Idiomarina fontislapidosi sp. nov. and *Idiomarina ramblicola* sp. nov., isolated from inland hypersaline habitats in Spain

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

Correspondence
Emilia Quesada
equesada@ugr.es

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

Two bacterial strains, F23^T and R22^T, have been isolated from hypersaline habitats in Málaga (S. Spain) and Murcia (E. Spain). The novel strains, similar to previously described *Idiomarina* species, are slightly curved rods, Gram-negative, chemo-organotrophic, strictly aerobic and motile by a single polar flagellum. Both strains produce catalase and oxidase. They hydrolyse aesculin, gelatin, casein, Tween 20, Tween 80 and DNA but not starch or tyrosine. The strains differ from the hitherto described *Idiomarina* species in their capacity to produce extracellular polysaccharides and their different patterns of carbon sources and antimicrobial susceptibility. They are moderate halophiles capable of growing in NaCl concentrations of 0.5 to 25 % w/v, the optimum being 3–5 % w/v. Cellular fatty acids are predominantly iso-branched. The main fatty acids in strain F23^T are 15:0 iso (26.75 %), 16:1 ω 7c (11.33 %) and 16:0 (11.73 %) whilst 15:0 iso (24.69 %), 17:0 iso (12.92 %) and 17:1 ω 9c (11.03 %) are predominant in strain R22^T. The DNA G + C composition is 46.0 mol% in strain F23^T and 48.7 mol% in strain R22^T. Phylogenetic analyses indicate conclusively that the two strains belong to the genus *Idiomarina*. DNA–DNA hybridization revealed that they represent novel species. In the light of the polyphasic evidence accumulated in this study, it is proposed that they be classified as novel species of the genus *Idiomarina*, with the names *Idiomarina fontislapidosi* sp. nov. (type strain F23^T = CECT 5859^T = LMG 22169^T) and *Idiomarina ramblicola* sp. nov. (type strain R22^T = CECT 5858^T = LMG 22170^T).

The genus *Idiomarina*, a member of the family *Alteromonadaceae* (Ivanova & Mikhailov, 2001) within the γ -subclass of the *Proteobacteria*, was proposed by Ivanova *et al.* (2000) to accommodate two strains isolated from sea-water samples collected at a depth of 4000–5000 m from the north-western Pacific Ocean. These strains were described as two different species, *Idiomarina abyssalis* (type species) and *Idiomarina zobellii*. Since then two more species have been added to the genus: *Idiomarina loihiensis*, which was isolated from the Lō‘ihi submarine volcano in Hawaii (Donachie *et al.*, 2003), and *Idiomarina baltica*, which was found in the central Baltic Sea (Brettar *et al.*, 2003). Members of *Idiomarina* share many phenotypic characteristics with other heterotrophic, oxidative, marine and halophilic members of the γ -*Proteobacteria*. Nevertheless, phylogenetic studies based on 16S rRNA gene sequence

analysis have proved that *Idiomarina* represents a distinct evolutionary line. One eminent feature of the genus *Idiomarina* is its uniquely high content of iso-branched fatty acids, which is atypical of *Proteobacteria* with the sole exception of the *Xanthomonas* branch (Finkmann *et al.*, 2000). They can also be distinguished from other marine bacteria by their physiological properties, being able to grow within a broad range of temperatures, pH values and NaCl concentrations.

In this study we describe two strains, for which we propose the names *Idiomarina fontislapidosi* sp. nov. and *Idiomarina ramblicola* sp. nov. These two strains are the only representatives of the genus *Idiomarina* identified so far that have not been isolated from sea-water samples.

