Salirhabdus euzebyi gen. nov., sp. nov., a Gram-positive, halotolerant bacterium isolated from a sea salt evaporation pond

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A low-G + C, Gram-positive bacterium, designated CVS-14^T, was recovered from a sea salt evaporation pond on the island of Sal in the Cape Verde Archipelago. This organism was catalase- and oxidase-positive. Cells were motile, spore-forming aerobic rods, with an optimum growth temperature of about 35–40 °C and optimum pH between 7.0 and 8.5. Optimal growth occurred in media containing 4–6 % (w/v) NaCl, although the organism was able to grow in medium without added NaCl and in medium containing 16 % NaCl. The cell-wall peptidoglycan was of A1 γ type and the major respiratory quinone was menaquinone 7 (MK-7). Major fatty acids were iso-15:0, anteiso-15:0, iso-17:0 and anteiso-17:0. The DNA G + C content was 37.0 mol%. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain CVS-14^T formed a distinct new branch within the radiation of the moderately halophilic bacilli group, forming a separate lineage from species of the genera *Salinibacillus*, *Paucisalibacillus*, *Oceanobacillus*, *Lentibacillus* and *Virgibacillus*. Strain CVS-14^T showed 16S rRNA gene pairwise similarity values of ~95 % with species of the genus *Salinibacillus*. On the basis of morphological, physiological, chemotaxonomic and phylogenetic characteristics, strain CVS-14^T is considered to represent a novel species in a new genus, for which the name *Salirhabdus euzebyi* gen. nov., sp. nov. is proposed. The type strain is CVS-14^T (=LMG 22839^T = CIP 108577^T).

Gram-positive rod-shaped, aerobic or facultatively anaerobic, spore-forming bacteria that grow optimally in media containing NaCl are currently assigned to 19 genera with similar characteristics and close phylogenetic relationships (Nunes *et al.*, 2006). During a survey of bacterial diversity present in sea salt evaporation pond samples collected on the island of Sal in the Cape Verde Archipelago, several slightly halophilic Gram-positive bacteria were isolated and characterized further. One of these isolates shared several physiological and biochemical characteristics with other species of halophilic bacilli and was phylogenetically most closely related to species of the genus *Salinibacillus* (Ren & Zhou, 2005). However, this organism had a distinctly lower NaCl requirement for optimal growth and a distinctive fatty acid content. Furthermore, 16S rRNA gene sequence analysis indicated that this organism represents a novel

phylogenetic lineage within the radiation of the genus *Bacillus* and related genera. Based on morphological, physiological, chemotaxonomic and phylogenetic characteristics, we describe a novel species in a new genus to accommodate strain CVS-14^T.

Strain CVS-14^T was isolated from a sea salt evaporation pond on solid R3A-V medium (Tiago *et al.*, 2006), pH 7.0, containing 2 % NaCl (w/v). Soil samples (3 g) were resuspended on sterile blenders with sterile 2 % NaCl. One drop of the homogenate was spread on R3A-V agar plates. These preparations were incubated at 37 °C for up to 5 days. The R3A-V medium contained the following components (l⁻¹): 1.0 g yeast extract (Difco), 1.0 g proteose peptone (Difco no. 3), 1.0 g Casamino acids, 1.0 g glucose, 0.6 g K₂HPO₄, 0.1 g MgSO₄·7H₂O, 0.05 g Na pyruvate, 15.0 g agar (Difco), 50 ml of a 10-fold concentrated macronutrients solution, 5 ml of a 100-fold concentrated trace elements solution and 100 ml of 1 M phosphate buffer (pH 7.0); macronutrients, trace elements and the phosphate buffer were autoclaved separately and added to the cooled R3A agar. The 10-fold concentrated macronutrients

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CVS-14^T is AM292417.

A figure showing the effect of salt on the growth of strain CVS-14^T and a monodimensional TLC of polar lipids of strain CVS-14^T are available with the online version of this paper.

solution contained (l^{-1}): 1.0 g nitrilotriacetic acid, 0.6 g $CaSO_4 \cdot 2H_2O$, 1.0 g $MgSO_4 \cdot 7H_2O$, 0.8 g NaCl, 1.03 g KNO_3 , 6.89 g $NaNO_3$ and 1.11 g $NaHPO_4$. The 100-fold concentrated trace elements solution contained (l^{-1}): 0.22 g $MnSO_4 \cdot H_2O$, 0.05 g $ZnSO_4 \cdot 7H_2O$, 0.05 g H_3BO_3 , 0.0025 g $CuSO_4 \cdot 5H_2O$, 0.0025 g $Na_2MoO_4 \cdot 2H_2O$ and 0.0046 g $CoCl_2 \cdot 6H_2O$.

