Salipiger mucescens gen. nov., sp. nov., a moderately halophilic, exopolysaccharide- producing bacterium isolated from hypersaline soil, belonging to the α -*Proteobacteria*

M. José Martínez-Cánovas, Emilia Quesada, Fernando Martínez-Checa,
Ana del Moral and Victoria Béjar

Correspondence
Victoria Béjar
vbejar@ugr.es

Microbial Exopolysaccharide Research Group, Department of Microbiology, Faculty of
Pharmacy, Cartuja Campus, University of Granada, 18071 Granada, Spain

Salipiger mucescens gen. nov., sp. nov. is a moderately halophilic, exopolysaccharide-producing, Gram-negative rod isolated from a hypersaline habitat in Murcia in south-eastern Spain. The bacterium is chemoheterotrophic and strictly aerobic (i.e. unable to grow under anaerobic conditions either by fermentation or by nitrate or fumarate respiration). It does not synthesize bacteriochlorophyll *a*. Catalase and phosphatase are positive. It does not produce acids from carbohydrates. It cannot grow with carbohydrates or amino acids as sole sources of carbon and energy. It grows best at 9–10 % w/v NaCl and requires the presence of Na⁺ but not Mg²⁺ or K⁺, although they do stimulate its growth somewhat when present. Its major fatty-acid component is 18:1 ω 7c (78.0 %). The predominant respiratory lipoquinone found in strain A3^T is ubiquinone with ten isoprene units. The G+C content is 64.5 mol%. Phylogenetic analyses strongly indicate that this strain forms a distinct line within a clade containing the genus *Roseivivax* in the subclass α -*Proteobacteria*. The similarity value with *Roseivivax halodurans* and *Roseivivax halotolerans* is 94 %. In the light of the polyphasic evidence gathered in this study it is proposed that the isolate be classified as representing a new genus and species, *Salipiger mucescens* gen. nov., sp. nov. The proposed type strain is strain A3^T (=CECT 5855^T=LMG 22090^T=DSM 16094^T).

Moderately halophilic bacteria are widely distributed throughout hypersaline habitats and require from 3 to 15 % w/v NaCl for satisfactory growth (Kushner & Kamekura, 1988). In recent years it has been found that several products of these bacteria, such as exopolysaccharides (EPSs), halophilic enzymes and compatible solutes, may have very useful applications in biotechnology (Ventosa, 2004; Ventosa *et al.*, 1998). During an extensive search of many different hypersaline habitats in Spain and Morocco designed to obtain new EPSs we discovered that the commonest halophilic EPS producers were various novel species of the genus *Halomonas*, most importantly *Halomonas maura* and *Halomonas eurihalina* (Bouchotroch *et al.*, 2001; Martínez-Cánovas *et al.*, 2004c; Quesada *et al.*, 1990, 2004), together, to a lesser extent, with *Halomonas ventosae* (Martínez-Cánovas *et al.*, 2004a) and *Halomonas*

anticariensis (Martínez-Cánovas *et al.*, 2004b). We describe and classify here a hitherto unassigned halophilic EPS-producing strain that was also isolated in these studies. On the basis of its phenotypic features, comparative studies of its 16S rRNA gene sequence and investigations into its polar-lipid and isoprenoid quinone contents, together with its salt requirements and its inability to synthesize bacteriochlorophyll *a*, we propose that this bacterium should be assigned to a new genus, *Salipiger*, with a single species *Salipiger mucescens* gen. nov., sp. nov.

The strain named A3^T was isolated from a saline soil bordering a saltern on the Mediterranean coast at Calblanque (Murcia, south-eastern Spain) (Martínez-Cánovas *et al.*, 2004c). The strain was routinely grown at 32 °C in MY medium (Quesada *et al.*, 1993) supplemented with a 7.5 % w/v sea-salt solution (Rodríguez-Valera *et al.*, 1981).

