Bacillus velezensis is a later heterotypic synonym of *Bacillus amyloliquefaciens*

Li-Ting Wang, Fwu-Ling Lee, Chun-Ju Tai and Hsiao-Ping Kuo

Correspondence

Fwu-Ling Lee

fll@firdi.org.tw

Bioresource Collection and Research Center, Food Industry Research and Development Institute, PO Box 246, Hsinchu 30099, Taiwan, ROC

Strain BCRC 14193, isolated from soil, shared more than 99 % 16S rRNA gene sequence similarity with *Bacillus amyloliquefaciens* BCRC 11601^T and *Bacillus velezensis* BCRC 17467^T. This strain was previously identified as *B. amyloliquefaciens*, based on DNA–DNA hybridization, but its DNA relatedness value with *B. velezensis* BCRC 17467^T was 89 %. To investigate the relatedness of strain BCRC 14193, *B. amyloliquefaciens* and *B. velezensis*, the partial sequence of the gene encoding the subunit B protein of DNA gyrase (*gyrB*) was determined. *B. velezensis* BCRC 17467^T shared high *gyrB* gene sequence similarity with *B. amyloliquefaciens* BCRC 14193 (98.4 %) and all of the *B. amyloliquefaciens* strains available (95.5–95.6 %). DNA–DNA hybridization experiments revealed high relatedness values between *B. velezensis* BCRC 17467^T and *B. amyloliquefaciens* BCRC 11601^T (74 %) and the *B. amyloliquefaciens* reference strains (74–89 %). Based on these data and the lack of phenotypic distinctive characteristics, we propose *Bacillus velezensis* as a later heterotypic synonym of *Bacillus amyloliquefaciens*.

Bacillus velezensis, isolated from the mouth of the river Vélez in Málaga (southern Spain), has been described recently by Ruiz-García *et al.* (2005). The type strain BCRC 17467^T (CR-502^T=LMG 22478^T) and strain CR-14b had identical 16S rRNA gene sequences and the topology of the phylogenetic tree confirmed that they were members of the *Bacillus subtilis* group. The 16S rRNA gene sequences of these strains shared 99 % similarity with *Bacillus subtilis* subsp. *subtilis* (Nakamura *et al.*, 1999), *Bacillus amyloliquefaciens* (Priest *et al.*, 1987) and *Bacillus vallismortis* (Roberts *et al.*, 1996), and 98 % similarity with *Bacillus mojavensis* (Roberts *et al.*, 1994) and *Bacillus atrophaeus* (Nakamura, 1989). DNA–DNA hybridization analysis confirmed that *B. velezensis* BCRC 17467^T and CR-14b show less than 20 % relatedness with the species mentioned above. The two strains were characterized by their capacity to produce acids from glycogen, lactose, methyl α -D-glycoside and raffinose, but not from turanose, and produce β -galactosidase (determined by ONPG reaction), but not arginine dihydrolase. Strain CR-502^T showed a specific fatty acid profile characterized mainly by its high C_{16:0} content and the presence of C_{16:1} ω 11c, iso-C_{13:0}, C_{14:0} and iso-C_{17:1} ω 10c. Genomic and phenotypic data demonstrated that *B. velezensis* represents a novel species of *Bacillus* (Ruiz-García *et al.*, 2005).

B. amyloliquefaciens, isolated from soil, was first proposed by Fukumoto (1943a, b). This taxon was not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), but was later revived by Priest *et al.* (1987). *B. amyloliquefaciens* is responsible for much of the world production of α -amylase and protease (Harwood, 1992). *B. amyloliquefaciens* is closely related to *B. subtilis* and there is a need to distinguish them in the enzyme industry to avoid confusion with *B. subtilis*, which differs metabolically and secretes different enzymes (Priest, 1977). *B. amyloliquefaciens* can be distinguished from *B. subtilis* by a few phenotypic traits and DNA similarity (Logan & Berkeley, 1984; Nakamura, 1987; O'Donnell *et al.*, 1980).

