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

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Two strains of strictly aerobic, moderately halophilic Gram-positive rods were isolated from fermented shrimp paste ('ka-pi') produced in Thailand. They produced a red pigment and grew optimally in the presence of 5–30 % NaCl. The diagnostic diamino acid in the cell-wall peptidoglycan was *meso*-diaminopimelic acid. The predominant menaquinone was MK-7. The major cellular fatty acid was anteiso-C_{15:0}. Phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids were found to be the major polar lipid components. The DNA G + C content was 41.2–41.6 mol%. Comparative 16S rRNA gene sequence analyses showed that strain PN7-6^T was most closely related to *Lentibacillus salarius* KCTC 3911^T with 96.5 % sequence similarity. On the basis of phenotypic and molecular properties, the two isolates represent a novel species of the genus *Lentibacillus*, for which the name *Lentibacillus kapialis* sp. nov. is proposed. The type strain is PN7-6^T (=JCM 12580^T=PCU 259^T=TISTR 1551^T).

Moderately halophilic, alkaliphilic, and related aerobic endospore-forming, Gram-positive, rod-shaped bacteria, including cocci, have been isolated from various salty environments. These isolates have included members of the genera *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*, *Halolactibacillus* (Ishikawa *et al.*, 2002, 2005), *Cerasibacillus* (Nakamura *et al.*, 2004), *Pontibacillus* (Lim *et al.*, 2005a), *Tenuibacillus* (Ren & Zhou, 2005a), *Salinibacillus* (Ren & Zhou, 2005b), *Alkalibacillus* (Jeon *et al.*, 2005b) and *Thalassobacillus* (García *et al.*, 2005). Several novel species of the genus *Lentibacillus* have been described recently, *Lentibacillus salicampi*, *Lentibacillus*

juripiscarius, *Lentibacillus salarius*, *Lentibacillus lacisalsi* and *Lentibacillus halophilus* (Yoon *et al.*, 2002; Namwong *et al.*, 2005; Jeon *et al.*, 2005a; Lim *et al.*, 2005b; Tanasupawat *et al.*, 2006). A fermented shrimp paste ('ka-pi'), produced in Thailand, contains a high concentration of NaCl which permits the growth of various halophilic micro-organisms (Phithakpol *et al.*, 1995; Tanasupawat & Komagata, 2001). In this study, a red-pigmented novel species isolated from ka-pi was identified as belonging to the genus *Lentibacillus* on the basis of phenotypic and chemotaxonomic characterization and 16S rRNA gene sequence analysis.

The halophilic bacteria investigated in this work were isolated from samples of fermented shrimp paste collected from a market in Nakhonsrithammarat Province in southern Thailand. The spread-plate technique was used on agar plates of JCM medium no. 168; incubation was performed at 37 °C for 7 days. Liquid cultures were cultivated in Erlenmeyer flasks containing the same medium and incubated on a rotary shaker. All media contained 20 % (w/v) NaCl, except when NaCl tolerance was being investigated. Cell shape, size and arrangement and colony size were examined using cells grown on JCM medium no. 168 agar at 37 °C for 5 days. The Hucker–Conn modification was used for Gram staining (Hucker & Conn, 1923). Spore formation was examined using Gram-stained

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PN7-6^T is AB231905.

A scanning electron micrograph of endospore-containing cells of strain PN7-6^T and a table showing strain designations, isolation sources, DNA G + C contents and levels of DNA–DNA relatedness for strains PN5-2 and PN7-6^T and related *Lentibacillus* species are available as supplementary material in IJSEM Online.

specimens and colonies subjected to a temperature of 80 °C for 30 min. Critical-point-dried cells were observed under a scanning electron microscope. The presence of flagella was determined as described by Forbes (1981) and observed by using transmission electron microscopy. The physiological and biochemical characteristics were determined as described by Thornley (1960), Leifson (1963) and Barrow & Feltham (1993), using medium supplemented with 15 % (w/v) NaCl. Growth under anaerobic conditions on agar plates with or without nitrate (1 %, w/v) was investigated using a GasPak (BBL) anaerobic jar. Growth at various temperatures (10–50 °C), pH values (5.0, 6.0, 7.0, 7.5, 8.0 and 9.0) and NaCl concentrations (0–30 %, w/v) was assessed. At the lower NaCl concentrations (0.0–2.0 %, w/v), MgSO₄·7H₂O was omitted from the test medium, while KCl and C₆H₅Na₃O₇·2H₂O (trisodium citrate dehydrate) were added. Growth was monitored by measuring culture turbidity at 660 nm (Namwong *et al.*, 2005).

