NOTE

Reclassification of *Bacillus marismortui* as *Salibacillus marismortui* comb. nov.

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Recently, the features of a group of strains isolated from Dead Sea enrichments obtained in 1936 by one of us (B. E. Volcani) were described. They were Gram-positive, moderately halophilic, spore-forming rods, and were placed in a new species, *Bacillus marismortui*. At the same time, the new genus *Salibacillus* was proposed for the halophilic species *Bacillus salexigens*. *B. marismortui* and *Salibacillus salexigens* have similar phenotypic characteristics and the same peptidoglycan type. Phylogenetic analysis based on 16S rRNA sequence comparisons showed that they are sufficiently closely related (96.6% similarity) as to warrant placement in the same genus. However, DNA–DNA hybridization experiments showed that they constitute two separate species (41% DNA similarity). Therefore the reclassification of *Bacillus marismortui* as *Salibacillus marismortui* comb. nov. is proposed.

Keywords: *Bacillus*, *Salibacillus marismortui*, moderately halophilic bacteria, 16S rRNA sequence, taxonomy

In a recent study, we characterized taxonomically 91 moderately halophilic, Gram-positive, rod-shaped strains isolated from 57-year-old enrichments obtained from Dead Sea water samples by B. E. Volcani (Arahal *et al.*, 1999). All of the isolates grouped in a single phenon according to the results of the numerical analysis, since they had very similar phenotypic characteristics. At the same time, we observed significant differences relative to previously described moderately halophilic, Gram-positive rods (Fendrich *et al.*, 1990; Garabito *et al.*, 1997; Spring *et al.*, 1996; Ventosa *et al.*, 1989). All strains were Gram-positive, motile, endospore-forming rods and all were strictly aerobic. Their cell wall peptidoglycan contained *meso*-diaminopimelic acid and they had a DNA G+C content between 39.0 and 42.8 mol% (Arahal *et al.*, 1999). Thus, they can be considered as members of the genus *Bacillus sensu lato* (Claus & Berkeley, 1986).

The DNA–DNA relatedness found between the strains chosen as representatives of the group was $\geq 70\%$,

while only low values (0–45%) were obtained between those strains and the reference strains used in the study. All these results, together with the phylogenetic analysis, supported the inclusion of the 91 isolates into a new species for which the name *Bacillus marismortui* was proposed (Arahal *et al.*, 1999).

The high levels of 16S rRNA sequence similarity found between *B. marismortui* and its closest relatives corresponded to those for *Virgibacillus pantothenicus* (97.8% similarity) and *Bacillus salexigens* (96.6% similarity). *V. pantothenicus* (formerly *Bacillus pantothenicus*) was recently proposed as the single member of the new genus *Virgibacillus*, mainly on the basis of its phylogenetic differences (Heyndrickx *et al.*, 1998). *B. marismortui* cannot be included in this genus because of the many phenotypic and genotypic differences that can be found between this species and *V. pantothenicus*. For instance, whereas *B. marismortui* is a moderately halophilic bacterium (with optimal growth at 10% salts) which needs aerobic conditions for growth and has a DNA G+C content of 39.0–42.8 mol%, *V. pantothenicus* grows well in media without NaCl, in anaerobic conditions and has a lower G+C content (36.9–38.3 mol%) (Arahal *et al.*, 1999; Heyndrickx *et al.*, 1998) (Table 1).

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The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of *Salibacillus marismortui* strain 123^T is AJ009793.

Table 1. Differential phenotypic features among the species *Salibacillus marismortui*, *Salibacillus salexigens*, *Virgibacillus pantothenicus* and *Virgibacillus proomii*

Data are from Arahal *et al.* (1999), Garabito *et al.* (1997) and Heyndrickx *et al.* (1999). +, Positive; –, negative; ND, not determined.

Characteristic	<i>Salibacillus marismortui</i>	<i>Salibacillus salexigens</i>	<i>Virgibacillus pantothenicus</i>	<i>Virgibacillus proomii</i>
Spore shape	Ellipsoidal	Ellipsoidal	Spherical or ellipsoidal	Spherical or ellipsoidal
Pigmentation	Cream	None	Creamy grey	Creamy grey
Anaerobic growth	–	–	+	+
Growth at 0% salts	–	–	+	+
Optimal growth in 10% (w/v) salts	+	+	–	–
Growth at 50 °C	+	–	+	+
Growth at pH 10.0	–	+	+	ND
H ₂ S production	+	+	–	–
Nitrate reduction	+	–	+	–
Hydrolysis of:				
Starch	–	–	+	ND
Tween 80	–	–	+	ND
Phosphatase	+	–	+	ND
Methyl red	+	–	–	ND
Acid production from:				
D-Arabinose	–	ND	+	–
D-Galactose	–	–	+	+
Glycerol	+	+	+	–
D-Mannose	–	+	+	+
D-Trehalose	–	–	+	+
G + C content (mol %)	40.7	39.5	36.9	37.0