Strain BCRC 14193 was isolated from soil taken from Hsinchu, Taiwan. For the isolation of strain BCRC 14193, a 10 g soil sample was vortexed with 50 ml sterile distilled water for 5 min. Serial 50-fold dilutions were prepared and 100 μ l of the mixture was spread onto trypticase soy agar (TSA; Difco) at 30 °C for 24 h under aerobic conditions. Morphologically distinct single colonies that developed were picked up and restreaked onto fresh TSA at least twice before being considered pure. Pure strains were preserved at –80 °C in trypticase soy broth or by lyophilization. Strain BCRC 14193 was identified as *B. amyloliquefaciens*, based mainly on DNA–DNA hybridization (77 %), in our previous study (Wang *et al.* 2007b). In addition to strain BCRC 14193, *B. amyloliquefaciens* strains BCRC 11601^T, BCRC 14711 and BCRC 17038 and other related taxa were selected for further comparative study. *B. velezensis* BCRC 17467^T was also included in the study. All strains were

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of *Bacillus velezensis* strain BCRC 17467^T are EF433407 and DQ903176.

Additional phylogenetic trees and fatty acid composition and phenotypic characteristic tables are available with the online version of this paper.

cultivated in nutrient agar or broth (Difco) at 30 °C for 24 h under aerobic conditions.

Genomic DNA was isolated using the Qiagen Blood & Cell Culture DNA kit. The 16S rRNA gene sequence was PCR-amplified and sequenced using the MicroSeq Full Gene 16S rDNA Bacterial Identification kit (Applied Biosystems). The partial sequence of the gene encoding the subunit B protein of DNA gyrase (*gyrB*) was included in the phylogenetic analysis for comparative purposes. The *gyrB* gene was PCR-amplified and sequenced using a set of primers described by Wang *et al.* (2007b). Sequencing was performed using a 3730 DNA sequencer (Applied Biosystems and Hitachi). Sequence assembly was performed using the ABI PRISM DNA Sequencing Analysis software (PE Applied Biosystems). Sequence similarity was calculated and DNA sequences were translated using the Wisconsin Package version 10.1 (Accelrys). The sequences obtained in this study and from GenBank were aligned using the CLUSTAL_X program, version 1.8 (Thompson *et al.*, 1997), and phylogenetic analysis including neighbour-joining, maximum-parsimony and maximum-likelihood trees of DNA and protein sequence alignments were performed with the PHYLIP computer program package (Felsenstein, 2002). Bootstrap analysis was made with 1000 replicates, except for maximum-likelihood, where only 100 replicates were generated. Tree figures were drawn with MEGA3 (Kumar *et al.*, 2004). Phylogenetic analysis by the neighbour-joining (Fig. 1), maximum-parsimony and maximum-likelihood methods (see Supplementary Figs S1 and S2, available in IJSEM Online) produced similar trees, with the exception of minor differences in the tree topology of the basal branches.

The phylogenetic tree of the 16S rRNA gene sequences between positions 35 and 1431 (*Escherichia coli* K-12 numbering) showed that *B. velezensis* and *B. amyloliquefaciens* strains are more closely related to *B. vallismortis* (Fig. 1a). *B. velezensis* BCRC 17467^T shared 99.9% similarity with *B. vallismortis* BCRC 17183^T and 99.7% similarity with all of the *B. amyloliquefaciens* strains available (Table 1). Although strain BCRC 14193 is on the same branch as the *B. velezensis* type strain, all *Bacillus* species among this cluster showed more than 99% 16S rRNA gene sequence identity, indicating that it is not sufficient to discriminate one species from another. Comparatively, the tree based on the *gyrB* gene sequences between positions 316 and 1480 (*E. coli* K-12 numbering) clearly showed that *B. velezensis* BCRC 17467^T and the *B. amyloliquefaciens* strains investigated formed a phylogenetic grouping with 100% bootstrap support (Fig. 1b). *B. velezensis* BCRC 17467^T shared 98.4% *gyrB* gene sequence similarity with *B. amyloliquefaciens* BCRC 14193 and 95.5–95.6% similarity with the other *B. amyloliquefaciens* strains, whereas the sequence similarities between *B. velezensis* and other members of the related cluster were less than 81%. High sequence similarity of the *gyrB* gene of *B. velezensis* BCRC 17467^T and *B. amyloliquefaciens* BCRC 14193 meant that they yielded a branch with the *B.*

Fig. 1. Neighbour-joining phylogenetic trees based on 16S rRNA (a) and *gyrB* (b) gene sequences of *Bacillus* strains. Genetic distances were computed by Kimura's two-parameter model. Only bootstrap percentages above 50% are shown. Bars, 0.005 (a) or 0.05 (b) substitutions per nucleotide position.

