Oceanobacillus kapialis sp. nov., from fermented shrimp paste in Thailand

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A Gram-positive, rod-shaped, strictly aerobic, spore-forming, moderately halophilic bacterium, designated strain SSK2-2^T, was isolated from fermented shrimp paste (ka-pi) produced in Thailand. It contained MK-7 as the predominant menaquinone and meso-diaminopimelic acid in the cell-wall peptidoglycan. The isolate grew at 8–43 °C, pH 6–9 and in 0.5–24 % (w/v) NaCl (optimum, 6–14 % NaCl). The major cellular fatty acids were anteiso-C_{15:0} and anteiso-C_{17:0}. Phosphatidylglycerol and diphosphatidylglycerol were the major polar lipid components. The DNA G + C content was 39.7 mol%. Comparative 16S rRNA gene sequence analyses showed that strain SSK2-2^T was most closely related to *Oceanobacillus picturae* KCTC 3821^T with 98.7 % sequence similarity. Based on phenotypic and molecular features combined with DNA–DNA hybridization results (≤ 24.9 % with *O. picturae* KCTC 3821^T), this strain represents a novel species of the genus *Oceanobacillus* for which the name *Oceanobacillus kapialis* sp. nov. is proposed; the type strain is SSK2-2^T (=KCTC 13177^T=PCU 300^T=TISTR 1858^T).

The genus *Oceanobacillus* was first described by Lu *et al.* (2001) to accommodate an aerobic, rod-shaped, spore-forming, halophilic bacterium, *Oceanobacillus iheyensis*. At the time of writing, the genus *Oceanobacillus* comprised: *O. iheyensis* from a deep-sea environment (Lu *et al.*, 2001); *Oceanobacillus caeni* from wastewater (Nam *et al.*, 2008); *Oceanobacillus picturae* from a painting (Heyrman *et al.*, 2003; Lee *et al.*, 2006); *Oceanobacillus profundus* from deep-sea sediment (Kim *et al.*, 2007); *Oceanobacillus chironomi* from a chironomid egg mass (Raats & Halpern, 2007); *Oceanobacillus oncorhynchi* subsp. *oncorhynchi* from the skin of a rainbow trout (Yumoto *et al.*, 2005); and *O. oncorhynchi* subsp. *incaldanensis* from an algal mat (Romano *et al.*, 2006). To date, the numerous reports on halophilic bacteria have revealed novel isolates with distinct properties from various salty environments resulting in a proliferation in the number of genera of moderately halophilic bacteria, including *Marinococcus*

(Hao *et al.*, 1984), *Bacillus* (Ventosa *et al.*, 1989), *Amphibacillus* (Niimura *et al.*, 1990; Zhilina *et al.*, 2001), *Halobacillus* (Spring *et al.*, 1996), *Tetragenococcus* (Satomi *et al.*, 1997), *Virgibacillus* (Heyndrickx *et al.*, 1998), *Gracilibacillus* (Wainø *et al.*, 1999), *Filobacillus* (Schlesner *et al.*, 2001), *Oceanobacillus* (Lu *et al.*, 2001), *Lentibacillus* (Yoon *et al.*, 2002), *Paraliobacillus* (Ishikawa *et al.*, 2002), *Halolactibacillus* (Ishikawa *et al.*, 2005), *Cerasibacillus* (Nakamura *et al.*, 2004), *Pontibacillus* (Lim *et al.*, 2005), *Tenuibacillus* (Ren & Zhou, 2005a), *Salinibacillus* (Ren & Zhou, 2005b), *Alkalibacillus* (Jeon *et al.*, 2005), *Thalassobacillus* (García *et al.*, 2005) and *Piscibacillus* (Tanasupawat *et al.*, 2007). Here, the isolation, phenotypic and chemotaxonomic characteristics, DNA–DNA relatedness and phylogenetic properties of a novel moderately halophilic isolate, designated SSK2-2^T, from fermented shrimp paste (ka-pi), which is fermented mainly from shrimp with a high concentration of NaCl (20–30 %, w/v) (Phithakpol *et al.*, 1995; Tanasupawat & Komagata, 2001), are described.