The strains were found during two different samplings made in 1998 (Martínez-Cánovas *et al.*, 2004). F23^T was isolated from a soil sampled at Fuente de Piedra (Málaga, S. Spain), an inland, hypersaline wetland. Strain R22^T was isolated from a water sample taken in Rambla Salada (Murcia, E. Spain), a hypersaline rambla (a steep-sided river bed, normally dry but subject to flash flooding). The isolation

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medium was MY (Moraine & Rogovin, 1966) supplemented with 7.5% w/v salts (Rodríguez-Valera *et al.*, 1981); its composition is the following (g l⁻¹): NaCl, 51.3; MgCl₂·6H₂O, 9; MgSO₄·7H₂O, 13; CaCl₂·2H₂O, 0.2; KCl, 1.3; NaCO₃H, 0.05; NaBr, 0.15; FeCl₃·6H₂O, traces; glucose, 10; yeast extract, 3; malt extract, 3; proteose-peptone, 5 (pH 7). Both strains were kept and routinely grown in MH medium (Quesada *et al.*, 1983) at 32 °C; its composition is the following (g l⁻¹): NaCl, 51.3; MgCl₂·6H₂O, 9; MgSO₄·7H₂O, 13; CaCl₂·2H₂O, 0.2; KCl, 1.3; NaCO₃H, 0.05; NaBr, 0.15; FeCl₃·6H₂O, traces; glucose, 1; yeast extract, 10; proteose-peptone, 5 (pH 7). Bacto agar (2 g l⁻¹) was added for the preparation of solid media.

The strains were originally characterized phenotypically by Martínez-Cánovas *et al.* (2004) according to the methods described by Mata *et al.* (2002). Phenotypic data are given in the species description. Table 1 shows the main phenotypic differences between the strains F23^T (*I. fontislapidosi* sp. nov.) and R22^T (*I. ramblicola* sp. nov.) and the other four species of the genus *Idiomarina*. The same table contains the G + C contents of both strains estimated from the midpoint value (*T*_m) of the DNA thermal denaturation profile, as described in Martínez-Cánovas *et al.* (2004).

Phylogenetic analyses were made according to Bouchotroch *et al.* (2001). We determined almost the complete sequence of the 16S rRNA genes of strains F23^T (1455 bp) and R-22^T

Table 1. Characteristics that distinguish between *I. fontislapidosi* sp. nov., *I. ramblicola* sp. nov. and other species of the genus *Idiomarina*

Data from Brettar *et al.* (2003), Donachie *et al.* (2003) and Ivanova *et al.* (2000).

Characteristic	<i>I. fontislapidosi</i>	<i>I. ramblicola</i>	<i>I. abyssalis</i>	<i>I. zobellii</i>	<i>I. loihiensis</i>	<i>I. baltica</i>
Cell size (µm)	3–4 × 0.75	2–3 × 0.75	1–1.8 × 0.7–0.9	1–1.8 × 0.7–0.9	0.7–1.8 × 0.35–0.45	0.7–1.6 × 0.4–0.7
Cell morphology	Slightly curved	Slightly curved	Slightly curved	Slightly curved	Straight to slightly curved	Slightly curved
Pigmentation	Cream	Cream	Non-pigmented	Lightish yellow	Beige to yellow	Non-pigmented
Exopolysaccharide	+	+	–	–	–	–
Flagellum	Polar	Polar	Polar	Polar	Subpolar or polar	Polar
NaCl range (% w/v)	0.5–20	0.5–15	0.6–15.0	1–10	0.5–20	0.8–10
NaCl optimum (% w/v)	3–5	3–5	3–6	3–6	7.5–10	3–6
pH range	5–10	5–10	5.5–9.5	5.5–9.5	ND	ND
Temperature range (°C)	4–45	15–40	4–30	4–30	4–46	8–46
Temperature optimum (°C)	32	32	20–22	20–22	ND	30–40
Acid from D-glucose	–	–	–	–	–	+*
Growth on:†						
L-Arabinose	–	–	–	–	ND	+
Maltose	–	–	–	–	+	–
Acetate	–	–	+	–	+	+
Citrate	–	–	–	–	+	–
Lactate	–	–	–	–	+	–
Malonate	–	–	–	–	+	–
Propionate	–	–	+	–	+	–
Succinate	–	–	+	+	–	–
Glycerol	–	–	+	–	+	–
myo-Inositol	–	–	ND	ND	+	ND
L-Alanine	–	–	+	+	+	–
L-Serine	–	–	–	–	+	–
Susceptibility to:						
Ampicillin (10 µg)	+	+	–	–	ND	ND
Kanamycin (30 µg)	+	–	–	–	ND	ND
Streptomycin (10 µg)	+	–	+	–	ND	ND
Sulphamide (250 µg)	–	+	ND	ND	ND	ND
Tobramycin (10 µg)	+	–	ND	ND	ND	ND
G + C content (mol%)	46.0	48.7	50.49	48	47.4	49.4–49.9
Habitat	Saline wetland (soil), Spain	Saline rambla (water), Spain	N. W. Pacific Ocean (depth)	N. W. Pacific Ocean (depth)	Hydrothermal vent, Hawaii	Central Baltic Sea (surface)

*Weak.

