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REVIEW

Antonio Ventosa · M. Carmen Márquez
María J. Garabito · David R. Arahal

Moderately halophilic gram-positive bacterial diversity in hypersaline environments

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Abstract Moderately halophilic bacteria are microorganisms that grow optimally in media containing 3%–15% (w/v) salt. They are represented by a heterogeneous group of microorganisms included in many different genera. Gram-negative moderately halophilic bacteria have been studied in more detail, but studies on gram-positive species are more scarce. Recent studies carried out by our research group on gram-positive moderate halophiles have permitted clarifying their taxonomic and phylogenetic position and describing new species. Thus, we have isolated six strains from ponds of salterns that show phenotypic and genotypic characteristics similar to those of *Nesterenkonia halobia* (formerly *Micrococcus halobius*), a moderately halophilic gram-positive coccus that was described on the basis of a single strain. Our data demonstrate quite clearly that they are members of this species and contribute to a better description of these moderately halophilic cocci. Similarly, a study of a large number of gram-positive moderately halophilic rods that were able to produce endospores led us to describe a new species, designated *Bacillus salexigens*. Further, isolates grouped in other three phenons, obtained by numerical taxonomy analysis and showing phenotypic features quite similar to those of this species, represent different genomovars, with very low DNA-DNA homology. Although they might represent additional new species, it will be necessary to determine new phenotypic features to differentiate them from previously described *Bacillus* species. We have also studied the viability of some old enrichments provided by B.E. Volcani, which were set up in 1936. We isolated 31 gram-positive motile endospore-forming rods that, according to their phenotypic characteristics, could represent a new species of the genus *Bacillus*.

Key words Moderately halophilic bacteria · Hypersaline environments · Taxonomy · Phylogeny · DNA base composition

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A. Ventosa (✉) · M.C. Márquez · M.J. Garabito · D.R. Arahal
Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain.
Tel. +34-5-4556765; Fax +34-5-4628162
e-mail: ventosa@cica.es

Introduction

Halobacteria are a group of microorganisms that belong to the archaeal lineage and are very well adapted to live in environments with high salinities. They are able to grow optimally in media containing between 15% and 25% NaCl and grow at saturated salt concentrations (Kushner and Kamekura 1988; Rodriguez-Valera 1988). Although in hypersaline habitats Archaea constitute the predominant organisms, other prokaryotes, the moderate halophiles, are able to grow under these conditions. They possess haloadaptation mechanisms to grow and survive in hypersaline environments (Ventosa et al. 1998).

Moderately halophilic bacteria are microorganisms that grow optimally in media containing between 3% and 15% (w/v) salt (Kushner and Kamekura 1988). Because of the wide range of salinity in which this group of microorganisms can grow optimally, they are widely distributed in different saline habitats such as hypersaline lakes, desert and saline soils, saltern ponds, salt mines, salted foods, and others (Ventosa 1988; Javor 1989).

Although the early studies on moderately halophilic bacteria were based, in most cases, on strains isolated from salted foods because they may contaminate or spoil these products, the majority of recent studies concerning this group of microorganisms have been carried out in hypersaline lakes as well as ponds of marine salterns. The aspects that attracted the interest of researchers were mainly those related to their physiological adaptation to highly saline concentrations and their ecology (Kushner and Kamekura 1988; Ventosa et al. 1998).

Taxonomy of moderately halophilic bacteria

Our current knowledge about moderately halophilic bacteria contrasts with early studies a decade ago. In fact, in 1980 only six moderately halophilic species were included in the Approved Lists of Bacterial Names (Skerman et al. 1980). However, during the past decade, extensive studies on hypersaline habitats have permitted the isolation and taxonomic characterization of a large number of moderately halophilic species. Thus, moderate halophiles are distributed among diverse groups in the domains Bacteria and Archaea. Moderately halophilic Archaea are represented by a few methanogenic species (Ventosa 1994). There have been many descriptions of gram-negative, moderately halophilic species belonging to the Bacteria, whereas there have been only a few reports about gram-positive moderately halophilic Bacteria (Ventosa 1994; Ventosa et al. 1998).

