Gracilibacillus kekensis sp. nov., a moderate halophile isolated from Keke Salt Lake

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A novel moderately halophilic bacterium, designated strain K170^T, was isolated from Keke Salt Lake in Qinghai, China. The strain grew with 0–22% (w/v) NaCl, at 4–50 °C and at pH 6–11, with optimum growth in 3% (w/v) NaCl, at 40 °C and at pH 8. The predominant respiratory quinone was menaquinone 7 (MK-7). The polar lipids included diphosphatidylglycerol, phosphatidylglycerol, unidentified phospholipids, aminolipids and glycolipids. The major cellular fatty acids were anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. The DNA G+C content was 35.8 mol%. Phylogenetic analysis based on the full-length 16S rRNA gene sequence revealed that strain K170^T was a member of the genus *Gracilibacillus*. High levels of 16S rRNA gene sequence similarity were found between strain K170^T and *Gracilibacillus boracitolerans* DSM 17256^T (97.3%) and *Gracilibacillus thailandensis* JCM 15569^T (97.1%). 16S rRNA gene sequence similarities between strain K170^T and the type strains of other recognized members of the genus *Gracilibacillus* were below 97%. The DNA–DNA hybridization values of strain K170^T with *G. boracitolerans* DSM 17256^T and *G. thailandensis* JCM 15569^T were 21.9% and 34.3%, respectively. On the basis of these results, strain K170^T is considered to represent a novel species of the genus *Gracilibacillus*, for which the name *Gracilibacillus kekensis* sp. nov. is proposed; the type strain is K170^T (=CGMCC 1.10681^T=DSM 23178^T).

The genus *Gracilibacillus* was first proposed by Wainø *et al.* (1999), based on the type species *Gracilibacillus halotolerans*, and *Bacillus dipsosauri* (Lawson *et al.*, 1996) was reclassified into the genus as *Gracilibacillus dipsosauri* at the same time. At the time of writing, the genus comprised 10 species: *Gracilibacillus halotolerans* (Wainø *et al.*, 1999), *G. dipsosauri* (Wainø *et al.*, 1999), *G. orientalis* (Carrasco *et al.*, 2006), *G. boracitolerans* (Ahmed *et al.*, 2007), *G. laciisali* (Jeon *et al.*, 2008), *G. halophilus* (Chen *et al.*, 2008a), '*G. quinghaiensis*' (Chen *et al.*, 2008b), *G. saliphilus* (Tang *et al.*, 2009), *G. ureilyticus* (Huo *et al.*, 2010) and *G. thailandensis* (Chamroensaksri *et al.*, 2010). In this study, we describe the isolation and classification of a strain (designated K170^T)

that represents a novel moderately halophilic species of the genus *Gracilibacillus* on the basis of phenotypic, chemotaxonomic characterization and 16S rRNA gene sequence analysis.

Strain K170^T was isolated from sediment of Keke Salt Lake (36° 50'–37° 06' N 97° 58'–98° 30' E) in Qinghai, China. At the time of sampling, the temperature of the sediment was 17 °C, salinity was 8% (w/v) NaCl and pH was 6.75. For isolation, 0.1 g sample was suspended in 10 ml modified S-G liquid medium (Sehgal & Gibbons, 1960), stirred for 30 min, serially diluted and spread on modified S-G agar medium. The modified S-G liquid medium contained (l⁻¹): 10 g tryptone, 5 g yeast extract, 5 g Casamino acids, 2 g KCl, 3 g trisodium citrate, 20 g MgSO₄·7H₂O and 80 g NaCl, adjusted to pH 8.0. After 3 days of incubation at 30 °C, strain K170^T was selected and a pure culture was isolated after repeated purification. The pure culture was maintained at –80 °C in 30% glycerol. Morphological, physiological and biochemical studies were performed in modified S-G medium supplied

Abbreviations: SEM, scanning electron microscopy; TEM, transmission electron microscopy.

The GenBank/EMBL/DBJ accession number for 16S rRNA of *Gracilibacillus kekensis* K170^T is HQ439902.

Four supplementary figures and one supplementary table are available with the online version of this paper.

with 3 % (w/v) NaCl at pH 8.0 and 30 °C. *G. boracitolerans* DSM 17256^T and *G. thailandensis* JCM 15569^T were used as reference strains.