An initial phenotypic study including 135 tests was undertaken by Martínez-Cánovas *et al.* (2004c). Salt requirements and optimum salt concentration were determined in MY medium according to the methods described by Bouchotroch

Published online ahead of print on 9 July 2004 as DOI 10.1099/ijs.0.63166-0.

Abbreviations: EPS, exopolysaccharide; PHA, poly- β -hydroxyalkanoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A3^T is AY527274.

et al. (2001). The salt concentrations assayed ranged from 0.5 to 30 % w/v and were prepared from a mixture of sea salts according to Rodríguez-Valera *et al.* (1981). We also tested to see whether strain A3^T could survive with NaCl alone or whether it required other magnesium and/or potassium salts.

Bacteriochlorophyll *a* was analysed spectrophotometrically using the procedure of Cohen-Bazire *et al.* (1957) following the recommendations of Allgaier *et al.* (2003). Two micro-litres of a liquid culture of strain A3^T incubated in the dark was centrifuged and the pellet resuspended in a drop of the remaining medium. A 1.5 ml volume of an ice-cold (−20 °C) acetone/methanol solution (7:2 v/v) was added, mixed thoroughly and incubated at room temperature in the dark for 12 h. After centrifugation, spectrophotometric measurements were made at 600–900 nm.

Fatty acids and quinones were identified by high-resolution GLC and HPLC respectively at the DSMZ.

Transmission electron micrographs were made using the methods described by Bouchotroch *et al.* (2001).

The 16S rRNA gene was amplified by PCR using standard protocols (Saiki *et al.*, 1988). The forward primer, 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3'), annealed at positions 8–27 and the reverse primer, 16R1488 (5'-CGGTT-ACCTTGTTAGGACTTCACC-3') (both from Pharmacia), annealed at the complement of positions 1511–1488 (*Escherichia coli* numbering according to Brosius *et al.*, 1978). The PCR products were purified using the QIAquick spin-gel extraction kit (Qiagen). Direct sequence determinations of PCR-amplified DNAs were carried out with the ABI PRISM dye-terminator cycle-sequencing ready-reaction kit (Perkin-Elmer) and an ABI PRISM 377 sequencer (Perkin-Elmer) according to the manufacturer's instructions. The sequences obtained were compared to reference 16S rRNA gene sequences available in the GenBank, EMBL and DDBJ databases obtained from the National Center of Biotechnology Information database using the BLAST search. Phylogenetic analysis was performed using the software MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignments of data by CLUSTAL X (Thompson *et al.*, 1997). Distances and clustering were determined using the neighbour-joining and maximum-parsimony methods. The stability of clusters was ascertained by performing a bootstrap analysis (1000 replications).

The strain described here was isolated during a wide research programme, the main objective of which was to identify EPS-producing bacteria in different hypersaline habitats (Martínez-Cánovas *et al.*, 2004c; Quesada *et al.*, 2004). Numerical analysis of its phenotypic characteristics demonstrated that strain A3^T was not related to other halophilic EPS-producing strains isolated from these habitats (Martínez-Cánovas *et al.*, 2004c). A3^T was also found to contain at least seven plasmids (550, 467, 184, 140.8, 110.6, 98.2 and 30.8 kb). It did not however contain

the 600 kb megaplasmid found in other halophilic micro-organisms (Argandoña *et al.*, 2003). All these facts led us to characterize this strain further and eventually to propose its assignment to a new genus.

Strain A3^T is strictly halophilic, being unable to grow in the absence of sea salts. It is an aerobic chemo-organotroph, unable to grow under anaerobic conditions either by fermenting sugars or by reducing nitrate, nitrite or fumarate. Bacteriochlorophyll *a* was not detected. It is characterized by its low nutritional and biochemical versatility. Its phenotypic characteristics appear in the species description. Phenotypic features that differentiate strain A3^T from the two species of *Roseivivax* and other members of the family 'Rhodobacteraceae' related to it phylogenetically are shown in Table 1. The data included in this table demonstrate that there is no phenotypic similarity between A3^T and the other strains concerned. The G+C content of strain A3^T is 64.5 mol% (Martínez-Cánovas *et al.*, 2004c), which is similar to the value of 64.4 mol% obtained for *Roseivivax halodurans* (Suzuki *et al.*, 1999).