Strain SSK2-2^T was isolated from fermented shrimp paste samples collected from a market in Samutsakorn Province in the central part of Thailand. The spread-plate technique was used on agar plates of *Lentibacillus* medium [JCM medium no. 377, pH 7.2, composed of (1⁻¹): 100 g NaCl,

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SSK2-2^T is AB366005.

A table showing the cellular fatty acid compositions of strain SSK2-2^T and related taxa is available as supplementary material with the online version of this paper.

5 g Casamino acids, 5 g yeast extract, 1 g glutamic acid, 2 g KCl, 3 g trisodium citrate, 20 g MgSO₄·7H₂O, 36 mg FeCl₂·4H₂O, 0.36 mg MnCl₂·4H₂O and 20 g agar] (Namwong *et al.*, 2005), which were incubated at 37 °C for 7 days. Liquid cultures of strain SSK2-2^T and *O. picturae* KCTC 3821^T were cultivated in Erlenmeyer flasks containing the same medium and incubated on a rotary shaker. Cell shape, cell size, cell arrangement and colony size were examined using cells grown on JCM medium no. 377 agar at 37 °C for 5 days. The Hucker–Conn modification was used for Gram staining (Hucker & Conn, 1923). Spore formation was examined on Gram-stained specimens. Critical-point-dried cells were observed under a scanning electron microscope. Flagella were examined as described by Forbes (1981). Physiological and biochemical characteristics were analysed as described by Thornley

(1960), Leifson (1963) and Barrow & Feltham (1993). Growth under anaerobic conditions on agar plates with or without nitrate (1 %, w/v) was investigated using a GasPak (BBL) anaerobic jar. Growth was determined at various temperatures (8–50 °C), pH (5.0, 6.0, 7.0, 7.5, 8.0, 9.0 and 10.0) and NaCl concentrations (0–30 %, w/v). At low NaCl concentrations (0–2.0 %, w/v), MgSO₄·7H₂O and KCl were omitted from the test medium. Growth was monitored by measuring culture turbidity at 660 nm (Namwong *et al.*, 2005). Diaminopimelic acid (DAP) type in the peptidoglycan and menaquinones were analysed as described previously (Komagata & Suzuki, 1987). Polar lipids were determined according to the methods of Minnikin *et al.* (1984) and Albert *et al.* (2005). Quantitative analysis of cellular fatty acids was carried out as described previously (Sasser, 1990; Kämpfer &

Table 1. Differential characteristics of strain SSK2-2^T (*O. kapialis* sp. nov.) and strains of *Oceanobacillus* species

Strains: 1, SSK2-2^T (data from this study); 2, *O. picturae* KCTC 3821^T (this study, except where marked); 3, *O. iheyensis* JCM 11309^T (Lu *et al.*, 2001); 4, *O. oncorhynchi* subsp. *oncorhynchi* JCM 12661^T (Yumoto *et al.*, 2005); 5, *O. profundus* KCCM 42318^T (Kim *et al.*, 2007); 6, *O. caeni* KCTC 13061^T (Nam *et al.*, 2008); 7, *O. oncorhynchi* subsp. *incaldanensis* DSM 16557^T (Romano *et al.*, 2006); 8, *O. chironomi* DSM 18262^T (Raats & Halpern, 2007). +, Positive; –, negative; w, weak; NA, not available.