Cell morphology was observed by light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For endospore observation, cells were grown on endospore-forming medium for 3–4 days and then examined by SEM. The endospore-forming medium contained (l⁻¹): 1.0 g yeast extract, 5.0 g tryptone, 10.99 g MgCl₂, 4.06 g Na₂SO₄, 1.51 g CaCl₂·2H₂O, 0.69 g KCl, 0.2 g NaHCO₃, 0.1 g KBr, 0.1 g FeCl₃, 0.027 g H₃BO₃, 0.042 g SrCl₂·6H₂O, 0.003 g NaF, 0.01 g MnCl₂·4H₂O, 0.002 g NH₄NO₃, 0.005 g Na₂SiO₃·H₂O and 30 g NaCl, adjusted to pH 7.0. For the observation of flagella, cells were grown on modified S-G agar medium for 1 day and then examined by TEM (Heimbrook *et al.*, 1989). Gram staining was carried out by using the standard Gram reaction combined with the KOH lysis test (Gregersen, 1978). Cell motility was determined by using two methods, i.e. directly by light microscopy and by observation of the spread of growth in test tubes containing semisolid modified S-G medium with 0.6 % agar after 3 days incubation at 30 °C. To determine aerotactic ability, bacterial cells were inoculated by mixing them in test tubes with semisolid medium at 40–50 °C followed by growth at 30 °C for 3 days.

Growth conditions (pH, salinity and temperature ranges) were determined in liquid media by monitoring the increase in OD₆₀₀. Growth at different salt concentrations (0, 1, 3, 5, 8, 10, 12, 15, 18, 20, 22 and 25 % NaCl) and temperatures (4, 25, 30, 37, 40 and 50 °C) was tested in modified S-G medium at pH 8.0. To determine the pH range for growth, the medium was adjusted to various pH values (pH 2–12, at intervals of 0.5 pH units) by the addition of HCl or KOH.

Activities of catalase, oxidase and urease, nitrate reduction, hydrolysis of aesculin, production of indole, acid production from carbohydrates, utilization of carbon and nitrogen sources, Methyl red and Voges–Proskauer reactions, and hydrolysis of Tweens 20 and 80 were determined as recommended by Smibert & Krieg (1994); Ventosa *et al.* (1982) and Harrigan & McCance (1976). Hydrolysis of casein, gelatin, starch and ONPG were determined as described by Atlas (1993) and Barrow & Feltham (1993).

Amino acids in whole-cell hydrolysates were determined as described by Hasegawa *et al.* (1983). Polar lipids were extracted according to the method of Minnikin *et al.* (1984). They were separated by two-dimensional TLC and identified by spraying the chromatogram with the appropriate detection reagents (Collins & Jones, 1980). Lipoquinones were purified by TLC and analysed by HPLC as described by Collins & Jones (1980). For determination of cellular fatty acid composition, strain K170^T was incubated in trypticase soy broth (Difco) at 30 °C for 2 days. The cellular fatty acid pattern was analysed using the Microbial Identification System (MIDI, Sherlock version 6.0) as described by Sasser (1990).

The 16S rRNA gene was obtained from a PCR product amplified from strain K170^T by using the universal forward primer P₁ and the universal reverse primer P₆. Primer P₁ (5'-AGAGTTTGTATCCTGGTCAGAACGCT-3') corresponds to positions 8–37 and primer P₆ (5'-TACGGCTACCTTGT-TACGACTTCACCCC-3') corresponds to positions 1479–1506 in the *Escherichia coli* 16S rRNA gene (Yanagi & Yamasato, 1993). Phylogenetic analysis was performed using the software package MEGA 4.1 after multiple alignment of the sequence data with CLUSTAL_X. Phylogenetic trees based on the neighbour-joining, maximum-parsimony and maximum-likelihood methods were constructed by using the bootstrap phylogeny test with 1000 replications. Total genomic DNA was extracted and purified according to the method described by Yoon *et al.* (1996) with some modifications. The DNA G+C content was determined by the method of Tamaoka & Komagata (1984). DNA–DNA hybridization was performed according to the method of Ziemke *et al.* (1998).