amyloliquefaciens strains. This tree topology can be observed in the *B. licheniformis* cluster group. *B. licheniformis* BCRC 11702^T shared 99.8% similarity with *B. licheniformis* BCRC 14353 and 95.5–95.7% similarity with the other *B. licheniformis* strains (BCRC 11702^T and the *B. licheniformis* strains investigated showed high DNA–DNA relatedness values of 72–91%). Phylogenetic analysis based on GyrB protein sequence trees led to the same overall conclusions (these trees are provided as Supplementary Figs S3–S5 in IJSEM Online). *B. velezensis* BCRC 17467^T

Table 1. Levels of *gyrB* and 16S rRNA gene sequence similarity and DNA relatedness values between *Bacillus* strains

<i>Bacillus</i> strains compared	<i>gyrB</i> /16S rRNA gene sequence similarity (%)	DNA relatedness value (%)
<i>B. velezensis</i> BCRC 17467 ^T compared with:		
<i>B. velezensis</i> BCRC 17710 ^T	100 / 100	100
<i>B. amyloliquefaciens</i> BCRC 14193	98.4 / 99.7	89
<i>B. amyloliquefaciens</i> BCRC 14711	95.6 / 99.7	74
<i>B. amyloliquefaciens</i> BCRC 17038	95.5 / 99.7	76
<i>B. amyloliquefaciens</i> BCRC 11601 ^T	95.6 / 99.7	74
<i>B. licheniformis</i> BCRC 11702 ^T	77.8 / 97.9	11
<i>B. subtilis</i> subsp. <i>subtilis</i> BCRC 10255 ^T	79.8 / 99.7	19
<i>B. subtilis</i> subsp. <i>spizizenii</i> BCRC 17366 ^T	80.7 / 99.6	20
<i>B. mojavensis</i> BCRC 17124 ^T	80.9 / 99.4	16
<i>B. atrophaeus</i> BCRC 17123 ^T	81.0 / 99.3	18
<i>B. sonorensis</i> BCRC 17416 ^T	78.6 / 97.8	10
<i>B. vallismortis</i> BCRC 17183 ^T	80.8 / 99.9	12
<i>B. amyloliquefaciens</i> BCRC 11601 ^T compared with:		
<i>B. amyloliquefaciens</i> BCRC 14711	100 / 100	100
<i>B. amyloliquefaciens</i> BCRC 17038	99.9 / 100	97
<i>B. amyloliquefaciens</i> BCRC 14193	96.0 / 99.7	84
<i>B. velezensis</i> BCRC 17467 ^T	95.6 / 99.7	82
<i>B. velezensis</i> BCRC 17710 ^T	95.6 / 99.7	83
<i>B. licheniformis</i> BCRC 11702 ^T	77.8 / 98.2	10
<i>B. subtilis</i> subsp. <i>subtilis</i> BCRC 10255 ^T	79.8 / 99.5	20
<i>B. subtilis</i> subsp. <i>spizizenii</i> BCRC 17366 ^T	80.2 / 99.5	19
<i>B. mojavensis</i> BCRC 17124 ^T	80.6 / 99.5	21
<i>B. atrophaeus</i> BCRC 17123 ^T	80.9 / 99.3	19
<i>B. sonorensis</i> BCRC 17416 ^T	78.7 / 98.2	10
<i>B. vallismortis</i> BCRC 17183 ^T	79.8 / 99.7	12
<i>B. licheniformis</i> BCRC 11702 ^T compared with:		
<i>B. licheniformis</i> BCRC 12826	95.5 / 99.5	72
<i>B. licheniformis</i> BCRC 14353	99.8 / 99.8	91
<i>B. licheniformis</i> BCRC 15413	95.7 / 99.5	73
<i>B. subtilis</i> subsp. <i>subtilis</i> BCRC 10255 ^T	76.4 / 98.3	13
<i>B. subtilis</i> subsp. <i>spizizenii</i> BCRC 17366 ^T	77.1 / 98.4	13
<i>B. mojavensis</i> BCRC 17124 ^T	77.2 / 98.3	14
<i>B. atrophaeus</i> BCRC 17123 ^T	78.5 / 98.2	12
<i>B. sonorensis</i> BCRC 17416 ^T	89.9 / 99.7	31
<i>B. vallismortis</i> BCRC 17183 ^T	76.4 / 98.2	14
<i>B. subtilis</i> subsp. <i>subtilis</i> BCRC 10255 ^T compared with:		
<i>B. subtilis</i> subsp. <i>subtilis</i> BCRC 14716	98.8 / 99.9	75
<i>B. subtilis</i> subsp. <i>subtilis</i> BCRC 17435	98.3 / 99.8	74
<i>B. subtilis</i> subsp. <i>spizizenii</i> BCRC 17366 ^T	94.9 / 99.8	67
<i>B. mojavensis</i> BCRC 17124 ^T	88.8 / 99.6	38
<i>B. atrophaeus</i> BCRC 17123 ^T	80.2 / 99.1	24
<i>B. sonorensis</i> BCRC 17416 ^T	76.7 / 98.0	14
<i>B. vallismortis</i> BCRC 17183 ^T	91.9 / 99.6	46
<i>B. subtilis</i> subsp. <i>spizizenii</i> BCRC 17366 ^T compared with:		
<i>B. subtilis</i> subsp. <i>spizizenii</i> BCRC 10447	96.8 / 99.9	71
<i>B. mojavensis</i> BCRC 17124 ^T	89.8 / 99.7	38
<i>B. atrophaeus</i> BCRC 17123 ^T	79.5 / 99.1	17
<i>B. sonorensis</i> BCRC 17416 ^T	76.9 / 98.0	11
<i>B. vallismortis</i> BCRC 17183 ^T	93.9 / 99.8	52
<i>B. mojavensis</i> BCRC 17124 ^T compared with:		
<i>B. mojavensis</i> BCRC 17531	97.0 / 99.9	89
<i>B. atrophaeus</i> BCRC 17123 ^T	79.2 / 99.7	23
<i>B. sonorensis</i> BCRC 17416 ^T	77.9 / 98.1	18
<i>B. vallismortis</i> BCRC 17183 ^T	88.7 / 99.5	41

