

## Molecular taxonomy of Gram-positive moderately halophilic cocci

A. Ventosa

Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, E-41012 Seville (Spain)

**Abstract.** The Gram-positive moderately halophilic cocci validly described up to date are *Micrococcus halobius*, *Sporosarcina halophila*, *Marinococcus halophilus*, *Marinococcus albus*, *Marinococcus hispanicus*, and the recently proposed *Salinicoccus roseus*. Our chemotaxonomic studies on these bacteria have revealed that *Marinococcus hispanicus* should be placed in the genus *Salinicoccus*. DNA–DNA hybridization studies showed that this species should be considered as a separate species of *S. roseus*. Thus, we have proposed transferring it to the genus *Salinicoccus*, as *S. hispanicus*. Besides, phenotypic and chemotaxonomic features of the culture collection moderate halophiles *Micrococcus* sp. strains CCM 168 and CCM 1405 indicate that they should be included in the species *Salinicoccus roseus*. The description of this species was based only on one strain.

**Key words.** Moderate halophiles; taxonomy; *Salinicoccus roseus*; *Marinococcus hispanicus*.

### Introduction

Moderately halophilic bacteria are microorganisms able to grow optimally in media containing 0.5–2.5 M (about 3–15%) salt<sup>21</sup>. The wide range of salinity in which they grow optimally make evident that they are well adapted to very diverse saline habitats, including hypersaline lakes (as for example the Dead Sea, the Great Salt Lake, Wadi Natrun, Lake Magadi or the hypersaline lakes in the Antarctica)<sup>10,13,19,38,42,43</sup>, marine and inland salterns, utilized for the production of salt by evaporation of seawater or saline water<sup>38,39</sup>, salt mines<sup>13</sup>, saline soils<sup>35,38</sup> and, although in low proportions, seawater<sup>51</sup>. Traditionally, they have been isolated from salted foods, specially salted fishes and meats<sup>12,43</sup>.

The aspects that attracted the interest of researchers were mainly those related to their physiological adaptation to highly saline concentrations<sup>21</sup>. Their ecology and distribution have also been studied although in a very limited number of habitats<sup>13,19,38</sup>. With respect to their taxonomic distribution, moderately halophilic bacteria are a very heterogeneous physiological group of microorganisms that include a great variety of species belonging both to the Archaea and the Bacteria. In the Approved Lists of Bacterial Names<sup>41</sup>, only six moderately halophilic bacteria were included as valid species: *Flavobacterium halmephilum*, *Micrococcus halobius*, *Paracoccus halodenitrificans*, *Planococcus halophilus*, *Spirochaeta halophila* and *Vibrio costicola*. However, the extensive studies carried out by researchers during the last decade have led to the description of many new species and genera isolated from very different hypersaline habitats. Table 1 presents the validly described bacterial species belonging to the moderately halophilic category. Besides, two families have been proposed: Halomonadaceae, in order to accommodate the species belonging to the genera *Halomonas* and *Deleya*<sup>11</sup>,

and the family Haloanaerobiaceae, which includes the species belonging to *Haloanaerobium*, *Halobacteroides*, *Sporohalobacter* and the recently proposed *Haloicola*<sup>30,57</sup>. It is noteworthy that these families were proposed with a phylogenetic criterion, based on the comparison of 16S rRNA oligonucleotide catalogues.

With respect to the Gram-positive moderately halophilic cocci, until recently only three species were accepted: *Micrococcus halobius*, isolated from salt samples<sup>27</sup>, *Planococcus halophilus*, from salted mackerel<sup>25</sup> and *Sporosarcina halophila*, a sporeforming motile coccus isolated from salt marsh habitats<sup>5</sup>. Ventosa et al.<sup>50</sup> carried out a study of 38 isolates from hypersaline soils and ponds of marine salterns, and obtained three different biogroups: group I included 25 strains with characteristics very similar to *Planococcus halophilus*; group II was assigned to *Sporosarcina halophila*, and finally, group III showed characteristics quite different from those of the species previously described, and which in the absence of other molecular data, were designated as *Planococcus* sp. In 1984, Hao et al.<sup>15</sup> proposed accommodating *Planococcus halophilus* in a new genus, as *Marinococcus halophilus*, and strains of group III of the study of Ventosa et al.<sup>50</sup> in a new species of this genus, as *Marinococcus albus*. The genus *Marinococcus* was characterized by the typical murein type, cell walls with meso-diaminopimelic acid in the murein, as well as by some phenotypic features: Gram-positive moderately halophilic motile cocci, non-sporeforming, catalase positive, aerobic, showing optimal growth at about 10% salt<sup>15</sup>.

