Halomonas ventosae sp. nov., a moderately halophilic, denitrifying, exopolysaccharide-producing bacterium

M. José Martínez-Cánovas, Emilia Quesada, Inmaculada Llamas and Victoria Béjar

Correspondence

Victoria Béjar
vbejar@ugr.es

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

Halomonas ventosae sp. nov. includes three moderately halophilic, exopolysaccharide-producing strains isolated from saline soils in Jaén (south-eastern Spain). These strains can grow anaerobically using either nitrate or nitrite as terminal electron acceptor and hydrolyse both tyrosine and phenylalanine. Their G+C content varies between 72.6 and 74.3 mol%. The affiliation of the isolates with the genus *Halomonas* was confirmed by 16S rRNA gene sequence comparison. DNA–DNA hybridization shows 70.4–82.7 % relatedness among the three strains. Nevertheless, their relatedness is less than 43 % compared to related reference strains. The proposed type strain for *Halomonas ventosae* is strain Al12^T (= CECT 5797^T = DSM 15911^T). It grows best at 8 % (w/v) sea salts and requires the presence of Na⁺. Its major fatty acids are 18:1 ω 7c, 16:0, 16:1 ω 7c, and 15:0 iso 2-OH. The predominant respiratory lipoquinone found in strain Al12^T is ubiquinone with nine isoprene units (Q-9).

The *Halomonadaceae* include four genera of halophilic bacteria: *Halomonas*, *Chromohalobacter*, *Alcanivorax* and the recently described *Cobetia*, plus two genera of non-halophilic bacteria, *Zymobacter* and *Carnimonas* (Arahal *et al.*, 2002; Dobson & Franzmann, 1996; Garriga *et al.*, 1998; Yakimov *et al.*, 1998). The genus *Halomonas* was originally proposed to accommodate one species, *Halomonas elongata* (Vreeland *et al.*, 1980). It now contains a large number of recently discovered species (Heyrman *et al.*, 2002; Romanenko *et al.*, 2002), as well as already known bacteria which were initially assigned to other genera, such as *Deleya*, *Volcaniella* and *Halovibrio* (Mellado *et al.*, 1995; Quesada *et al.*, 1984, 1990; Valderrama *et al.*, 1991; Ventosa *et al.*, 1998), but have since been included in *Halomonas*.

Halomonas species are widely distributed throughout hypersaline environments. Some of them have been recognized for their potential use in biotechnology (Margesin & Schinner, 2001; Ventosa & Nieto, 1995). *Halomonas eurihalina* and *Halomonas maura* were isolated by our group from hypersaline soils and found to produce exopolysaccharides (EPSs) (Quesada *et al.*, 1990; 1993; Bouchotroch

et al., 2001), which have since been characterized and evaluated for potential applications in industry (Calvo *et al.*, 1995, 1998; Béjar *et al.*, 1998; Martínez-Checa *et al.*, 2002; Arias *et al.*, 2003; Quesada *et al.*, 2004).

We classify here three unassigned EPS-producing strains, which are characterized in particular by their capacity to denitrify. On the basis of phenotypic features, DNA–DNA hybridization and comparison of 16S rRNA gene sequences, as well as an investigation for strain Al12^T of polar lipids, isoprenoid quinones and salt requirements, we propose a novel species, *Halomonas ventosae* sp. nov.

The strains, referred to as Al12^T, Al15 and Al16, were all isolated from a saline soil in Jaén, south-eastern Spain (Quesada *et al.*, 2004). All the strains were routinely grown in MY medium (Quesada *et al.*, 1993) at 32 °C. For comparison, we used the strains from culture collections that appear in the figures and tables below.

Phenotypic studies (135 tests) and numerical analyses were made as described in previous publications (Mata *et al.*, 2002; Quesada *et al.*, 1983; Ventosa *et al.*, 1982). The data were submitted to cluster analysis using the simple matching coefficient (S_{SM}) (Sokal & Michener, 1958) and clustering was achieved by the unweighted-pair-group method of association (UPGMA) (Sneath & Sokal, 1973). Computer analysis was made with the TAXAN program (Information Resources Group, Maryland Biotechnology Institute). Test error was evaluated by examining 10 strains in duplicate (Sneath & Johnson, 1972).

