Halococcus thailandensis sp. nov., from fish sauce in Thailand

Sirilak Namwong,[1] Somboon Tanasupawat,[1] Wonnop Visessanguan,[2] Takuji Kudo[3] and Takashi Itoh[3]

Correspondence
Somboon Tanasupawat
Somboon.T@chula.ac.th

[1]Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

[2]National Center for Genetic Engineering and Biotechnology, Pathumthani 12120, Thailand

[3]Japan Collection of Microorganisms, RIKEN BioResource Center, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan

Fifteen strains of red-pigmented, strictly aerobic, coccoid, extremely halophilic archaea were isolated from fish sauce (nam-pla) produced in Thailand. They grew optimally at 37 °C, pH 6–8 and in the presence of 20–30 % (w/v) NaCl. The DNA G+C contents of the isolates were 60.0–61.8 mol%. They had MK-8(H_2) as a major menaquinone component and $C_{20}C_{20}$ and $C_{20}C_{25}$ derivatives of phosphatidylglycerol, phosphatidylglycerol methylphosphate and a sulfated glycolipid, S-DGA-1, as major polar lipid components. 16S rRNA gene sequence comparisons revealed that a representative strain, HDB5-2^T, was affiliated with *Halococcus dombrowskii* JCM 12289^T, *Halococcus qingdaonensis* JCM 13587^T and *Halococcus morrhuae* JCM 8876^T (levels of similarity of 98.2–98.7 %). Based on data from DNA–DNA hybridization experiments, the 15 strains represented a single species, showing hybridization values of >78.9 % to representative strain HDB5-2^T, but were unrelated to either *Halococcus dombrowskii* JCM 12289^T or *Halococcus morrhuae* JCM 8876^T, with levels of relatedness of <50 %. Moreover, a comparison of phenotypic properties discriminated these new isolates from recognized species of the genus *Halococcus*. The 15 strains are thus considered to represent a novel species of the genus *Halococcus*, for which the name *Halococcus thailandensis* sp. nov. is proposed. The type strain is HDB5-2^T (=BCC 20213^T =JCM 13552^T =PCU 278^T).

The genus *Halococcus* was first proposed by Schoop (1935) to accommodate red, extremely halophilic cocci that thrive in hypersaline environments, and the generic name was implemented by Kocur & Hodgkiss (1973) with the proposal of a single species, *Halococcus morrhuae*. In addition to *Hcc. morrhuae*, the genus *Halococcus* currently comprises five other recognized species, *Halococcus saccharolyticus*, *Hcc. salifodinae*, *Hcc. dombrowskii*, *Hcc. hamelinensis* and *Hcc. qingdaonensis* (Montero *et al.*, 1989; Denner *et al.*, 1994; Stan-Lotter *et al.*, 2002; Goh *et al.*, 2006; Wang *et al.*, 2007). Fish sauce (nam-pla) fermentation in Thailand has also provided isolation sources of novel halophilic archaea and bacteria, for example *Halobacterium salinarum*, *Halobacillus thailandensis*, *Tetragenococcus halophilus*, *Tetragenococcus muriaticus*, *Lentibacillus salicampi*, *Lentibacillus juripiscarius* and *Lentibacillus halophilus* (Chaiyanan *et al.*, 1999; Tanasupawat & Komagata, 2001; Thongthai *et al.*, 1992; Thongsanit *et al.*, 2002; Namwong *et al.*, 2005; Tanasupawat *et al.*, 2006). The present study describes the isolation of extremely halophilic archaeal cocci isolated from fish sauce fermentation and their characterization based on phenotypic, chemotaxonomic, DNA–DNA relatedness and 16S rRNA gene sequencing data.