The presence of *meso*-diaminopimelic acid in the cell wall peptidoglycan and menaquinone profiles were analysed as described previously (Komagata & Suzuki, 1987). Polar lipid profiles were determined according to the methods of Minnikin *et al.* (1984) and Albert *et al.* (2005). Quantitative analysis of the cellular fatty acids was performed as described previously (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). DNA was isolated from cells grown in JCM medium no. 168 broth, supplemented with 15 % (w/v) NaCl and purified according to the method of Saito & Miura (1963). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984), with reversed-phase HPLC. DNA–DNA hybridization was conducted in microdilution-well plates, as reported by Ezaki *et al.* (1989), and was detected by using the colorimetric method described by Tanasupawat *et al.* (2000). The 16S rRNA gene of the novel isolate was amplified, purified and sequenced according to the methods of Seearunruangchai *et al.* (2004). The

Table 1. Characteristics that differentiate strain PN7-6^T from related *Lentibacillus* species

Strains: 1, PN7-6^T; 2, *L. salarius* KCTC 3911^T (data from Jeon *et al.*, 2005a); 3, *L. salicampi* JCM 11462^T (Yoon *et al.*, 2002; Namwong *et al.*, 2005); 4, *L. juripiscarius* JCM 12147^T (Namwong *et al.*, 2005); 5, *L. lacisalsi* KCTC 3915^T (Lim *et al.*, 2005b); 6, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006). +, Positive; –, negative; W, weak; NA, no data available.

Characteristic	1	2	3	4	5	6
Spore shape	Spherical	Spherical/oval	Spherical/oval	Oval	Spherical	Spherical
Pigmentation	Red	–	–	–	–	–
Motility	–	+	+	–	+	+
Colony diameter (mm)	1.2–3.0	NA	0.2–1.3	1.3–3.2	NA	0.2–0.6
Maximum temp. for growth (°C)	45	50	40	45	40	40
NaCl range for growth (%)	5–30	1–20	3–25	3–30	5–25	12–30
Oxidase activity	+	–	+	+	+	+
Nitrate reduction	+	+	+	+	+	–
Hydrolysis of:						
Aesculin	–	+	–	–	–	–
Casein	–	–	+	+	–	–
Tween 80	–	–	+	+	–	–
Acid production from:						
Cellobiose	–	NA	W	–	NA	–
D-Fructose	+	+	–	+	+	–
D-Galactose	+	NA	W	–	NA	–
D-Glucose	+	+	+	+	–	–
Maltose	–	+	W	–	–	–
D-Mannitol	+	W	–	–	–	–
D-Mannose	W	+	W	–	–	–
D-Ribose	+	+	–	+	+	–
Salicin	–	–	W	–	–	–
Sucrose	W	NA	–	W	NA	–
D-Trehalose	–	W	–	–	–	–
D-Xylose	–	+	–	+	W	–
Major fatty acids	anteiso-C _{15:0} , iso-C _{16:0}	anteiso-C _{15:0} , iso-C _{16:0}	anteiso-C _{15:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , iso-C _{16:0}	anteiso-C _{15:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , anteiso-C _{17:0}
DNA G+C content (mol%)	41.6	43	42.4	43.4	44	42

Table 2. Cellular fatty acid contents (%) of strain PN7-6^T and related taxa

Strains: 1, PN7-6^T; 2, *L. salarius* KCTC 3911^T (data from Jeon *et al.*, 2005a); 3, *L. salicampi* JCM 11462^T (Yoon *et al.*, 2002; Namwong *et al.*, 2005); 4, *L. juripiscarius* JCM 12147^T (Namwong *et al.*, 2005); 5, *L. lacisalsi* KCTC 3915^T (Lim *et al.*, 2005b); 6, *L. halophilus* JCM 12149^T (Tanasupawat *et al.*, 2006). NA, No data available.