With respect to the gram-positive moderately halophilic cocci, seven species are currently recognized as valid species: *Marinococcus albus*, *M. halophilus* (Hao et al. 1984), *Salinicoccus roseus* (Ventosa et al. 1990), *S. hispanicus* (Ventosa et al. 1992), *Nesterenkonia halobia* (Stackebrandt et al. 1995), *Halobacillus halophilus* (Spring et al. 1996), and *Tetragenococcus muriaticus* (Satomi et al. 1997). The majority of these species are motile, *Marinococcus halophilus* being the predominant coccus found in most hypersaline environments studied (Ventosa et al. 1983; Márquez et al. 1992), whereas nonmotile species are less common (Ventosa et al. 1982, 1984, 1990, 1992; Stackebrandt et al. 1995).

Nesterenkonia halobia is a moderately halophilic gram-positive nonmotile coccus that was isolated from unrefined solar salt of unknown origin obtained from Noda, Japan, and described originally as *Micrococcus halobius* by Onishi and Kamekura (1972). This bacterium produces an extracellular amylase that depends on divalent cations and a high concentration of NaCl or KCl for activity and stability (Onishi 1972). Since its description in 1972, very few studies had been carried out in this species. Monteoliva-Sanchez et al. (1989) determined the fatty acid composition of *M. halobius*, showing that the predominant fatty acid of this organism was the branched br-C15:0; in addition, elevated amounts of br-C17:0 were also present. In 1994, Koch et al. analyzed the 16S ribosomal DNA (rDNA) sequence of *M. halobius* and showed that this species did not group phylogenetically with the type species of the genus *Micrococcus*, *M. luteus.* Latterly, Stackebrandt et al. (1995) carried out a phylogenetic and chemotaxonomic analysis of the genus *Micrococcus* and confirmed the results previously obtained by Koch et al. (1994). They proposed the inclusion of *M. halobius* in the new genus *Nesterenkonia*, as *N. halobia*. The description of this species was based on a single strain, and it had not been characterized in detail (Onishi and Kamekura 1972; Stackebrandt et al. 1995).

Recently, Mota et al. (1997) have isolated and characterized in depth six cocci from ponds and salterns located in Huelva (Spain). Their phenotypic features and DNA base composition were very similar to those described for *N. halobia* (Stackebrandt et al. 1995). In addition, DNA-DNA hybridization experiments showed a high degree of homology (72%–100%) among the six isolates and the type strain *N. halobia* ATCC 21727, and the 16S rDNA sequence analysis of one representative isolate showed that it was phylogenetically quite close to *N. halobia*. On the basis of these results, it was proposed that the isolates should be classified within the species *N. halobia.* This work has contributed to a more complete description of this species, especially with respect to its nutritional capabilities.

Until 1989 there were not any gram-positive moderately halophilic rods recognized as valid species. In 1980, Onishi et al. reported the isolation of a moderately halophilic gram-positive rod from rotting wood from the Pacific Ocean. This organism produced an extracellular nuclease in saline medium and was designated *Bacillus* sp. strain N23-2 (Onishi et al. 1983). Latterly, García et al. (1987) studied this strain to determine its taxonomic position. It is an endospore-forming gram-positive rod, strictly aerobic and motile by peritrichous flagella. It has *meso*-diaminopimelic acid in the cell wall and a DNA base composition of 51.5 mol%. On the basis of phenotypic and chemotaxonomic data, they proposed its inclusion in a new taxon of the genus *Bacillus*, with the name *Bacillus halophilus*.

In 1996, Spring et al. isolated two moderately halophilic, gram-positive, heterotrophic bacterial strains from hypersaline sediments of the Great Salt Lake in Utah. These two strains were motile, spore-forming, strictly aerobic rods, and contained peptidoglycan of the Orn-D-Asp type in their cell wall. A detailed investigation of the phenotypic and phylogenetic characteristics of these strains showed that each isolate represents a new species. They created a new genus, *Halobacillus*, which included two new species named *Halobacillus litoralis* and *Halobacillus trueperi*. This study also showed that *Sporosarcina halophila*, a moderately halophilic gram-positive endospore-forming coccus, belongs to this genus, and proposed the transfer of *S. halophila* to *Halobacillus*, as *H. halophilus*.

Recent chemotaxonomic studies on moderately halophilic bacteria

Very recently, Garabito et al. (1997) proposed the new species *Bacillus salexigens*, based on the characteristics of six strains isolated from salterns and hypersaline soils located in different geographical areas of Spain. These strains were moderately halophilic, gram-positive, endospore-forming, motile rods. They had *meso*-diaminopimelic acid in the cell wall and a DNA base composition ranging between 36.3 and 39.5 mol%. These results, together with those obtained from DNA-DNA homology studies and determination of the sequence of the 16S rDNA of a representative of this group of isolates, justified the inclusion of the six strains in the genus *Bacillus* and the proposal of this as a new species.

