

## *Halomonas anticariensis* sp. nov., from Fuente de Piedra, a saline-wetland wildfowl reserve in Málaga, southern Spain

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

Correspondence  
Emilia Quesada  
equesada@ugr.es

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

Three *Halomonas* strains, FP34, FP35<sup>T</sup> and FP36, which were isolated from soil samples taken from Fuente de Piedra, a saline wetland in the province of Málaga in southern Spain, are described. Phylogenetic analyses based on 16S rRNA gene sequences show that the three isolates belong to the genus *Halomonas* in the  $\gamma$ -*Proteobacteria* and form an independent genetic line. Phenotypically, they share the characteristics of *Halomonas* and differ from the most closely related species, *Halomonas campisalis*, in the following features: they are strictly aerobic and, because of their production of exopolysaccharides, form cream-coloured, mucoid colonies; they produce phosphatase and grow within narrow pH and temperature ranges; and they are susceptible to kanamycin and streptomycin. Their G+C content varies between 60.0 and 61.4 mol%. The name *Halomonas anticariensis* sp. nov. is proposed for these isolates. Strain FP35<sup>T</sup> (= LMG 22089<sup>T</sup> = CECT 5854<sup>T</sup>) is the type strain. The bacterium grows best in 7.5% (w/v) NaCl and does not require magnesium or potassium salts for growth, although they do stimulate growth somewhat when present. Its major fatty acids are 18:1 $\omega$ 7c, 16:0, 16:1 $\omega$ 7c, 15:0 iso 2-OH, 12:0 3-OH, 12:0, 10:0 and 19:0 cyclo  $\omega$ 8c. Its predominant respiratory lipiquinone is ubiquinone with nine isoprene units (Q-9).

The genus *Halomonas* currently contains about 30 species of halophilic bacteria, most of which have been isolated from saline environments (Dobson & Franzmann, 1996; Heyrman *et al.*, 2002; Kaye *et al.*, 2004; Martínez-Cánovas *et al.*, 2004a; Mata *et al.*, 2002; Reddy *et al.*, 2003; Romanenko *et al.*, 2002; Ventosa *et al.*, 1998; Vreeland *et al.*, 1980). Within this genus we have described three species that produce exopolysaccharides (EPSs) with potential applications in biotechnology: *Halomonas eurihalina* (Quesada *et al.*, 1990), *Halomonas maura* (Bouchotroch *et al.*, 2001) and *Halomonas ventosae* (Martínez-Cánovas *et al.*, 2004a). The taxonomic relationship between EPS-producing bacterial strains gathered from 18 hypersaline

habitats has recently been investigated (Martínez-Cánovas *et al.*, 2004b; Quesada *et al.*, 2004). Our results show that besides the three species cited above, there exist in hypersaline habitats other EPS-producing bacterial strains that cannot be assigned to any of the currently recognized halophilic species.

The aim of this communication is to describe a novel species of the genus *Halomonas*, with the proposed name of *Halomonas anticariensis* sp. nov.

The bacterial strains in question, FP34, FP35<sup>T</sup> and FP36, were isolated from samples of soil taken from the temporally emerged banks of the Laguna Redonda in the Fuente de Piedra saline-wetland wildfowl reserve in the area of Antequera in the province of Málaga, southern Spain. All three strains were initially identified as *Halomonas* species by Martínez-Cánovas *et al.* (2004b). They were maintained and routinely grown in MH medium (Quesada *et al.*, 1983) at 32 °C.

The procedures followed for phenotypic characterization have been described by Mata *et al.* (2002). Characteristics common to all three strains are given in the species description. Phenotypic features differentiating between the three strains are given in Table A, available as

Published online ahead of print on 9 February 2004 as DOI 10.1099/ijms.0.63108-0.

Abbreviation: EPS, exopolysaccharide.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains FP35<sup>T</sup>, FP34 and FP36 are AY489405, AY489406 and AY489407, respectively.