In 1990, Márquez et al.<sup>22</sup> studied five Gram-positive cocci isolated from salterns and hypersaline soils. They were non-motile, non-sporeforming, strict aerobes, catalase and oxidase positive, reddish to orange pigmented and able to grow between 0.5 and 25% salt, showing optimal growth at 10% salt. The DNA base

Table 1. Moderately halophilic bacteria described as valid species

Species	Type strain	References
Archaea:		
<i>Methanohalophilus mahii</i>	ATCC 35705	33
<i>Methanohalophilus zhilinae</i>	DSM 4017	23
<i>Methanohalophilus halophilus</i>	DSM 3094	53
<i>Halomethanococcus doii</i>	ATCC 43619	55
Bacteria:		
A. Phototrophic:		
<i>Rhodospirillum salexigens</i>	DSM 2132	7
<i>Rhodospirillum salinarum</i>	ATCC 35394	24
<i>Ectothiorhodospira vacuolata</i>	DSM 2111	18
<i>Ectothiorhodospira marismortui</i>	DSM 4180	29
<i>Chromatium salexigens</i>	DSM 4395	2
<i>Thiocapsa halophila</i>	DSM 6210	3
B. Chemoorganotrophic:		
Gram-negative aerobic or facultatively anaerobic:		
<i>Vibrio costicola</i>	NCMB 701	12
<i>Halomonas elongata</i>	ATCC 33173	52
<i>Halomonas subglaciescola</i>	UQM 2927	10
<i>Halomonas halodurans</i>	ATCC 29696	16
<i>Halomonas halmophila</i>	ATCC 19717	6
<i>Deleya halophila</i>	CCM 3662	36
<i>Deleya salina</i>	ATCC 49509	44
<i>Spirochaeta halophila</i>	ATCC 29478	14
<i>Paracoccus halodenitrificans</i>	ATCC 13511	20
<i>Halovibrio variabilis</i>	DSM 3051	8
<i>Pseudomonas halophila</i>	DSM 3050	8
<i>Chromohalobacter marismortui</i>	ATCC 17056	46
<i>Volcaniella eurihalina</i>	ATCC 49336	34
Gram-negative anaerobic:		
<i>Haloanaerobium praevalens</i>	ATCC 33744	56
<i>Halobacteroides halobius</i>	ATCC 35273	32
<i>Halobacteroides acetothylicus</i>	ATCC 43120	37
<i>Sporohalobacter lortetii</i>	ATCC 35059	28, 31
<i>Sporohalobacter marismortui</i>	ATCC 35420	31
<i>Haloicola saccharolytica</i>	DSM 6643	57
Gram-positive:		
<i>Micrococcus halobius</i>	ATCC 21727	27
<i>Sporosarcina halophila</i>	DSM 2266	5
<i>Marinococcus halophilus</i>	ATCC 27964	15, 25
<i>Marinococcus albus</i>	CCM 3517	15
<i>Marinococcus hispanicus</i>	ATCC 49259	22
<i>Salinicoccus roseus</i>	ATCC 49258	49
<i>Bacillus halophilus</i>	ATCC 49085	45
<i>Clostridium halophilum</i>	DSM 5387	9
Budding prosthecate bacteria:		
<i>Dichotomicrobium thermohalophilum</i>	DSM 5002	17
Actinomycete:		
<i>Actinopolyspora mortivallis</i>	JCM 7550	54
Sulfate-reducing bacteria:		
<i>Desulfohalobium rebaense</i>	DSM 5692	26
<i>Desulfovibrio halophilus</i>	DSM 5663	4