Table 1. Phenotypic characteristics of the five phenons that grouped the gram-positive moderately halophilic endospore-forming rods

Characteristics	A (12)	B (10)	C (8)	D (5)	E (5)
Spore shape					
Oval[a]	83	80	100	100	0
Spherical[a]	17	20	0	0	100
Spore position					
Central	0	10	25	20	ND
Terminal[a]	67	70	0	0	ND
Subterminal[a]	35	20	75	80	ND
Salt growth at (%):					
0.9	25	10	12	40	0
3[a]	50	10	25	80	100
5[a]	50	20	37	80	100
7[a]	100	20	62	100	100
25[a]	0	40	50	40	100
30	0	20	0	20	40
Temperature: growth at (°C)					
10[a]	0	0	0	0	100
45	100	100	75	100	100
50[a]	100	70	62	0	80
55[a]	100	70	0	0	80
pH: growth at					
5[a]	42	80	0	0	100
6	100	100	50	100	100
10[a]	92	100	100	20	80
11	67	20	62	60	20
Oxidase	100	100	100	80	40
Anaerobic growth	8	20	37	60	20
Hydrolysis of					
Gelatin	58	100	81	80	40
Starch	25	10	0	20	20
Casein	67	50	62	0	40
Esculin	50	50	37	40	60
Tween 80	17	10	0	0	0
Tyrosine	17	0	0	0	40
Methyl red	60	20	0	0	0
Nitrate reduction[a]	75	30	12	60	80
Nitrite reduction[a]	17	10	0	0	40
Simmon's citrate	0	0	0	0	100
DNAse	75	80	100	80	60
Phosphatase	75	60	62	80	100
Lecitinase	42	40	0	20	0
Arginine dehydrolase	0	0	12	0	0
H_2S production	67	60	81	80	80
Acid production from:					
D-Galactose	25	10	12	40	20
D-Fructose	100	90	81	60	40
Lactose	17	10	25	0	0
Maltose	92	100	50	60	60
D-Mannitol	17	10	50	60	40
Sucrose	25	20	0	60	20
Glycerol	100	80	81	60	20
D-Trehalose	17	10	12	80	0
D-Xylose	0	0	25	20	60
D-Mannose	100	90	81	80	40
L-Rhamnose	8	10	12	20	40
Utilization of:					
α-Ciclodextrin	75	60	12	0	0
β-Ciclodextrin[a]	92	90	37	0	20
Dextrin	92	90	100	100	100
Glycogen[a]	83	10	100	100	20
Inulin[a]	8	80	12	20	20
Mannan	8	0	0	0	0
Tween 40[a]	75	40	12	60	80
Tween 80[a]	0	0	0	0	80
N-Acetyl-D-glucosamine	92	90	100	100	80
N-Acetyl-D-mannosamine[a]	92	50	87	100	20
Amygdalin[a]	8	0	0	0	80
L-Arabinose	33	80	37	100	100
D-Arabitol[a]	0	0	12	0	80

