Marinomonas alcarazii sp. nov., *M. rhizomae* sp. nov., *M. foliarum* sp. nov., *M. posidonica* sp. nov. and *M. aquiplantarum* sp. nov., isolated from the microbiota of the seagrass *Posidonia oceanica*

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Five novel Gram-reaction-negative aerobic marine bacterial strains with DNA G+C contents <50 mol% were isolated from the seagrass *Posidonia oceanica*. 16S rRNA sequence analysis indicated that they belonged to the genus *Marinomonas*. Major fatty acid compositions, comprising C_{10:0} 3-OH, C_{16:0}, C_{16:1ω7c} and C_{18:1ω7c}, supported the affiliation of these strains to the genus *Marinomonas*. Strains IVIA-Po-14b^T, IVIA-Po-145^T and IVIA-Po-155^T were closely related to *Marinomonas pontica* 46-16^T, according to phylogenetic analysis. However, DNA–DNA hybridization values <35% among these strains revealed that they represented different species. Further differences in the phenotypes and minor fatty acid compositions were also found among the strains. Another two strains, designated IVIA-Po-181^T and IVIA-Po-159^T, were found to be closely related to *M. dokdonensis* DSW10-10^T but DNA–DNA relatedness levels <40% in pairwise comparisons, as well as some additional differences in phenotypes and fatty acid compositions supported the creation of two novel species. Accordingly, strains IVIA-Po-14b^T (=CECT 7730^T =NCIMB 14671^T), IVIA-Po-145^T (=CECT 7377^T =NCIMB 14431^T), IVIA-Po-155^T (=CECT 7731^T =NCIMB 14672^T), IVIA-Po-181^T (=CECT 7376^T =NCIMB 14433^T) and IVIA-Po-159^T (=CECT 7732^T =NCIMB 14673^T) represent novel species, for which the names *Marinomonas alcarazii* sp. nov., *Marinomonas rhizomae* sp. nov., *Marinomonas foliarum* sp. nov., *Marinomonas posidonica* sp. nov. and *Marinomonas aquiplantarum* sp. nov. are proposed, respectively.

The genus *Marinomonas* is composed of Gram-negative bacterial strains that have been found distributed across many different marine environments (Sanchez-Amat & Solano, 2005). The number of species in this genus has increased during recent years, after the initial description of the genus by Van Landschoot & De Ley (1983). At the time of writing, the list of species with validly published names in the genus *Marinomonas* included *M. communis* and *M. vaga* (formerly *Alteromonas communis* and *A. vaga*; Van Landschoot & De Ley, 1983; Baumann *et al.*, 1972), *M. mediterranea* (Solano & Sanchez-Amat, 1999), *M. primoryensis* (Romanenko *et al.*, 2003), *M. pontica*

(Ivanova *et al.*, 2005), *M. dokdonensis* (Yoon *et al.*, 2005), *M. aquimarina* (Macián *et al.*, 2005), *M. ushuaiensis* (Prabakaran *et al.*, 2005), *M. polaris* (Gupta *et al.*, 2006), *M. ostreistagni* (Lau *et al.*, 2006), *M. arctica* (Zhang *et al.*, 2008), *M. basaltis* (Chang *et al.*, 2008), *M. arenicola* (Romanenko *et al.*, 2009), *M. balearica* and *M. pollencensis* (Espinosa *et al.*, 2010). *M. brasiliensis* has since been added to the genus and *M. communis* and *M. basaltis* have been recognized as heterotypic synonyms (Chimetto *et al.*, 2011).

It has been reported that the microbiota of the marine seagrass *Posidonia oceanica* contains a diverse range of species of the genus *Marinomonas*, namely, *M. balearica*, *M. pollencensis* and *M. mediterranea* (Espinosa *et al.*, 2010). Here, we report the characterization of five novel strains of the genus *Marinomonas* isolated from *Posidonia oceanica*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains IVIA-Po-181^T, IVIA-Po-159^T, IVIA-Po-14b^T, IVIA-Po-145^T and IVIA-Po-155^T are EU188445, EU188447, EU188442, EU188443 and EU188444, respectively.

Samples of seagrass were collected from different meadows on the coast of the Balearic Islands, Spain, and processed as described previously (Espinosa *et al.*, 2010). After plating on Marine Agar 2216 and incubating for 24–72 h at 25 °C, several hundreds of strains were isolated. In a preliminary step, partial 16S rRNA gene sequences, corresponding approximately to the fragment between position 70 and 660 of the 16S rRNA gene of *Escherichia coli*, were obtained from all the isolated strains by the Molecular Diagnosis Center (MDC, Orihuela, Spain) following the method of Martínez-Murcia *et al.* (1999). DNA sequences were determined by direct sequencing of the PCR products using an ABI 3100 Avant sequencer (Applied Biosystems).