Characteristic	1	2	3	4	5	6	7	8
Colony size (mm)	0.3–2.7	0.1–1.5	NA	2–3	NA	NA	NA	NA
Spores*								
Shape	O	ES†	E	E	E	E	–	E
Position	T	T	T, S	S	T	C	–	T, S
Temperature range for growth (°C)	8–43	8–40	15–42	15–40	15–42	20–45	10–40	12–46
pH range for growth	6.0–9.0	6.0–9.0	6.5–10.0	9.0–10.0	6.5–9.5	6.0–9.0	6.5–9.5	6.5–10.0
NaCl concentration for growth (w/v, %)								
Optimum	6–14	6–12	3	7	1–3	2–5	10	1–3
Range	0.5–24.0	0–22	0–21	0–22	0–14	0–10	5–20	0–11
Hydrolysis of:								
Aesculin	–	–	+	+‡	+	–	+§	w§
Gelatin	+	w	+	–	+	–	+	+
Casein	+	–	+	–	+	NA	+	–
Acid from:								
L-Arabinose	+	–	–	NA	–	+	NA	NA
D-Fructose	+	–	+	+	+	+	NA	NA
Glycerol	–	+	+	NA	+	+	NA	NA
Maltose	+	–	+	+	+	+	NA	NA
D-Mannitol	+	–	w‡	–‡	+	w	NA	NA
D-Mannose	+	+	+	+	+	NA	+§	–§
Melibiose	–	–	–	+	–	w	NA	NA
Methyl α-D-glucoside	–	+	NA	NA	NA	NA	NA	NA
Trehalose	–	–	–‡	+	+	NA	NA	NA
DNA G + C content (mol%)	39.7	40†	36	38.5	40.2	33.6	40.1	38.1

*Abbreviations: E, ellipsoidal; ES, ellipsoidal or spherical endospores; O, oval; C, central; S, subterminal; T, terminal.

†Data from Lee *et al.* (2006).

‡Data from Kim *et al.* (2007).

§Data from Nam *et al.* (2008).

||Data from Romano *et al.* (2006).

Kroppenstedt, 1996). DNA was purified as described by Saito & Miura (1963). The DNA G+C content was determined by using reversed-phase HPLC (Tamaoka & Komagata, 1984). DNA–DNA hybridization was conducted in microdilution-well plates, as reported by Ezaki *et al.* (1989), and detected by using the colorimetric method reported by Tanasupawat *et al.* (2000). The 16S rRNA gene was sequenced as described previously (Seearunruangchai *et al.*, 2004). The determined sequence (1487 bases) was aligned with selected sequences obtained from GenBank/EMBL/DDBJ by using CLUSTAL_X version 1.83 (Thompson *et al.*, 1997). The alignment was edited manually to remove gaps and ambiguous nucleotides prior to the construction of the phylogenetic tree using the neighbour-joining method (Saitou & Nei, 1987) in MEGA version 2.1 (Kumar *et al.*, 2001). The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

The phenotypic and chemotaxonomic characteristics of strain SSK2-2^T, a moderately halophilic, rod-shaped bacterium, were determined. Results are listed in the species description, Table 1 and in Supplementary Table S1 (available in IJSEM Online). Strain SSK2-2^T produced oval terminal endospores in swollen sporangia (Fig. 1) and contained *meso*-DAP as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant isoprenoid quinone was MK-7. The major cellular fatty acids of the strain were anteiso-C_{15:0} (41.2 %) and anteiso-C_{17:0} (31.3 %). The fatty acid profile (see Supplementary Table S1 available in IJSEM Online) comprised C_{14:0} (0.7 %), C_{16:0} (3.9 %), C_{16:1}ω7c alcohol (0.5 %), iso-C_{14:0} (3.8 %), iso-C_{15:0} (1.4 %), iso-C_{16:0} (14.3 %), iso-C_{17:0} (0.8 %) and trace amounts of various summed features. The fatty acid profile was the same as that of *O. picturae* KCTC 3821^T; however, the amounts of C_{16:0}, iso-C_{14:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0} were different from those observed in *O. iheyensis* KCTC 3954^T and *O. oncorhynchi* subsp. *oncorhynchi* JCM 12661^T as reported by Lee *et al.* (2006). The difference in the amounts of fatty acids may be due to

Fig. 1. Scanning electron micrograph of cells of strain SSK2-2^T grown on JCM medium no. 377 at 37 °C. Bar, 1 µm.

