***Paenibacillus jamilae* sp. nov., an exopolysaccharide-producing bacterium able to grow in olive-mill wastewater**

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Endospore-forming strains were isolated from corn-compost treated with olive-mill wastewater ('alpechín'). The strains were taxonomically studied and proposed as a novel *Paenibacillus* species. These organisms (strains B.3^T, B.7 and B.9) were particularly distinguishable from other aerobic spore-forming species by their ability to grow optimally in 100% (v/v) olive-mill wastewater at 30 °C and pH 7.0 and concomitant production of an interesting exopolysaccharide. Chemotaxonomic analysis revealed that MK-7 was the predominant menaquinone, the major fatty acid was anteiso C_{15:0} and the cell wall contained meso-diaminopimelic acid. The DNA G+C content was 40.7 mol%. Comparative sequence analysis of 16S rDNA with different reference species from the genera *Bacillus*, *Paenibacillus*, *Brevibacillus*, *Aneurinibacillus*, *Alicyclobacillus*, *Halobacillus*, *Virgibacillus*, *Amphibacillus*, *Coprobacillus* and *Gracilibacillus* indicated that the isolated strains were highly related to the genus *Paenibacillus*. Strain B.3^T formed an evolutionary lineage distinct from other species within the evolutionary radiation encompassing the genus *Paenibacillus*. Strain B.3^T was a close relative of *Paenibacillus polymyxa*, but DNA–DNA relatedness data with this species was very low (relative binding ratio < 16%). Based on the morphological and physiological characteristics, as well as on the phylogenetic position determined by 16S rDNA analysis and DNA–DNA relatedness data, it is concluded that these strains should be designated a novel species, for which the name *Paenibacillus jamilae* sp. nov. is proposed. The type strain is B.3^T (= CECT 5266^T = DSM 13815^T).

Keywords: *Paenibacillus jamilae* sp. nov., olive-mill wastewater, exopolysaccharide, 16S rDNA analysis

INTRODUCTION

Olive-mill wastewater (OMWW), known as 'alpechín', is a toxic aqueous residue produced in olive oil extraction. The most important olive oil-producing countries are Spain and Italy. Bioremediation of alpechín by using the residue as a culture medium for some micro-organisms can also provide new resources, such as exopolysaccharides (EPSs), which are highly

interesting biopolymers due to their potential applications (Ramos-Cormenzana *et al.*, 1995). Developments in molecular biological methods have suggested that the genus *Bacillus* is a phylogenetically heterogeneous taxon. For example, analyses showed a wide range of G+C contents (32–69 mol%) among the DNAs of *Bacillus* species (Claus & Berkeley, 1986; Slepecky & Hemphill, 1991; Stackebrandt & Liesack, 1993). DNA reassociation studies have shown that many *Bacillus* species are composites of several genetically unrelated species (Priest, 1981; Slepecky & Hemphill, 1991; Nakamura, 1996; Shida *et al.*, 1997b). Resulting phylogenetic studies by 16S rRNA gene sequencing revealed that the genus *Bacillus* could be

Abbreviations: EPS, exopolysaccharide; OMWW, olive-mill wastewater; RBR, relative binding ratio.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain B.3^T is J271157

separated into several phylogenetically distinct genera such as *Alicyclobacillus* (Wisotzkey *et al.*, 1992), *Paenibacillus* (Ash *et al.*, 1993), *Aneurinibacillus* (Shida *et al.*, 1996), *Brevibacillus* (Shida *et al.*, 1996), *Halobacillus* (Spring *et al.*, 1996), *Gracilibacillus* and *Salibacillus* (Wainø *et al.*, 1999), and *Coprobacillus* (Kageyama & Benno, 2000).

In the present study, three strains (B.3^T, B.7 and B.9) of Gram-variable, spore-forming bacilli were isolated from a corn-compost treated with OMWW. The strains were able to grow in 100% (v/v) OMWW and to produce an heteropolysaccharide consisting of fucose, xylose, rhamnose, arabinose, mannose, galactose and glucose as sugar components (V. Guerra, M. Monteoliva-Sánchez, M. Aguilera & A. Ramos-Cormenzana, unpublished data). This EPS has been shown to enhance the immune response in mice (Ramos-Cormenzana & Monteoliva-Sánchez, 2000). Based on phenotypic properties and DNA G+C content, these organisms were tentatively identified as *Bacillus* species, closely related to *Bacillus firmus*, *Brevibacillus laterosporus*, *Bacillus lentus* and *Paenibacillus polymyxa*. To establish their correct taxonomic position and to rectify the tenuous classification, strains B.3^T, B.7 and B.9 were further characterized by 16S rDNA sequencing. Data suggested that the strains B.3^T, B.7 and B.9 could be a *Paenibacillus* species. After carefully studying the data obtained by phenotypic characterization, DNA G+C content, DNA–DNA relatedness and 16S rDNA analysis, a novel species, *Paenibacillus jamilae* sp. nov., is proposed.