Supplementary tables giving phenotypic characteristics and figures showing a dendrogram, a phylogenetic tree including *Halomonas* species and other Gram-negative halophilic species, and a transmission electron micrograph of strain FP35<sup>T</sup> are available as supplementary material in IJSEM Online.

supplementary material in IJSEM Online. We compared the novel strains with 22 species of *Halomonas* and six other related taxa of Gram-negative halophilic bacteria by numerical analysis based on data derived from 122 phenotypic characteristics carried out as described by Martínez-Cánovas *et al.* (2004b). Computer analysis was made with the program TAXAN (Information Resources Group, Maryland Biotechnology Institute, University of Maryland, College Park, USA). A dendrogram based on the simple matching coefficient and UPGMA method (Fig. A, available as supplementary material in IJSEM Online) shows that, at 88 % similarity, the three strains group into one phenon, which shares less than 80 % similarity with the rest of the species of the genus *Halomonas*. Table B (available as supplementary material in IJSEM Online) shows the main phenotypic differences between the three strains assigned to *Halomonas anticariensis* and other related species of the genus.

The G+C DNA content of strains FP36 and FP34 was estimated from the midpoint value ( $T_m$ ) of the thermal denaturation profile, as described by Martínez-Cánovas *et al.* (2004b) for strain FP35<sup>T</sup>. The values were 60.0 mol% for strain FP36 and 60.2 mol% for strain FP34.

Phylogenetic analyses based on 16S rRNA gene sequences were made as described by Bouchotroch *et al.* (2001). The almost complete 16S rDNA sequences of the three strains were determined (FP34, 1447 bp; FP35<sup>T</sup>, 1464 bp; and FP36, 1428 bp). The fragment analysed contained the 15 signature nucleotides defined for *Halomonadaceae* (Dobson & Franzmann, 1996). Phylogenetic trees constructed using the neighbour-joining and maximum-parsimony algorithms are available as supplementary material in IJSEM Online (Figs B1 and B2). The three sequences share a high degree of similarity and are on the same separate phylogenetic branch. The taxa included in the tree in Fig. 1(a) and (b) represent only the nearest neighbours.

A chemotaxonomic study of strain FP35<sup>T</sup> included analyses of its fatty acids and quinones. They were identified by high-resolution GLC and HPLC, respectively, at Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. The results are given in the species description. Strain FP35<sup>T</sup> shows a combination of fatty acids found in other species of *Halomonas* (Dobson & Franzmann, 1996), although it also contains relatively high proportions of C10 and C12 fatty acids.

Salt requirements and growth rate under optimum conditions were determined for strain FP35<sup>T</sup> in MY medium at 32 °C according to the methods described by Bouchotroch *et al.* (2001). Strain FP35<sup>T</sup> grew between 0.5 and 15 % (w/v) NaCl, with optimum growth at 7.5 % (w/v) NaCl (growth rate of 0.22 h<sup>-1</sup>). The bacterium did not require additional magnesium or potassium salts for growth, although it did grow faster in their presence [7.5 % (w/v) NaCl plus 0.1 % (w/v) K<sup>+</sup> plus 0.34 % (w/v) Mg<sup>2+</sup>], reaching a growth rate of 0.30 h<sup>-1</sup>.

The cell size and morphology of strain FP35<sup>T</sup>, as well as its EPS, are shown in Fig. C, available as supplementary material in IJSEM Online. The transmission electron micrograph was made as described by Bouchotroch *et al.* (2001).