Table 1. cont.

<i>Bacillus</i> strains compared	<i>gyrB</i> /16S rRNA gene sequence similarity (%)	DNA relatedness value (%)
<i>B. atrophaeus</i> BCRC 17123 ^T compared with:		
<i>B. atrophaeus</i> BCRC 17530	99.8 / 99.9	90
<i>B. sonorensis</i> BCRC 17416 ^T	79.3 / 98.1	15
<i>B. vallismortis</i> BCRC 17183 ^T	79.1 / 99.4	21
<i>B. sonorensis</i> BCRC 17416 ^T compared with:		
<i>B. sonorensis</i> BCRC 17532	100 / 99.8	92
<i>B. vallismortis</i> BCRC 17183 ^T	75.4 / 98.1	13

and the *B. amyloliquefaciens* strains investigated formed a phylogenetic grouping and are closely related to the group of *B. atrophaeus*.

DNA base composition and DNA relatedness values were determined as described previously (Tamaoka & Komagata, 1984; Ezaki *et al.*, 1989; Goris *et al.*, 1998; Tai *et al.*, 2006). DNA G+C contents of *B. velezensis* BCRC 17467^T and *B. amyloliquefaciens* strains BCRC 11601^T, BCRC 14193 and BCRC 17038 were 45.7, 45.4, 45.9 and 45.2 mol%, respectively. These values were close to the value of 44–46 mol% which was described for the strains of *B. amyloliquefaciens* (Priest *et al.*, 1987). The relatedness of the genomic DNA of the strains tested is shown in Table 1. *B. velezensis* BCRC 17467^T and *B. amyloliquefaciens* strains BCRC 11601^T, BCRC 14193, BCRC 14711 and BCRC 17038 showed high DNA–DNA relatedness values in the range 74–89 %, indicating clearly that these taxa belong to the same genospecies. Previous data by Ruiz-García *et al.* (2005) reported DNA–DNA relatedness values for *B. velezensis* CR-502^T and *B. amyloliquefaciens* ATCC 23350^T of 18.6 %, which is much lower than the value determined in our study. We presume that a mixed culture may possibly have been used in the DNA–DNA hybridization experiments of Ruiz-García *et al.* (2005). This kind of result was frequently reported by other taxonomists (Naser *et al.*, 2006a, b; Kostinek *et al.*, 2005; Wang *et al.*, 2007a). The correlation between *gyrB* gene sequence similarity values and levels of DNA–DNA relatedness in the species of the *B. subtilis* group were studied previously. Strains with approximately 95 % or more *gyrB* gene sequence similarity in the cluster exhibited DNA–DNA relatedness of >70 %, an acceptable value for proposal of a single species. The levels of *gyrB* gene sequence similarity (>95.5 %) and DNA–DNA relatedness (>74 %) shared by *B. velezensis* BCRC 17467^T and all *B. amyloliquefaciens* strains available indicated that they represent the same species in our previous study (Wang *et al.*, 2007b).