composition ranged from 45.7 to 49.3 mol%. These strains were placed in a new species, named *Marinococcus hispanicus*<sup>22</sup>. Recently, Ventosa et al.<sup>48</sup> proposed a new genus, *Salinicoccus*, with a single species *S. roseus* to accommodate a Gram-positive moderately halophilic coccus able to grow between 0.9 and 25% salt, with optimal growth at 10% salt. This organism was non-motile, non-sporeforming, pink to red pigmented, strictly aerobic, catalase and oxidase positive, with a

guanine plus cytosine (G + C) content of 51.2 mol% and murein of the L-Lys-Gly<sub>5</sub> type.

#### Recent studies on Gram-positive moderately halophilic cocci

Since there were some phenotypic similarities between the species *Marinococcus hispanicus* and *Salinicoccus roseus*, and the DNA base composition was not very different, we carried out a comparative taxonomic study of these two species using chemical and molecular methods. For this purpose, we determined the content of respiratory quinones, polar lipids, fatty acids, the murein type and, finally, we performed DNA-DNA hybridization studies. Later on, the results of all these features are concisely summarized. Both moderately halophilic cocci, *Marinococcus hispanicus* and *Salinicoccus roseus*, have menaquinones with six isoprene units (MK-6) as the major respiratory lipoquinone present. This feature is characteristic of these two moderate halophiles, since *Marinococcus halophilus*, *M. albus* and *Sporosarcina halophila* have MK-7 (refs 5 and 15), and *Micrococcus halobius* has MK-8, MK-7 and MK-6 (ref. 27). The polar lipids of both halophiles are essentially identical and included phosphatidylglycerol, diphosphatidylglycerol, and a glycolipid of unknown structure. With respect to the fatty acid composition, they showed a similar pattern, with predominantly straight and branched chain fatty acids. This is similar to fatty acids present in other moderate halophiles. The cell walls of *S. roseus* and *M. hispanicus* contain murein of the L-Lys-Gly<sub>5</sub> type. This murein type is found only in these two microorganisms and some members of the genus *Staphylococcus*<sup>49</sup>. All of these chemotaxonomic features supported the transfer of *M. hispanicus* to the genus *Salinicoccus*. However, nucleic acid hybridization analysis was necessary in order to determine whether *M. hispanicus* and *S. roseus* constituted two different species. DNA-DNA homology between these two halophiles was very low (31%) and they should thus be considered as two species of the genus *Salinicoccus*. In conclusion, our results support the transfer of *Marinococcus hispanicus* to the genus *Salinicoccus*, as *Salinicoccus hispanicus*<sup>49</sup>.

Another study has recently been carried out by our group in collaboration with Drs M. Kocur and B. J. Tindall, on two red-pigmented, culture collection, moderately halophilic eubacteria: *Micrococcus* sp. strain CCM 168, isolated from salted hide, and *Micrococcus* sp. strain CCM 1405, isolated from salted meat. The G + C content of their DNA was 49.4 mol%<sup>1</sup>, a low value compared with that reported for the species of the genus *Micrococcus*. On the other hand, the murein composition of these strains was of the L-Lys-Gly<sub>5</sub>-L-Ala type<sup>40</sup>, which is also different from the typical *Micrococcus*. These features led us to investigate in

Table 2. Characteristics useful in distinguishing the 6 validly described moderately halophilic Gram-positive cocci