The halophilic archaeal strains were isolated from fish sauce samples (nam-pla) collected from factories in Thailand during the early, middle and late stages of the fermentation process by using a spread-plate technique on JCM medium 169 agar plates [consisting of (per litre): 250 g NaCl, 7.5 g Casamino acids, 10 g yeast extract, 2 g KCl, 3 g trisodium citrate, 20 g $MgSO_4.7H_2O$, 0.05 g $FeSO_4.4H_2O$, 0.2 g $MnSO_4.4H_2O$, 20 g agar, pH 7.2] incubated at 37 °C for 1–2 weeks. Unless otherwise stated, the test strains were grown in liquid or on agar medium of JCM medium 169.

Cell morphology of the isolates was observed by light microscopy and scanning electron microscopy for cells grown on agar plates at 37 °C for 7 days. Tests for general physiological and biochemical characteristics were performed as specified by Oren *et al.* (1997). Tests for catalase

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HDB5-2^T is AB220647.

and oxidase activities, indole production, nitrate reduction and hydrolysis of casein, gelatin, arginine and Tween 80 were performed as described by Barrow & Feltham (1993). Carbon utilization and acid production from carbohydrates were determined in modified Leifson medium (Leifson, 1963) supplemented with 0.01 % yeast extract and 20 % (w/v) NaCl. Casitone was omitted for the carbon utilization tests and Tris/HCl was omitted for the acid production tests. Anaerobic growth was observed in standard growth medium with 0.5 % nitrate or arginine by using a Gaspak (BBL) anaerobic jar. The temperature range for growth was examined by incubating cultures on agar plates at 20, 28, 37, 45 and 50 °C. Effects of NaCl concentration for growth were tested in medium containing 0–30 % (w/v) NaCl. At lower NaCl concentrations (0–2 %), $MgSO_4.7H_2O$, KCl and trisodium citrate were omitted from the test medium. Similarly, the requirement for Mg^{2+} was tested in JCM 169 medium by omitting $MgSO_4.7H_2O$ but supplementing with 2 % (w/v) Na_2SO_4 and 0–10 % (w/v) $MgCl_2$. Growth was determined by measuring culture turbidity at 660 nm. The antibiotic susceptibility of the strains was tested as described by Stan-Lotter *et al.* (2002). Menaquinones were analysed as described by Komagata & Suzuki (1987). Polar lipids were determined according to the method of Minnikin *et al.* (1984).

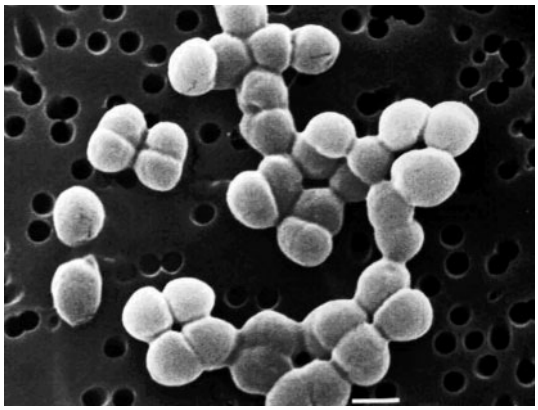

Fig. 1. Scanning electron micrograph of cells of strain HDB5-2^T grown on JCM 169 medium at 37 °C. Bar, 1 μm.

DNA was isolated and purified according to the method of Saito & Miura (1963). The DNA G+C content was determined as described by Tamaoka & Komagata (1984) by using reversed-phase HPLC. DNA–DNA hybridization tests were performed as described by Ezaki *et al.* (1989) and levels of relatedness were determined according to

Table 1. Differential characteristics between strain HDB5-2^T and related *Halococcus* species

Strains: 1, HDB5-2^T (identical results produced for 14 other strains); 2, *Hcc. dombrowskii* JCM 12289^T; 3, *Hcc. morrhuae* JCM 8876^T; 4, *Hcc. saccharolyticus* JCM 8878^T; 5, *Hcc. hamelinensis* JCM 12892^T (data from Goh *et al.*, 2006); 6, *Hcc. salifodinae* JCM 9578^T (data from Denner *et al.*, 1994); 7, *Hcc. qingdaonensis* JCM 13587^T (data from Wang *et al.*, 2007). +, Positive; −, negative; W, weak; ND, no data.