Despite repeated attempts to isolate additional strains, only one isolate was obtained. Cultures were purified by sub-culturing on the same medium and maintained at $-70^\circ C$ in Degryse medium 162 containing 3 % NaCl and 15 % (w/v) glycerol (Degryse *et al.*, 1978). The organism was also routinely cultured in Degryse medium 162 containing 5 % NaCl at $37^\circ C$ for up to 5 days, unless stated otherwise (Albuquerque *et al.*, 2005). *Paucisolibacillus globulus* B22^T was used for comparative purposes; the type strains of *Salinibacillus kushneri* and *Salinibacillus aidingensis* were not available from the Japanese Collection of Microorganisms (JCM).

The growth temperature range of strain CVS-14^T was examined in liquid medium in a reciprocal water bath shaker between 10 and $55^\circ C$; the pH range for growth was determined at $37^\circ C$ in the same medium buffered at pH values between 5.5 and 10.5 by using 20 mM MES, Tris and CAPSO (Sigma). The NaCl range for growth was determined in liquid medium without additional NaCl and with up to 20 % NaCl. Enzymic activities were determined by using the API ZYM system (bioMérieux) at $37^\circ C$. Anaerobic growth was assessed in solidified Degryse medium with 5.0 % NaCl and 1.0 g $KNO_3 l^{-1}$ in anaerobic chambers (GENbox anaer; bioMérieux). Single carbon source assimilation tests were performed in a defined medium composed of Degryse basal salts containing 5.0 % NaCl, 0.1 g yeast extract l^{-1} , 0.5 g ammonium sulfate l^{-1} , 2.0 g single carbon sources l^{-1} and 2 % deionized water-washed agar (Oxoid). Cultures (24 h) from plates were resuspended in the basal salts medium (turbidity equivalent to a McFarland no. 1 standard) and a single drop of the suspension was placed on plates, each of which contained a single carbon source. Growth was examined visually for up to 5 days. Negative control plates did not include a carbon source. Carbon source assimilation profiles were also investigated in liquid media with the same composition indicated above, but this proved unsuccessful owing to the absence of growth or clumping of cells. Acid production from carbohydrates was determined by using the API 50 CH system (bioMérieux) according to the manufacturer's instructions, with API 50 CHB/E medium containing 5.0 % NaCl. Results were recorded after 24 h, 48 h and 5 days incubation at $37^\circ C$.

Peptidoglycan analysis was performed according to the method of Schleifer & Kandler (1972) and Schleifer (1985); respiratory quinone analysis was performed according to the method of Tindall (1989). Cultures for fatty acid analyses were grown on solidified Degryse medium 162 containing 5 % NaCl incubated in sealed plastic bags submerged in a water-bath at $37^\circ C$ for 48 h. Fatty acid methyl esters

were extracted as described by Moreira *et al.* (2000). Identification and quantification of fatty acid methyl esters, as well as numerical analysis of the fatty acid profiles, were performed by using the standard MIS Library Generation Software (Microbial ID). Polar lipid analyses were performed as described by Prado *et al.* (1988).

The G + C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989). The 16S rRNA gene was amplified by PCR and sequenced as described by Rainey *et al.* (1996). Phylogenetic analyses were performed using the ARB software package (Ludwig *et al.*, 2004) and a phylogenetic tree was constructed by using the neighbour-joining (Saitou & Nei, 1987) algorithm. Tree topologies were evaluated by performing bootstrap analysis (Felsenstein, 1985) of 1000 resamplings of the data set.