METHODS

Bacterial strains and culture conditions. Strains B.3^T, B.7 and B.9 were isolated from a sample of corn-compost treated with OMWW. The following reference strains were included in the study: *Bacillus firmus* CCM 2213^T, *Bacillus lentus* CCM 2214^T, *Brevibacillus laterosporus* CCM 2116^T and *P. polymyxa* CECT 153. All strains were grown on YM broth (Difco) and 15 g agar (Difco) l⁻¹ (if needed). Strains B.3^T, B.7 and B.9 were cultivated at 30 °C for 24 h on TSA (Difco) and TSB (Difco) supplemented with 0.5% (w/v) glycine for the isolation of DNA.

Morphological and physiological tests. The morphology of cells, motility, shape of spores, swollen sporangia and parasporal crystal were examined by phase-contrast microscopy. The presence of EPS and size of the cells were examined by transmission electron microscopy using cells from a 48 h culture that were stained with 2.5% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in a 0.1 M cacodylate–sodium salt buffer which contained 0.075% (w/v) ruthenium red. The grids were examined using a model Zeiss 902 transmission electron microscope. Catalase activity was determined by bubble production in a 10% (v/v) H₂O₂ solution. Oxidase activity was determined by oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine. Utilization and production of gases by substrates as sole carbon source was performed with API 50CHB (bio-Mérieux) using *P. polymyxa* as control strain. The following characteristics were determined as previously described (Claus & Berkeley, 1986): hydrolysis of gelatin, casein and starch; anaerobic growth; Voges–Proskauer; utilization of

citrate and propionate; reduction of nitrate; production of indole; growth at pH 5, 6, 7, 8, 9, 10, 11 and 12; growth at 2, 5, 7 and 10% (w/v) NaCl; growth at 5, 10, 30, 40, 50, 55 and 65 °C; and growth in the presence of 0.001% (w/v) lysozyme. Other tests were completed with API 20E (bio-Mérieux).

Chemotaxonomic characterization

Analysis of isoprenoid quinones. Isoprenoid quinones were extracted by treating 500 mg lyophilized cells grown in YM broth with 150 ml chloroform:methanol (2:1, v/v) for 2 h, using a reciprocal shaker (120 strokes min⁻¹) at room temperature. The extracted solution was concentrated using liquid N₂ and transferred by re-dissolving in acetone. The resulting solution was evaporated, separated by TLC using n-hexane:dimethyl ether (85:15, v/v) as the solvent and recovered from TLC plates with acetone. Isoprenoid quinones thus obtained were analysed by HPLC (Waters) as described by Yumoto *et al.* (1998).

Analyses of cellular fatty acids and the cell wall. For cellular fatty acid analysis, cells were cultivated in YM broth until the late exponential phase of growth. Methods for fatty acid extraction, methyl ester preparation, methyl ester separation by GC and identification of fatty acids were previously described (Monteoliva-Sánchez *et al.*, 1993).

Identification of *meso*-diaminopimelic acid in the cell wall was performed by TLC (DC-Alufoline Cellulose; Merck) as described by Yamada & Komagata (1970).

Isolation of DNA and determination of G+C content. Chromosomal DNA was isolated by the method of Marmur (1961). DNA preparations were analysed by electrophoresis through 0.8% (w/v) agarose gel, spectrophotometrically quantified at 260 nm (Biophotometer-Eppendorf) and stored at –20 °C.

The G+C content was determined by the method of Marmur & Doty (1962), based on the determination of DNA *T_m*. DNA from *Escherichia coli* was included in each set of analyses as a reference standard. The G+C content of *E. coli* DNA was regarded as 51.7 mol%.

DNA–DNA reassociation and 16S rDNA sequencing. The hybridization of genomic DNA from strain B.3^T with B.7, B.9, *Bacillus firmus*, *Bacillus lentus*, *Brevibacillus laterosporus* and *P. polymyxa* was performed according to the method of Ziemke *et al.* (1998).