Fatty acid	1	2	3	4	5	6
Saturated fatty acids:						
C _{14:0}	0.15	0.2	0.2	0.2	NA	1.0
C _{15:0}	0.16	0.3	0.1	0.1	NA	0.4
C _{16:0}	0.71	1.0	0.9	0.8	1.3	5.4
Unsaturated fatty acids:						
C _{16:1} ω7c alcohol	NA	NA	NA	0.6	1.5	NA
Branched fatty acids:						
iso-C _{14:0}	14.8	13.9	7.9	10.2	5.7	1.0
iso-C _{15:0}	6.9	16.5	4.1	4.9	8.0	4.7
anteiso-C _{15:0}	38.3	25.3	48.6	45.1	50.8	53.9
iso-C _{16:0}	23.4	26.5	16.3	20.2	12.0	4.3
iso-C _{17:0}	0.9	4.4	0.9	0.5	1.9	0.8
anteiso-C _{17:0}	14.1	11.5	20.9	16.7	18.2	25.3

sequence determined (1516 bases) was aligned with selected sequences (obtained from GenBank) by using CLUSTAL W, version 1.81 (Thompson *et al.*, 1994). The alignment was manually edited to remove gaps and ambiguous nucleotides prior to the construction of the phylogenetic tree. The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) in MEGA, version 2.1 (Kumar *et al.*, 2001). The confidence values for the branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Two strains of moderately halophilic rods were characterized on the basis of their phenotypic and chemotaxonomic characteristics; the results are listed in the species description and in Tables 1 and 2. In the case of strain PN7-6^T, endospores were observed after high-temperature treatment (80 °C) of a culture for 30 min (see Supplementary Fig. S1 available in IJSEM Online). Flagella were not observed. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The predominant isoprenoid quinone found was MK-7. Polar lipid analysis revealed the presence of phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids, whereas *L. salicampi* JCM 11462^T contained phosphatidylglycerol,

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain PN7-6^T and related bacterial species. Bootstrap percentages above 50 %, based on 1000 replications, are shown at the nodes. Bar, 1 substitution per 100 nucleotide positions.

diphosphatidylglycerol and one unidentified glycolipid (result not shown).

The cellular fatty acids of strain PN7-6^T included anteiso-C_{15:0} (38.3 %), iso-C_{16:0} (23.4 %) and iso-C_{14:0} (14.8 %) (Table 2). On the basis of these characteristics and the data from 16S rRNA gene sequencing, strain PN7-6^T was included in a monophyletic cluster consisting of the *Lentibacillus* species, as shown in Fig. 1 (Yoon *et al.*, 2002; Jeon *et al.*, 2005a; Lim *et al.*, 2005b; Namwong *et al.*, 2005). The values for 16S rRNA gene sequence similarity between strain PN7-6^T and *L. salarius* KCTC 3911^T, *L. salicampi* JCM 11462^T, *L. juripiscarius* JCM 12147^T, *L. lacisalsi* KCTC 3915^T and *L. halophilus* JCM 12149^T were 96.5, 96.3, 96.3, 95.6 and 94.7 %, respectively. In addition, strain PN7-6^T showed 94.5–95.1 % 16S rRNA gene sequence similarity to members of the genus *Virgibacillus* and 90.8–94.2 % sequence similarity to the other related bacteria (Fig. 1). The DNA–DNA hybridization study revealed that strains PN5-2 and PN7-6^T were closely related, exhibiting 98–118 % similarity, but they showed only low levels of DNA–DNA relatedness to *L. salicampi* JCM 11462^T (2.3–16.5 %) and *L. juripiscarius* JCM 12147^T (5.9–16.2 %) (see Supplementary Table S1 available in IJSEM Online), indicating that the two novel strains are unrelated to these *Lentibacillus* species (Wayne *et al.*, 1987). The DNA G + C contents of strains PN5-2 and PN7-6^T were in the range 41.2–41.6 mol%. The two novel strains could be differentiated from *L. salarius* KCTC 3911^T and related species on the basis of pigmentation, motility, maximum growth temperature, NaCl tolerance, oxidase activity, hydrolysis of aesculin, casein and Tween 80, acid production from maltose, D-trehalose and D-xylose and DNA G + C content, as shown in Table 1. Thus, strains PN5-2 and PN7-6^T represent a novel species within the genus *Lentibacillus*, for which we propose the name *Lentibacillus kapialis* sp. nov.