Characteristic	1	2	3	4	5	6	7
Optimum NaCl (%, w/v)	20–30	20–30	20–30	20–30	15	20–30	18
Optimum $MgCl_2$ (%, w/v)	0–0.8	0.6–5	0.8–5	ND	ND	ND	ND
Growth at pH 9.0	+	−	+	−	+	+	+
Growth at 45 °C	+	−	−	ND	ND	ND	ND
Oxidase	+	+	+	+	−	+	−
Nitrate reduction	+/no gas	+/no gas	+/no gas	+	+/no gas	+	−
Indole formation	−	ND	+	ND	−	ND	ND
Hydrolysis of gelatin	−	+	−	v	−	+	−
Acid from:							
L-Arabinose	+	W	−	−	ND	ND	ND
Cellobiose	+	−	−	−	ND	ND	ND
D-Glucose	+	−	−	−	+	ND	ND
Lactose	+	−	−	−	ND	ND	ND
D-Mannitol	+	−	−	−	ND	ND	ND
Melibiose	−	−	−	−	ND	ND	ND
Trehalose	+	−	−	−	ND	ND	ND
Utilization of:							
L-Arabinose	W	+	−	+	ND	+	ND
D-Fructose	+	+	−	+	ND	+	ND
D-Glucose	W	−	−	+	+	+	+
D-Galactose	−	+	−	+	+	ND	ND
D-Xylose	W	+	−	−	+	ND	ND
Glutamic acid	+	−	−	+	ND	ND	ND
Serine	−	−	−	+	ND	ND	ND

Tanasupawat *et al.* (2000). The almost-complete 16S rRNA gene sequence of a representative strain, designated HDB5-2^T, was amplified by PCR with primers D30F (5′-ATTCCGGTTCATCCTGC, positions 6–22 according to the *Escherichia coli* numbering system) and D56R (5′-GYTACCTTGTTACGACTT, positions 1492–1509). The amplified DNA fragment was separated by agarose gel electrophoresis and recovered by using a GenElute Minus EtBr spin column (Sigma). The sequence was determined by using the BigDye Terminator cycle sequencing ready reaction kit (version 3.0; Applied Biosystems) in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) with the following primers: D30F, D33R (5′-TCGCGCCTG-CGCCCCGT, positions 344–360), D34R (5′-GGTCTC-GCTCGTTGCCTG, positions 1096–1113), D56R, B99R (5′-GTGTTACCGCGGCTGCTG, positions 519–536), B36R (5′-GGACTACCAGGGTATCTA, positions 789–806) and X10R (5′-ACGGGCGGTGTGTRC, positions 1392–1406). The phylogenetic tree was constructed as described by Thompson *et al.* (1994), Saitou & Nei (1987), Kumar *et al.* (2001) and Felsenstein (1985).

Fifteen extremely halophilic, Gram-negative cocci were isolated from various stages of the fish sauce fermentation process. Cells of these isolates were non-motile, 0.8–1.2 μm in diameter, occurring singly, in pairs or in tetrads (Fig. 1) during both exponential and stationary phases of growth. In addition, the cells did not lyse when suspended in distilled water for 1–2 h. They formed small, red-pigmented colonies (1–2 mm in diameter) on agar plates. The isolates grew in medium containing a high concentration of NaCl [at least 15 % (w/v) and optimally 20–30 % (w/v)]. Their growth temperature range was 15–45 °C (optimum growth at 37 °C) and pH range was 6–10 (optimum growth at pH 6–8). The physiological and biochemical properties of strain HDB5-2^T are given in the species description below and in Table 1. A thin-layer chromatogram of the polar lipid fraction from four representative strains, HDS4-1, HDB5-2^T, HDS7-4 and HIS10-2, revealed $C_{20}C_{20}$ and $C_{20}C_{25}$ diether lipids of phosphatidylglycerol, phosphatidylglycerol methylphosphate, a sulfated diglycosyl diether (S-DGA-1) and unidentified glycolipids, by comparing the profiles with those of *Hcc. dombrowskii* JCM 12289^T and *Hcc. morrhuae* JCM 8876^T. Phosphatidylglycerol sulfate was not detected. The novel strains had MK-8(H_2) as the major menaquinone component. The DNA G+C contents of the novel strains were 60.0–61.8 mol% (Table 2).