Abbreviation: EPS, extracellular polysaccharide.

The GenBankEMBL/DDBJ accession number for the 16S rDNA sequence of strain Al12^T is A4268080.

Supplementary data relating to the phenotypic characteristics of *Halomonas ventosae* sp. nov., the results of DNA–DNA hybridization experiments, and dendrograms of phylogenetic relationships are available at IJSEM Online.

DNA was purified using the technique of Marmur (1961). The G+C content of the DNA was estimated from the midpoint value (T_m) of the thermal denaturation profile (Marmur & Doty, 1962). T_m was determined by the graphic method described by Ferragut & Leclerc (1976) and the G+C content was calculated from this temperature using the equation of Owen & Hill (1979). The T_m value of reference DNA from *Escherichia coli* NCTC 9001^T was taken to be 74.6 °C in 0.1 × SSC (Owen & Pitcher, 1985).

The 16S rRNA gene was amplified by PCR using standard protocols (Saiki *et al.*, 1988). The forward primer, 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3'), annealed at position 8–27, and the reverse primer, 16R1488 (5'-CGGT-TACCTTGTTAGGACTTCACC-3'), annealed at position 1511–1488 (*E. coli* numbering according to Brosius *et al.*, 1978). Both primers were obtained from Pharmacia. PCR products were purified using a Qiaquick spin gel extraction kit (Qiagen). Direct sequence determination of PCR-amplified DNAs was 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 and EMBL databases, obtained from the National Center for Biotechnology Information database using BLAST. Phylogenetic analysis was carried out using MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001), after multiple alignments of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances and clustering with the neighbour-joining and maximum-parsimony methods were determined by using bootstrap values based on 1000 replications.

DNA–DNA hybridization was conducted following the methods of Lind & Ursing (1986) with the modifications of Ziemke *et al.* (1998) and Bouchotroch *et al.* (2001).

The fatty-acid analysis of strain Al12^T was carried out by Microbial Identification Systems (Williston, VT, USA), using the MID/Hewlett Packard Microbial Identification System (MIS), which employs high-resolution GC to obtain the fatty-acid profile. Isoprenoid quinones were identified by HPLC by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH).

Transmission and scanning electron micrographs were obtained by the methods of Bouchotroch *et al.* (2001).

Salt requirements and optimum concentrations for strain Al12^T were determined in MY medium according to the methods described by Bouchotroch *et al.* (2001). The salt concentrations assayed ranged from 1 to 15 % (w/v) and were prepared from a mixture of sea salts (Rodríguez-Valera *et al.*, 1981). We also tested to see whether strain Al12^T needed NaCl alone or whether it required other magnesium and/or potassium salts.

The three strains described here were strictly halophilic,

being unable to grow in the absence of sea salts. They were characterized by their capacity to produce EPS. Phenotypic characteristics common to all three strains are given in the species description; differential phenotypic features among the three strains and the dendrogram obtained from numerical analysis are available as Supplementary Table A and Fig. A, respectively, in IJSEM Online. The estimated error in the phenotypic study was no more than 3 % in any of the tests, which would not affect the cluster analysis significantly. No test was found to be so non-reproducible (test variance ≥ 0.2) as to be excluded from the analysis. The test responsible for most error was gluconate oxidation. At an 85 % similarity level, the novel strains grouped into a phenon sharing the highest similarity (about 80 %) with the species *Halomonas halodenitrificans* and *Halomonas maura*. The novel EPS-producing strains were characterized by their denitrifying capability: they were able to reduce nitrate and nitrite under anaerobic conditions; this capacity is uncommon among halophilic bacteria and may prove very useful for biotechnological applications (Mormile *et al.*, 1999; Peyton *et al.*, 2001). They also hydrolyse tyrosine and produce the enzyme phenylalanine deaminase; the latter capacity has only been observed hitherto in *Halomonas desiderata* (Mata *et al.*, 2002). They differ from *H. halodenitrificans*, which is non-motile and does not produce EPS or phenylalanine deaminase.