†As sole source of carbon and energy; L-alanine and L-serine were tested as sole sources of carbon, nitrogen and energy.

Fig. 1. Phylogenetic tree deriving from a neighbour-joining analysis of the 16S rRNA gene sequences of *Idiomarina* species and other species belonging to related genera of the γ -Proteobacteria. Bar, 1% estimated sequence divergence.

(1459 bp). The phylogenetic tree obtained by neighbour-joining is shown in Fig. 1. Maximum-parsimony algorithms gave a similar result. The analyses confirmed the affiliation of the novel strains to *Idiomarina* and their relationship with *I. baltica* (strain F23^T) and *I. loihiensis* (strain R22^T). Nevertheless, Stackebrandt & Pukall (1999) advise that even a 16S rRNA gene sequence similarity of over 99.5% is insufficient evidence to affiliate an isolate to a particular species. We have demonstrated that the two new isolates can be distinguished at the species level through their phenotypic features, fatty-acid contents and by DNA–DNA hybridization. The next closest neighbours were *Thalassomonas*, described by Macián *et al.* (2001), and *Colwellia*, another member of the family *Alteromonadaceae* (Ivanova & Mikhailov, 2001).

The fatty acids were analysed at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) by GLC. Both profiles (Table 2) display a predominance of iso-branched fatty acids, which is characteristic of *Idiomarina*. The taxonomic significance of the fatty-acid composition of *Idiomarina* species is discussed in Brettar *et al.* (2003). 15:0 iso (26.75%), 16:1 ω 7c (11.33%) and 16:0 (11.73%) form the main peaks of the fatty-acid pattern in strain F23^T whilst 15:0 iso (24.69%), 17:0 iso (12.92%) and 17:1 ω 9c (11.03%) are the most abundant fatty acids in strain R22^T. As can be seen in Table 2, the fatty-acid pattern of the new isolates differs to some extent from those of the previously described species of *Idiomarina*.

DNA–DNA hybridization was carried out according to Lind and Ursing's method with the modifications employed by Ziemke *et al.* (1998), all of which is fully described in Bouchotroch *et al.* (2001). The results (Table 3) prove that strains F23^T and R22^T should be accepted as two novel species of the genus *Idiomarina*.

On the basis of phylogeny, DNA–DNA hybridization, fatty-acid profiles and phenotypic differences between the novel and previously described species we conclude that strains F23^T and R22^T represent novel species within the genus *Idiomarina*.

Description of *Idiomarina fontislapidosi* sp. nov.

Idiomarina fontislapidosi (fon'tis.la.pi.do'si. L. sb. masc. gen. *fontis* of the spring; L. masc. adj. *lapidosi* stony; N.L. sb. masc. gen. *fontislapidosi* of the stony spring, i.e. from Fuente de Piedra, the site from which the type strain was isolated).