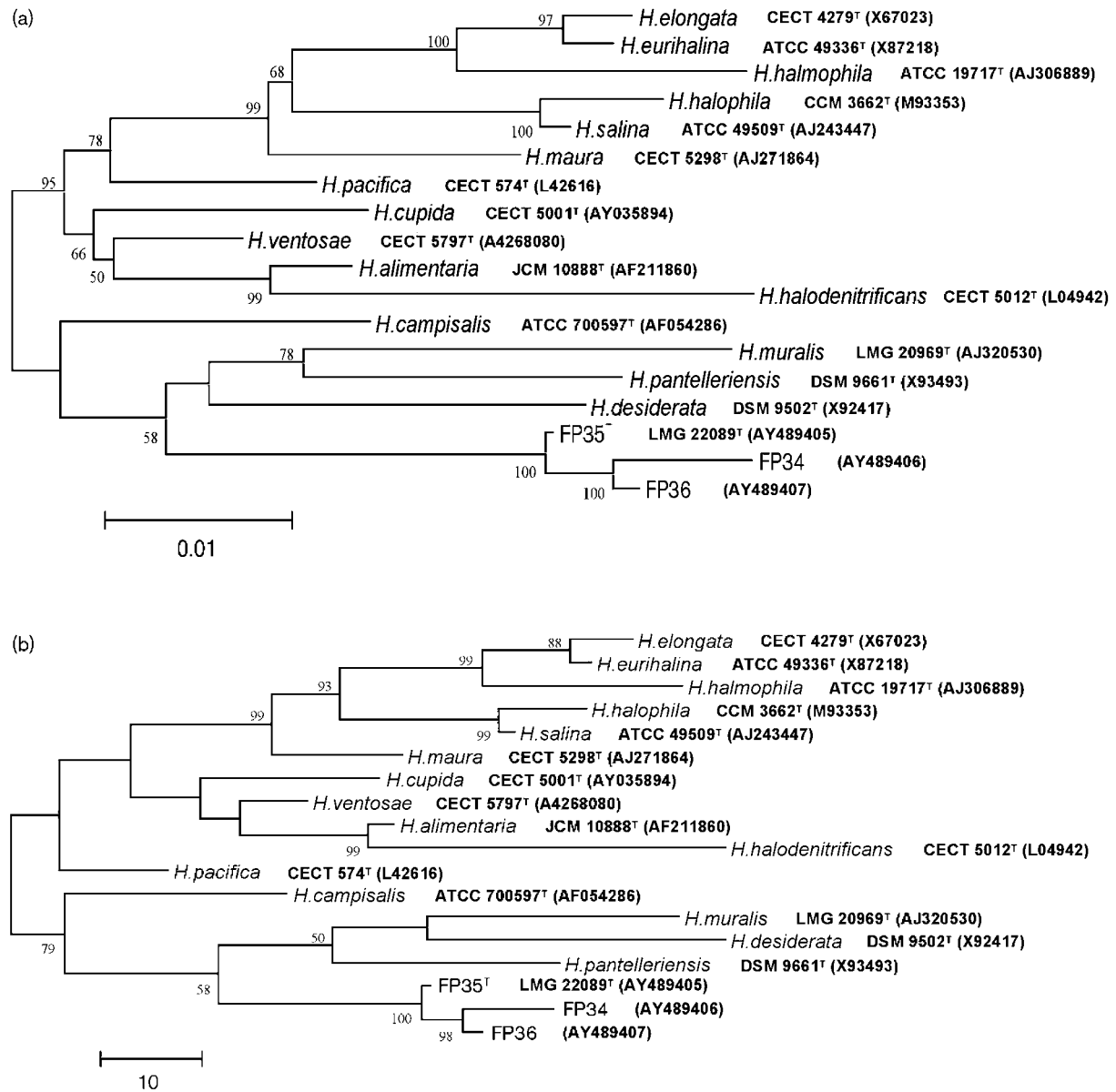
On the basis of the data discussed and the full description provided below, we propose that a novel species of the genus *Halomonas*, named *Halomonas anticariensis* sp. nov., should be admitted to include the EPS-producing strains FP34, FP35<sup>T</sup> and FP36.

### Description of *Halomonas anticariensis* sp. nov.

*Halomonas anticariensis* (an.ti.ca.ri.en'sis. N.L. fem. adj. *anticariensis*, pertaining to Antequera, originally the Roman city of Anticaria, in the province of Málaga, southern Spain, where the strains were isolated).

Straight, Gram-negative rods, 3.00–3.50 × 0.75–1.00 μm, appearing either singly or in pairs. Cells are encapsulated and motile by peritrichous flagella. Accumulates poly-β-hydroxyalkanoates and does not form endospores. Colonies are circular, convex, cream-coloured and mucoid. Their growth pattern is uniform in a liquid medium. Moderately halophilic and capable of growth in 0.5–15.0 % (w/v) NaCl; optimum growth observed at 7.5 % (w/v) NaCl. Grows at 20–45 °C and pH 6–9. Chemo-organotrophic. Metabolism is respiratory with oxygen as terminal electron acceptor. Does not grow anaerobically in the presence of nitrate, nitrite or fumarate. Catalase and oxidase are produced. Does not produce acids from sugars. Indole, methyl red and Voges–Proskauer are negative. Reduces selenite and nitrate, but not nitrite. Does not hydrolyse starch, casein, gelatin, Tween 80, aesculin or lecithin. Produces phosphatase, but not phenylalanine deaminase. Gluconate is oxidized. Does not produce H<sub>2</sub>S from L-cysteine, grow on cetrimide agar or haemolyse blood. Acetate, L-arabinose, citrate, fumarate, DL-glycerol, D-gluconate, D-glucose, *myo*-inositol, D-mannitol, D-mannose and D-trehalose are acceptable as sole carbon and energy sources, whereas D-cellobiose, formate, lactate, lactose, malonate, propionate and salicin are unacceptable. L-Alanine, L-histidine, L-isoleucine and L-serine are used as sole sources of carbon, nitrogen and energy, whereas L-cysteine and L-methionine are not. Susceptible to amoxicillin (25 μg), ampicillin (10 μg), carbenicillin (100 μg), cefotaxime (30 μg), cefoxitin (30 μg), chloramphenicol, (30 μg), kanamycin (30 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), polymyxin B (300 IU), rifampicin (30 μg), sulfamide (250 μg), streptomycin (10 μg), tobramycin (10 μg) and trimethoprim-sulfamethoxazole (1.25–23.75 μg).

