Reclassification of *Bacillus axarquiensis* Ruiz-García *et al.* 2005 and *Bacillus malacitensis* Ruiz-García *et al.* 2005 as later heterotypic synonyms of *Bacillus mojavensis* Roberts *et al.* 1994

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The *Bacillus subtilis* group encompasses the taxa *Bacillus subtilis* subsp. *subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. atrophaeus*, *B. mojavensis*, *B. vallismortis*, *B. subtilis* subsp. *spizizenii*, *B. sonorensis*, *B. velezensis*, *B. axarquiensis* and *B. malacitensis*. In this study, the taxonomic relatedness between the species *B. axarquiensis*, *B. malacitensis* and *B. mojavensis* was investigated. Sequence analysis of the 16S rRNA gene and the gene for DNA gyrase subunit B (*gyrB*) confirmed the very high similarities between these three type strains and a reference strain of *B. mojavensis* (>99 and >97 %, respectively). DNA–DNA hybridization experiments revealed high relatedness values between the type strains of *B. axarquiensis*, *B. malacitensis* and *B. mojavensis* and between these strains and a reference strain of *B. mojavensis* (83–98 %). Based on these molecular taxonomic data and the lack of phenotypic distinctive characteristics, *Bacillus axarquiensis* and *Bacillus malacitensis* should be reclassified as later heterotypic synonyms of *Bacillus mojavensis*.

The species *Bacillus subtilis* (subsp. *subtilis* and subsp. *spizizenii*) (Smith *et al.*, 1964; Nakamura *et al.*, 1999), *B. licheniformis* (Skerman *et al.*, 1980), *B. amyloliquefaciens* (Priest *et al.*, 1987), *B. atrophaeus* (Nakamura, 1989), *B. mojavensis* (Roberts *et al.*, 1994), *B. vallismortis* (Roberts *et al.*, 1996), *B. sonorensis* (Palmisano *et al.*, 2001), *B. velezensis* (Ruiz-García *et al.*, 2005a), *B. axarquiensis* and *B. malacitensis* (Ruiz-García *et al.*, 2005b), Gram-positive, rod-shaped, endospore-forming, aerobic and fermentative bacteria, are difficult to distinguish from each other by phenotypic characteristics alone, but can be differentiated from one another reliably by using DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *B. axarquiensis* strains CIP 108772^T and LMG 22476^T and *B. malacitensis* strains CECT 5687^T and LMG 22477^T are DQ993670–DQ993673, respectively, and the accession numbers of the *gyrB* gene sequences of these four strains are DQ903177–DQ903180, respectively.

Maximum-likelihood and maximum-parsimony trees based on 16S rRNA and *gyrB* gene sequences and details of fatty acid profiles and differential phenotypic characteristics of the studied strains are available with the online version of this paper.

In the present study, we provide evidence, on the basis of sequence similarities of the 16S rRNA and *gyrB* genes and DNA–DNA hybridization data, that the type strains of *B. axarquiensis* and *B. malacitensis* are very closely related to the type strain of *B. mojavensis*, suggesting that the three species represent the same species.

The bacterial strains used in this study were cultivated in nutrient agar or broth (Difco) and incubated aerobically at 30 °C. Genomic DNA was isolated and purified using the Qiagen Blood & Cell Culture DNA kit. The complete sequence of a 1.5 kb 16S rRNA gene fragment was determined using the MicroSeq Full Gene 16S rDNA bacterial identification kit (Applied Biosystems). Sequencing was performed using an ABI 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 using the programs of the Wisconsin package version 10.1 (Accelrys Inc.). 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 was performed using the PHYLIP computer program package

(Felsenstein, 2002). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis was made with 1000 replicates except in maximum-likelihood, where only 100 replicates were generated. Tree figures were drawn by TreeView software (Page, 1996).

Sequences of highly conserved housekeeping or other protein-encoding genes provide higher resolution than 16S rRNA gene sequences and may supplant DNA–DNA reassociation or 16S rRNA gene sequence data at the species level for taxonomic analysis (Stackebrandt *et al.*, 2002; Zeigler, 2003). The partial sequence for the gene encoding the subunit B protein of DNA gyrase (*gyrB*) was included in the phylogenetic analysis for comparative purposes. An approximately 1.2 kb fragment of the *gyrB* gene was amplified by PCR and sequenced using a set of primers described by Yamamoto & Harayama (1995). Using the method described above, the *gyrB* gene sequences were also aligned and phylogenetic trees were constructed. 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 16S rRNA gene sequences (positions 35–1431; *Escherichia coli* K-12 numbering) of *B. axarquiensis* strains LMG 22476^T and CIP 108772^T and *B. malacitensis* strains LMG 22477^T and CECT 5687^T shared 99.9% similarity with each other and shared 99.9% similarity with sequences of *B. mojavensis* strains BCRC 17124^T and BCRC 17531 (Fig. 1). Sequences of the *B. axarquiensis* and *B. malacitensis* type strains shared 99.6 and 99.5% similarity, respectively, with those of *B. subtilis* subsp. *subtilis* BCRC 10255^T and *B. amyloliquefaciens* BCRC 11601^T. The 16S rRNA gene sequences (positions 35–1431) of *B. axarquiensis* LMG 22476^T and CIP 108772^T and *B. axarquiensis* CR-119^T (GenBank accession no. AY603657) shared 99.9, 99.9 and 99.5% similarity, respectively, with that of *B. mojavensis* BCRC 17124^T. In contrast, the similarity between *B. axarquiensis* CR-119^T and *B. mojavensis* reported by Ruiz-García *et al.* (2005b) was noticeably lower, at 97.4%. The *gyrB* gene sequences (positions 316–1480; *E. coli* K-12 numbering) for *B. axarquiensis* LMG 22476^T and CIP 108772^T and *B. malacitensis* LMG 22477^T and CECT 5687^T showed high similarities (>97%) with the investigated *B. mojavensis* strains and significantly lower similarities (ranging from 80 to 90%) with their nearest neighbour (Fig. 1b). The similarities of the 16S rRNA and *gyrB* gene sequences indicate that *B. axarquiensis*, *B. malacitensis* and *B. mojavensis* cannot be differentiated and may represent the same species.