Characteristic:	<i>Salinicoccus roseus</i>	<i>Salinicoccus hispanicus</i>	<i>Marinococcus halophilus</i>	<i>Marinococcus albus</i>	<i>Micrococcus halobius</i>	<i>Sporosarcina halophila</i>
Motility	—	—	+	+	—	+
Pigmentation	Reddish orange	Pink red	Yellowish orange	Creamy white	Cream	Orange
Spore production	—	—	—	—	—	+
Oxidase	+	+	—	+	+	+
Acid produced from						
D-Glucose	—	+	+	—	+	—
Nitrate reduction	+	+	—	+	—	—
Hydrolysis of gelatin	+	+	+	—	—	+
Hydrolysis of esculin	—	+	+	—	N.D.	N.D.
Menaquinone system	MK-6	MK-6	MK-7	MK-7	MK-8, MK-7, MK-6	MK-7
Murein type	L-Lys-Gly <sub>5</sub>	L-Lys-Gly <sub>5</sub>	m-Dpm	m-Dpm	Ala, Glu, Gly, Lys	Orn-D-Asp
DNA base composition (mol%)	51.2	45.6–49.3	46.4	44.9	70–71.5	40.1–40.9

N.D., not determined; MK-6, MK-7, MK-8, menaquinone with 6, 7, and 8 isoprene units, respectively; m-Dpm, meso-Diaminopimelic acid.

detail these two strains and to postulate that they could belong to the genus *Salinicoccus*<sup>47</sup>. In fact, we observed that their phenotypic features were very similar to those of *S. roseus*, and an extensive chemotaxonomic study revealed that they have MK-6, and polar lipid and fatty acid patterns similar to those of *S. roseus*. DNA homology experiments showed that they are very closely related to *S. roseus* (with DNA-DNA relatedness of 70 and 95%, for strains CCM 168 and CCM 1405 respectively). Our data support the placement of these two strains in the species *Salinicoccus roseus*. Thus the description of this species should be based not only on the single strain CCM 3516 (type strain), but also on these two additional isolates<sup>47</sup>. Future studies based on molecular features should permit the correct classification of other moderate halophiles. Table 2 summarizes some differential characteristics that may be useful for the identification of the Gram-positive moderately halophilic cocci.

Acknowledgments. I should like to thank Drs M. C. Marquez, B. J. Tindall and M. Kocur for permitting me to use some unpublished data and for stimulating discussion, and the Dirección General de Investigación Científica y Técnica (grant PB90-0907) and the Junta de Andalucía for financial support.