Purified genomic DNA was used for the amplification of 16S rRNA genes via PCR with the forward primer 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3') and the reverse primer 16R1525 (5'-AAGGAGGTGWTCCARCC-3'). PCR reactions were carried out under the conditions described by Saiki *et al.* (1988). Amplification products were purified with Microcon-100 concentrators (Amicon) and directly sequenced using an Applied Biosystems 373A DNA sequencer and the manufacturer's protocols for *Taq* cycle-sequencing with fluorescent dye-labelled dideoxynucleotides (Perkin-Elmer). The primers used for the sequencing reactions were previously described (Lane, 1991).

Phylogenetic analysis. To establish the relatives of strain B.3^T based on 16S rDNA sequence similarity, preliminary searches in different databases were performed with the program BLAST. Sequences of close relatives (*Paenibacillus*) were retrieved from databases and were aligned with the sequences of other *Bacillus*, *Lactobacillus*, *Alicyclobacillus*, *Aneurinibacillus*, *Virgibacillus*, *Brevibacillus*, *Amphibacillus*, *Halobacillus*, *Gracilibacillus* and *Coprobacillus* species using

CLUSTAL W software (Thompson *et al.*, 1994). The 16S rDNA similarity values were calculated from the alignments. The evolutionary distances were calculated using the DNADIST program of PHYLIP 3.5 software package (Felsenstein, 1989) with the model of Jukes & Cantor (1969). A phylogenetic tree was constructed using the FITCH program, which uses the least-squares algorithm of Fitch & Margoliash (1967). The tree was drawn with TREEVIEW program (Page, 1996).

RESULTS AND DISCUSSION

Morphological and physiological characteristics

The cells of strains B.3^T, B.7 and B.9 were rod-shaped measuring 0.5–1.2 × 4.5–6.5 µm after growing for 48 h at 30 °C and pH 7.0. They produced ellipsoidal spores

Table 1. Differentiation of strain B.3^T from closely related *Paenibacillus* species

Strains: 1, B.3^T; 2, *P. polymyxa*; 3, *P. peoriae*; 4, *P. azotofixans*. +, Positive; –, negative; v, variable; ND, not determined.

Characteristic	1	2*	3*	4*
Growth in 0.001 % lysozyme	+	v	+	–
Acid from:				
Arabinose	+	+	+	–
Xylose	+	+	+	–
Glycerol	+	+	–	–
Trehalose	+	+	–	ND
Methyl β-xyloside	–	+	ND	ND
Gas from carbohydrate	–	+	+	+
Starch hydrolysis	+	+	+	–
Citrate utilization	–	+	–	–
Nitrate reduced to nitrite	+	+	+	–
Casein hydrolysis	+	+	+	–
G + C content (mol %)	40–43	43–46	45–47	48–53

* Data from Montefusco *et al.* (1993).

in swollen sporangia. Colonies were convex, mucoid and opaque. The colonies of strain B.7 were greater in size (> 4 mm) than those of strains B.9 and B.3^T (< 4 mm). All strains formed motile microcolonies while growing on wet agar plates. The strains were facultatively anaerobic, Gram-variable and motile by means of peritrichous flagella. Strains had catalase activity, but no oxidase activity. These strains did not grow in the presence of 5 % (w/v) NaCl, which is the case with other *Paenibacillus* species (Shida *et al.*, 1997a). Gelatin, casein and starch were hydrolysed. The optimum growth temperature was 30 °C, as it is for all *Paenibacillus* species except *Paenibacillus macquariensis* which has an optimum growth temperature of 20–23 °C (Shida *et al.*, 1997a). The optimum pH for growth was 7.0, but it was able to grow in a wide range of pH (5–12). Members of the genus *Paenibacillus* grow optimally at the same pH (Shida *et al.*, 1997a). The strains B.3^T, B.7 and B.9 grew in 100 % (v/v) OMWW, which was an important phenotypic difference that distinguished our strains from other known *Paenibacillus* species. All the physiological and biochemical characteristic which are identical among the isolated B.3^T, B.7 and B.9 are given below in the description of the novel species. Only strain B.3^T was positive for ONPG hydrolysis and results obtained for acid production from several carbohydrates showed that utilization of methyl α-D-mannoside was positive only for strain B.3^T, methyl α-D-glucoside was positive for strains B.3^T and B.7, and utilization of both inulin and gentiobiose was positive for strains B.7 and B.9. *Paenibacillus* strain B.3^T can be differentiated from other related *Paenibacillus* species as shown in Table 1.