Searches using BLAST (Altschul *et al.*, 1997) were performed with the sequences obtained and those showing higher similarity to 16S rRNA sequences of species of the genus *Marinomonas* than to those of any other species were selected. Strains whose 16S rRNA gene sequence was identical to that of previously described species of the genus *Marinomonas* were disregarded so that the study focused on potential novel species. Here, we report the characterization of strains IVIA-Po-14b^T, IVIA-Po-145^T, IVIA-Po-155^T, IVIA-Po-181^T and IVIA-Po-159^T.

Strains that showed novel partial 16S rRNA gene sequences were selected as potential novel species. Genomic DNA was isolated from these strains and the corresponding almost complete 16S rRNA genes were amplified and sequenced by the MDC; the sequences were then deposited in the GenBank database. The 16S rRNA gene sequences of all the previously described species of the genus *Marinomonas*, as well as those of some related marine gammaproteobacteria and *Vibrio cholerae*, as an outgroup, were included in the analysis. Phylogenetic analyses based on the sequences of the 16S rRNA genes were conducted using MEGA version 4 (Tamura *et al.*, 2007). All the sequences were aligned using the CLUSTAL W program within the MEGA software and distance matrices were generated to calculate the p-distances. Phylogenetic trees were reconstructed using the neighbour-joining method. The reliability of the trees was evaluated using bootstrap analysis with 1000 resamplings.

DNA–DNA hybridizations were performed by the DSMZ identification service. DNA was isolated using a French pressure cell and purified by chromatography as described by Cashion *et al.* (1977). DNA–DNA hybridizations were carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe. To determine the G + C content of the DNA, DNA was isolated, digested with nuclease P1 and dephosphorylated as described by Johnson (1994). The nucleosides obtained were analysed by HPLC (Kumura *et al.*, 1991).

Unless otherwise indicated, the biochemical, microscopic and physiological tests were performed as described by Baumann *et al.* (1972), Solano & Sanchez-Amat (1999) and

Espinosa *et al.* (2010). The utilization of different compounds as carbon sources was assayed in BM mineral medium (Baumann *et al.*, 1972; Solano & Sanchez-Amat, 1999). Fatty acid analysis was performed using the same conditions as described by Espinosa *et al.* (2010). In all the tests performed in this study, *M. pontica* 46-16^T, generously gifted by E. Ivanova, and *M. dokdonensis* DSW10-10^T, obtained from the DSMZ, were used as reference strains since they were the species that proved to be most closely related to the novel strains.

The phylogenetic analysis confirmed the results of the BLAST searches, indicating that the novel strains characterized in this study belonged to the genus *Marinomonas* (Fig. 1). Taking into consideration that *M. mediterranea*, *M. balearica* and *M. pollencensis* were also detected in the culturable microbiota of *P. oceanica*, Fig. 1 shows that this plant harbours a great phylogenetic diversity of strains of the genus *Marinomonas*, including strains from most of the branches within the genus.

Strains IVIA-Po-14b^T, IVIA-Po-145^T and IVIA-Po-155^T were most closely related to *M. pontica* 46-16^T (Fig. 1); the 16S rRNA gene sequence similarities between these strains and *M. pontica* 46-16^T were 99.5 ± 0.2, 98.2 ± 0.2 and 99.0 ± 0.2 %, respectively. In order to determine whether they belonged to the same species, DNA–DNA hybridization studies were performed comparing these four strains. It was found that, in all cases, these values were much lower than the value of 70 %, recommended as the threshold for the delineation of bacterial species (Wayne *et al.*, 1987). The highest DNA–DNA relatedness value was 31 % between *M. pontica* 46-16^T and strain IVIA-Po-155^T. The DNA–DNA relatedness between *M. pontica* 46-16^T and strain IVIA-Po-14b^T was 26.8 % in spite of a 99.5 % 16S rRNA gene sequence similarity.