Strain A3^T contains a large quantity (78.0 %) of *cis*-11 octadecenoic acid (18:1 ω 7c) in combination with 16:0, 18:0, 11-methyl-branched *cis*-9 octadecenoic acid (11-methyl 18:1 ω 7c) and 3-hydroxy 12:0 (12.4, 2.0, 1.9 and 2.3 %, respectively). The presence of 18:1 ω 7c as the predominant fatty acid is a feature characteristic of several major taxa within the α -Proteobacteria (Table 1). Nevertheless, strain A3^T also contains cyclo-substituted fatty acids (2.3 %), which are not widely present in the family 'Rhodobacteraceae'. The fatty acids of the *Roseivivax* species, which are most phylogenetically related to A3^T, have not been thoroughly described, although in a study published before the taxonomical description of *Roseivivax halodurans* and *Roseivivax halotolerans* Nishimura *et al.* (1994) reported that the main cellular fatty-acid component in these bacteria was 18:1. The only respiratory lipoquinone detected was ubiquinone 10. The presence of ubiquinone 10 as the dominant respiratory lipoquinone is characteristic of members of the α -Proteobacteria.

Fig. 1 shows the cell morphology of strain A3^T. Thin sections reveal a typical Gram-negative cell-envelope profile; the cell contains poly- β -hydroxyalkanoate (PHA) granules. EPS appears associated with the cell surface.

According to the recommendations of Stackebrandt *et al.* (2002) we determined the almost complete 16S rRNA gene sequence of strain A3^T (1364 bp), corresponding to positions 46–1445 of the *Escherichia coli* 16S rRNA gene. The phylogenetic tree obtained via the neighbour-joining method is shown in Fig. 2. The maximum-parsimony algorithm gave a similar result (data not shown). Together with the sequence of A3^T, our phylogenetic analysis also included all the representatives of the family 'Rhodobacteraceae' described to date plus four halophilic bacteria belonging to the γ -Proteobacteria as an outgroup. Strain A3^T belongs to a clade containing *Roseivivax halodurans*

Table 1. Characteristics that distinguish *Salipiger mucescens* gen. nov., sp. nov. A3^T from other related members of the family 'Rhodobacteraceae'

Reference strains: 1 and 2, *Roseivivax halodurans* JCM 10272^T and *Roseivivax halotolerans* JCM 10271^T (Suzuki *et al.*, 1999; Nishimura *et al.*, 1994); 3, *Antarctobacter heliothermus* DSM 11445^T (Labrenz *et al.*, 1998); 4, *Ketogulonicigenium vulgare* DSM 405^T (Urbance *et al.*, 2001); 5, *Jannaschia helgolandensis* DSM 14858^T (Wagner-Döbler *et al.*, 2003); 6, *Leisingeria methylolithalivorans* ATCC BAA-92^T (Schaefer *et al.*, 2002); 7, *Octadecabacter arcticus* CIP 106731^T (Gosink *et al.*, 1997); 8, *Roseobacter litoralis* (Shiba, 1991; Labrenz *et al.*, 1998); 9, *Roseovarius tolerans* DSM 11457^T (Labrenz *et al.*, 1999); 10, *Ruegeria algicola* ATCC 51440^T (Lafay *et al.*, 1995; Labrenz *et al.*, 1998); 11, *Sagittula stellata* ATCC 700073^T (González *et al.*, 1997); 12, *Silicibacter lacuscaerulensis* DSM 11314^T (Petursdottir & Kristjansson, 1997); 13, *Sulfitobacter mediterraneus* DSM 12244^T (Pukall *et al.*, 1999); 14, *Staleyia guttiformis* DSM 11443^T (Labrenz *et al.*, 2000). +, Positive; −, negative; w, weak; ND, no data available.