Cells are slightly curved rods, 3–4 μ m long and 0.75 μ m wide, appearing either singly or in pairs, and often forming amorphous aggregates. They stain Gram-negative and are motile by one polar flagellum. No spores or polyhydroxy-alkanoate are observed under any conditions. Colonies are round, convex, mucoid and cream coloured. Exopolysaccharide is produced. The growth pattern is uniform in a liquid medium. The bacterium is chemo-organotrophic and strictly aerobic. Anaerobic respiration with nitrate, nitrite or fumarate is negative. It does not produce acid from any of the following sugars: adonitol, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, mannose, D-melezitose, L-rhamnose, sucrose, D-salicin, D-sorbitol, sorbose or D-trehalose. It is moderately halophilic, capable of growing in NaCl concentrations of 0.5 to 25% w/v, the optimum being 3–5% w/v. It does not require additional magnesium or potassium salts. It grows within the temperature range of 4 to 45 °C, the optimum being 32 °C, and at pH values of between 5 and 10, the optimum being 7–8. It shows positive activity for catalase, cytochrome oxidase, phosphatase, ONPG, selenite reduction, H₂S production from cysteine, growth on MacConkey and hydrolysis of aesculin, gelatin, casein, Tween 20, Tween 80 and DNA. It shows no activity for the hydrolysis of starch and tyrosine, nitrate and nitrite reduction, urease, lecithinase, phenylalanine deaminase, gluconate oxidation, growth on cetrimide agar, haemolysis, indole, methyl red and Voges–Proskauer. It does not grow in synthetic media supplemented with different sole sources of carbon and energy, or carbon, nitrogen and energy (aesculin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, L-rhamnose, D-salicin, starch, D-trehalose, acetate, citrate, formate, fumarate, gluconate, lactate,

Table 2. Main differences in cellular fatty-acid composition (%) between *I. fontislapidosi* sp. nov., *I. ramblicola* sp. nov. and previously described species of *Idiomarina*Data from Brettar *et al.* (2003), Donachie *et al.* (2003) and Ivanova *et al.* (2000). —, Negative or less than 1%.

Fatty acid	<i>I. fontislapidosi</i>	<i>I. ramblicola</i>	<i>I. baltica</i>	<i>I. loihiensis</i>	<i>I. abyssalis</i>	<i>I. zobellii</i>
10:0 3-OH	2.34	1.10	1.16–1.91	0.8	—	—
11:0 iso	2.77	3.43	2.48–2.22	2.0	—	—
11:0 iso 3-OH	2.56	5.64	3.65–2.74	4.1	—	—
13:0 iso	0.92	1.46	0.81–0.60	1.8	1	1.10
13:0 iso 3-OH	1.61	2.27	3.22–3.22	3.3	—	—
14:0	1.92	0.61	—	—	—	—
15:0 anteiso	0.62	1.16	—	—	—	—
15:1 isoF	1.46	1.91	1.53–0.92	1.3	2.30	1.60
15:0 iso	26.75	24.69	36.88–31.31	32.6	33.70	40.60
15:1 ω 8c	—	—	—	—	1.30	1.10
16:0	11.73	7.38	4.81–9.70	7.6	6.30	4.60
16:1 ω 7c	11.33*	5.21	8.4	6.0	7.0	8.3
17:0	0.62	1.69	—	—	—	—
17:0 iso	8.83	12.92	11.21–9.44	11.0	11.90	12.50
17:1 ω 6c	—	—	—	—	1.50	3.40
17:1 ω 8c	0.31	1.11	0.71–1.06	0.9	0.80	1.10
17:0 cyclo	1.22	2.53	0.61	1.7	—	—
17:1 iso ω 9c	4.04	11.03	9.99–5.62	11.9	—	—
18:1 ω 9c	1.13	1.22	0.87–0.95	1.0	1.40	0.90
18:1 ω 8c	—	—	0.87	0.95	1.40	0.90
18:1 ω 7c	9.32	5.92	5.98–9.17	5.5	6.70	5.90
18:0	4.94	3.00	0.94–1.83	1.6	1.80	0.80
11-Methyl 18:1 ω 7c	0.93	0.18	1.78–2.51	—	—	—

*16:1 ω 7c/15:0 iso 2-OH.

malonate, propionate, succinate, adonitol, ethanol, glycerol, *myo*-inositol, sorbitol, L-alanine, L-cysteine, L-histidine, DL-isoleucine, L-lysine, L-methionine, L-serine and L-valine). It is susceptible to amoxicillin (25 µg), ampicillin (10 µg), carbenicillin (100 µg), cefotaxime (30 µg), cefoxitin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), polymyxin B (300 UI), rifampicin (30 µg), streptomycin (10 µg), tobramycin (10 µg) and

trimetoprim/sulphamethoxazol (1.25/23.75 µg). It is resistant to sulphamide (250 µg). DNA G + C content of the type strain is 46.0 mol% (T_m method).