Chemotaxonomic characteristics

meso-Diaminopimelic acid was the diamino acid found in the cell-wall peptidoglycan of strains B.3^T, B.7 and B.9. This diamino acid is common to the members of the genus *Paenibacillus* and to other aerobic endospore formers such as *Bacillus*, *Sporolactobacillus* and *Amphi-*

Table 2. G + C content, 16S rDNA similarity and DNA–DNA hybridization data for some strains studied

Strain	<i>T</i> _m (°C)	G + C content (mol %)	16S rDNA similarity (%)	Hybridization to DNA from B.3 ^T (RBR; %)
B.3 ^T	85.98	40.6	100	100
B.7	85.87	40.5	98	75
B.9	86.05	40.8	98	73
<i>Bacillus firmus</i>	85.78	40.2	ND	6
<i>Bacillus lentus</i>	85.56	39.6	ND	13
<i>Brevibacillus laterosporus</i>	85.31	39.1	ND	21
<i>P. polymyxa</i>	87.5	44.5	98	15
<i>P. peoriae</i>	88.16	46.0	96	ND
<i>P. azotofixans</i>	90.01	50.5	95	ND

ND, Not determined.

Fig. 1. Phylogenetic tree showing the position of strain B.3^T in relation to *Paenibacillus* species and some rod-shaped, endospore-forming bacteria based on 16S rRNA gene sequences. EMBL accession numbers of reference sequences are given after the strain names. Bar, 1 nucleotide substitution per 10 nucleotides.

bacillus (Shida *et al.*, 1997a). The major isoprenoid quinone was MK-7, which is the major menaquinone generally found in aerobic, endospore-forming rods. Anteiso-C_{15:0}, the predominant fatty acid found in this genus (Shida *et al.*, 1997a), was also the major fatty acids component of strains B.3^T, B.7 and B.9.

DNA base composition and DNA–DNA reassociation

The G + C contents of strains B.3^T, B.7 and B.9 were 40.6, 40.7 and 40.8 mol %, respectively. These G + C contents lie within the range found in the members of genus *Paenibacillus* (Shida *et al.*, 1997a). DNA G + C contents of *Bacillus firmus*, *Bacillus lentus*, *Brevibacillus laterosporus* and *P. polymyxa*, *Paenibacillus*

peoriae, *Paenibacillus azotofixans* and their relative binding ratios (RBRs) of DNA–DNA hybridization are compiled in Table 2. Strains B.3^T, B.7 and B.9 belonged to the same species because their RBRs to each other were > 70 %. However, it was concluded that these strains were a separate species from *Bacillus firmus*, *Brevibacillus laterosporus*, *Bacillus lentus* and *Paenibacillus polymyxa* because the RBRs with these species were < 22 %.

Phylogenetic analysis

Although the preceding biochemical, chemotaxonomic, G + C content and hybridization analysis did not establish a definite taxonomic position for the

strains B.3^T, B.7 and B.9, these data suggested *Paenibacillus* as a possible classification. 16S rDNA was sequenced to verify the suggestion. An almost complete 16S rDNA sequence (1525 bp) of strain B.3^T, which corresponded to a region between positions 1 and 1524 by comparison with other eubacteria 16S rRNA genes, was obtained. The phylogenetic tree (Fig. 1) constructed from the sequence data showed that strain B.3^T appeared within the evolutionary radiation encompassing genus *Paenibacillus* and occupied a distinct phylogenetic position within the genus. The phylogenetic study clearly established that our strains were a *Paenibacillus* species. Moreover, there was a consensus signature sequence (PAEN515F) of *Paenibacillus* genus (5'-GAGTAACTGCTACAA-GAGTGACGGTACCTGAGAAGAAAGCCCC-3') within the 16S rRNA sequence of our strains (Shida *et al.*, 1997a).

The results of this polyphasic study supports strains B.3^T, B.7 and B.9 being the same species and belonging to the genus *Paenibacillus*. Their genetic distinctiveness inferred from the phylogenetic study warrants the proposal of these strains as a novel species, *Paenibacillus jamilae* sp. nov.

Description of *Paenibacillus jamilae* sp. nov.

Paenibacillus jamilae (ja.mi'lae. N.L. fem. n. *jamilae* residual water of olive oil production, from *jamilá* specific term of Arabic origin commonly used in Andalusia, Spain).