Characteristic	<i>S. mucescens</i>	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Morphology	Rods	Rods	Rods	Pointed rods. Buds and rosettes formed	Ovoid to rods	Irregular rods	Rods or ovoid rods	Long rods. Gas vacuolate bacteria	Rods or ovoid rods	Pointed and budded rods	Ovoid rods	Arrow rods with surface vesicles. Holdfast and rosettes formed	Long rods. Gas vacuolate bacteria	Rods. Rosettes formed	Short rods. Bud and rosettes formed
Source of isolation	Hypersaline soil	Charophyte sp. of a saline lake	Cyanobacterial mats of a saline lake	Hypersaline Antarctic lake	Soil enriched with L-sorbose	Sea water	Sea water enriched with methyl bromide	Antarctic sea ice	Green seaweeds	Hypersaline Antarctic lake	Marine dinoflagellate	Sea water	Geothermal silica saline lake	Sea water	Hypersaline Antarctic lake
Pigment	−	Pink	Pink	Brown-yellow	Brown	−	−	−	Pink	Red	Beige to pink	Cream	Tan	Cream	Beige to pink
Flagella*	−	S, SP	S, SP	SP	−	−	+	−	M, SP	+	SP	+	−	M, SP	S
PHA	+	ND	ND	+	ND	White inclusions	ND	ND	ND	+	−	+	−	+	+
Oxidase	+	+	+	+	+	W	+	−	+	W	+	+	+	+	+
Preferred metabolism†	CH, AE	CH, AE	CH, AE	CH, AE	CH, FA	CH, AE	M, AE	CH, MA	CH, AE	CH, AE	CH, AE	CH, AE	CH, AE	CH, AE	CH, AE
Anaerobic growth	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Bacteriochlorophyll <i>a</i>	−	+	+	−	−	−	ND	−	+	+	−	−	−	−	+
Growth factor requirement	−	−	−	+	−	−	−	+	+	+	+	W	−	−	+
Na ⁺ requirement	+	−	+	+	−	+	+	+	+	+	+	+	+	+	W
Optimum NaCl concentration (%)	9–10	ND	ND	2–6	0.01–0.2	ND	3	ND	ND	1–8	ND	ND	3–5	1.5–2	1
Salt growth range (%)	0.5–20	0–20	0.5–20	1–10	0–4	1–7	1–6	1.7–7	ND	1–15	0.5–12	ND	1.5–7	0.2–8	1–15
Optimum temperature (°C)	20–40	27–30	27–30	16–26	27–31	25–30	27	4–15	20–30	8.5–33.5	25–30	30	45	17–28	12–20
Nitrate to nitrite	−	+	−	+	ND	−	−	−	−	−	−	−	+	−	+
Growth on carbohydrates‡	−	+	+	+	+	+	−	W	+	+	+	+	−	+	W
Acids from glucose	−	+	W/−	−	ND	−	−	W	−	−	−	−	ND	−	−
Major fatty acids§ (%)	18:1ω7c (78.0), 16:0 (12.4), 18:0 (2.0), 16:1ω7c (1.3)	18:1 (not quantified)	18:1 (not quantified)	18:1 (83.2), 16:0 (2.5), 18:0 (1)	18:1ω7d/ω9t (52), ω12t (33.5), 16:1ω7c (2.7)	18:1ω7c (45.1), 18:0 (10.8), 12:1 (4.9), 17:0 (1)	ND	18:1ω7d/ω9t/ω12t (75), 16:1ω7c (8), 16:0 (6)	18:1 (88.8), 18:2 (1.4), 18:0 (1.3)	18:1 (70.2), 18:2 (10.6), 16:0 (6.2)	18:1ω7c (91.5), 18:0 (2.2), 18:2 (1.6), 16:0 (6.8)	18:1ω7c (not quantified), 16:0 (8.6), 18:0 (6.8)	ND	18:1ω11c (72.4), 16:0 (6.1), 16:1ω7c (1.9), 18:1ω9c (1.7)	18:1ω7c (79.7), 18:2 (5.3), 16:0 (3.9), 19:1 (1.4)
3-OH fatty acids	12:1 (2.3)	−	−	12:1 (3.1)	10:0 (3.6), 12:0 (2.9)	10:0 (5.6), 14:1 (1.3)	ND	10:0 (4)	10:0 (1.9)	12:1 (3.6)	−	12:1 (3.6)	ND	10:0 (2.5)	10:0 (5.9), 14:1 (2.1)
2-OH fatty acids	−	−	−	−	−	−	ND	−	14:0 (3.9)	12:0 (2.4)	−	−	ND	−	−
Methyl fatty acids	11-methyl 18:1ω7c (1.9)	−	−	−	−	Methyl 18:1 (5.7)	ND	−	−	−	−	−	ND	10-methyl 18:1 (2.6), 18-methyl 18:1 (1.4)	−
Cyclo-substituted fatty acids	19:0 cyclo ω8c (2.3)	−	−	19:0 cyclo (2.4)	−	19:0 cyclo (25.1)	ND	−	−	−	−	19:0 cyclo (1.8)	ND	−	−
Quinone type	Q10	Q10	Q10	Q10	ND	Q10	ND	ND	Q10	Q10	ND	ND	ND	ND	Q10
G + C content (mol%)	64.5	64.4	59.7	62.3–62.8	54.0	63–63.1	60.5	57	56.3–58.1	62.2–63.3	64–65	65	66.2	59	55–56.3