Comparative analyses of 1479 nt positions of the 16S rRNA gene sequence of strain CVS-14^T with those of other lineages within the low-G + C Gram-positive bacteria showed that the new isolate clustered with other moderately halophilic bacilli. Strain CVS-14^T showed highest 16S rRNA gene sequence similarities to species of the genus *Salinibacillus*, 95.7 % to *S. aidingensis* JCM 12389^T and 95.14 % to *S. kushneri* JCM 12390^T. The next closest relative was *P. globulus* B22^T with 94.9 % 16S rRNA gene sequence similarity. Similarity values in the range 90.3–95.2 % were found to members of the genera *Lentibacillus*, *Oceanobacillus* and *Virgibacillus*. Strain CVS-14^T grouped with species of the genus *Salinibacillus* but the branching point was not fully supported, with a bootstrap value of 69 % (Fig. 1). The G + C content of the DNA of strain CVS-14^T was 37.0 mol%.

Strain CVS-14^T formed non-pigmented colonies and Gram-positive, motile, long rod-shaped cells (0.3–0.4 μm in width by 2.9–8.6 μm in length) with oval endospores at a terminal position within a swollen sporangium, giving the cells the appearance of spermatozooids (Fig. 2). The optimum NaCl concentration for growth was 4–6 %, but good growth occurred both in the absence of additional NaCl and in medium containing up to 16 % NaCl (see Supplementary Fig. S1 in IJSEM Online). Species belonging to the genus *Salinibacillus*, in contrast with strain CVS-14^T, require 1 % NaCl for growth and have higher optimum and maximum NaCl ranges for growth (Table 1).

Strain CVS-14^T was oxidase-, catalase- and DNase-positive and hydrolysed gelatin, hippurate and Tweens 20, 40, 60 and 80. Aesculin, arbutin, casein, elastin, starch and xylan were not hydrolysed. Yeast extract was required for growth on single carbon sources. Strain CVS-14^T assimilated carbohydrates, polyols, organic acids and amino acids. Acid was produced from several carbohydrates. Nitrate and nitrite were reduced. Anaerobic growth in the presence of nitrate was not observed.

Strain CVS-14^T had a cell-wall type A1 γ peptidoglycan with *meso*-diaminopimelic acid as the diagnostic diamino acid, which is common in the majority of endospore-forming

Fig. 1. Phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequences of strain CVS-14^T and its closest phylogenetic relatives. The tree was created by using the neighbour-joining method. Numbers on the tree indicate bootstrap support (%), derived from 1000 replications. Bar, 1 inferred nucleotide substitution per 100 nt.

Fig. 2. Phase-contrast micrograph of cells of strain CVS-14^T cultivated for 24 h showing elongated cells and terminal spores within a swollen sporangium. Bar, 2 μm.

Gram-positive rods. The polar lipid pattern on TLC revealed the presence of diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol (see Supplementary Fig. S2 in IJSEM Online). The major respiratory lipoquinone was menaquinone 7 (MK-7). The fatty acid profile of strain CVS-14^T, as well as those of species of the genus *Salinibacillus*, was dominated by branched fatty acids, namely iso-15:0, anteiso-15:0, iso-17:0 and anteiso-17:0 (Table 2). However, strain CVS-14^T and species of the genus *Salinibacillus* could be distinguished based on the relative amounts of these fatty acids. Moreover, strain CVS-14^T possessed acyl compounds such as 16:1ω7c alcohol, iso-17:1ω10c and summed feature 4 (iso-17:1 I and/or anteiso-17:1 B) that are not detected in the type strains of the two other recognized species of the genus *Salinibacillus* (Ren & Zhou, 2005) or in the type strain of *P. globulus*. Strain CVS-14^T could also be distinguished from other slightly halophilic endospore-forming bacilli (Tables 1 and 2).

Despite similar characteristics and phylogenetic relatedness to several moderately halophilic bacilli, strain CVS-14^T could be clearly distinguished from the type strains of species of the related genera *Paucisalibacillus* and *Salinibacillus*, namely based on NaCl requirements for optimal growth, fatty acid content as well as other phenotypic characteristics and, for the genus *Paucisalibacillus*, a different cell-wall type.

On the basis of these findings, strain CVS-14^T is considered to represent a novel species in a new genus, for which the name *Salirhabdus euzebii* gen. nov., sp. nov. is proposed.