Table 1. *Continued*

Characteristics	A (12)	B (10)	C (8)	D (5)	E (5)
Arbutin[a]	17	10	50	100	80
Cellobiose	92	80	100	100	100
D-Fructose	92	100	100	100	100
D-Galactose	58	30	37	60	80
D-Galacturonic acid[a]	8	20	0	0	100
Gentiobiose[a]	92	90	100	0	100
D-Gluconic acid[a]	8	10	100	0	80
α-D-Glucose	92	100	100	100	100
m-Inositol[a]	0	0	0	40	80
α-D-Lactose	0	0	12	0	0
Lactulose	8	0	0	0	20
Maltose	92	90	100	100	100
Maltotriose	83	90	100	100	100
D-Mannitol[a]	8	0	87	0	100
D-Mannose	92	100	100	100	100
D-Melezitose	0	0	0	20	0
3-Methylglucose[a]	92	70	25	20	20
α-Methyl-D-glucoside[a]	0	0	62	100	80
β-Methyl-D-glucoside	0	0	0	0	20
α-Methyl-D-mannoside[a]	0	0	62	80	20
Palatinose	73	50	100	80	80
D-Psicose	83	100	100	100	100
D-Raffinose	8	0	0	20	0
L-Rhamnose	8	0	37	0	60
D-Ribose	92	100	100	100	60
Salicin	92	90	100	100	100
Psedoheptulosan	8	0	0	0	0
D-Sorbitol[a]	18	0	100	20	100
Sucrose[a]	0	0	62	100	100
D-Tagatose[a]	83	50	0	100	40
D-Trehalose	67	30	75	80	80
Turanose	92	90	100	100	80
Xylitol	8	20	0	0	20
Acetic acid	50	60	37	100	100
α-Hydroxybutyric acid[a]	0	30	37	100	20
β-Hydroxybutyric acid	100	60	62	100	80
γ-Hydroxybutyric acid	0	0	12	0	60
p-Hydroxyphenyl acetic acid	8	0	0	0	20
α-Ketoglutaric acid	8	10	0	0	0
α-Ketovaleric acid[a]	100	0	12	20	0
Lactamide[a]	8	10	12	80	20
D-Lactic acid methylester[a]	42	0	25	100	80
L-Lactic acid	75	80	87	100	80
D-Malic acid[a]	25	10	12	0	80
L-Malic acid[a]	33	20	75	20	80
Methyl pyruvate[a]	100	60	75	100	80
Methyl succinate	0	0	12	0	40
Propionic acid[a]	33	0	0	60	80
Succinamic acid	42	70	37	40	100
N-Acetyl-L-glutamic acid	100	90	87	100	60
Alaninamide	0	0	0	0	20
D-Alanine	90	50	100	100	100
L-Alanine[a]	100	30	100	60	100
L-Alanyl glycine	92	70	100	100	80
L-Asparagine[a]	0	0	0	60	80
L-Glutamic acid	100	90	62	100	80
Glycyl-L-glutamic acid	92	90	100	100	60
L-Pyroglutamic acid	17	0	0	0	0
L-Serine[a]	92	10	100	100	100
Putrescine[a]	0	0	0	0	80
2,3-Butanediol[a]	100	60	0	0	0
Glycerol	100	90	100	100	80
Adenosine	100	80	87	100	100
2′-Desoxyadenosine	100	90	100	100	100
Inosine	100	90	100	100	100
Thymidine	100	90	100	100	80
Uridine	100	90	100	100	100
Adenosine-5′-monophosphate[a]	92	10	0	20	20
Thymidine-5′-monophosphate	33	10	0	0	60

Table 1. *Continued*

Characteristics	A (12)	B (10)	C (8)	D (5)	E (5)
Uridine-5′-monophosphate[a]	100	20	12	0	40
Fructose-6-phosphate[a]	75	20	12	0	20
Glucose-1-phosphate	42	10	0	0	0
Glucose-6-phosphate[a]	100	30	25	0	20
DL-α-Glycerolphosphate[a]	100	80	12	100	20
Antibiotic susceptibility:					
Ampicillin	100	100	100	80	60
Bacitracin	25	40	62	60	0
Carbenicillin	100	100	87	100	100
Cephalotin	100	100	100	100	40
Erythromicin	100	100	62	100	60
Nalidixic acid	17	10	12	20	20
Novobiocin[a]	100	100	100	100	20
Rifampicin[a]	100	100	75	80	0
Penicillin G	100	100	87	80	40
Polymixin	0	10	12	20	0

ND, not determined.
All strains were gram-positive, motile, endospore-forming rods that grew at 10%, 15%, and 20% salts (w/v); at pH 7, 8, and 9; 15°, 25°, and 37°C; were catalase positive, produced acid from glucose, utilized pyruvic acid and D-xylose, and were susceptible to chloramphenicol. All strains were negative for the following features: growth without salts, growth at pH 4 and 12, growth at 5° and 60°C, indole production, phenylalanine deaminase, Voges-Proskauer test, lysine decarboxylase, ornithine decarboxylase, acid production from dulcitol and melibiose, and utilization of L-fructose, D-melibiose, β-methyl-D-galactoside, β-methyl-D-galactoside, estaquiose, and succinamic acid.
[a] Characteristics selected as differential features among phenons.