Strain K170^T was able to grow with 0–22 % (w/v) NaCl, at 4–50 °C and pH 6–11, with optimum growth at 3 % (w/v) NaCl, 40 °C and pH 8, respectively. Colonies on modified S-G medium were round, smooth, creamy white and 0.5–1.0 mm in diameter after 3 days incubation at 30 °C. Cells were rod-shaped, approximately 0.20–1.05 µm wide and 1.50–4.00 µm long, Gram-positive, motile and strictly aerobic. Methyl red test, H₂S production, hydrolysis of ONPG and casein were positive, whereas indole production, oxidase, Voges–Proskauer, tyrosine hydrolase, nitrate reduction test and phenylalanine dehydrogenase were negative. Starch, and Tweens 20 and 80 were hydrolysed, whereas gelatin and urea were not. Cells had peritrichous flagella. The physiological and biochemical characteristics of strain K170^T and some related type strains are presented in Table 1. SEM of cells of strain K170^T showed that round spores were located terminally in swollen sporangia (Fig. 1). TEM showed that cells have peritrichous flagella (Fig. S1, available in IJSEM Online).

The chemotaxonomic data of strain K170^T are consistent with assignment of the isolate to the genus *Gracilibacillus*. The cell-wall peptidoglycan type of K170^T was the same as that of *G. boracitolerans* DSM 17256^T and *G. thailandensis* JCM 15569^T. *meso*-Diaminopimelic acid was the predominant diamino acid of the cell-wall peptidoglycan of strain K170^T. The major respiratory quinone of strain K170^T was menaquinone 7 (MK-7); minor amounts of menaquinone 8 (MK-8) were also found. Profiles of the major fatty acids of strain K170^T and *G. boracitolerans* DSM 17256^T and *G. thailandensis* JCM 15569^T are shown in Table S1 (available in IJSEM Online). The major fatty acids of strain K170^T were anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}, which are the major fatty acids of members of the genus *Gracilibacillus*. The polar lipids of strain K170^T consist of diphosphatidylglycerol, phosphatidylglycerol, unidentified phospholipids, aminolipids and glycolipids (Fig. S2, available in IJSEM Online).

Table 1. Differential phenotypic characteristics of strain K170^T and the type strains of members of the genus *Gracilibacillus*

Strains: 1, *Gracilibacillus kekensis* sp. nov. K170^T (data from this study); 2, *G. boraciitolerans* DSM 17256^T (this study); 3, *G. thailandensis* JCM 15569^T (this study); 4, *G. ureilyticus* CGMCC 1.7727^T (Huo *et al.*, 2010); 5, *G. saliphilus* YIM 91119^T (Tang *et al.*, 2009); 6, *G. lacisalsi* DSM 19029^T (Jeon *et al.*, 2008); 7, *G. orientalis* CCM 7326^T (Carrasco *et al.*, 2006); 8, '*G. quinghaiensis*' DSM 17858 (Chen *et al.*, 2008b); 9, *G. halophilus* DSM 17856^T (Chen *et al.*, 2008a); 10, *G. halotolerans* JCM 10718^T (Wainø *et al.*, 1999); 11, *G. dipsosauri* DSM 11125^T (Wainø *et al.*, 1999). +, Positive; w, weakly positive; –, negative; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Spore shape*	E	S	O	S	S	S	S	S	E	E	S
Growth in NaCl (%)											
Range	0–22	0–15	1–22	0–15	1–22	1–18	3–20	0.5–8	7–30	0–20	0–15
Optimum	3	0.5–3	5–10	3	10–15	5–7	10	1–3	15	0	3
Growth temperature (°C)											
Range	4–50	4–40	15–55	10–45	4–45	15–50	4–45	4–45	28–60	6–50	28–50
Optimum	40	25	37	35–37	28–37	40	37	37	45–50	47	45
Growth pH											
Range	6–11	6–10	6–8	6.5–8.5	6–8	5.5–10	5–9	6.0–8.5	6–9	5–10	ND
Optimum	8	7.5–8.5	7	7	7	7.5–8	7.5	7–7.5	7	7.5	7.5
Acid production from:											
D-Glucose	+	+	+	+	+	+	+	+	w	+	+
D-Fructose	+	–	+	+	+	+	+	–	w	ND	ND
D-Mannitol	–	–	+	+	+	+	+	–	w	+	+
Sucrose	+	–	+	+	+	+	+	–	w	ND	ND
Hydrolysis of:											
Casein	+	+	–	–	–	–	–	–	–	–	–
Gelatin	–	–	+	+	–	ND	+	–	+	+	+
Starch	+	+	–	+	+	–	+	–	+	+	+
Tween 80	+	–	–	–	–	–	–	–	+	+	–
Urea	–	–	–	+	+	–	–	+	–	+	–
Indole test	–	–	–	–	–	ND	–	–	–	ND	–
Nitrate reduction	–	–	+	+	+	+	–	+	+	+	+
Voges–Proskauer test	–	+	–	+	+	ND	ND	–	–	ND	–
DNA G + C content (mol%)	35.8	35.8	37.6	35.3	40.1	38.8–39.0	36.1–37.1	40.9	42.3	38	39.4

*E, Ellipsoidal; O, oval; S, spherical.