Comparison of the 16S rRNA gene sequence (1384 bp) of strain HDB5-2^T with those of representative members of the family *Halobacteriaceae* revealed that it belonged to the genus *Halococcus* (Grant & Larsen, 1989; Grant, 2001). A phylogenetic tree showing the position of strain HDB5-2^T in the genus *Halococcus* was reconstructed as shown in Fig. 2. Strain HDB5-2^T was closely related to *Hcc. morrhuae* JCM 8876^T, *Hcc. qingdaonensis* JCM 13587^T and *Hcc. dombrowskii* JCM 12289^T, with 16S rRNA gene sequence

Table 2. DNA G+C contents and levels of DNA–DNA relatedness among the 15 novel strains, *Hcc. dombrowskii* JCM 12289^T and *Hcc. morrhuae* JCM 8876^T

Strain	Factory/ fermentation time (days)	DNA G+C content (mol%)	DNA–DNA relatedness (%) with labelled strain		
			JCM 12289^T	JCM 8876^T	HDB5-2^T
HIS10-2	A/10	60.7	21.6	30	91.3
HDB1-4	B/30	61.7	31.6	49.0	90.2
HKS35-3	A/35	61.2	45.6	49.4	78.9
HKS87-3	A/87	ND	32.7	49.9	91.5
HDS4-1	B/120	60.0	42.3	42.3	98.4
HDB5-2^T	B/150	60.2	41.0	36.1	100.0
HDS6-1A	B/180	61.6	40.3	41.5	84.5
HDS6-2	B/180	61.3	24.3	39.8	83.8
HDS6-6	B/180	ND	34.1	44.6	88.7
HDS7-4	B/210	ND	47.9	43.5	97.1
HDB8-2	B/240	61.8	36.7	38.6	81.1
HDB8-5	B/240	61.5	44.4	46.3	84.2
HDS10-5	B/300	ND	46.6	43.5	97.5
HDB10-5	B/300	ND	41.3	45.7	86.6
HKS333-2	A/333	61.7	35.6	43.0	85.9
Hcc. dombrowskii JCM 12289^T	–	61.3*	100.0	ND	42.3
Hcc. morrhuae JCM 8876^T	–	57.8†	34.1	100.0	25.7

ND, Not determined.

*Data from Stan-Lotter *et al.* (2002).

†Data from Bohácek *et al.* (1968).

similarities of 98.2–98.7 %, whereas it was more distantly related to *Hcc. hamelinensis* JCM 12892^T, *Hcc. saccharolyticus* JCM 8878^T and *Hcc. salifodinae* JCM 9578^T, with sequence similarities of 93.7–94.1 %. DNA–DNA hybridization experiments revealed that the 15 novel strains constituted a homogeneous genetic group with more than 78.9 % DNA–DNA relatedness (Wayne *et al.*, 1987). However, the novel strains showed low levels of DNA–DNA relatedness (<50 %) with *Hcc. morrhuae* JCM 8876^T and *Hcc. dombrowskii* JCM 12289^T (Table 2). In addition, the 15 strains could be differentiated from recognized species of the genus *Halococcus* based on intolerance to high concentrations of $MgCl_2$, growth at 45 °C, acid production from carbohydrates and utilization of various carbon sources (Table 1).

Based on their phenotypic and chemotaxonomic characteristics, including low levels of DNA–DNA relatedness to recognized *Halococcus* species, the novel strains described herein are considered to represent a novel species of this genus, for which the name *Halococcus thailandensis* sp. nov. is proposed.

Description of *Halococcus thailandensis* sp. nov.

Halococcus thailandensis (thai.lan.den′sis. N.L. masc. adj. *thailandensis* pertaining to Thailand, where the first strains were isolated).