Description of *Lentibacillus kapialis* sp. nov.

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

Cells are Gram-positive, slender-rod-shaped, strictly aerobic, red-pigmented, non-motile and approximately 0.2–0.4 × 0.8–2.5 µm in size. Spherical endospores are formed terminally in swollen sporangia. Flagella are not observed. Colonies are low convex, smooth and circular (0.2–1.3 mm in diameter). Growth occurs at temperatures between 15 and 45 °C (optimum at 37 °C), at pH 5–9 (optimum at pH 7.0) and with 5–30 % NaCl (optimum at 15 % NaCl). Anaerobic growth is not observed in the presence of 1 % (w/v) nitrate. Positive in tests for catalase, oxidase, and urease activity and nitrate reduction. Hydrolyses arginine and gelatin, but not aesculin, casein, Tween 80, tyrosine, starch, xanthine or hypoxanthine. Produces acid from D-fructose, D-galactose, D-glucose, glycerol, D-mannitol, D-mannose, D-ribose, sorbitol and

sucrose, but not from amygdalin, L-arabinose, cellobiose, aesculin, gluconate, *myo*-inositol, inulin, lactose, maltose, melibiose, melezitose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-trehalose or D-xylose. Contains *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. MK-7 is the major menaquinone. The fatty acid profile includes anteiso-C_{15:0} (38.3 %), iso-C_{16:0} (23.4 %) and iso-C_{14:0} (14.8 %). Phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids are predominant in the polar lipid profile. The DNA G + C content of the type strain is 41.6 mol%.

The type strain, PN7-6^T (=JCM 12580^T=PCU 259^T=TISTR 1551^T), was isolated from fermented shrimp paste ('ka-pi') produced in Thailand.

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References

- Albert, R. A., Archambault, J., Rossello-Mora, R., Tindall, B. J. & Matheny, M. (2005). *Bacillus acidicola* sp. nov., a novel mesophilic, acidophilic species isolated from acidic *Sphagnum* peat bogs in Wisconsin. *Int J Syst Evol Microbiol* 55, 2125–2130.
- Barrow, G. I. & Feltham, R. K. A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Forbes, L. (1981). Rapid flagella stain. *J Clin Microbiol* 13, 807–809.
- Garcia, M. T., Gallego, V., Ventosa, V. & Mellado, E. (2005). *Thalassobacillus devorans* gen. nov., sp. nov., a moderately halophilic, phenol-degrading, Gram-positive bacterium. *Int J Syst Evol Microbiol* 55, 1789–1795.
- Hao, M. V., Kocur, M. & Komagata, K. (1984). *Marinococcus* gen. nov., a new genus for motile cocci with *meso*-diaminopimelic acid in the cell wall; and *Marinococcus albus* sp. nov. and *Marinococcus halophilus* (Novitsky and Kushner) comb. nov. *J Gen Appl Microbiol* 30, 449–459.
- Heyndrickx, M., Lebbe, L., Kerster, K., De Vos, P., Forsyth, G. & Logan, N. A. (1998). *Virgibacillus*: a new genus to accommodate *Bacillus panthothenticus* (Proom and Knight 1995). Emended description of *Virgibacillus panthothenticus*. *Int J Syst Bacteriol* 48, 99–106.
- Hucker, G. J. & Conn, H. J. (1923). Method of Gram staining. *Tech Bull NY St Agric Exp Stn* 93, 3–37.
- Ishikawa, M., Ishizaki, S., Yamamoto, Y. & Yamasato, K. (2002). *Paraliobacillus ryukyuensis* gen. nov., sp. nov., a new Gram-positive, slightly halophilic, extremely halotolerant, facultative anaerobe isolated from a decomposing marine alga. *J Gen Appl Microbiol* 48, 269–279.