We have carried out a phenotypic and chemotaxonomic study of 63 strains of moderately halophilic endospore-forming rods isolated from different hypersaline sources in Spain (pond of salterns as well as saline soils), including in this study 6 strains previously isolated and described as *B. salexigens* by Garabito et al. (1997). These strains were examined for 178 morphological, physiological, biochemical, and nutritional tests. These results were analyzed by numerical taxonomy techniques. Using the Jaccard (S_J) coefficient and unweighted pair group method of association (UPGMA), five phenons (named A to E) were obtained at 64% similarity level, whereas 21 strains, 1 of which was a reference strain, did not cluster at this similarity level. *B. salexigens* strains clustered in phenon C (Fig. 1). All strains were gram-positive, motile, strictly aerobic, and catalase- and oxidase positive. Although we found some phenotypic differences among strains belonging to phenons A, B, C, and D, in general these isolates were phenotypically quite homogeneous and very similar to the species *Bacillus salexigens* (Garabito et al. 1997). However, phenotypic features of strains included in phenon E were very different from the other phenons and from those reported for the moderately halophilic gram-positive rods described previously. The most useful features for differentiating the five phenons are shown in Table 1.

Latterly, the murein composition, DNA base composition, and DNA-DNA homology of some representative strains selected from each phenon obtained by numerical taxonomy were determined. All strains examined had *meso*-diaminopimelic acid in their peptidoglycan, as found in *B. salexigens*, B. *halophilus*, and the majority of the species of the genus *Bacillus* (Garcia et al. 1987; Garabito et al. 1997).

The G+C content of the DNA from strains chosen as representative isolates of phenons A, B, C, and D ranged between 36.6 and 46.2 mol% (Table 2). These values are different from those described for *Bacillus halophilus* (51.5 mol%) (Garcia et al. 1987), but are similar to the values reported for *Halobacillus litoralis* (42 mol%), *Halobacillus trueperi* (43 mol%) (Spring et al. 1996), and *Bacillus salexigens* (36.9–39.5 mol%) (Garabito et al. 1997). Representative strains of phenon E had a DNA base composition between 64.6 and 65.7 mol% (Table 2). These values are very different from those reported for the moderately halophilic gram-positive rods previously described.

The term genomovar was recently introduced to denote phenotypically similar but genotypically distinct groups of strains that were previously referred to by a variety of different terms, including genomic species, genomic groups, genospecies, or genomospecies (Ursing et al. 1995). These genomovars share a low level of DNA hybridization and therefore represent distinct species for which an official binomial name is not proposed, pending the availability of differential diagnostic tests.

The DNA-DNA relatedness experiments showed a high degree of homology between the representative strains of each phenon and the strains of the same phenon (70%–100%) (Table 2). Those data indicate that strains belonging to each phenon form a single genomic group. On the other hand, low levels of DNA-DNA homology (0%–46%) were