The G+C content was determined using reversed-phase HPLC according to Tamaoka & Komagata (1984) with slight modifications. The nucleoside mixture was separated using a Cosmosil 5C₁₈ column (Waters, 4.0 × 150 mm;

Nacalai Tesque) in a mobile phase composed of 0.2 M (NH₄)H₂PO₄/acetonitrile (20:1, v/v) at a flow rate of 1 ml min⁻¹ at room temperature. DNA relatedness values between *B. axarquiensis* LMG 22476^T and CIP 108772^T, *B. malacitensis* LMG 22477^T and CECT 5687^T, *B. mojavensis* strains BCRC 17124^T and BCRC 17531, *B. subtilis* subsp. *subtilis* BCRC 10255^T and *B. amyloliquefaciens* BCRC 11601^T were determined using the fluorometric hybridization method in microdilution wells as described previously (Ezaki *et al.*, 1989; Goris *et al.*, 1998; Chern *et al.*, 2004). The hybridization temperature was 42 °C. DNA G+C contents of *B. axarquiensis* (LMG 22476^T and CIP 108772^T), *B. malacitensis* (LMG 22477^T and CECT 5687^T) and *B. mojavensis* strains BCRC 17124^T and BCRC 17531 were respectively 43, 44, 44 and 44 mol%. These values were close to the value of 43 mol% determined for the type strain of *B. mojavensis* by Roberts *et al.* (1994). The relatedness values of genomic DNA of the strains tested are showed in the Table 1. *B. axarquiensis* LMG 22476^T and CIP 108772^T, *B. malacitensis* LMG 22477^T and CECT 5687^T and *B. mojavensis* BCRC 17124^T and BCRC 17531 showed high DNA–DNA relatedness values, in the range 83–98%, indicating clearly that these taxa belong to the same genospecies. These strains showed less than 40% relatedness with *B. subtilis* subsp. *subtilis* BCRC 10255^T, *B. vallismortis* BCRC 17183^T and *B. amyloliquefaciens* BCRC 11601^T. The results contradict those obtained by Ruiz-García *et al.* (2005b), who reported a low hybridization value of less than 47% between the type strains of these taxa.

Cellular fatty acid composition was determined using the Sherlock Microbial Identification System (MIDI Inc.). Extracts of methylated fatty acids were prepared according to the protocol provided by the manufacturer and analysed with an Agilent 6890N gas chromatograph equipped with a flame-ionization detector and 7683 Automatic Liquid Sampler (Agilent). Identification of peaks was made by comparing the results with the built-in TSBA 50 database (MIDI Inc.). The fatty acid compositions of phylogenetically related species are listed in Supplementary Table S1 (available in IJSEM Online). In strains of *B. axarquiensis*, *B. malacitensis* and *B. mojavensis*, iso- and anteiso-fatty acids with 15 to 17 carbons predominated. Their fatty acid profiles showed high similarity, and could not be used to differentiate the strains from one another or from *B. subtilis*. The results differed from those obtained by Ruiz-García *et al.* (2005b), who reported a significant quantity (11.5%) of 16:0 fatty acid in *B. axarquiensis* CR-119^T and no unsaturated fatty acids in *B. axarquiensis* CR-119^T or *B. malacitensis* CR-95^T.

Additional biochemical tests were performed using the API CH50 and 20E kits (bioMérieux) according to the manufacturer's instructions. Supplementary Table S2 shows differential biochemical features between the tested *Bacillus* strains. Very few of these characteristics could be used to differentiate *B. axarquiensis* and *B. malacitensis* from one another or from the other species. Only a negative oxidase

Fig. 1. Neighbour-joining trees based on 16S rRNA (a) and *gyrB* (b) gene sequences of *Bacillus* strains. Genetic distances were computed by Kimura's two-parameter model. *Escherichia coli* K-12 was used as an outgroup. Only bootstrap percentages above 50% are shown. Bars, 0.01 (a) and 0.1 (b) substitutions per nucleotide position.