Cells are Gram-negative, non-motile, strictly aerobic cocci, 0.8–1.2 μm in diameter, occurring singly, in pairs, tetrads or sarcina packets. Colonies are small, red-pigmented and circular with entire margins (1–2 mm in diameter after 1 week of incubation at 37 °C) when grown on complex medium of neutral pH. No lysis in distilled water. Grows aerobically but not anaerobically even in the presence of nitrate or arginine. Grows between 15 and 45 °C (optimally at 37 °C) and between pH 6 and 10 (optimally at pH 6–8). Extremely halophilic; requires at least 15 % (w/v) NaCl for growth and grows optimally at 20–30 % (w/v) NaCl. Does not require $MgCl_2$ for growth. Catalase-, oxidase- and urease-positive. Nitrate is reduced but gas is not formed. Does not hydrolyse arginine, casein, gelatin, Tween 80 or starch. Produces acids from L-arabinose, cellobiose, D-glucose, lactose, D-mannitol, melibiose, sucrose and trehalose. Utilizes L-arabinose, D-fructose, D-glucose, D-xylose and glutamic acid, but not D-galactose or serine as the sole energy source. The type strain is susceptible to bacitracin, novobiocin and rifampicin but resistant to chloramphenicol, tetracycline, ampicillin, gentamicin, kanamycin, nalidixic acid and streptomycin. The DNA G+C content is 60.2–61.8 mol%. Possesses $C_{20}C_{20}$ and $C_{20}C_{25}$ diether core lipids. MK-8(H_2) is the predominant menaquinone component. Possesses phosphatidylglycerol and phosphatidylglycerol methylphosphate as major polar lipid components. S-DGD-1, a sulfated mannosylglucosylglycerol diether, and unidentified glycolipids are also present.

The type strain, HDB5-2^T (=BCC 20213^T =JCM 13552^T =PCU 278^T), was isolated from fish sauce fermentation in Thailand.

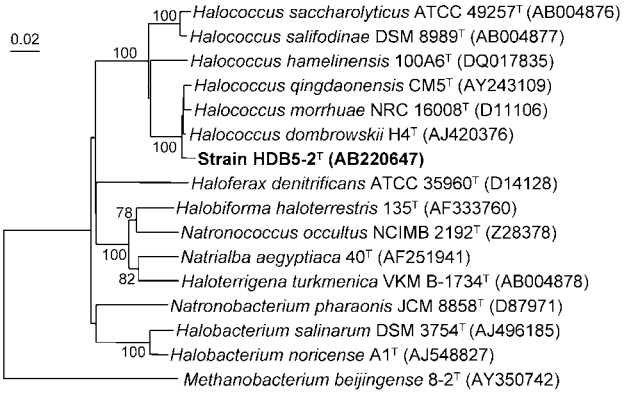

Fig. 2. Phylogenetic tree showing the relationships between strain HDB5-2^T and related bacterial species based on 16S rRNA gene sequences. The branching pattern was generated by using the neighbour-joining method. Bootstrap percentages ⩾78 %, based on 1000 replications, are shown at nodes. Bar, 2 substitutions per 100 nucleotide positions.

Acknowledgements

We are very grateful to the Sindhu Samuth Fish Sauce Factory (Squid Brand) Ltd, Samutprakarn, and the Thai Fish sauce factory (Squid Brand) Co., Ltd, Samutsongkram, for providing samples. This study was supported by the Thailand Research Fund for a 2001 Royal Golden Jubilee Scholarship as a research grant to S. N. and in part by a Ratchadapiseksomphot Research Grant, Chulalongkorn University (2002).

References

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.

Bohácek, J., Kocur, M. & Martinec, T. (1968). Deoxyribonucleic acid base composition of some marine and halophilic micrococci. *J Appl Bacteriol* **31**, 215–219.