Fig. 1. SEM of cells of strain K170^T grown on endospore-forming medium. Bar, 5 μm.

The DNA G + C content of strain K170^T was 35.8 mol%. Based on a comparison of 16S rRNA gene sequences, the highest similarities of strain K170^T are 97.3 % with *G. boraciitolerans* DSM 17256^T and 97.1 % with *G. thailandensis* JCM 15569^T. Levels of 16S rRNA gene sequence similarity between strain K170^T and the type strains of other recognized members of the genus *Gracilibacillus* were below 97 %. Neighbour-joining phylogenetic analysis based on 16S rRNA gene sequences showed that K170^T had the closest phylogenetic affinity to the type strain of *G. boraciitolerans*, with a high level of bootstrap support (Fig. 2). Topologies of phylogenetic trees built using the maximum-parsimony and maximum-likelihood methods also supported the results that strain 170^T formed a stable clade with *G. boraciitolerans* (Figs S3 and S4, available in IJSEM Online). The DNA–DNA hybridization values between strain K170^T and the two reference strains (*G. boraciitolerans* DSM 17256^T and *G. thailandensis* JCM 15569^T) were 21.9 and 34.3 %, respectively. It is well known that genomic relatedness below 70 % is a key marker for

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain K170^T among species of the genus *Gracilibacillus*. Sequences from the type strains of *Virgibacillus koreensis* and *Virgibacillus carmonensis* were used as the outgroup. Bootstrap analyses were determined based on 1000 resamplings; values >50 % are shown. Bar, 0.005 substitutions per nucleotide position.

the identification of a novel species (Wayne *et al.*, 1987). Thus, strain K170^T represents a novel species that can be differentiated from any recognized member of the genus *Gracilibacillus*.

Description of *Gracilibacillus kekensis* sp. nov.

Gracilibacillus kekensis (ke.ken'sis. N.L. masc. adj. *kekensis* of or belonging to Keke Salt Lake in China, where the bacterium was isolated).

Cells are Gram-positive, aerobic, motile, short rods (approximately 0.20–1.05 × 1.50–4.00 µm). Endospores are formed in a terminal position. Cells have peritrichous flagella. Colonies on modified S-G medium are round, smooth, creamy white and 0.5–1.0 mm in diameter after 3 days incubation at 30 °C. Growth occurs with 0–22 % (w/v) NaCl, at 4–50 °C and at pH 6–11, with optimum growth with 3 % (w/v) NaCl, at 37 °C and at pH 8, respectively. Methyl red test, H₂S production, ONPG and casein hydrolase are positive, whereas indole production, oxidase, Voges–Proskauer reaction, tyrosine hydrolase, nitrate reduction and phenylalanine dehydrogenase are negative. Starch and Tweens 20 and 80 are hydrolysed, whereas gelatin and urea are not. Acid is produced from inulin, arabinose, raffinose, maltose, D-galactose, ribose, D-xylose, glucose, lactose, mannose and glycerol, but not from rhamnose, inositol, mannitol, trehalose, aesculin or sorbitol. Fructose, galactose, mannose, raffinose, glycerol, maltose, lactose, glucose, sucrose, D-ribose, arabinose and L-threonine can be utilized as sole carbon and energy sources, whereas D-sorbitol, D-mannitol, D-xylose, L-aspartic acid, L-glutamic acid, L-ornithine and L-phenylalanine cannot be utilized. Cells are resistant to (µg ml⁻¹) kanamycin (30), ampicillin (30) and streptomycin (10), but susceptible to (µg ml⁻¹) chloramphenicol (10), tetracycline (30), gentamicin (60) and neomycin (10). *meso*-Diaminopimelic acid is the predominant diamino acid of the cell-wall peptidoglycan. The major respiratory quinone

is MK-7; minor amounts of MK-8 are found. The major fatty acids are anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. The polar lipids diphosphatidylglycerol, phosphatidylglycerol and unidentified lipids were detected.