Table 1. Phenotypic characteristics that differentiate strain CVS-14^T from the type strains of species of the genera *Salinibacillus*, *Paucisalibacillus* and other related genera

Taxa: 1, strain CVS-14^T; 2, *Salinibacillus* (data from the two recognized species of the genus *Salinibacillus* from Ren & Zhou, 2005); 3, *Paucisalibacillus globulus* B22^T (data from Nunes *et al.*, 2006); 4, *Virgibacillus* (data from 10 recognized species of the genus *Virgibacillus* from Yoon *et al.*, 2005; Lee *et al.*, 2006); 5, *Oceanobacillus* (data from five recognized species of the genus *Oceanobacillus* from Lu *et al.*, 2001; Lee *et al.*, 2006; Raats & Halpern, 2007; Kim *et al.*, 2007); 6, *Lentibacillus* (data from six recognized species of the genus *Lentibacillus* from Pakdeeto *et al.*, 2007). +, Positive; –, negative; V, variable; ND, not determined.

Characteristic	1	2	3	4	5	6
NaCl requirement for growth	–	+	–	+	–	+
NaCl range (% w/v)	0–16	1–30	0–8	0.5–25	0–22	1–30
NaCl optimum (% w/v)	4–6	12–15	1	4–10	3	4–26
Growth temperature range (°C)	15–50	20–50	20–40	5–50	15–42	15–50
Optimum pH	7.0–8.5	7.0–8.0	8.0–8.5	7–7.4	7.0–9.5	6.0–8.0
pH range	6–9.5	6–10	7.0–9.0	5.5–10	6.5–10.0	ND
Presence of:						
Oxidase	+	V	–	+	V	+
DNase	+	–	+	+	–	ND
Hydrolysis of:						
Starch	–	V	+	V	–	–
Aesculin	–	+	+	V	ND	V
Casein	–	+	+	+	+	V
Tween 20	+	–	+	ND	ND	ND
Tween 60	+	–	+	ND	+	ND
Tween 80	+	–	+	V	ND	V
Major fatty acids	iso-15:0, anteiso-15:0, anteiso-17:0, iso-17:0	anteiso-15:0, iso-15:0, anteiso-17:0, iso-16:0	anteiso-15:0, anteiso-17:0, iso-15:0	anteiso-15:0, iso-15:0, anteiso-17:0	anteiso-15:0, iso-15:0, anteiso-17:0	anteiso-15:0, iso-16:0, anteiso-17:0, iso-14:0
DNA G+C content (mol%)	37.0	37.2–37.4	37.9	37.0–40.0	36.0–40.2	41.6–44.0
Cell-wall peptidoglycan type	A1 γ	A1 γ	A4 α	A1 γ	A1 γ	A1 γ

Description of *Salirhabdus* gen. nov.

Salirhabdus [Sa.li.rhab'dus. L. n. *sal salis* salt; N.L. fem. n. *rhabdus* (from Gr. fem. n. *rhabdos*) a rod, wand; N.L. fem. n. *Salirhabdus* a rod that grows in salt].

Forms rod-shaped cells that stain Gram-positive and form terminal spores within a swollen sporangium. Strictly aerobic. Oxidase- and catalase-positive. NaCl is not required for growth but the addition of NaCl to the culture medium improves growth. Cell-wall peptidoglycan is of the A1 γ type. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol are the major polar lipids. Major respiratory quinone is MK-7. Fatty acids are predominantly saturated branched chain. The genus belongs to the family *Bacillaceae*. The type species is *Salirhabdus euzebyi*.

Description of *Salirhabdus euzebyi* sp. nov.

Salirhabdus euzebyi (eu.ze.by'i. N.L. gen. masc. n. *euzebyi* of Euzéby, in honour of the French bacteriologist Jean P. Euzéby).

Has the following characteristics in addition to those given for the genus. Forms rod-shaped cells 0.3–0.4 μ m in width

and 2.9–8.6 μ m in length. Cells are motile. Oval endospores are formed at a terminal position in a swollen sporangium. Colonies are cream-pigmented on Degryse medium 162 with 5 % NaCl. The optimum growth temperature is ~37–45 °C; growth does not occur at 10 or 55 °C. The optimum pH is between 7.0 and 8.5; growth does not occur at pH 5.5 or pH 10.0. Grows in media without added salt and in media containing 16 % NaCl; the optimum NaCl concentration for growth is between 4 and 6 %. Growth does not occur in media containing 20 % NaCl. The major fatty acids are iso-15:0, anteiso-15:0, iso-17:0 and anteiso-17:0; iso-14:0, 15:0, 16:1 ω 7c alcohol, iso-16:0, 16:0, iso-17:1 ω 10c, summed feature 4, 17:0, iso-18:0 and 19:0 are present as minor components. Yeast extract or growth factors are required for growth. Denitrifies. Gelatin, hippurate and Tweens 20, 40, 60 and 80 are hydrolysed, but aesculin, arbutin, casein, elastin, starch and xylan are not. Positive for DNase, alkaline phosphatase, leucine arylamidase, α -chymotrypsin and β -glucosidase; negative for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase and β -galactosidase. Glucose, mannose, fructose, D-arabinose, xylose, sucrose, maltose, trehalose, cellobiose, melibiose,