- Bohacek, J., Kocur, M., and Martinec, T., DNA base composition and taxonomy of some micrococci. *J. gen. Microbiol.* 46 (1967) 369–376.
- Caumette, P., Baulaigue, R., and Matheron, R., Characterization of *Chromatium salexigens* sp. nov., a halophilic Chromatiaceae isolated from Mediterranean salinas. *Syst. appl. Microbiol.* 10 (1988) 284–292.
- Caumette, P., Baulaigue, R., and Matheron, R., *Thiocapsa halophila* sp. nov., a new halophilic phototrophic purple sulfur bacterium. *Archs Microbiol.* 155 (1991) 170–176.
- Caumette, P., Cohen, Y., and Matheron, R., Isolation and characterization of *Desulfovibrio halophilus* sp. nov., a halophilic sulfate-reducing bacterium isolated from Solar Lake (Sinai). *Syst. appl. Microbiol.* 14 (1991) 33–38.
- Claus, D., Fahmy, F., Rolf, H. J., and Tosunoglu, N., *Sporosarcina halophila* sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. *Syst. appl. Microbiol.* 4 (1983) 496–506.
- Dobson, S. J., James, S. R., Franzmann, P. D., and McMeekin, T. A., Emended description of *Halomonas halophila* (NCMB 1971<sup>T</sup>). *Int. J. syst. Bact.* 40 (1990) 462–463.
- Dreus, G., *Rhodospirillum salexigens*, spec. nov., an obligate halophilic phototrophic bacterium. *Archs Microbiol.* 130 (1981) 325–327.
- Fendrich, C., *Halovibrio variabilis* gen. nov. sp. nov., *Pseudomonas halophila* sp. nov. and a new halophilic aerobic coccoid eubacterium from Great Salt Lake, Utah, USA. *Syst. appl. Microbiol.* 11 (1988) 36–43.
- Fendrich, C., Hippe, H., and Gottschalk, G., *Clostridium halophilum* sp. nov. and *C. litorale* sp. nov., an obligate halophilic and a marine species degrading betaine in the Stickland reaction. *Archs Microbiol.* 154 (1990) 127–132.
- Franzmann, P. D., Burton, H. R., and McMeekin, T. A., *Halomonas subglaciescola*, a new species of halotolerant bacteria isolated from Antarctica. *Int. J. syst. Bact.* 37 (1987) 27–34.
- Franzmann, P. D., Wehmeyer, U., and Stackebrandt, E., Halomonadaceae fam. nov., a new family of the class Proteobacteria to accommodate the genera *Halomonas* and *Deleya*. *Syst. appl. Microbiol.* 11 (1988) 16–19.
- García, M. T., Ventosa, A., Ruiz-Berraquero, F., and Kocur, M., Taxonomic study and amended description of *Vibrio costicola*. *Int. J. syst. Bact.* 37 (1987) 251–256.
- Grant, W. D., General view of halophiles, in: *Superbugs. Microorganisms in Extreme Environments*, pp. 15–37. Eds K. Horikoshi and W. D. Grant. Japan Scientific Societies Press, Tokyo 1991.
- Greenberg, E. P., and Canale-Parola, E., *Spirochaeta halophila* sp. n., a facultative anaerobe from a high-salinity pond. *Archs Microbiol.* 110 (1976) 185–194.
- Hao, M. V., Kocur, M., and Komagata, K., *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. Microbiol.* 30 (1984) 449–459.
- Herbert, A. M., and Vreeland, R. H., Phenotypic comparison of halotolerant bacteria: *Halomonas halodurans* sp. nov., nom. rev., comb. nov. *Int. J. syst. Bact.* 37 (1987) 347–350.
- Hirsch, P., and Hoffmann, B., *Dichotomicrobium thermohalophilum*, gen. nov., spec. nov., budding prosthecate bacteria from the Solar Lake (Sinai) and some related strains. *Syst. appl. Microbiol.* 11 (1989) 291–301.
- Imhoff, J. F., Tindall, B. J., Grant, W. D., and Trüper, H. G., *Ectothiorhodospira vacuolata* sp. nov., a new phototrophic bacterium from soda lakes. *Archs Microbiol.* 130 (1981) 238–242.
- Javor, B., *Hypersaline Environments. Microbiology and Biogeochemistry*. Springer Verlag, Berlin 1989.

