolated by a standard dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM). Flagellum type was examined by TEM using cells from exponentially growing cultures. Gram-reaction was determined by using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate,

both of which had been prepared anaerobically using nitrogen. The pH range for growth was determined in marine broth 2216 (MB; Difco) that was adjusted to various pH values (initial pH 4.5–9.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization by the addition of HCl or Na₂CO₃. Growth at various NaCl concentrations was investigated in MB or trypticase soy broth (Difco). Growth in the absence of NaCl was investigated in trypticase soy broth lacking NaCl. Growth at various temperatures (4–50 °C) was measured on MA. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tween 80, tyrosine and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were determined as described by Lanyi (1987). H₂S production was tested as described by Bruns *et al.* (2001). Acid production from carbohydrates was determined according to the method of Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981), with the substrates supplemented with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Enzyme activity was determined by using the API ZYM system (bioMérieux). Other physiological and biochemical tests were performed with the API 20E system (bioMérieux). Morphological, cultural, physiological and biochemical characteristics of strain SW-211^T are shown in Table 1 or are given in the species description.

Cell biomass for DNA extraction and for cell-wall and isoprenoid quinone analyses was obtained from cultivation

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SW-211^T is AY608605.

Table 1. Phenotypic characteristics of *Bacillus litoralis* sp. nov. and some related *Bacillus* species

Species: 1, *B. litoralis* sp. nov.; 2, *B. fastidiosus* (data from Claus & Berkeley, 1986); 3, *Bacillus cibi* (Yoon *et al.*, 2005); 4, *Bacillus indicus* (Suresh *et al.*, 2004); 5, *Bacillus horikoshii* (Nielsen *et al.*, 1995; Li *et al.*, 2002; Logan *et al.*, 2002); 6, *Bacillus halmapalus* (Nielsen *et al.*, 1995; Li *et al.*, 2002; Logan *et al.*, 2002); 7, *Bacillus cohnii* (Spanka & Fritze, 1993; Suresh *et al.*, 2004); 8, *Bacillus cereus* (Claus & Berkeley, 1986; Priest *et al.*, 1988); 9, *Bacillus flexus* (Claus & Berkeley, 1986; Suresh *et al.*, 2004); 10, *Bacillus megaterium* (Claus & Berkeley, 1986; Reva *et al.*, 2002; Täubel *et al.*, 2003); 11, *Bacillus simplex* (Priest *et al.*, 1988; De Clerck *et al.*, 2004). +, Positive; –, negative; ND, not determined; NG, no growth; v, variable. Data in parentheses are for the type strain. Cells of all species are rod-shaped and catalase-positive.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Spore shape*	E	E	E	ND	E	E	E	E	ND	E	E or C
Spore position†	C, S or T	ND	C or S	S	S	C or S	T	C	ND	C	C, S or T
Oxidase	+	ND	+	ND	ND	ND	+	V	ND	–	–
Anaerobic growth	–	–	–	–	–	–	ND	+	ND	–	–
Growth at/in:											
5 % (w/v) NaCl	+	+	+	–	+	–	+	+	+	+	+
10 % (w/v) NaCl	+	–	+	–	–	–	–	V	ND	–	–
10 °C	+	+	+	–	+	+	+	V	ND	+	ND
45 °C	+	ND	+	–	–	–	+	ND	ND	ND	ND
Nitrate reduction	–	–	–	–	–	–	+	+	–	–	+
Hydrolysis of:											
Aesculin	+	ND	+	+	+	+	(–)	+	–	+	–
Casein	+	–	+	ND	+	+	V(+)	+	ND	+	+
Gelatin	+	–	–	+	+	+	+	+	–	+	+
Starch	+	–	+	+	+	+	+	V	ND	+	+
Tween 80	+	ND	+	ND	–	–	V(+)	V	ND	ND	V
Growth on:											
D-Galactose	+	ND	+	ND	–	–	ND	ND	ND	(–)	ND
D-Cellobiose	+	ND	+	+	ND	ND	(–)	ND	+	(–)	V
D-Mannose	–	ND	+	+	V	+	(–)	ND	–	(–)	–
Sucrose	+	ND	+	+	ND	ND	(–)	ND	+	(–)	ND
Maltose	+	ND	+	+	ND	ND	(–)	ND	+	(+)	–
Pyruvate	+	ND	+	–	ND	ND	(+)	ND	–	(–)	ND
Acid production from:											
D-Glucose	+	NG	+	–	+	+	(–)	+	+	+	+
Lactose	+	ND	–	–	–	–	(–)	V	–	V	–
D-Mannitol	–	NG	–	–	+	–	(–)	–	–	+	V
D-Mannose	–	ND	+	+	V	+	(–)	V	–	V	V
D-Xylose	+	NG	–	–	V	V	(–)	–	–	+	–
DNA G + C content (mol%)	35.2	34.3–35.1 (35.1)	45	41.2	41.1–42.0	38.6	33.9–35.0	31.7–40.1 (35.7 or 36.2)	38.4	36.5–45.0 (37.3 or 37.6)	40–41

*C, Cylindrical; E, ellipsoidal or oval.