*M, Multiple; S, single; SP, subpolar.

†AE, Aerobic; CH, chemoheterotrophic; FA, facultatively anaerobic; M, methylotrophic; MA, microaerophilic.

‡Growth on minimum medium supplemented in some cases with growth factors.

§Only percentages higher than 1% are shown.

Fig. 1. Transmission electron micrograph of cells of strain A3^T stained with ruthenium red. Bar, 1 µm.

and *Roseivivax halotolerans* and shows 94 % similarity to both species. This value indicates the possibility of a new taxon of at least genus status. The physiology of strain A3^T is also clearly different from that of the *Roseivivax* species, which are pink-pigmented chemoheterotrophs that synthesize bacteriochlorophyll *a* under aerobic conditions, produce acids from sugars and are able to use different compounds as sole sources of carbon and energy. Thus, on the basis of phylogenetic evidence, differences in phenotypic characteristics and its inability to synthesize bacteriochlorophyll *a*, we are of the opinion that strain A3^T should be recognized as a new genus with a single species, for which we propose the name *Salipiger mucescens* gen. nov., sp. nov. The genus is in the same clade as that of *Roseivivax*, belonging to the α -3 group of the α -subclass of the *Proteobacteria*, within the family 'Rhodobacteraceae' (Garrity & Holt, 2001). Members of this taxon are in general non-sulfur, purple bacteria that carry out anoxygenic photosynthesis. This family includes a group of aerobic bacteriochlorophyll-containing bacteria (ABC), to which *Roseivivax* pertains (Imhoff & Madigan, 2002). *Roseivivax* is taxonomically related to the *Roseobacter* clade, a group of 11 genera of the family 'Rhodobacteraceae' (Allgaier *et al.*,

Fig. 2. Phylogenetic relationships amongst *Salipiger mucescens* A3^T and members of other genera of the family 'Rhodobacteraceae' plus other taxa of Gram-negative halophilic bacteria. The tree was constructed using the neighbour-joining algorithm. Only bootstrap values above 50 % are shown (1000 replications). Bar, 2 % estimated sequence divergence.

2003), which make up the most abundant populations in marine habitats (González & Moran, 1997).

Description of *Salipiger* gen. nov.

Salipiger (Sa.li.pi'ger. L. masc. sb. *sal* salt; L. masc. adj. *piger* lazy; N.L. masc. sb. *Salipiger* lazy halophile).