The G+C content of the three novel strains was 74.3 mol% for strain Al12^T, 74.2 for strain Al16 and 72.6 for strain Al15. These G+C values are very high and considerably above the typical G+C for *Halomonas* species of 52–68 mol% (Franzmann *et al.*, 1988). Nevertheless, the phenotypic data point to the conclusion that this cluster does indeed belong to *Halomonas* and this is supported by the chemotaxonomic, genotypic and phylogenetic analyses.

The results of DNA–DNA hybridization are shown in Supplementary Table B. In this respect, none of the three strains was closely related to the type strains of related species, showing less than 43 % hybridization with them. These reference strains were chosen because of their phenotypic or phylogenetic relationship to the new isolates. Additionally, some of them produce EPS (*H. eurihalina* and *H. maura*) or can grow anaerobically with nitrate as terminal electron acceptor (*H. halodenitrificans*, *Halomonas campisalis*, *H. desiderata* and *Marinobacter hydrocarbonoclasticus*). Nevertheless, our results show that the new strains were not closely related to any of them.

We determined almost complete 16S rDNA sequences for strains Al12^T (1395 bp), Al15 (1398 bp) and Al16 (1396 bp), corresponding to positions 46 and 1445 of the *E. coli* 16S rRNA gene. The fragment analysed contained the 15 signature nucleotides defined for the *Halomonadaceae*, as described by Dobson & Franzmann (1996). The three sequences share 99.7–100 % similarity among themselves and are on the same phylogenetic branch. The phylogenetic tree constructed using the neighbour-joining method is

shown in Supplementary Fig. B. The maximum-parsimony algorithm gave a similar result (data not shown).

Fig. 1 shows the cell morphology of strain Al12^T. Thin sections reveal a typical Gram-negative cell envelope profile; the cells also contain poly- β -hydroxyalkanoate (PHA) granules. EPS appears associated with the cell surface and also in the medium surrounding the bacterium.

Strain Al12^T is euryhaline and can grow in media containing between 1 and 15 % (w/v) sea salts, although it grew best under our experimental conditions in a concentration of 8 % (w/v), within which it required the presence of 1.38 M Na⁺. It did not require magnesium or potassium. Under optimum conditions, its growth rate was 1.47 h⁻¹.

The results of chemotaxonomic analysis are given in the species description.

Table 1 shows the features that distinguish *Halomonas ventosae* sp. nov. from other phenotypically or phylogenetically related taxa.

Accordingly, on the basis of differences in phenotypic characteristics, phylogenetic inference and genetic distinctiveness, strains Al12^T, Al15 and Al16 should be placed

as a novel species of the genus *Halomonas*, for which the name *Halomonas ventosae* sp. nov. is proposed.

Description of *Halomonas ventosae* sp. nov.

Halomonas ventosae (ven.to.sa'e. N.L. sb. gen. *ventosae* of *Ventosa*, named in honour of Professor A. Ventosa, a renowned expert on halophilic bacteria).

Cells are capsulated, motile, Gram-negative rods, 1.2–1.4 × 0.7–0.8 μ m, occurring either singly or in pairs. They accumulate PHA. On MY solid medium the bacteria grow in circular, convex, cream-coloured, mucoid colonies. In liquid medium their growth pattern is uniform. Moderately halophilic, capable of growing in salt concentrations (mixture of sea salts) of 3–15 % (w/v), whereas no growth occurs in the absence of salt. Grow within the temperature range 15–50 °C and at pH values between 6 and 10. Chemoorganotrophic. Metabolism is respiratory, with oxygen, nitrate or nitrite as terminal electron acceptor. Cells do not grow anaerobically in the presence of fumarate. Catalase and oxidase are produced. They do not produce acids from sugars. Indol, methyl-red and Voges-Proskauer are negative. Selenite is reduced, phenylalanine deaminase is produced and tyrosine and Tween 20 are hydrolysed, but not Tween 80, starch, urea, gelatin, DNA, lecithin or casein. There is no growth on MacConkey agar and cetrimide agar. Blood is not haemolysed. The following can be used as sole carbon and energy sources: acetate, citrate, D-galactose, D-gluconate, D-glucose, lactate, malonate, maltose, propionate, D-sorbitol and succinate, but not L-arabinose, D-cellobiose, ethanol and formate. The following are not used as sole carbon, nitrogen and energy sources: L-alanine, L-cysteine, L-histidine, isoleucine, L-lysine, L-methionine and L-valine. Cells are susceptible to amoxycillin (25 μ g), ampicillin (10 μ g), carbenicillin (100 μ g), cefotaxime (30 μ g), cefoxitin (30 μ g), chloramphenicol (30 μ g), kanamycin (30 μ g), nalidixic acid (30 μ g), nitrofurantoin (300 μ g), polymyxin B (300 UI), rifampicin (30 μ g), sulfonamide (250 μ g) and trimethoprim/sulfamethoxazole (1.25–23.75 μ g). The three strains were isolated from a saline soil in Jaén, south-eastern Spain. The DNA G + C content is 72.6–74.3 mol% (T_m method).