Table 2. Fatty acid content (%) of strain CVS-14^T and the type strains of species of the genera *Salinibacillus*, *Paucisolibacillus* and other related genera

Taxa: 1, strain CVS-14^T; 2, *Salinibacillus* (data from the two recognized species of the genus *Salinibacillus* from Ren & Zhou, 2005); 3, *Paucisolibacillus globulus* B22^T (data from Nunes *et al.*, 2006); 4, *Virgibacillus* (data from ten recognized species of the genus *Virgibacillus* from Yoon *et al.*, 2005; Lee *et al.*, 2006); 5, *Oceanobacillus* (data from five recognized species of the genus *Oceanobacillus* from Lu *et al.*, 2001; Lee *et al.*, 2006; Raats & Halpern, 2007; Kim *et al.*, 2007); 6, *Lentibacillus* (data from four recognized species of the genus *Lentibacillus* from Pakdeeto *et al.*, 2007). Data are mean $\pm$ SD percentages of each fatty acid. —, Not detected; tr, trace (<0.5%).

Fatty acids	1	2	3	4	5	6
iso-13:0	—	—	tr	1.2 $\pm$ 0.1	—	tr
anteiso-13:0	—	—	tr	0.4 $\pm$ 0.1	—	—
iso-14:0	2.5	2.6 $\pm$ 0.1	1.4	3.3 $\pm$ 1.6	5.7 $\pm$ 4.1	8.9 $\pm$ 5.2
14:0	—	—	0.5	—	1.6 $\pm$ 2.8	tr
iso-15:0	25.1	23.2 $\pm$ 6.8	15.4	26.1 $\pm$ 14.2	19.1 $\pm$ 14.7	7.5 $\pm$ 4.6
anteiso-15:0	18.9	31.5 $\pm$ 10.7	58.9	39.5 $\pm$ 9.3	42.6 $\pm$ 15.1	43.7 $\pm$ 10.5
15:0	1.7	—	—	0.5 $\pm$ 0.1	0.3 $\pm$ 0.5	tr
16:1 ω 7c alcohol	4.9	—	—	—	—	1.1 $\pm$ 0.6
iso-16:0	7.8	10.9 $\pm$ 1.5	2.5	6.4 $\pm$ 1.3	7.0 $\pm$ 3.6	17.1 $\pm$ 8.1
16:1 ω 11c	0.8	—	—	—	—	—
16:0	1.8	2.3 $\pm$ 1.1	1.1	0.9 $\pm$ 0.2	5.8 $\pm$ 7.4	1.7 $\pm$ 1.7
iso-17:1 ω 10c	3.3	—	—	—	—	—
Summed feature 4*	2.5	—	—	—	—	—
iso-17:0	11.7	7.6 $\pm$ 5.4	1.1	5.0 $\pm$ 2.5	2.7 $\pm$ 2.3	1.6 $\pm$ 1.5
anteiso-17:0	15.5	21.1 $\pm$ 3.7	18.3	16.0 $\pm$ 10.6	12.3 $\pm$ 7.0	17.8 $\pm$ 4.9
17:0	1.0	—	—	—	—	—
iso-18:0	0.8	—	—	—	—	—
iso-19:0	0.5	—	—	—	—	—

*A summed feature represents groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 comprises iso-17:1 I and/or anteiso-17:1 B.