Gram-negative, non-motile rods, 2–2.5 µm long by 0.75–1 µm wide. Chemoheterotrophic, strictly aerobic, being unable to grow under anaerobic conditions either by fermentation, nitrate or fumarate reduction or photoheterotrophy. PHA, cytochrome oxidase and catalase are present. Low nutritional and biochemical versatility. Strictly halophilic, requiring Na⁺ ions for growth. The principal cellular fatty acids are 18:1ω7c and 16:0. It has ubiquinone with ten isoprene units. The type species is *Salipiger mucescens*.

Description of *Salipiger mucescens* sp. nov.

Salipiger mucescens (mu.ces'cens. L. masc. ppl. adj. *mucescens* slimy).

In addition to the traits reported for the genus, the species grows on MY solid medium in the form of circular, convex, cream-coloured, mucoid colonies. In liquid medium its growth pattern is uniform. The cells are encapsulated. It is moderately halophilic, capable of growing in salt concentrations (mixture of sea salts) from 0.5 to 20 % w/v, the optimum range being between 3 and 6 % w/v. Nevertheless, if NaCl is substituted for sea salts, the concentration required for optimum growth reaches values of 9 to 10 % w/v. It grows within the temperature range of 20 to 40 °C and at pH values of between 6 and 10. It produces H₂S from L-cysteine. Selenite reduction, gluconate oxidation and phosphatase are positive. Urea and Tween 20 are hydrolysed. It does not produce acids from the following sugars: adonitol, D-cellobiose, D-fructose, D-galactose, D-glucose, myo-inositol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, L-rhamnose, sucrose, D-salicin, D-sorbitol, sorbose and D-trehalose. O/F, ONPG, indole, methyl-red and Voges–Proskauer are negative. Phenylalanine deaminase is not produced. Tyrosine, Tween 80, starch, aesculin, gelatin, DNA, lecithin and casein are not hydrolysed. Growth on either MacConkey or cetrimide agar is unviable. Blood is not haemolysed. Neither nitrate nor nitrite is reduced. The following compounds are not acceptable as sole carbon and energy sources: L-arabinose, D-cellobiose, aesculin, D-fructose, D-glucose, D-galactose, lactose, maltose, D-mannose, D-salicin, acetate, citrate, formate, fumarate, glycerol, lactate, malonate, propionate and succinate. The following compounds are not used as sole carbon, nitrogen and energy sources: L-alanine, L-cysteine, L-histidine, isoleucine, L-lysine, L-methionine, L-serine and L-valine. It is susceptible to (µg) amoxicillin (25), ampicillin (10), carbenicillin (100), cefotaxime (30), cefoxitin (30), chloramphenicol (30), erythromycin (15), kanamycin (30), nitrofurantoin (300), rifampicin (30), sulfonamide (250), tobramycin (10) and trimetoprim/sulphametoxazol

(1.25/23.7), and is resistant to nalidixic acid (30) and polymixin B (300). The major fatty acids (%) are 18:1ω7c (78.0), 16:0 (12.4), 12:1 3-OH (2.3), 19:0 cyclo ω8c (2.3), 18:0 (2.0) and 16:1ω7c/15:0 iso 2-OH (1.3). Its DNA G+C content is 64.5 mol% (T_m method).

The type strain is strain A3^T (=CECT 5855^T=LMG 22090^T=DSM 16094^T), isolated from a hypersaline soil taken from a solar saltern in Calblanche (Murcia, south-eastern Spain).

Acknowledgements

This research was supported by grants from the Dirección General de Investigación Científica y Técnica (BOS2000-1519) and from the Plan Andaluz de Investigación, Spain. The authors are very grateful to C. Fernández and D. Porcel for their expertise in the electron-microscope studies and to our colleague Dr J. Trout for revising the English text. Thanks also go to Professor H. G. Trüper of the University of Bonn for his help with the genus and species names.