Table 1. DNA–DNA relatedness values between *Bacillus* strains

ATCC, American Type Culture Collection (Manassas, VA, USA); BCRC, Bioresource Collection and Research Center at Food Industry Research and Development Institute (FIRDI) (Hsinchu, Taiwan); CECT, Colección Española de Cultivos Tipo, Universidad de Valencia (Valencia, Spain); CIP, Collection Bactérienne de l'Institut Pasteur (Paris, France); DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany); LMG, Culture Collection of the Laboratorium voor Microbiologie Gent, Universiteit Gent (Gent, Belgium).

Source of unlabelled DNA	Relatedness (%) to labelled DNA from:		
	<i>B. axarquiensis</i> LMG 22476 ^T	<i>B. malacitensis</i> LMG 22477 ^T	<i>B. mojavensis</i> BCRC 17124 ^T
<i>B. axarquiensis</i> LMG 22476 ^T (=BCRC 17501 ^T)	100	85	98
<i>B. axarquiensis</i> CIP 108772 ^T (=BCRC 17653 ^T)	99	90	97
<i>B. malacitensis</i> LMG 22477 ^T (=BCRC 17502 ^T)	87	100	86
<i>B. malacitensis</i> CECT 5687 ^T (=BCRC 17654 ^T)	84	99	83
<i>B. mojavensis</i> BCRC 17124 ^T (=DSM 9205 ^T)	97	84	100
<i>B. mojavensis</i> BCRC 17531 (=DSM 9206)	84	83	83
<i>B. subtilis</i> subsp. <i>subtilis</i> BCRC 10255 ^T (=ATCC 6051 ^T)	31	39	31
<i>B. vallismortis</i> BCRC 17183 ^T (=DSM 11031 ^T)	35	37	35
<i>B. amyloliquefaciens</i> BCRC 11601 ^T (=ATCC 23350 ^T)	15	17	15

test and positive reactions for acid production from gentio-
biose and inulin differentiated *B. axarquiensis* from *B. malacitensis* and *B. mojavensis*. Additionally, *B. axarquiensis* LMG 22476^T and CIP 108772^T are both negative for arginine dihydrolase, lysine decarboxylase and ornithine decarboxy-
lase activities (not shown in Supplementary Table S2), which differed from the results obtained by Ruiz-García *et al.* (2005b).

Although RAPD analysis with primers OPA3 and OPL12 could be used to distinguish *B. axarquiensis* and *B. mala-
citensis* from one another and from *B. mojavensis* (Ruiz-
García *et al.*, 2005b), genetic heterogeneity in the same
species has also been reported by some authors (Tynkynen
et al., 1999; Matarante *et al.*, 2004; Naser *et al.*, 2006).

Previous data reported by Ruiz-García *et al.* (2005b) gave
DNA–DNA relatedness values for *B. axarquiensis* and *B. malacitensis* strains with strains of other closely related
species that were lower than 46.9 %, which is much lower
than the values (83–99 %) determined in our study. Our
results show that the strains submitted to the culture collec-
tions are pure, and our DNA–DNA hybridization experi-
ments clearly identify the *B. axarquiensis* and *B. malacitensis*
type strains, as deposited in the culture collections, as
B. mojavensis. The DNA–DNA hybridization experiments
confirmed the results of our other investigation methods
(16S rRNA and *gyrB* gene sequencing) used to differentiate
between the species. In addition, these methods could clearly
distinguish between type or reference strains of other closely
related species. Our investigation shows clearly that the
B. axarquiensis and *B. malacitensis* type strains can not be
distinguished from the *B. mojavensis* type strain or another
B. mojavensis reference strain at the species level. The data
from the present study show that no significant phenotypic
or genotypic differences exist to justify the separation of the

type strains of *B. axarquiensis* and *B. malacitensis* and that
of *B. mojavensis*. Consequently, it is proposed that *Bacillus
axarquiensis* Ruiz-García *et al.* 2005 and *Bacillus malacitensis*
Ruiz-García *et al.* 2005 should be considered as later hetero-
typic synonyms of *Bacillus mojavensis* Roberts *et al.* 1994.

**Emended description of *Bacillus mojavensis*
Roberts *et al.* 1994**

The description is the same as that given by Roberts *et al.*
(1994) except for the following traits. Oxidase, citrate utiliz-
ation and nitrite reduction are variable. Does not hydrolyse
L-rhamnose or D-galactose. Acid production from D-xylose,
inositol, inulin, D-raffinose, gentiobiose, amygdalin and
methyl α-D-glucoside is strain-dependent. Major fatty acids
are 15:0 anteiso, 15:0 iso, 17:0 iso and 17:0 anteiso. The
DNA G + C content ranges from 43 to 44 mol%. Known
habitats are desert soils and brackish sediments. Additional
chemotaxonomic data and results of biochemical tests are
given in Supplementary Tables S1 and S2.

The type strain is strain RO-H-1^T (=NRRL B-14698^T =
DSM 9205^T = LMG 17797^T = CIP 104095^T = ATCC
51516^T = BCRC 17124^T = NBRC 15718^T).

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