The type strain is strain Al12^T (=CECT 5797^T=DSM 15911^T). The description of the type strain is the same as that of the species. Additionally, this strain is able to grow in a medium containing 1 % (w/v) mixture of sea salts, although it grows best in 8 % (w/v). It requires sodium but not magnesium or potassium; the optimum NaCl concentration is 1.38 M. It produces H₂S from L-cysteine. Phosphatase and aesculin are not hydrolysed. Gluconate is oxidized. The following can be used as sole carbon and energy sources: adonitol, D-fructose, fumarate, DL-glycerol, *myo*-inositol, lactose, D-mannitol and D-trehalose, but not D-mannose, D-salicin, L-serine and starch. Al12^T is resistant to tobramycin (10 μ g). The major fatty acids (up to 83 %) are 18:1 ω 7c, 16:0, 16:1 ω 7c and 15:0 iso 2-OH, and it

Fig. 1. Electron micrographs of strain Al12^T stained with ruthenium red: (A) transmission electron micrograph of an ultrathin section; (B) scanning electron micrograph. Bars, 1 μ m.

Table 1. Phenotypic characteristics that distinguish *H. ventosae* from other related type-strains of the genus *Halomonas*

Data from Mata *et al.* (2002), Ventosa *et al.* (1998) and Yoon *et al.* (2001). Species: 1, *H. ventosae*; 2, *H. alimentaria*; 3, *H. campisalis*; 4, *H. cupida*; 5, *H. desiderata*; 6, *H. eurihalina*; 7, *H. halodenitrificans*; 8, *H. maura*; 9, *H. pacifica*; 10, *H. salina*; 11, *H. variabilis*. ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Morphology	Short rod	Coccus or short rod	Rod	Rod	Rod	Rod	Short rod	Long rod	Rod	Short rod	Curved rod
Colony pigmentation	Cream	Cream-yellow	White	White	Cream	Cream	Cream	Cream	Cream	Cream	Cream
EPS	+	—	—	—	—	+	—	+	—	+	—
Motility	+	—	+	+	+	—	—	—	+	—	+
Acid from glucose	—	—	—	+	—	—	—	—	+	—	+
Oxidase	+	+	+	—	+	—	+	+	+	+	+
Sea-salt range (% w/v)	3–15	0.5–23	0.5–15	0–15	0–20	0.5–25	3–20	1–20	0–20	2–20	1–25
Sea-salt optimum (% w/v)	6–9	1–13	5	7.5–10	1–5	7.5	5–9	9	0.5–3	5	10
pH range	6–10	6.5–7.5	8–11	5–10	7–11	5–10	5–10	5–10	5–10	5–10	6–9
Temperature range (°C)	15–50	4–45	4–50	15–37	10–45	4–45	5–37	10–45	4–45	4–45	15–37
Nitrate reduction	+	+	+	+	+	+	+	+	—	+	—
Nitrite reduction	+	ND	+	—	+	—	+	+	—	—	—
Respiration on nitrate	+	ND	+	—	+	—	+	+	—	—	—
Respiration on nitrite	+	ND	+	—	+	—	+	—	—	—	—
Hydrolysis of:											
DNA	—	ND	+	+	+	—	—	—	+	—	—
Gelatin	—	—	—	+	—	+	—	—	—	—	—
ONPG	—	ND	—	—	—	+	—	—	—	—	—
Phenylalanine	+	ND	—	—	+	—	—	—	—	+	—
Tween 80	—	—	—	—	+	+	—	—	—	—	—
Tyrosine	+	—	—	—	+	+	+	—	—	+	—
Urea	—	+	—	—	+	+	—	—	+	+	+
G + C content (mol%)	72.6–74.6	63	66	60–63	66	59.1–65.7	64–66	62.2–64.1	67–68	60.7–64.2	61