Chaiyanan, S., Chaiyanan, S., Maugel, T., Huq, A., Robb, F. T. & Colwell, R. R. (1999). Polyphasic taxonomy of a novel *Halobacillus*, *Halobacillus thailandensis* sp. nov. isolated from fish sauce. *Syst Appl Microbiol* **22**, 360–365.

Denner, E. B. M., McGenity, T. J., Busse, H.-J., Grant, W. D., Wanner, G. & Stan-Lotter, H. (1994). *Halococcus salifodinae* sp. nov., an archaeal isolate from an Austrian salt mine. *Int J Syst Bacteriol* **44**, 774–780.

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.

Goh, F., Leuko, S., Allen, M. A., Bowman, J. P., Kamekura, M., Neilan, B. A. & Burns, B. P. (2006). *Halococcus hamelinensis* sp. nov., a novel

halophilic archaeon isolated from stromatolites in Shark Bay, Australia. *Int J Syst Evol Microbiol* **56**, 1323–1329.

Grant, W. D. (2001). Genus *Halococcus* Schoop 1935a, 871^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 311–314. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Grant, W. D. & Larsen, H. (1989). Group III. Extremely halophilic archaeobacteria. Order *Halobacteriales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2216–2219. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

Kocur, M. & Hodgkiss, W. (1973). Taxonomic status of the genus *Halococcus* Schoop. *Int J Syst Bacteriol* **23**, 151–156.

Komagata, K. & Suzuki, K. (1987). Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Kumar, S., Tamura, K., Jakobson, I.-B. & Nei, M. (2001). MEGA2: molecular evolution analysis software. *Bioinformatics* **17**, 1244–1245.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.

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.

Montero, C. G., Ventosa, A., Rodríguez-Valera, F., Kates, M., Moldoveanu, N. & Ruiz-Berraquero, F. (1989). *Halococcus saccharolyticus* sp. nov., a new species of extremely halophilic non-alkaliphilic cocci. *Syst Appl Microbiol* **12**, 167–171.

Namwong, S., Tanasupawat, S., Smitinont, T., Visessanguan, W., Kudo, T. & Itoh, T. (2005). Isolation of *Lentibacillus salicampi* strains and *Lentibacillus juripiscarius* sp. nov. isolated from fish sauce in Thailand. *Int J Syst Evol Microbiol* **55**, 315–320.

Oren, A., Ventosa, A. & Grant, W. D. (1997). Proposed minimal standards for description of new taxa in the order *Halobacteriales*. *Int J Syst Bacteriol* **47**, 233–238.

Saito, H. & Miura, K. (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.

Schoop, G. (1935). *Halococcus litoralis*, ein obligat halophiler Farbstoffbildner. *Dtsch Tierarztl Wochenschr* **43**, 817–820 (in German).

Stan-Lotter, H., Pfaffenhuemer, M., Legat, A., Busse, H.-J., Radax, C. & Gruber, C. (2002). *Halococcus dombrowskii* sp. nov., an archaeal isolate from a Permian alpine salt deposit. *Int J Syst Evol Microbiol* **52**, 1807–1814.

Tamaoka, J. & 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.

Thongsanit, J., Tanasupawat, S., Keeratipibul, S. & Jatikavanich, S. (2002). Characterization and identification of *Tetragenococcus halophilus* and *Tetragenococcus muriaticus* strains from fish sauce (nam-pla). *Jpn J Lactic Acid Bacteria* **13**, 46–52.

Thongthai, C., McGenity, T. J., Suntinanalert, P. & Grant, W. D. (1992). Isolation and characterization of an extremely halophilic archaeobacterium from traditionally fermented Thai fish sauce (nam pla). *Lett Appl Microbiol* **14**, 111–114.

Wang, Q.-F., Li, W., Yang, H., Liu, Y.-L., Cao, H.-H., Dornmayr-Phaffenhuemer, M., Stan-Lotter, H. & Guo, G.-Q. (2007). *Halococcus qingdaonensis* sp. nov., a halophilic archaeon isolated from a crude sea-salt sample. *Int J Syst Evol Microbiol* **57**, 600–604.

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.