L-rhamnose, raffinose, ribitol, xylitol, sorbitol, mannitol, myo-inositol, succinate, 2-oxoglutarate, lactate, malate, pyruvate, cysteine, histidine, lysine, proline, arginine, serine, valine and ornithine are assimilated, but galactose, sorbose, L-arabinose, ribose, lactose, melezitose, glycerol, citrate, acetate, aspartate, glutamate, alanine, asparagine, phenylalanine, glycine, isoleucine, methionine, glutamine and threonine are not. Acid is produced from glycerol, ribose, glucose, fructose, mannose, L-sorbose, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, trehalose, tagatose, potassium 2-ketogluconate and potassium 5-ketogluconate, but not from erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, adonitol (ribitol), methyl β -D-xylopyranoside, galactose, rhamnose, dulcitol (galactitol), inositol, mannitol, sorbitol, methyl α -D-mannopyranoside, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol or potassium gluconate. The G+C content of the DNA is 37.0 mol% (HPLC method).

The type strain, CVS-14^T (= LMG 22839^T = CIP 108577^T), was isolated from a sea salt evaporation pond on the island of Sal in the Cape Verde Archipelago.

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References

- Albuquerque, L., Simões, C., Nobre, M. F., Pino, N. M., Battista, J. R., Silva, M. T., Rainey, F. A. & da Costa, M. S. (2005). *Truepera radiovictrix* gen. nov., sp. nov., a new radiation-resistant species and the proposal of *Trueperaceae* fam. nov. *FEMS Microbiol Lett* **247**, 161–169.
- Degryse, E., Glansdorff, N. & Pierard, A. (1978). A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. *Arch Microbiol* **117**, 189–196.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Kim, Y. G., Choi, D. H., Hyun, S. & Cho, B. C. (2007). *Oceanobacillus profundus* sp. nov., isolated from a deep-sea sediment core. *Int J Syst Evol Microbiol* **57**, 409–413.
- Lee, J.-S., Lim, J.-M., Lee, K. C., Lee, J.-C., Park, Y.-H. & Kim, C.-J. (2006). *Virgibacillus koreensis* sp. nov., a novel bacterium from a salt field and transfer of *Virgibacillus picturae* to the genus

Oceanobacillus as *Oceanobacillus picturae* comb. nov. with emended descriptions. *Int J Syst Evol Microbiol* **56**, 251–257.

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.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Mesbah, M., Premachandran, U. & Whitman, W. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Moreira, C., Rainey, F. A., Nobre, M. F., da Silva, M. T. & da Costa, M. S. (2000). *Tepidimonas ignava* gen. nov., sp. nov., a new chemolithoheterotrophic and slightly thermophilic member of the β -Proteobacteria. *Int J Syst Evol Microbiol* **50**, 735–742.

Nunes, I., Tiago, I., Pires, A. L., da Costa, M. S. & Verissimo, A. (2006). *Paucisolibacillus globulus* gen. nov., sp. nov., a Gram-positive bacterium isolated from potting soil. *Int J Syst Evol Microbiol* **56**, 1841–1845.

Pakdeeto, A., Tanasupawat, S., Thawai, C., Moonmangmee, S., Kudo, T. & Itoh, T. (2007). *Lentibacillus kapialis* sp. nov., from fermented shrimp paste in Thailand. *Int J Syst Evol Microbiol* **57**, 364–369.

Prado, A., da Costa, M. S. & Madeira, V. M. C. (1988). Effect of growth temperature on the lipid composition of two strains of *Thermus* sp. *J Gen Microbiol* **134**, 1653–1660.

Raats, D. & Halpern, M. (2007). *Oceanobacillus chironomi* sp. nov., a halotolerant and facultatively alkaliphilic species isolated from a chironomid egg mass. *Int J Syst Evol Microbiol* **57**, 255–259.

Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.

Ren, P. G. & Zhou, P. J. (2005). *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.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Tiago, I., Mendes, V., Pires, C., Morais, P. V. & Verissimo, A. (2006). *Chimaericella alkaliphila* gen. nov., sp. nov., a Gram-negative alkaliphilic bacterium isolated from a nonsaline alkaline ground-water. *Syst Appl Microbiol* **29**, 100–108.

Tindall, B. J. (1989). Fully saturated menaquinones in the archaeobacterium *Pyrobaculum islandicum*. *FEMS Microbiol Lett* **60**, 251–254.

Yoon, J. H., Kang, S. J., Lee, S. Y., Lee, M. H. & Oh, T. K. (2005). *Virgibacillus dokdonensis* sp. nov., isolated from a Korean island, Dokdo, located at the edge of the East Sea in Korea. *Int J Syst Evol Microbiol* **55**, 1833–1837.