†C, Central or paracentral; S, subterminal; T, terminal.

in MB at 37 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the modification 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 by Yoon *et al.* (1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003a). The isomer type of diamino acid of the cell-wall peptidoglycan was determined by the method of Komagata & Suzuki (1987). Isoprenoid quinones were extracted and

analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. For fatty acid methyl ester analysis, a loop of cell mass was harvested from MA plates after cultivation for 2 days at 37 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

An almost complete 16S rRNA gene sequence of strain SW-211^T comprising 1505 nt (approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence) was analysed. Comparative 16S rRNA gene sequence analyses showed that strain SW-211^T is phylogenetically affiliated to *Bacillus* species. The phylogenetic tree based on the neighbour-joining algorithm showed that strain SW-211^T fell within the radiation of the cluster comprising *Bacillus* species and joined *Bacillus fastidiosus* with a bootstrap resampling value of 98.0 % (Fig. 1). Strain SW-211^T exhibited 16S rRNA gene sequence similarity levels of 96.2 % to the type strain of *B. fastidiosus* and lower than 95.3 % with respect to the type strains of other recognized *Bacillus* species. Sequence similarities to all other species included in the phylogenetic analysis were below 91.4 % (Fig. 1). Strain SW-211^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant isoprenoid quinone found in strain SW-211^T was unsaturated menaquinone with seven isoprene units (MK-7). Major components (>5 %) of the fatty acids detected in strain SW-211^T were branched fatty acids anteiso-C_{15:0} (34.8 %), iso-C_{15:0} (15.6 %), iso-C_{16:0} (12.5 %), iso-C_{14:0} (10.0 %) and anteiso-C_{17:0} (6.4 %) and straight-chain fatty acid C_{16:0} (5.5 %). The DNA G + C content of strain SW-211^T was 35.2 mol%.

The results obtained from the phenotypic and phylogenetic characterizations indicated that strain SW-211^T belongs to the genus *Bacillus*. The phylogenetic distinctiveness (16S

rRNA gene sequence similarity of <97 %) confirms that strain SW-211^T represents a species distinct from recognized *Bacillus* species (Stackebrandt & Goebel, 1994). There were some phenotypic differences between strain SW-211^T and several phylogenetically related *Bacillus* species (Table 1). Therefore, on the basis of the data presented, strain SW-211^T should be classified in the genus *Bacillus* as a member of a novel species, for which the name *Bacillus litoralis* sp. nov. is proposed.

Description of *Bacillus litoralis* sp. nov.

Bacillus litoralis (li.to.ra'lis. L. masc. adj. *litoralis* of the shore).

Cells are aerobic rods, 0.8–1.0 × 2.5–4.5 µm in size. Gram-variable. Motile by means of peritrichous flagella. Central, subterminal or terminal ellipsoidal endospores are observed in swollen sporangia. Colonies grown on MA are circular to slightly irregular, smooth, slightly raised, yellowish white in colour and 2–3 mm in diameter after 2 days of incubation at 37 °C; colony colour becomes dark from the centre as cultures age. Optimal growth temperature is 37 °C; growth occurs at 4 and 45 °C but not above 46 °C. Optimal pH for growth is 7.5. Growth is observed at pH 5.5 but not at pH 5.0. Optimal growth occurs in the presence of 2–3 % (w/v) NaCl; growth does not occur without NaCl or in the presence of >11 % (w/v) NaCl. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. Catalase-positive. Urease-negative. Hypoxanthine, tyrosine and xanthine are not hydrolysed. H₂S and indole are not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Acid is produced from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, sucrose and D-trehalose. Acid is not produced from adonitol, D-sorbitol, *myo*-inositol or maltose. The following substrates are utilized for growth: L-arabinose, D-fructose, D-glucose, D-trehalose, D-xylose, lactose, acetate and L-malate. Benzoate, citrate, formate, succinate or L-glutamate are not utilized. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acids are anteiso-C_{15:0} (34.8 %), iso-C_{15:0} (15.6 %), iso-C_{16:0} (12.5 %) and iso-C_{14:0} (10.0 %). The DNA G + C content is 35.2 mol% (determined by HPLC).

The type strain, SW-211^T (= KCTC 3898^T = DSM 16303^T), was isolated from a tidal flat near Daepo beach of the Yellow Sea in Korea.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of SW-211^T, some *Bacillus* species and representatives of other related taxa based on 16S rRNA gene sequence analysis. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

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