- Ishikawa, M., Nakajima, K., Itamiya, Y., Furukawa, S., Yamamoto, Y. & Yamasato, K. (2005). *Halolactibacillus halophilus* gen. nov., sp. nov. and *Halolactibacillus miurensis* sp. nov., halophilic and alkaliphilic marine lactic acid bacteria constituting a phylogenetic lineage in *Bacillus* rRNA group 1. *Int J Syst Evol Microbiol* **55**, 2427–2439.
- Jeon, C. O., Lim, J.-M., Lee, G. S., Lee, J.-M., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2005a). *Lentibacillus salarius* sp. nov., isolated from saline sediment in China, and emended description of the genus *Lentibacillus*. *Int J Syst Evol Microbiol* **55**, 1339–1343.
- Jeon, C. O., Lim, J.-M., Lee, J.-M., Xu, L.-H., Jiang, C.-L. & Kim, C.-J. (2005b). Reclassification of *Bacillus haloalkaliphilus* Fritze 1996 as *Alkalibacillus haloalkaliphilus* gen. nov., comb. nov. and the description of *Alkalibacillus salilacus* sp. nov., a novel halophilic bacterium isolated from a salt lake in China. *Int J Syst Evol Microbiol* **55**, 1891–1896.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Komagata, K. & Suzuki, K.-I. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Kumar, S., Tamura, K., Jakobson, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary analysis software. *Bioinformatics* **17**, 1244–1245.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Lim, J.-M., Jeon, C. O., Song, S. M. & Kim, C.-J. (2005a). *Pontibacillus chungwhensis* gen. nov., sp. nov., a moderately halophilic Gram-positive bacterium from a solar saltern in Korea. *Int J Syst Evol Microbiol* **55**, 165–170.
- Lim, J.-M., Jeon, C. O., Song, S. M., Lee, J.-C., Ju, Y. J., Xu, L. H., Jiang, C.-L. & Kim, C.-J. (2005b). *Lentibacillus lacisalsi* sp. nov., a moderately halophilic bacterium isolated from a saline lake in China. *Int J Syst Evol Microbiol* **55**, 1805–1809.
- Lu, J., Nogi, Y. & Takami, H. (2001). *Oceanobacillus iheyensis* gen. nov., sp. nov., a deep-sea extremely halotolerant and alkaliphilic species isolated from a depth of 1050 m on the Iheya Ridge. *FEMS Microbiol Lett* **205**, 291–297.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Nakamura, K., Haruta, S., Ueno, S., Ishii, M., Yokota, A. & Igarashi, Y. (2004). *Cerasibacillus quisquiliarum* gen. nov., sp. nov., isolated from a semi-continuous decomposing system of kitchen refuse. *Int J Syst Evol Microbiol* **54**, 1063–1069.
- Namwong, S., Tanasupawat, S., Smitinont, T., Visessanguan, W., Kudo, T. & Itoh, T. (2005). Isolation of *Lentibacillus salicampi* strains and *Lentibacillus juripiscarius* sp. nov. from fish sauce in Thailand. *Int J Syst Evol Microbiol* **55**, 315–320.
- Niimura, Y., Koh, E., Yanagida, F., Suzuki, K.-I., Komagata, K. & Kozaki, M. (1990). *Amphibacillus xylanus* gen. nov., sp. nov., a facultatively anaerobic sporeforming xylan-digesting bacterium with lacks cytochrome, quinone, and catalase. *Int J Syst Bacteriol* **40**, 297–301.
- Phithakpol, B., Varanyanond, W., Reungmaneeapaitoon, S. & Wood, H. (1995). *The Traditional Fermented Foods of Thailand*. Kuala Lumpur: ASEAN Food Handling Bureau Level 3.
- Ren, P.-G. & Zhou, P.-J. (2005a). *Tenuibacillus multivorans* gen. nov., sp. nov., a moderately halophilic bacterium isolated from saline soil in Xin-Jiang, China. *Int J Syst Evol Microbiol* **55**, 95–99.