Cells are rod-shaped (0.5–1.2 × 4.5–6.5 µm) and motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Colonies are convex, mucoid and opaque. Forms motile microcolonies on wet agar plates. Facultatively anaerobic and Gram-variable. Catalase reaction is positive, whereas oxidase activity is negative. Gelatin, casein and starch are hydrolysed. Acid but not gas is produced from glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, D-mannitol, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, raffinose, glycogen and D-turanose. Erythritol, D-arabinose, L-xylose, adonitol, methyl β-xyloside, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, N-acetylglucosamine, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-gluconate and 5-keto-gluconate are not utilized as sole carbon sources. Citrate and propionate are not utilized. Acetylmethylcarbinol is positive (as determined by the Voges-Proskauer reaction). Nitrate is reduced to nitrite. Growth occurs at temperatures of 30–40 °C and at pH 5–12. Optimum growth occurs at 30 °C and at pH 7.0. Growth occurs in the presence of 2% NaCl and 0.001% lysozyme, whereas it is inhibited by 5% NaCl. The major fatty acid is anteiso-C_{15:0}. The major quinone is MK-7. The mean DNA G+C content is 40.6–40.8 mol%. The type strain is B.3^T (= CECT 5266^T = DSM 13815^T).

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REFERENCES

- Ash, C., Priest, F. G. & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Wallbanks and Collins) using a PCR probe test. *Antonie Leeuwenhoek* **64**, 253–260.
- Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872, 174^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Felsenstein, J. (1989). PHYLIP – Phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability. *Science* **155**, 279–284.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kageyama, A. & Benno, Y. (2000). *Coprobacillus cateniformis* gen. nov., sp. nov., a new genus and species isolated from human faeces. *Microbiol Immunol* **44**, 23–28.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: John Wiley.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **4**, 109–118.
- Montefusco, A., Nakamura, L. K. & Labeda, D. P. (1993). *Bacillus peoriae* sp. nov. *Int J Syst Bacteriol* **43**, 388–390.
- Monteoliva-Sánchez, M., Ramos-Cormenzana, A. & Russell, N. J. (1993). The effect of salinity and compatible solutes on the biosynthesis of cyclopropane fatty acids in *Pseudomonas halosaccharolytica*. *J Gen Microbiol* **139**, 1877–1884.
- Nakamura, L. K. (1996). *Paenibacillus apiarius* sp. nov. *Int J Syst Bacteriol* **46**, 688–693.
- Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Priest, F. G. (1981). DNA homology in the genus *Bacillus*. In *The Aerobic Endospore-forming Bacteria*, pp. 33–57. Edited by R. C. W. Berkeley & M. Goodfellow. London: Academic Press.
- Ramos-Cormenzana, A. & Monteoliva-Sánchez, M. (2000). Biopharmaceutical potential of olive industrial wastes. *ARS Pharm* **41**, 129–136 (in Spanish).
- Ramos-Cormenzana, A., Monteoliva Sánchez, M. & López, M. J. (1995). Bioremediation of alpechin. *Int Biodeterior Biodegrad* **35**, 249–268.
- Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.

- Shida, O., Takagi, H., Kadowaki, K. & Komagata, K. (1996). Proposal for two new genera. *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. *Int J Syst Bacteriol* **46**, 939–946.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997a). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis* and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* **47**, 289–298.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997b). Emended description of *Paenibacillus amylolyticus* and description of *Paenibacillus illinoisensis* sp. nov. and *Paenibacillus chibensis* sp. nov. *Int J Syst Bacteriol* **47**, 299–306.
- Slepecky, R. A. & Hemphill, H. E. (1991). The genus *Bacillus* – nonmedical. In *The Prokaryotes*, pp. 1663–1696. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- 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 truperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. *Int J Syst Bacteriol* **46**, 492–496.
- Stackebrandt, E. & Liesack, W. (1993). Nucleic acids and classification. In *Handbook of New Bacterial Systematics*, pp. 152–189. Edited by M. Goodfellow & A. G. O'Donnell. London: Academic Press.
- 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.
- 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.
- Wisotzkey, J. D., Jurtshuk, P., Jr, Fox, G. E., Deinhard, G. & Poralla, K. (1992). Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int J Syst Bacteriol* **42**, 263–269.
- Yamada, K. & Komagata, K. (1970). Taxonomic studies on coryneform bacteria. II. Principal amino acids in the cell wall and their taxonomic significance. *J Gen Appl Microbiol* **16**, 103–113.
- Yumoto, I., Yamazaki, K., Sawabe, T., Nakano, K., Kawasaki, K., Ezura, Y. & Shinano, H. (1998). *Bacillus horti* sp. nov., a new Gram-negative alkaliphilic bacillus. *Int J Syst Bacteriol* **48**, 565–571.
- 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.