References

- Allgaier, M., Uphoff, H., Feelske, A. & Wagner-Döbler, I. (2003). Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microbiol* **69**, 5051–5059.
- Argandoña, M., Martínez-Checa, F., Llamas, I., Quesada, E. & Del Moral, A. (2003). Megaplasmids in Gram-negative, moderately halophilic bacteria. *FEMS Microbiol Lett* **227**, 81–86.
- Bouchotroch, S., Quesada, E., del Moral, A., Llamas, I. & Béjar, V. (2001). *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **51**, 1625–1632.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by non-sulfur purple bacteria. *J Cell Comp Physiol* **49**, 25–68.
- Garrity, G. M. & Holt, J. G. (2001). The road map to the *Manual*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 119–166. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- González, J. M. & Moran, M. A. (1997). Numerical dominance of a group of marine bacteria in the α-subclass of the class *Proteobacteria* in coastal seawater. *Appl Environ Microbiol* **63**, 4237–4242.
- González, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997). *Sagittula stellata* gen. nov., sp. nov., a lignin-transforming bacterium from a coastal environment. *Int J Syst Bacteriol* **47**, 773–780.
- Gosink, J. J., Herwig, R. P. & Staley, J. T. (1997). *Octadecabacter arcticus* gen. nov., sp. nov., and *O. antarcticus*, sp. nov., non-pigmented, psychrophilic gas vacuolate bacteria from polar sea ice and water. *Syst Appl Microbiol* **20**, 356–365.
- Imhoff, J. F. & Madigan, M. T. (2002). International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of phototrophic bacteria. Minutes of the meetings, 28 August 2000, Barcelona, Spain. *Int J Syst Evol Microbiol* **52**, 2335–2336.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: Molecular Evolutionary Genetics Analysis software. *Bioinformatics* **17**, 1244–1245.