has a ubiquinone with nine isoprene units. Its DNA G + C content is 74.3 mol% (T_m method).

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. We thank Dr J. Trout for revising our English text.

References

- Arahal, D. R., Castillo, A. M., Ludwig, W., Schleifer, K. H. & Ventosa, A. (2002). Proposal of *Cobetia marina* gen. nov., comb. nov., within the family *Halomonadaceae*, to include the species *Halomonas marina*. *Syst Appl Microbiol* **25**, 207–211.
- Arias, S., Del Moral, A., Ferrer, M. R., Quesada, E. & Béjar, V. (2003). Mauran, an exopolysaccharide produced by the halophilic bacterium *Halomonas maura*, with a novel composition and interesting properties for biotechnology. *Extremophiles* **7**, 319–326.
- Béjar, V., Llamas, I., Calvo, C. & Quesada, E. (1998). Characterization of exopolysaccharides produced by 19 halophilic strains of the species *Halomonas eurihalina*. *J Biotechnol* **61**, 135–141.
- 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.
- Calvo, C., Ferrer, M. R., Martínez-Checa, F., Béjar, V. & Quesada, E. (1995). Some rheological properties of the extracellular polysaccharide produced by *Volcaniella eurihalina* F2-7. *Appl Biochem Biotechnol* **55**, 45–54.
- Calvo, C., Martínez-Checa, F., Mota, A., Béjar, V. & Quesada, E. (1998). Effect of cations, pH and sulfate content on the viscosity and emulsifying activity of the *Halomonas eurihalina* exopolysaccharide. *J Ind Microbiol Biotechnol* **20**, 205–209.
- Dobson, S. J. & Franzmann, P. D. (1996). Unification of the genera *Deleya* (Bauman *et al.*, 1993), *Halomonas* (Vreeland *et al.*, 1980), and *Halovibrio* (Fendrich, 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons, 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family *Halomonadaceae*. *Int J Syst Bacteriol* **46**, 550–558.
- Ferragut, C. & Leclerc, H. (1976). Étude comparative des méthodes de détermination du T_m de l'ADN bactérien. *Ann Microbiol* **127**, 223–235.
- Franzmann, P. D., Wehmeyer, U. & Stackebrandt, E. (1988). *Halomonadaceae* fam. nov., a new family of the class *Proteobacteria* to accommodate the genera *Halomonas* and *Deleya*. *Syst Appl Microbiol* **11**, 16–19.
- Garriga, M., Ehrmann, M. A., Arnau, J., Hugas, M. & Vogel, R. F. (1998). *Carnimonas nigrificans* gen. nov., sp. nov., a bacterial