Phenotypic features were investigated for *B. velezensis* BCRC 17467^T and phylogenetically related species. Cellular fatty acid composition was determined according to the methods described by Tai *et al.* (2006). Their fatty acid compositions are listed in Supplementary Table S1 (available in IJSEM Online). *B. velezensis* BCRC 17467^T and *B. amyloliquefaciens* BCRC 14193 showed similar fatty acid profiles, characterized mainly by their high C_{16:0} fatty

acid content, low anteiso-C_{17:0} fatty acid content and the presence of C_{14:0}. However, fatty acid profiles have individual differences in all strains of *B. amyloliquefaciens*, indicating that these data are insufficient for species identification. Additional biochemical tests were performed using API 50CH and 20E (bioMérieux) following the manufacturer's instructions. Distinguishing biochemical characteristics are shown in Supplementary Table S2 (available in IJSEM Online). *B. velezensis* BCRC 17467^T had few differences from *B. amyloliquefaciens* BCRC 11601^T and other *Bacillus* strains. These species can be accurately differentiated from one another by DNA–DNA hybridization analysis, but it is quite difficult to distinguish members of the *B. subtilis* group by phenotypic characteristics.

Although the phenotypic characteristics have individual differences, these data are insufficient for species identification in the members of *Bacillus* closely related to the *B. subtilis* group, thus necessitating DNA–DNA hybridization tests. Based on the evidence from similarities of *gyrB* gene sequences and results from DNA–DNA hybridization studies, it is proposed that *B. velezensis* Ruiz-García *et al.* 2005 should be considered as a later heterotypic synonym of *B. amyloliquefaciens* Priest *et al.* 1987.

Emended description of *Bacillus amyloliquefaciens* (ex Fukumoto 1943) Priest *et al.* 1987

The description is the same as that given by Priest *et al.* (1987) except for the following traits. Catalase and oxidase are produced. Strains are capable of growing in NaCl concentrations up to 12 % w/v. Citrate utilization and Tween 20 hydrolysis are variable. They are able to produce dihydroxyacetone, but do not produce lecithinase. Acid is produced from aesculin, amygdalin, L-arabinose, arbutin, glycogen, inositol, lactose, methyl α -D-glycoside, raffinose, D-ribose, salicin, sorbitol, starch, D-xylose, galactose and inulin. H₂S production and ONPG reaction are strain-dependent. Vogues–Proskauer test is positive. Tests for degradation of indole, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and phenylalanine deaminase are negative. Major fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0}, according to this study and Ruiz-García *et al.* (2005). The DNA G+C content ranges from 44 to 46 mol%. Strains have been

isolated from desert soils and brackish sediments. Additional chemotaxonomic data and biochemical tests are given in Supplementary Tables S1 and S2. The type strain is Fukumoto strain F^T=ATCC 23350^T=DSM 7^T=BCRC 11601^T=CIP 103265^T=IFO (now NBRC) 15535^T=LMG 12234^T=NCIMB 12077^T=NRRL B-14393^T.