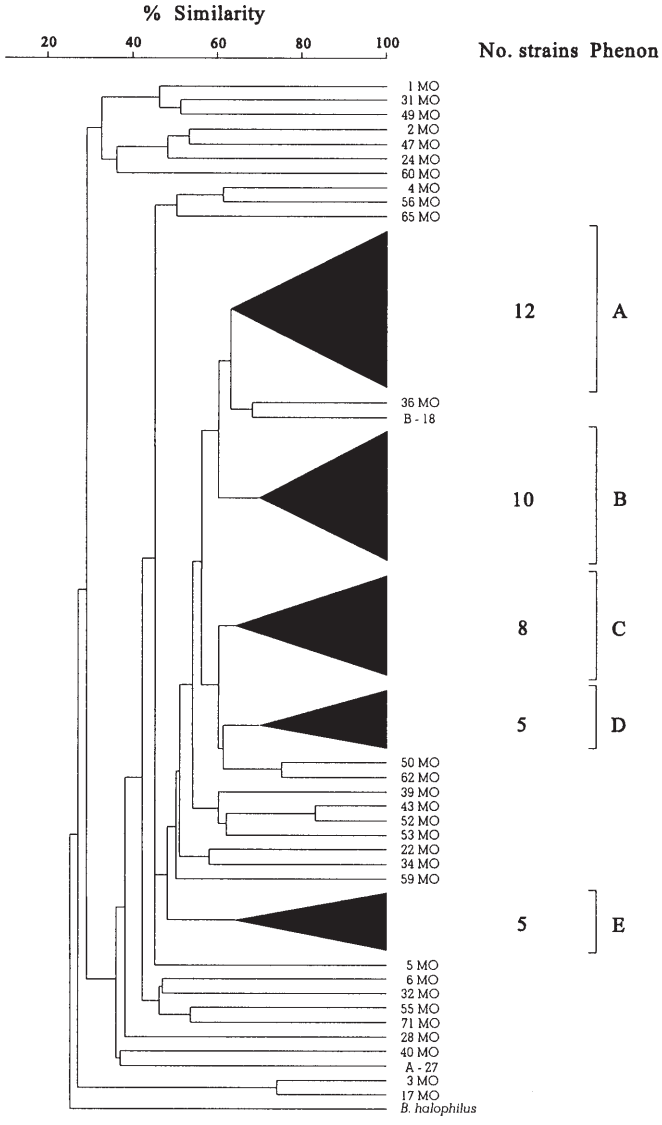

Fig. 1. Simplified dendrogram showing the clustering of strains into five phenons based on the S_J coefficient and UPGMA clustering for 69 moderately halophilic gram-positive endospore-forming rods isolated in this study

obtained between the strains included in different phenons. In addition, the isolates included in phenon C showed a high degree of homology (73%–100%) with the type strain of *Bacillus salexigens* (strain C20MO), also included in this phenon. However, low levels (0%–50%) of homology were obtained between the representative strains of each phenon and the other moderately halophilic, marine, or nonhalophilic bacteria that are members of the genus *Bacillus*, which we studied. All these results indicate that isolates included in phenon A, B, C, and D, should be assigned to the species *Bacillus salexigens*, and, in the absence of differential classical phenotypic features, we propose that these strains should be referred to as genomovar I (phenon C, which includes the type strain), genomovar II (phenon A), genomovar III (phenon B), and genomovar IV (phenon D), in accordance with the guidelines and recommendations for the delineation of new species (Wayne et al. 1987; Ursing et al. 1995). Strains belonging to phenon E might constitute a new species, but additional molecular data are necessary to determine its correct taxonomic position.

Our group also tested the viability of some old enrichments kindly provided by B.E. Volcani. These enrichments were prepared with Dead Sea water samples taken by Volcani in 1936 and stored for more than 50 years at room temperature in closed bottles. In a preliminary study, by using appropriate media and growth conditions, we isolated and characterized 22 extremely halophilic microorganisms that have been classified as members of the genera *Haloferax, Haloarcula*, and *Halobacterium* (Arahal et al. 1996). By reducing the total salt concentration of the media used to final values ranging from 10% to 15%, we isolated 31 gram-positive moderately halophilic bacteria. Phenotypic characterization of these isolates showed that they are members of the genus *Bacillus* (Claus and Berkeley 1986). They are gram-positive motile aerobic endospore-forming rods, growing in 5%–20% total salts (optimal growth at 10%). The endospores are oval in shape, located at the terminal position, and form a swollen sporangium. The strains show growth when incubated at temperatures from 15° to 50°C and at pH 6.0 to 9.0, and are positive for the following biochemical tests: oxidase, catalase, hydrolysis of gelatin, phosphatase, DNAse, methyl red, nitrate reduction, and urease and acid production from fructose, glycerol, glucose, and maltose. Negative results are obtained for the following tests: Voges-Proskauer, indole production, phenylalanine deaminase, hydrolysis of starch and Tween 80, and acid production from arabinose, galactose, lactose, mannose, sucrose, trehalose, and xylose (D.R. Arahal, M.C. Márquez, and A.Ventosa, unpublished results). According to these phenotypic traits our isolates could represent a new moderately halophilic species of the genus *Bacillus*, but additional characterization at the molecular and biochemical level is needed to complete the description of this microbial group.

It is interesting to note that during his studies Volcani isolated ten strains of sporulating gram-positive bacteria resembling *Bacillus* species. These were considered as contaminants carried into the lake with the inflow of freshwater (the Jordan River is the main contributor) whose spores were remarkably resistant to the salt even for long periods without losing the ability to germinate in salt-free media (Volcani 1940). However, the strains that we have isolated are not halotolerant, because they grow optimally at 10% salts (and are able to grow in a wide range of salt concentrations), and thus they should be considered as moderate halophiles, adapted to the high salinity of the Dead Sea.