- causative agent for black spot formation on cured meat products. *Int J Syst Bacteriol* **48**, 677–686.
- Heyrman, J., Balcaen, A., De Vos, P. & Swings, J. (2002). *Halomonas muralis* sp. nov., isolated from microbial biofilms colonizing the walls and murals of the Saint-Catherine chapel (Castle Herberstein, Austria). *Int J Syst Evol Microbiol* **52**, 2049–2054.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: Molecular Evolutionary Genetics Analysis software. *Bioinformatics* **17**, 1244–1245.
- Lind, E. & Ursing, J. (1986). Clinical strains of *Enterobacter agglomerans* (Synonyms, *Erwinia herbicola*, *Erwinia milletiae*) identified by DNA-DNA hybridization. *Acta Pathol Microbiol Immunol Scand* **94**, 205–213.
- Margesin, R. & Shinner, F. (2001). Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles* **5**, 75–83.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–212.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Martínez-Checa, F., Toledo, F. L., Vilchez, R., Quesada, E. & Calvo, C. (2002). Yield production, chemical composition and functional properties of emulsifier H28 synthesized by *Halomonas eurihalina* strain H-28 in media containing various hydrocarbons. *Appl Microbiol Biotechnol* **58**, 358–363.
- Mata, J. A., Martínez-Cánovas, M. J., Quesada, E. & Béjar, V. (2002). A detailed phenotypic characterization of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.
- Mellado, E., Moore, E. R. B., Nieto, J. J. & Ventosa, A. (1995). Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina*, and *Deleya salina* and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. *Int J Syst Bacteriol* **45**, 712–716.
- Mormile, M. R., Romine, M. F., García, M. T., Ventosa, A., Baisey, T. J. & Peyton, B. M. (1999). *Halomonas campisalis* sp. nov., a denitrifying, moderately haloalkaliphilic bacterium. *Syst Appl Microbiol* **22**, 551–558.
- Owen, R. J. & Hill, L. R. (1979). The estimation of base compositions, base pairing and genome size of bacterial deoxyribonucleic acids. In *Identification Methods for Microbiologists*, pp. 277–296, 2nd edn. Edited by F. A. Skinner & D. W. Lovelock. London: Academic Press.
- Owen, R. J. & Pitcher, D. (1985). Current methods for estimating DNA composition and levels of DNA–DNA hybridization. In *Chemical Methods in Bacterial Systematics*, pp. 67–93. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Peyton, B. M., Mormile, M. R. & Petersen, J. N. (2001). Nitrate reduction with *Halomonas campisalis*. Kinetics of denitrification at pH 9 and 12.5% NaCl. *Water Res* **35**, 4237–4242.
- Quesada, E., Ventosa, A., Rodríguez-Valera, F., Megías, L. & Ramos-Cormenzana, A. (1983). Numerical taxonomy of moderately halophilic Gram-negative bacteria from hypersaline soils. *J Gen Microbiol* **129**, 2649–2657.
- Quesada, E., Ventosa, A., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1984). *Deleya halophila*, a new species of moderately halophilic bacteria. *Int J Syst Bacteriol* **34**, 287–292.
- Quesada, E., Valderrama, M. J., Béjar, V., Ventosa, A., Gutiérrez, 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.
- Romanenko, L. A., Schumann, P., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2002). *Halomonas halocynthiae* sp. nov., isolated from the marine ascidian *Halocynthia aurantium*. *Int J Syst Evol Microbiol* **52**, 1767–1772.
- 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.
- Sneath, P. H. A. & Johnson, R. (1972). The influence on numerical taxonomic similarities of errors in microbiological tests. *J Gen Microbiol* **72**, 377–392.
- Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy. The Principles and Practice of Numerical Classification*. San Francisco: Freeman, Williams & Wilkins.
- Sokal, R. R. & Michener, C. D. (1958). A statistical method for evaluating systematic relationships. *Univ Kansas Sci Bull* **38**, 1409–1438.
- 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.
- Ventosa, A. & Nieto, J. J. (1995). Biotechnological applications and potentialities of halophilic microorganisms. *World J Microbiol Biotechnol* **11**, 85–94.
- 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., Nieto, J. J. & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.
- Vreeland, R. H., Litchfield, C. D., Martin, E. L. & Elliot, E. (1980). *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. *Int J Syst Bacteriol* **30**, 485–495.
- Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R., Abraham, W. R., Lunsdorf, H. & Timmis, K. N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int J Syst Bacteriol* **48**, 339–348.
- Valderrama, M.J., Quesada, E., Béjar, V., Ventosa, A., Gutiérrez, M.C., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1991). *Deleya salina* sp. nov., a moderately halophilic Gram-negative bacterium. *Int J Syst Bacteriol* **41**, 377–384.
- Yoon, J.-H., Choi, J. H., Lee, K.-C., Kho, Y. H., Kang, K. H. & Park, Y.-H. (2001). *Halomonas marisflavae* sp. nov., a halophilic bacterium isolated from the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **51**, 1171–1177.
- Ziemke, F., Manfred, G. H., Lalucat, J. & Roselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.