Recently, Wainø *et al.* (1999), proposed that two other species of *Bacillus* rRNA group 1 (*Bacillus sensu stricto*), namely *Bacillus dipsosauri* and *B. salexigens*, should be transferred to two new genera under the names *Gracilibacillus dipsosauri* (i.e. within the same genus as the newly described species *Gracilibacillus halotolerans*) and *Salibacillus salexigens*, respectively. More recently, a new species, for which the name *Virgibacillus proomii* was chosen, was proposed for the genus *Virgibacillus* (Heyndrickx *et al.*, 1999), now comprising two species.

Since the description of *B. marismortui* was published at the same time as the two above-mentioned studies, it was obviously not included in those reports. Therefore, it is necessary to evaluate the taxonomic status of this species, taking into account the recent changes in the group. *S. salexigens* and *B. marismortui* share many phenotypic characteristics and have overlapping values for the G + C content of the DNA (Table 1). They cannot be considered as the same species because their 16S rRNA sequence similarity is lower than 97%, the value considered to be the limit for species separation (Stackebrandt & Goebel, 1994); in addition, their DNA–DNA similarity is only 41% (Arahal *et al.*, 1999), which is too low for strains of the same species, which should have similarity values

Fig. 1. Phylogenetic tree based on 16S rRNA sequence comparisons showing the relationship of *Salibacillus marismortui* strain 123^T to other related species of the genera *Salibacillus*, *Virgibacillus*, *Bacillus* and *Halobacillus*. The values on the branches indicate the levels of support derived from 1000 bootstrapped trees.

≥ 70% (Owen & Pitcher, 1985; Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

On the other hand, the phylogenetic analysis of both strains and their closest relatives clearly indicates that *B. marismortui* should be included in the genus *Salibacillus* (Fig. 1). The phylogenetic tree shown was obtained by comparison of the 16S rRNA sequences

using the ARB software package (Ludwig & Strunk, 1996). Different treeing methods were applied using the appropriate tools of the ARB software package and consistent results were obtained. To check the robustness of the topologies, a bootstrap analysis of the neighbour-joining data was performed in which a total of 1000 trees were generated (Fig. 1). All of the sequences used in this study are almost complete sequences corresponding to the type strain of each one of the following species (the accession numbers of the sequences are provided in parentheses): *Bacillus cereus* (X55060), *Bacillus cohnii* (X76437), *Bacillus licheniformis* (X68416), *Bacillus marismortui* (AJ009793), *Bacillus megaterium* (X60629), *Bacillus subtilis* (X60646), *Gracilibacillus dipsosauri* (X82436), *Halobacillus halophilus* (X62174), *Halobacillus litoralis* (X94558), *Salibacillus salexigens* (Y11603), *Virgibacillus pantothenicus* (D78477) and *Virgibacillus proomii* (AJ012667). The node that groups *B. marismortui* and *S. salexigens* has a bootstrap percentage value of 96%, which indicates a strong stability supporting the topology of this branch. The neighbouring branches are formed by recently reclassified or newly described species (Heyndrickx *et al.*, 1998, 1999; Wainø *et al.*, 1999) separated from the *Bacillus sensu stricto* species (rRNA group 1). Thus it is clear that the correct taxonomic position of *B. marismortui* should no longer be within the genus *Bacillus* but should instead be within *Salibacillus*, in view of all the data presented in this study. Therefore we propose the transfer of *Bacillus marismortui* to the genus *Salibacillus* as *Salibacillus marismortui* comb. nov.

Description of *Salibacillus marismortui* comb. nov. (basonym *Bacillus marismortui*)

Salibacillus marismortui (ma.ris.mor'tu.i. L. gen. n. *maris* of the sea; L. adj. *mortuus* dead; M.L. gen. n. *marismortui* of the Dead Sea).

The description of the species is identical to that given by Arahall *et al.* (1999). The type strain is strain 123^T (= DSM 12325^T = ATCC 700626^T = CIP 105609^T = CECT 5066^T).

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