- 20 Kocur, M., Genus *Planococcus*, in: Bergey's Manual of Systematic Bacteriology Vol. 1, pp. 339–402. Eds N. R. Krieg, and J. G. Holt, Williams & Wilkins, Baltimore 1984.
- 21 Kushner, D. J., and Kamekura, M., Physiology of halophilic eubacteria, in: Halophilic Bacteria, Vol. 1, pp. 109–140. Ed. F. Rodriguez-Valera. CRC Press, Boca Raton, Florida 1988.
- 22 Márquez, M. C., Ventosa, A., and Ruiz-Berraquero, F., *Marinococcus hispanicus*, a new species of moderately halophilic Gram-positive cocci. Int. J. syst. Bact. 40 (1990) 165–169.
- 23 Mathrani, I. M., Boone, D. R., Mah, R. A., Fox, G. E., and Lau, P. P., *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int. J. syst. Bact. 38 (1988) 139–142.
- 24 Nissen, H., and Dundas, I. D., *Rhodospirillum salinarum* sp. nov., a halophilic photosynthetic bacterium isolated from a portuguese saltern. Archs Microbiol. 138 (1984) 251–256.
- 25 Novitsky, T. J., and Kushner, D. J., *Planococcus halophilus* sp. nov., a facultatively halophilic coccus. Int. J. syst. Bact. 26 (1976) 53–57.
- 26 Ollivier, B., Hatchikian, C. E., Prensier, G., Guezennec, J., and Garcia, J. L., *Desulfohalobium retbaense* gen. nov., sp. nov., a halophilic sulfate-reducing bacterium from sediments of a hypersaline lake in Senegal. Int. J. syst. Bact. 41 (1991) 74–81.
- 27 Onishi, H., and Kamekura, M., *Micrococcus halobius* sp. n. Int. J. syst. Bact. 22 (1972) 233–236.
- 28 Oren, A., *Clostridium lortetii* sp. nov., a halophilic obligatory anaerobic bacterium producing endospores with attached gas vacuoles. Archs Microbiol. 136 (1983) 42–48.
- 29 Oren, A., Kessel, M., and Stackebrandt, E., *Ectothiorhodospira marismortui* sp. nov., an obligately anaerobic, moderately halophilic purple sulfur bacterium from a hypersaline sulfur spring on the shore of the Dead Sea. Archs Microbiol. 151 (1989) 524–529.
- 30 Oren, A., Paster, B. J., and Woese, C. R., Haloanaerobiaceae: a new family of moderately halophilic, obligatory anaerobic bacteria. Syst. appl. Microbiol. 5 (1984) 71–80.
- 31 Oren, A., Pöhla, H., and Stackebrandt, E., Transfer of *Clostridium lortetii* to a new genus *Sporohalobacter* gen. nov. as *Sporohalobacter lortetii* comb. nov., and description of *Sporohalobacter marismortui* sp. nov. Syst. appl. Microbiol. 9 (1987) 239–246.
- 32 Oren, A., Weisburg, W. G., Kessel, M., and Woese, C. R., *Halobacteroides halobius* gen. nov., sp. nov., a moderately halophilic anaerobic bacterium from the bottom sediments of the Dead Sea. Syst. appl. Microbiol. 5 (1984) 58–70.
- 33 Paterek, J. R., and Smith, P. H., *Methanohalophilus mahii* gen. nov., sp. nov., a methylotrophic halophilic methanogen. Int. J. syst. Bact. 38 (1988) 122–123.
- 34 Quesada, E., Valderrama, M. J., Bejar, V., Ventosa, A., Gutierrez, M. C., Ruiz-Berraquero, F., and Ramos-Cormenzana, A., *Volcaniella eurihalina* gen. nov., sp. nov., a moderately halophilic nonmotile Gram-negative rod. Int. J. syst. Bact. 40 (1990) 261–267.
- 35 Quesada, E., Ventosa, A., Rodriguez-Valera, F., and Ramos-Cormenzana, A., Types and properties of some bacteria isolated from hypersaline solis. J. appl. Bact. 53 (1982) 155–161.
- 36 Quesada, E., Ventosa, A., Ruiz-Berraquero, F., and Ramos-Cormenzana, A., *Deleya halophila*, a new species of moderately halophilic bacteria. Int. J. syst. Bact. 34 (1984) 287–292.
- 37 Rengpipat, S., Langworthy, T. A., and Zeikus, J. G., *Halobacteroides acetothyliscus* sp. nov., a new obligately anaerobic halophile isolated from deep subsurface hypersaline environments. Syst. appl. Microbiol. 11 (1988) 28–35.
- 38 Rodriguez-Valera, F., Characteristics and microbial ecology of hypersaline environments, in: Halophilic Bacteria, Vol. 1, pp. 3–30 Ed. F. Rodriguez-Valera. CRC Press, Boca Raton, Florida 1988.