- Ren, P.-G. & Zhou, P.-J. (2005b). *Salinibacillus aidingensis* gen. nov., sp. nov. and *Salinibacillus kushneri* sp. nov., moderately halophilic bacteria isolated from a neutral saline lake in Xin-Jiang, China. *Int J Syst Evol Microbiol* **55**, 949–953.
- Saito, H. & Miura, K. I. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim Biophys Acta* **72**, 619–629.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI.
- Satomi, M., Kimura, B., Mizoi, M., Sato, T. & Fujii, T. (1997). *Tetragenococcus muriaticus* sp. nov., a new moderately halophilic lactic acid bacterium isolated from fermented squid liver sauce. *Int J Syst Bacteriol* **47**, 832–836.
- Schlesner, H., Lawson, P. A., Collins, M. D., Weiss, N., Wehmeyer, U., Volker, H. & Thomm, M. (2001). *Filobacillus milensis* gen. nov., sp. nov., a new halophilic spore-forming bacterium with Orn-D-Glu-type peptidoglycan. *Int J Syst Evol Microbiol* **51**, 425–431.
- Seearunruangchai, A., Tanasupawat, S., Keeratipibul, S., Thawai, C., Itoh, T. & Yamada, Y. (2004). Identification of acetic acid bacteria isolated from fruits collected in Thailand. *J Gen Appl Microbiol* **50**, 47–53.
- Spring, S., Ludwig, W., Marquez, M. C., Ventosa, A. & Schleifer, K.-H. (1996). *Halobacillus* gen. nov., with description of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophila* comb. nov. *Int J Syst Bacteriol* **46**, 492–496.
- Tamaoka, J. S. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tanasupawat, S. & Komagata, K. (2001). Lactic acid bacteria in fermented foods in Southeast Asia. In *Microbial Diversity in Asia. Technology and Prospects*, pp. 43–59. Edited by B. H. Nga, H. M. Tan & K. Suzuki. Singapore: World Scientific Publishing.
- Tanasupawat, S., Shida, O., Okada, S. & Komagata, K. (2000). *Lactobacillus acidipiscis* sp. nov. and *Weissella thailandensis* sp. nov., isolated from fermented fish in Thailand. *Int J Syst Evol Microbiol* **50**, 1479–1485.
- Tanasupawat, S., Pakdeeto, A., Namwong, S., Thawai, C., Kudo, T. & Itoh, T. (2006). *Lentibacillus halophilus* sp. nov., from fish sauce in Thailand. *Int J Syst Evol Microbiol* **56**, 1859–1863.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Thornley, M. J. (1960). The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. *J Appl Bacteriol* **23**, 37–52.
- Ventosa, A., García, M. T., Kamekura, M., Onishi, H. & Ruiz-Berraquero, M. (1989). *Bacillus halophilus* sp. nov., a moderately halophilic *Bacillus* species. *Syst Appl Microbiol* **12**, 162–165.
- Wainø, M., Tindall, B. J., Schumann, P. & Ingvorsen, K. (1999). *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int J Syst Bacteriol* **49**, 821–831.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology.

Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2002). *Lentibacillus salicampi* gen. nov., sp. nov., a moderately halophilic bacterium isolated from a salt field in Korea. *Int J Syst Evol Microbiol* **52**, 2043–2048.

Zhilina, T. N., Garnova, E. S., Tourova, T. P., Kostrikina, N. A. & Zavarzin, G. A. (2001). *Amphibacillus fermentum* sp. nov., *Amphibacillus tropicus* sp. nov., new alkaliphilic, facultatively anaerobic, saccharolytic bacilli from Lake Magadi. *Microbiology* (English translation of *Mikrobiologiya*) **70**, 711–722.