the difference in NaCl concentration (0–10 %, w/v, NaCl) in the culture medium as reported by Valderrama *et al.* (1998). Polar lipid analysis revealed the presence of phosphatidylglycerol, diphosphatidylglycerol, an unidentified phospholipid and two unidentified glycolipids. The DNA G+C content was 39.7 mol%. Phenotypic and chemotaxonomic characteristics of strain SSK2-2^T were as described in the genus description (Heyrman *et al.*, 2003; Lee *et al.*, 2006) and, based on the 16S rRNA gene sequence analysis, it was included in a monophyletic cluster consisting of strains of *Oceanobacillus* species as shown in Fig. 2. The 16S rRNA gene sequence similarity values between strain SSK2-2^T and *O. picturae* KCTC 3821^T, *O. profundus* KCCM 42318^T, *O. iheyensis* KCTC 3954^T, *O. oncorhynchi* subsp. *oncorhynchi* JCM 12661^T, *O. oncorhynchi* subsp. *incaldanensis* DSM 16557^T, *O. caeni* KCTC 13061^T and *O. chironomi* DSM 18262^T were 98.7, 96.2, 95.1, 95.0, 94.5, 94.2 and 93.1 %, respectively (Kim *et al.*, 2007; Lee *et al.*, 2006; Lu *et al.*, 2001; Romano *et al.*, 2006; Yumoto *et al.*, 2005; Nam *et al.*, 2008). Furthermore, DNA–DNA hybridization analysis revealed that strain SSK2-2^T showed low DNA–DNA relatedness with *O. picturae* KCTC 3821^T (22.4 %); in the reciprocal analysis, the relatedness between *O. picturae* KCTC 3821^T and strain SSK2-2^T was 24.9 %, indicating that strain SSK2-2^T was unrelated to *O. picturae* KCTC 3821^T (Wayne *et al.*, 1987). On the basis of its colony size, growth temperature, pH range, NaCl tolerance, ability to hydrolyse aesculin, casein and gelatin, ability to produce acid from L-arabinose, D-fructose, glycerol, maltose, D-mannitol, D-mannose and methyl α-D-glucoside, DNA G+C content and DNA–DNA relatedness values, strain SSK2-2^T can be distinguished from *O. picturae* KCTC 3821^T. Therefore, strain SSK2-2^T represents a novel species in the genus *Oceanobacillus*, for which the name *Oceanobacillus kapialis* sp. nov. is proposed.

Description of *Oceanobacillus kapialis* sp. nov.

Oceanobacillus kapialis (ka.pi.a'lis. Korean n. *ka-pi* shrimp paste; N.L. n. *kapium* shrimp paste; L. suff. *-alis* adjectival suffix meaning pertaining to; N.L. masc. adj. *kapialis* pertaining to shrimp paste, the source of isolation).

Cells are Gram-positive, strictly aerobic rods, approximately 0.4–0.5 × 0.8–3.0 µm. Oval endospores are formed terminally in swollen sporangia. Motile by means of peritrichous flagella. Colonies are low convex, smooth, circular and white to cream (0.3–2.7 mm in diameter). Growth occurs at 8–43 °C (optimum, 37 °C), between pH 6 and 9 (optimum, pH 8.0), and in 0.5–24.0 % (w/v) NaCl (optimum, 6–14 % NaCl). Anaerobic growth is not observed in the presence of 1 % (w/v) nitrate. Positive for catalase and oxidase activities and hydrolysis of gelatin and casein, but negative for nitrate reduction, urease, DNase, and hydrolysis of aesculin, Tween 80, tyrosine, starch, xanthine and hypoxanthine. Produces acid from L-arabinose, D-fructose, D-glucose, D-mannitol, D-mannose and maltose, but not from amygdalin, cellobiose, aesculin, D-gluconic acid, glycerol, D-galactose, *myo*-inositol, inulin, lactose, melibiose, melezitose, methyl α-D-glucoside, raffi-

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain SSK2-2^T and strains of related bacterial species. The branching pattern was generated by the neighbour-joining method. Bootstrap values above 60 %, based on 1000 replications, are shown at the nodes. Bar, 0.01 substitutions per nucleotide position.

nose, D-ribose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylose. The diagnostic diamino acid in the cell-wall peptidoglycan is *meso*-DAP. MK-7 is the major menaquinone. The predominant fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. Contains phosphatidylglycerol, diphosphatidylglycerol, an unidentified phospholipid and two unidentified glycolipids.

The type strain is SSK2-2^T (=KCTC 13177^T=PCU 300^T=TISTR 1858^T), isolated from fermented shrimp paste (ka-pi) produced in Thailand. The DNA G+C content of the type strain is 39.7 mol%.

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