The type strain is FP35<sup>T</sup> (=LMG 22089<sup>T</sup>=CECT 5854<sup>T</sup>). The description of the type strain is the same as that of the species, with the following additional features. Does not grow at pH 10 or on MacConkey agar. Hydrolyses DNA, urea and Tween 20, but not tyrosine. Adonitol, aesculin and D-fructose are acceptable as sole carbon and energy



**Fig. 1.** Phylogenetic relationships among the three strains of *Halomonas anticariensis* and the nearest *Halomonas* species. The trees were constructed using the neighbour-joining (a) and maximum-parsimony (b) algorithms. Only bootstrap values above 50% are shown (1000 replications).

sources. Does not grow on ethanol, D-galactose, L-lysine, maltose, starch, D-sorbitol or succinate. Resistant to erythromycin (15 µg). Principal fatty acids are (%): 18:1 $\omega$ 7c (47.59); 16:0 (25.49); 16:1 $\omega$ 7c/15:0 iso 2-OH (12.48); 12:0 3-OH (5.24); 12:0 (3.36); 10:0 (2.72); and 19:0 cyclo  $\omega$ 8c (1.07). The predominant respiratory lipoquinone is ubiquinone with nine isoprene units (Q-9). DNA G+C content is 61.4 mol% ( $T_m$  method).

## Acknowledgements

This research was supported by grants from the Direcció General de Investigaci6 Científica y T6cnica (BOS2000-1519) and from the Plan

Andaluz de Investigaci6n, Spain. Thanks go to Manuel Rend6n Martos, head of wildlife management of the Fuente de Piedra nature reserve (belonging to the environmental council of the Andalusian regional government) for his co-operation in our research. We also thank our colleague Dr J. Trout for revising our English text and Professor Hans Trüper (University of Bonn, Germany) for his help with the species name.

## References

- Bouchotroch, S., Quesada, E., Del Moral, A., Llamas, I. & B6jar, V. (2001). *Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium. *Int J Syst Evol Microbiol* 51, 1625–1632.

- 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.
- 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.
- Kaye, J. Z., Márquez, C., Ventosa, A. & Baross, J. A. (2004).** *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov., and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *Int J Syst Evol Microbiol* **54**, 499–511.
- 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., Quesada, E., Martínez-Checa, F. & Béjar, V. (2004b).** A taxonomic study to establish the relationship between exopolysaccharide-producing bacterial strains living in diverse hypersaline habitats. *Curr Microbiol* **48**, 348–353.
- Mata, J. A., Martínez-Cánovas, M. J., Quesada, E. & Béjar, V. (2002).** A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.
- 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., 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., Del Moral, A. & 8 other authors (2004).** Moderately halophilic, exopolysaccharide-producing bacteria. In *Halophilic Microorganisms*, pp. 297–314. Edited by A. Ventosa. Berlin, Heidelberg: Springer.
- Reddy, G. S. N., Raghavan, P. U. M., Sarita, N. B., Prakash, J. S. S., Nagesh, N., Delille, D. & Shivaji, S. (2003).** *Halomonas glaciei* sp. nov. isolated from fast ice of Adelie Land, Antarctica. *Extremophiles* **7**, 55–61.
- Romanenko, L. A., Schumann, P., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2002).** *Halomonas halocynthiae* sp. nov., isolated from the marine ascidian *Halocynthiae aurantium*. *Int J Syst Evol Microbiol* **52**, 1773–1782.
- 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.