The type strain is K170^T (=CGMCC 1.10681^T=DSM 23178^T), isolated from Keke Salt Lake in Qinghai, China. The DNA G + C content of strain K170^T is 35.8 mol%.

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References

- Ahmed, I., Yokota, A. & Fujiwara, T. (2007). *Gracilibacillus boracitolerans* sp. nov., a highly boron-tolerant and moderately halotolerant bacterium isolated from soil. *Int J Syst Evol Microbiol* **57**, 796–802.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Barrow, G. I. & Feltham, R. K. A. (editors) (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.
- Carrasco, I. J., Márquez, M. C., Yanfen, X., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2006). *Gracilibacillus orientalis* sp. nov., a novel moderately halophilic bacterium isolated from a salt lake in Inner Mongolia, China. *Int J Syst Evol Microbiol* **56**, 599–604.
- Chamroensaksri, N., Tanasupawat, S., Akarachanya, A., Visessanguan, W., Kudo, T. & Itoh, T. (2010). *Gracilibacillus thailandensis* sp. nov., from fermented fish (pla-ra). *Int J Syst Evol Microbiol* **60**, 944–948.
- Chen, Y. G., Cui, X. L., Zhang, Y. Q., Li, W. J., Wang, Y. X., Xu, L. H., Peng, Q., Wen, M. L. & Jiang, C. L. (2008a). *Gracilibacillus halophilus* sp. nov., a moderately halophilic bacterium isolated from saline soil. *Int J Syst Evol Microbiol* **58**, 2403–2408.
- Chen, Y. G., Cui, X. L., Zhang, Y. Q., Li, W. J., Wang, Y. X., Xu, L. H., Peng, Q., Wen, M. L. & Jiang, C. L. (2008b). *Gracilibacillus*

- quinghaiensis* sp. nov., isolated from salt-lake sediment in the Qaidam Basin, north-west China. *Syst Appl Microbiol* **31**, 183–189.
- Collins, M. & Jones, D. (1980).** Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Microbiol* **48**, 459–470.
- Gregersen, T. (1978).** Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.
- Harrigan, W. & McCance, M. (1976).** *Laboratory Methods in Foods and Dairy Microbiology*. London: Academic Press.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983).** A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Heimbrook, M. E., Wang, W. L. L. & Campbell, G. (1989).** Staining bacterial flagella easily. *J Clin Microbiol* **27**, 2612–2615.
- Huo, Y. Y., Xu, X. W., Cui, H. L. & Wu, M. (2010).** *Gracilibacillus ureilyticus* sp. nov., a halotolerant bacterium from a saline-alkaline soil. *Int J Syst Evol Microbiol* **60**, 1383–1386.
- Jeon, C. O., Lim, J. M., Jang, H. H., Park, D. J., Xu, L. H., Jiang, C. L. & Kim, C. J. (2008).** *Gracilibacillus lacisalsi* sp. nov., a halophilic Gram-positive bacterium from a salt lake in China. *Int J Syst Evol Microbiol* **58**, 2282–2286.
- Lawson, P. A., Deutch, C. E. & Collins, M. D. (1996).** Phylogenetic characterization of a novel salt-tolerant *Bacillus* species: description of *Bacillus dipsosauri* sp. nov. *J Appl Bacteriol* **81**, 109–112.
- 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.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Sehgal, S. N. & Gibbons, N. E. (1960).** Effect of some metal ions on the growth of *Halobacterium cutirubrum*. *Can J Microbiol* **6**, 165–169.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Manual of Methods for General and Microbiology Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tang, S. K., Wang, Y., Lou, K., Mao, P. H., Jin, X., Jiang, C. L., Xu, L. H. & Li, W. J. (2009).** *Gracilibacillus saliphilus* sp. nov., a moderately halophilic bacterium isolated from a salt lake. *Int J Syst Evol Microbiol* **59**, 1620–1624.
- Ventosa, A., Quesada, E., Rodriguez-Valera, F., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1982).** Numerical taxonomy of moderately halophilic Gram-negative rods. *J Gen Microbiol* **128**, 1959–1968.
- 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.
- Yanagi, M. & Yamasato, K. (1993).** Phylogenetic analysis of the family *Rhizobiaceae* and related bacteria by sequencing of 16S rRNA gene using PCR and DNA sequencer. *FEMS Microbiol Lett* **107**, 115–120.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996).** Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998).** Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.