Table 2. DNA base composition and DNA relatedness between representative strains of phenons A, B, C, D, and E and other related *Bacillus* species

Unlabeled DNA source	G+C content (mol%)[a]	Homology with ^{3}H-labeled DNA (%)[b]				
		A11MO	B57MO	C20MO	D68MO	E16MO
Phenon A						
A11MO	39.1	100	18	ND	46	2
A8MO	38.6	95	ND	ND	ND	ND
A19MO	39.9	88	ND	ND	ND	ND
A35MO	38.2	100	ND	ND	ND	ND
Phenon B						
B57MO	44.6	5	100	19	15	16
B27MO	43.4	ND	99	ND	ND	ND
B58MO	46.2	ND	100	ND	ND	ND
Phenon C						
C20MO	39.5	20	20	100	38	0
C29MO	36.6	ND	ND	81	ND	ND
C69MO	37.8	ND	ND	73	ND	ND
C26MO	38.2	ND	ND	100	ND	ND
Phenon D						
D68MO	38.2	5	5	19	100	18
D65MO	37.8	ND	ND	ND	70	ND
D61MO	39.4	ND	ND	ND	70	ND
D70MO	38.0	ND	ND	ND	76	ND
Phenon E						
E16MO	65.2	3	11	0	29	100
E23MO	64.6	ND	ND	ND	ND	89
E15MO	65.7	ND	ND	ND	ND	88
Bacillus alcalophilus DMS 485^{T}	ND	0	4	0	16	3
Bacillus badius ATCC 14574^{T}	ND	2	9	6	7	0
Bacillus circulans PCM 1398	ND	5	9	12	24	28
Bacillus globisporus DSM 4^{T}	ND	4	9	8	18	0
Bacillus halophilus DSM 4771^{T}	ND	2	13	0	0	9
Bacillus insolitus DSM 5^{T}	ND	33	4	22	21	0
Bacillus laterosporus DSM 25^{T}	ND	0	0	0	16	ND
Bacillus lentus ATCC 10840	ND	4	9	15	32	10
Bacillus marinus DSM 1297^{T}	ND	5	4	10	26	0
Bacillus megaterium DSM 32^{T}	ND	0	4	13	18	10
Bacillus pallidus DSM 3670^{T}	ND	2	3	0	20	0
Bacillus pantothenticus PCM 454	ND	26	15	15	50	8
Bacillus pumilus ATCC 7061^{T}	ND	17	0	0	15	0
Bacillus smithii DSM 4216^{T}	ND	0	12	1	35	7
Bacillus sp. DSM 578	ND	19	0	0	0	0
Bacillus subtilis ATCC 6051^{T}	ND	15	ND	ND	ND	ND

ND, not determined.
[a] Means of three determinations, obtained by the Tm method (Owen and Pitcher 1985).
[b] These results are the average of three different experiments, following the competition method (Johnson 1981).

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References

Arahal DR, Dewhirst FE, Paster BJ, Volcani BE, Ventosa A (1996) Phylogenetic analyses of some extremely halophilic Archaea isolated from Dead Sea water, determined on the basis of their 16S rRNA sequences. Appl Environ Microbiol 62:3779–3786

Claus D, Berkeley RCW (1986) Genus *Bacillus* Cohn 1872. In: Sneath PHA (ed) Bergey's manual of systematic bacteriology, vol 2. Williams & Wilkins, Baltimore, pp 1105–1139

Garabito MJ, Arahal DR, Mellado E, Márquez MC, Ventosa A (1997) *Bacillus salexigens* sp. nov., a new moderately halophilic *Bacillus* species. Int J Syst Bacteriol 47:735–741

García MT, Ventosa A, Ruiz-Berraquero F, Kocur M (1987) Taxonomic study and amended description of *Vibrio costicola*. Int J Syst Bacteriol 37:251–256

Hao MV, Kocur M, Komagata K (1984) *Marinococcus* gen. nov., a new genus for motile cocci with *meso*-diaminopimelic acid in the cell wall; and *Marinococcus albus* sp. nov. and *Marinococcus halophilus* (Novitsky and Kushner) comb. nov. J Gen Appl Microbiol 30:449–459

Javor BJ (1989) Hypersaline environments. Microbiology and biogeochemistry. Springer, Berlin

Johnson JL (1981) Genetic characterization. In: Gerhardh P (ed) Manual of methods for general bacteriology. American Society for Microbiology, Washington, DC, pp 450–472

Koch C, Rainey FA, Stackebrandt E (1994) 16S rDNA studies on members of *Arthrobacter* and *Micrococcus*: an aid for their future taxonomic restructuring. FEMS Microbiol Lett 123:167–172

Kushner DJ, Kamekura M (1988) Physiology of halophilic eubacteria. In: Rodriguez-Valera F (ed) Halophilic bacteria, vol I. CRC Press, Boca Ratón, pp 109–140

Márquez MC, Ventosa A, Ruiz-Berraquero F (1992) Phenotypic and chemotaxonomic characterization of *Marinococcus halophilus.* Syst Appl Microbiol 15:63–69