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References

- 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. (2002). PHYLIP (Phylogeny Inference Package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fukumoto, J. (1943a). Studies on the production of bacterial amylase. I. Isolation of bacteria secreting potent amylase and their distribution. *Nippon Nogei Kagakukai* **19**, 487–503 (in Japanese).
- Fukumoto, J. (1943b). Studies on the production of bacterial amylase. II. Bacterial and physiological nature. *Nippon Nogei Kagakukai* **19**, 643–650 (in Japanese).
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Harwood, C. R. (1992). *Bacillus subtilis* and its relatives: molecular biological and industrial workhorses. *Trends Biotechnol* **10**, 247–256.
- Kostinek, M., Pukall, R., Rooney, A. P., Schillinger, U., Hertel, C., Holzapfel, W. H. & Franz, C. M. (2005). *Lactobacillus arizonensis* is a later heterotypic synonym of *Lactobacillus plantarum*. *Int J Syst Evol Microbiol* **55**, 2485–2489.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Logan, N. A. & Berkeley, R. C. (1984). Identification of *Bacillus* strains using the API system. *J Gen Microbiol* **130**, 1871–1882.
- Nakamura, L. K. (1987). Deoxyribonucleic acid relatedness of lactose-positive *Bacillus subtilis* strains and *Bacillus amyloliquefaciens*. *Int J Syst Bacteriol* **37**, 444–445.
- Nakamura, L. K. (1989). Taxonomic relationship of black-pigmented *Bacillus subtilis* strains and a proposal for *Bacillus atrophaeus* sp. nov. *Int J Syst Bacteriol* **39**, 295–300.
- Nakamura, L. K., Roberts, M. S. & Cohan, F. M. (1999). Relationship of *Bacillus subtilis* clades associated with strains 168 and W23: a proposal for *Bacillus subtilis* subsp. *subtilis* subsp. nov. and *Bacillus subtilis* subsp. *spizizenii* subsp. nov. *Int J Syst Bacteriol* **49**, 1211–1215.
- Naser, S. M., Hagen, K. E., Vancanneyt, M., Cleenwerck, I., Swings, J. & Tompkins, T. A. (2006a). *Lactobacillus suntoryeus* Cachat and Priest 2005 is a later synonym of *Lactobacillus helveticus* (Orla-Jensen 1919) Bergey et al. 1925 (Approved Lists 1980). *Int J Syst Evol Microbiol* **56**, 355–360.
- Naser, S. M., Vancanneyt, M., Hoste, B., Snauwaert, C., Vandemeulebroecke, K. & Swings, J. (2006b). Reclassification of *Enterococcus flavescens* Pompei et al. 1992 as a later synonym of *Enterococcus casseliflavus* (ex Vaughan et al. 1979) Collins et al. 1984 and *Enterococcus saccharominimus* Vancanneyt et al. 2004 as a later synonym of *Enterococcus italicus* Fortina et al. 2004. *Int J Syst Evol Microbiol* **56**, 413–416.
- O'Donnell, A. G., Norris, J. R., Berkeley, R. C. W., Kaneko, Logan, N. A. & Nozaki, R. (1980). Characterization of *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, and *Bacillus amyloliquefaciens* by pyrolysis gas-liquid chromatography, deoxyribonucleic acid-deoxyribonucleic acid hybridization, biochemical tests, and API systems. *Int J Syst Bacteriol* **30**, 448–459.
- Priest, F. G. (1977). Extracellular enzyme synthesis in the genus *Bacillus*. *Bacteriol Rev* **41**, 711–753.
- Priest, F. G., Goodfellow, M., Shute, L. A. & Berkeley, R. C. W. (1987). *Bacillus amyloliquefaciens* sp. nov., nom. rev. *Int J Syst Bacteriol* **37**, 69–71.
- Roberts, M. S., Nakamura, L. K. & Cohan, F. M. (1994). *Bacillus mojavensis* sp. nov., distinguishable from *Bacillus subtilis* by sexual isolation, divergence in DNA sequence, and differences in fatty acid composition. *Int J Syst Bacteriol* **44**, 256–264.
- Roberts, M. S., Nakamura, L. K. & Cohan, F. M. (1996). *Bacillus vallismortis* sp. nov., a close relative of *Bacillus subtilis*, isolated from soil in Death Valley, California. *Int J Syst Bacteriol* **46**, 470–475.
- Ruiz-Garcia, C., Bejar, V., Martinez-Checa, F., Llamas, I. & Quesada, E. (2005). *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river Vélez in Málaga, southern Spain. *Int J Syst Evol Microbiol* **55**, 191–195.
- Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* **30**, 225–420.
- Tai, C. J., Kuo, H. P., Lee, F. L., Chen, H. K., Yokota, A. & Lo, C. C. (2006). *Chryseobacterium taiwanense* sp. nov., isolated from soil in Taiwan. *Int J Syst Evol Microbiol* **56**, 1771–1776.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wang, L. T., Lee, F. L., Tai, C. J., Yokota, A. & Kuo, H. P. (2007a). Reclassification of *Bacillus axarquensis* Ruiz-García et al. 2005 and *Bacillus malacitensis* Ruiz-García et al. 2005 as later heterotypic synonyms of *Bacillus mojavensis* Roberts et al. 1994. *Int J Syst Evol Microbiol* **57**, 1663–1667.
- Wang, L. T., Lee, F. L., Tai, C. J. & Kasai, H. (2007b). Comparison of *gyrB* gene sequences, 16S rRNA gene sequences and DNA–DNA hybridization in the *Bacillus subtilis* group. *Int J Syst Evol Microbiol* **57**, 1846–1850.