- 39 Rodriguez-Valera, F., Ventosa, A., Juez, G., and Imhoff, J. F., Variation of environmental features and microbial populations with salt concentrations in a multi-pond saltern. Microb. Ecol. 11 (1985) 107–115.
- 40 Schleifer, K. H., and Kandler, O., Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bact. Rev. 36 (1972) 407–477.
- 41 Skerman, V. B. D., McGowan, V., and Sneath, P. H. A., Approved lists of bacterial names. Int. J. syst. Bact. 30 (1980) 225–420.
- 42 Tindall, B. J., Prokaryotic life in the alkaline, saline, athalastic environment, in: Halophilic bacteria, Vol. 1, pp. 31–67. Ed. F. Rodriguez-Valera. CRC Press, Boca Raton, Florida 1988.
- 43 Trüper, H. G., and Galinski, E. A., Concentrated brines as habitats for microorganisms. Experientia 42 (1986) 1182–1187.
- 44 Valderrama, M. J., Quesada, E., Bejar, V., Ventosa, A., Gutierrez, M. C., Ruiz-Berraquero, F., and Ramos-Cormenzana, A., *Deleya salina* sp. nov., a moderately halophilic Gram negative bacterium. Int. J. syst. Bact. 41 (1991) 377–384.
- 45 Ventosa, A., García, M. T., Kamekura, M., Onishi, H., and Ruiz-Berraquero, F., *Bacillus halophilus* sp. nov., a moderately halophilic *Bacillus* species. Syst. appl. Microbiol. 12 (1989) 162–166.
- 46 Ventosa, A., Gutierrez, M. C., García, M. T., and Ruiz-Berraquero, F., Classification of "*Chromobacterium marismortui*" in a new genus, *Chromohalobacter* gen. nov., as *Chromohalobacter marismortui* comb. nov., nom. rev. Int. J. syst. Bact. 39 (1989) 382–386.
- 47 Ventosa, A., Márquez, M. C., Kocur, M., and Tindall, B. J., Comparative study of "*Micrococcus* sp." strains CCM 168 and CCM 1405 and members of the genus *Salinicoccus*. Int. J. syst. Bact. 43 (1993) 245–248.
- 48 Ventosa, A., Márquez, M. C., Ruiz-Berraquero, F., and Kocur, M., *Salinicoccus roseus* gen. nov., sp. nov., a new moderately halophilic Gram-positive coccus. Syst. appl. Microbiol. 13 (1990) 29–33.
- 49 Ventosa, A., Márquez, M. C., Weiss, N., and Tindall, B. J., Transfer of *Marinococcus hispanicus* to the genus *Salinicoccus* as *Salinicoccus hispanicus* comb. nov. Syst. appl. Microbiol. 15 (1992) 530–534.
- 50 Ventosa, A., Ramos-Cormenzana, A., and Kocur, M., Moderately halophilic Gram-positive cocci from hypersaline environments. Syst. appl. Microbiol. 4 (1983) 564–570.
- 51 Ventosa, A., Rodriguez-Valera, F., Poindexter, J. S., and Reznikoff, W. S., Selection for moderately halophilic bacteria by gradual salinity increases. Can. J. Microbiol. 30 (1984) 1279–1282.
- 52 Vreeland, R. H., Litchfield, C. D., Martin, E. L., and Elliot, E., *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. Int. J. syst. Bact. 30 (1980) 485–495.
- 53 Wilharm, T., Zhilina, T. N., and Hummel, P., DNA-DNA hybridization of methylotrophic halophilic methanogenic bacteria and transfer of *Methanococcus halophilus*<sup>VP</sup> to the genus *Methanohalophilus* as *Methanohalophilus halophilus* comb. nov. Int. J. syst. Bact. 41 (1991) 558–562.
- 54 Yoshida, M., Matsubara, K., Kudo, T., and Horikoshi, K., *Actinopolyspora mortivallis* sp. nov., a moderately halophilic actinomycete. Int. J. syst. Bact. 41 (1991) 15–20.
- 55 Yu, I. K., and Kawamura, F., *Halomethanococcus doii* gen. nov., sp. nov.: an obligately halophilic methanogenic bacterium from solar salt ponds, J. gen. appl. Microbiol. 33 (1987) 303–310.
- 56 Zeikus, J. G., Hegge, P. W., Thompson, T. E., Phelps, T. J., and Langworthy, T. A., Isolation and description of *Haloanaerobium praevalens* gen. nov. and sp. nov., an obligately anaerobic halophile common to Great Salt Lake sediments. Curr. Microbiol. 9 (1983) 225–234.
- 57 Zhilina, T. N., Zavarzin, G. A., Bulygina, E. S., Osipov, G. A., and Chumakov, K. M., Ecology, physiology and taxonomic studies on a new taxon of Haloanaerobiaceae, *Haloicola saccharolytica* gen. nov., sp. nov. Syst. appl. Microbiol. 15 (1992) 275–284.