Monteoliva-Sánchez M, Ventosa A, Ramos-Cormenzana A (1989) Cellular fatty acid composition of moderately halophilic cocci. Syst Appl Microbiol 12:141–144

Mota RR, Márquez MC, Arahal DR, Mellado E, Ventosa A (1997) Polyphasic taxonomy of *Nesterenkonia halobia.* Int J Syst Bacteriol 47:1231–1235

Onishi H (1972) Halophilic amylase from a moderately halophilic *Micrococcus*. J Bacteriol 109:570–574

Onishi H, Kamekura M (1972) *Micrococcus halobius* sp. n. Int J Syst Bacteriol 22:233–236

Onishi H, Fuchi H, Konomi K, Hidaka O, Kamekura M (1980) Isolation and distribution of a variety of halophilic bacteria and their classification by salt-response. Agric Biol Chem 44:1253–1258

Onishi H, Mori T, Takeuchi S, Tani K, Kobayashi T, Kamekura M (1983) Halophilic nuclease of a moderately halophilic *Bacillus* sp. production, purification and characteristics. Appl Environ Microbiol 45:24–30

Owen RJ, Pitcher D (1985) Current methods for estimating DNA base composition and levels of DNA-DNA hybridization. In: Goodfellow M, Minnikin DE (eds) Identification methods in bacterial systematics. Academic Press, London, pp 67–93

Rodriguez-Valera F (1998) The ecology and taxonomy of aerobic chemoorganotrophic halophilic eubacteria. FEMS Microbiol Rev 39:17–22

Satomi M, Kimura B, Mizoi M, Sato T, Fujii T (1997) *Tetragenococcus muriaticus* sp. nov., a new moderately halophilic lactic acid bacterium isolated from fermented squid liver sauce. Int J Syst Bacteriol 47:832–836

Skerman VBD, McGowan V, Sneath PHA (1980) Approved lists of bacterial names. Int J Syst Bacteriol 30:225–420

Spring S, Ludwig W, Márquez MC, Ventosa A, Schleifer K-H (1996) *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. Int J Syst Bacteriol 46:492–496

Stackebrandt E, Koch C, Gvozdiak O, Schumann P (1995) Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. Int J Syst Bacteriol 45:682–692

Ursing JB, Roselló-Mora RA, García-Valdés E, Lalucat J (1995) Taxonomic note: a pragmatic approach to the nomenclature of phenotypically similar genomic groups. Int J Syst Bacteriol 45:604

Ventosa A (1988) Taxonomy of moderately halophilic heterotrophic eubacteria. In: Rodriguez-Valera F (ed) Halophilic bacteria, vol I. CRC Press, Boca Raton, pp 71–84

Ventosa A (1994) Taxonomy and phylogeny of moderately halophilic bacteria. In: Priest FG, Ramos-Cormenzana A, Tindall BJ (eds) Bacterial diversity and systematics. Plenum Press, New York, pp 231–242

Ventosa A, Márquez MC, Ruiz-Berraquero F, Kocur M (1990) *Salinicoccus roseus* gen. nov. sp. nov., a new moderately halophilic gram-positive coccus. Syst Appl Microbiol 13:29–33

Ventosa A, Márquez MC, Weiss N, Tindall BJ (1992) Transfer of *Marinococcus hispanicus* to the genus *Salinicoccus* as *Salinicoccus hispanicus* comb. nov. Syst Appl Microbiol 15:530–534

Ventosa A, Nieto JJ, Oren A (1998) The biology of aerobic moderately halophilic bacteria. Microbiol Mol Biol Rev 62:504–544

Ventosa A, Quesada E, Rodríguez-Valera F, Ruiz-Berraquero F, Ramos-Cormenzana A (1982) Numerical taxonomy of moderately halophilic gram-negative rods. J Gen Microbiol 128:1959–1968

Ventosa A, Ramos-Cormenzana A, Kocur M (1983) Moderately halophilic gram-positive cocci from hypersaline environments. Syst Appl Microbiol 4:564–570

Ventosa A, Rodríguez-Valera F, Poindexter JS, Reznikoff WS (1984) Selection for moderately halophilic bacteria by gradual salinity increases. Can J Microbiol 30:1279–1282

Volcani BE (1940) Studies on the microflora of the Dead Sea. PhD thesis, The Hebrew University, Jerusalem, Israel

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murrey RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464