The type strain, F23^T (= CECT 5859^T = LMG 22169^T), was isolated from a sample of soil taken from the temporally emerged banks of a hypersaline pool in the Fuente de Piedra wetland wild-fowl reserve in the province of Málaga (S. Spain).

Description of *Idiomarina ramblicola* sp. nov.

Idiomarina ramblicola [ram.bli.co'la. Spanish sb. fem. *rambla* from Arabic sb. *ramla* lit. sandy ground; L. suff. [*in*]cola denizen of; N.L. sb. fem. nom. *ramblicola* denizen of a rambla. A rambla is a steep-sided watercourse, often dry but subject to flash flooding (cf. OED, 2nd edn, 1989, vol. XIII, p. 153), not synonymous with Arabic *wadi*].

The cells are slightly curved rods, 2–3 µm long and 0.75 µm wide, appearing either singly or forming amorphous aggregates. They stain Gram-negative and are motile by one polar flagellum. No spores or polyhydroxyalkanoate are observed under any conditions. Colonies are round, convex, mucoid and cream coloured. Exopolysaccharide

Table 3. DNA–DNA hybridization between *I. fontislapidosi* sp. nov., *I. ramblicola* sp. nov. and previously described species of *Idiomarina*

Strain	Hybridization (%) with DNA from:	
	<i>I. fontislapidosi</i> F23 ^T	<i>I. ramblicola</i> R22 ^T
<i>I. fontislapidosi</i> F23 ^T	100	35.91
<i>I. ramblicola</i> R-22 ^T	35.39	100
<i>I. loihiensis</i> L2-TR ^T	ND	43.68
<i>I. baltica</i> OS 145 ^T	37.30	38.96

is produced. The growth pattern is uniform in a liquid medium. The bacterium is chemo-organotrophic and strictly aerobic. Anaerobic respiration with nitrate, nitrite or fumarate is negative. It does not produce acid from any of the following sugars: adonitol, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, mannose, D-melezitose, L-rhamnose, sucrose, D-salicin, D-sorbitol, sorbose or D-trehalose. It is moderately halophilic, capable of growing in NaCl concentrations of 0.5 to 15 % w/v, the optimum being 3–5 % w/v. It does not require additional magnesium or potassium salts. It grows within the temperature range of 15 to 40 °C, the optimum being 32 °C, and at pH values of between 5 and 10, the optimum being 7–8. It shows positive activity for catalase, cytochrome oxidase, phosphatase, selenite reduction, gluconate oxidation, haemolysis, H₂S production from cysteine and the hydrolysis of aesculin, gelatin, casein, Tween 20, Tween 80 and DNA, and no activity for the hydrolysis of starch and tyrosine, reduction of nitrate and nitrite, oxidation of urease, lecithinase and phenylalanine and growth on cetrimide agar, haemolysis, indole, methyl red and Voges–Proskauer. It does not grow in synthetic media supplemented with different sole sources of carbon and energy, or carbon, nitrogen and energy (aesculin, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, L-rhamnose, D-salicin, starch, D-trehalose, acetate, citrate, formate, fumarate, gluconate, lactate, malonate, propionate, succinate, adonitol, ethanol, glycerol, *myo*-inositol, sorbitol, L-alanine, L-cysteine, L-histidine, DL-isoleucine, L-lysine, L-methionine, L-serine and L-valine). It is susceptible to amoxicillin (25 µg), ampicillin (10 µg), carbenicillin (100 µg), cefotaxime (30 µg), cefoxitin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), polymyxin B (300 UI), rifampicin (30 µg), sulphamide (250 µg) and trimetoprim/sulphamethoxazol (1.25/23.75 µg). It is resistant to kanamycin (30 µg), streptomycin (10 µg) and tobramycin (10 µg). DNA G+C content of the type strain is 48.7 mol% (*T_m* method).

The type strain, R22^T (=CECT 5858^T=LMG 22170^T), was isolated from a hypersaline water sample taken from Rambla Salada (Murcia, S.E. Spain).

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