- Kushner, D. J. & Kamekura, M. (1988). Physiology of halophilic bacteria. In *Halophilic Bacteria*, pp. 109–138. Edited by F. Rodríguez-Valera. Boca Raton, FL: CRC Press.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Braker, G. & Hirsch, P. (1998). *Antarctobacter heliothermus* gen. nov., sp. nov., a budding bacterium from hypersaline and heliothermal Ekho Lake. *Int J Syst Bacteriol* **48**, 1363–1372.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Schumann, P. & Hirsch, P. (1999). *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho lake. *Int J Syst Bacteriol* **49**, 137–147.
- Labrenz, M., Tindall, B. J., Lawson, P. A., Collins, M. D., Schumann, P. & Hirsch, P. (2000). *Staleyia guttiformis* gen. nov., sp. nov. and *Sulfitobacter brevis* sp. nov., α -3-Proteobacteria from hypersaline, heliothermal and meromictic antarctic Ekho Lake. *Int J Syst Evol Microbiol* **50**, 303–313.
- Lafay, B., Ruimy, R., Rausch de Traubenberg, C., Breittmayer, V., Gauthier, M. J. & Christen, R. (1995). *Roseobacter algicola* sp. nov., a new marine bacterium isolated from the phycosphere of the toxin-producing dinoflagellate *Prorocentrum lima*. *Int J Syst Bacteriol* **45**, 290–296.
- Martínez-Cánovas, M. J., Quesada, E., Llamas, I. & Béjar, V. (2004a). *Halomonas ventosae* sp. nov., a moderately halophilic, denitrifying, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* **54**, 733–737.
- Martínez-Cánovas, M. J., Béjar, V., Martínez-Checa, F. & Quesada, E. (2004b). *Halomonas anticariensis* sp. nov., from Fuente de Piedra, a saline-wetland wildfowl reserve in Málaga, southern Spain. *Int J Syst Evol Microbiol* **54**, 1329–1332.
- Martínez-Cánovas, M. J., Quesada, E., Martínez-Checa, F. & Béjar, V. (2004c). A taxonomic study to establish the relationship between exopolysaccharide-producing bacterial strains living in diverse hypersaline habitats. *Curr Microbiol* **48**, 348–353.
- Nishimura, Y., Muroga, Y., Saito, S., Shiba, T., Takamiya, K. & Shioi, Y. (1994). DNA relatedness and chemotaxonomic feature of aerobic bacteriochlorophyll-containing bacteria isolated from coasts of Australia. *J Gen Appl Microbiol* **40**, 287–296.
- Petursson, S. K. & Kristjánsson, J. K. (1997). *Silicibacter lacuscaerulensis* gen. nov., sp. nov., a mesophilic moderately halophilic bacterium characteristic of the Blue lagoon geothermal lake in Iceland. *Extremophiles* **1**, 94–99.
- Pukall, R., Buntetu, D., Frühling, A., Rohde, M., Kroppenstedt, R. M., Burghardt, J., Lebaron, P., Bernard, L. & Stackebrandt, E. (1999). *Sulfitobacter mediterraneus* sp. nov., a new sulfite-oxidizing member of the α -Proteobacteria. *Int J Syst Bacteriol* **49**, 513–519.
- Quesada, E., Valderrama, M. J., Béjar, V., Ventosa, A., Gutierrez, M. C., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1990). *Volcaniella eurihalina* gen. nov., sp. nov., a moderately halophilic nonmotile gram-negative rod. *Int J Syst Bacteriol* **40**, 261–267.
- Quesada, E., Béjar, V. & Calvo, C. (1993). Exopolysaccharide production by *Volcaniella eurihalina*. *Experientia* **49**, 1037–1041.
- Quesada, E., Béjar, V., Ferrer, M. R. & 8 other authors (2004). Moderately halophilic, exopolysaccharide-producing bacteria. In *Halophilic Microorganisms*, pp. 297–314. Edited by A. Ventosa. Heidelberg: Springer.
- Rodríguez-Valera, F., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1981). Characteristics of the heterotrophic bacterial populations in hypersaline environments of different salt concentrations. *Microb Ecol* **7**, 235–243.
- Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science* **239**, 487–491.
- Schaefer, J. K., Goodwin, K. D., McDonald, I. R., Murrell, J. C. & Oremland, R. S. (2002). *Leisingera methylohalidivorans* gen. nov., sp. nov., a marine methylotroph that grows on methyl bromide. *Int J Syst Evol Microbiol* **52**, 851–859.
- Shiba, T. (1991). *Roseobacter litoralis* gen. nov., and *Roseobacter denitrificans* sp. nov., aerobic pink-pigmented bacteria which contain bacteriochlorophyll *a*. *Syst Appl Microbiol* **14**, 140–145.
- Stackebrandt, E., Fredricksen, W., Garrity, G. M. & 10 other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Suzuki, T., Muroga, Y., Takahama, M. & Nishimura, Y. (1999). *Roseivivax halodurans* gen. nov., sp. nov. and *Roseivivax halotolerans* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake. *Int J Syst Bacteriol* **49**, 629–634.
- Thompson, J. D., Gibson, T. J., Plewniak, K., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignments aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Urbance, J. W., Bratina, B. J., Stoddard, S. F. & Schmidt, T. M. (2001). Taxonomic characterization of *Ketogulonigenium vulgare* gen. nov., sp. nov. and *Ketogulonigenium robustum* sp. nov., which oxidize L-sorbose to 2-keto-L-gulonic acid. *Int J Syst Evol Microbiol* **51**, 1059–1070.
- Ventosa, A. (editor) (2004). *Halophilic Microorganisms*. Heidelberg: Springer.
- Ventosa, A., Nieto, J. J. & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.
- Wagner-Döbler, I., Rheims, H., Felske, A., Pukall, R. & Tindall, B. J. (2003). *Jannaschia helgolandensis* gen. nov., sp. nov., a novel abundant member of the marine *Roseobacter* clade from the North Sea. *Int J Syst Evol Microbiol* **53**, 731–738.