M. pontica 46-16^T differed from the strains studied in that it was oxidase-positive, able to use *m*-hydroxybenzoate as carbon source (Table 1) and did not contain the unknown fatty acid of ECL 11.799 that was detected in other strains (Table 2). It is important to point out that, in spite of the differences indicated, the major fatty acid profiles of all the strains analysed in this study agreed with those reported for other members of the genus *Marinomonas* (Espinosa *et al.*, 2010). Strain IVIA-Po-14b^T was very similar to *M. pontica* 46-16^T in many respects but differed from it, in addition to the characteristics mentioned above, in its capacity to use galactose but not *m*-hydroxybenzoate as sole carbon source (Tables 1 and 2). Strains IVIA-Po-145^T and IVIA-Po-155^T differed from strains IVIA-Po-14b^T and *M. pontica* 46-16^T in their cell shape, being curved rods. The capacity to use L-tyrosine as sole carbon and energy source was a distinctive character of strain IVIA-Po-145^T (Table 1).

These results indicate that strains IVIA-Po-14b^T, IVIA-Po-145^T and IVIA-Po-155^T can be considered as novel species of the genus *Marinomonas*, for which the names *M. alcarazii* sp. nov., *M. rhizomae* sp. nov. and *M. foliarum* sp. nov. are proposed.

Fig. 1. Neighbour-joining tree based on 16S rRNA sequences showing the phylogenetic position of strains IVIA-Po-14b^T, IVIA-Po-145^T, IVIA-Po-155^T, IVIA-Po-181^T and IVIA-Po-159^T in relation to type strains of previously described species of the genus *Marinomonas* and representative marine bacteria. Numbers at branches indicate bootstrap values >70 % (based on 1000 resamplings). Asterisks indicate branches also identified by the maximum-parsimony method. Bar, 0.01 substitutions per nucleotide position.

Strains IVIA-Po-181^T and IVIA-Po-159^T were phylogenetically related to *M. dokdonensis* DSW10-10^T (Fig. 1). However, these two strains formed a well-defined separate cluster. This cluster represents the most abundant culturable group of species of the genus *Marinomonas* isolated from the microbiota of *P. oceanica* since 35 out of the 43 strains that were initially isolated belong to it. Strains IVIA-Po-181^T and IVIA-Po-159^T showed 16S rRNA gene sequence similarities in the range of 98.3–98.5 % with *M. dokdonensis* DSW10-10^T and although the similarity value between the two strains was higher than 99.4 %, they showed a DNA–DNA relatedness of only 42.4 % to each other, suggesting that they represent different species. This

assumption was supported by the fact that strains IVIA-Po-181^T and IVIA-Po-159^T showed DNA–DNA relatedness values of 10.5 and 25.9 %, respectively, to their closest relative, *M. dokdonensis* DSW 10-10^T.

In terms of their phenotypic characteristics, cells of strains IVIA-Po-181^T and IVIA-Po-159^T differed from *M. dokdonensis* DSW 10-10^T, by being helical in shape and oxidase-negative (Table 1) and by containing the fatty acid C_{12:1} 3-OH but not C_{12:0} (Table 2). The detection of an unknown fatty acid of ECL 11.799 in strain IVIA-Po-181^T was the main differential characteristic between these two novel strains (Table 2). Accordingly, strains IVIA-Po-181^T and

Table 1. Phenotypic characteristics of strains IVIA-Po-14b^T, IVIA-Po-145^T, IVIA-Po-155^T, IVIA-Po-181^T, IVIA-Po-159^T and the type strains of closely related species of the genus *Marinomonas*

Strains: 1, *M. pontica* 46-16^T (data from Ivanova *et al.*, 2005, unless indicated otherwise); 2, IVIA-Po-14b^T; 3, IVIA-Po-145^T; 4, IVIA-Po-155^T; 5, *M. dokdonensis* DSW10-10^T (data from Yoon *et al.*, 2005, unless indicated otherwise); 6, IVIA-Po-181^T; 7, IVIA-Po-159^T. All other data are from this study. All strains required NaCl for growth and utilized glucose, glutamate, α -ketoglutarate, fructose, maltose, sorbitol, acetate and malate as carbon sources. None of the strains were able to utilize lactose, butyrate or valerate, showed gelatinase or laccase activities, synthesized melanins or produced antimicrobial compounds in the conditions assayed. +, Positive; –, negative; w, weak reaction.

Characteristics	1	2	3	4	5	6	7
DNA G + C content (mol%)	46	46.2	45.5	46.2	45.5	44.7	47.8
Cell shape	Helical*	Helical	Curved	Curved	Straight	Helical	Helical
Oxidase	+	–	–	–	+	–	–
NaCl tolerance (%)	0.5–10	0.5–10	0.5–10	0.5–10	2–10	0.5–10	0.5–10
Temperature range (°C)	4–33	5–37	5–25	5–25	4–37	5–25	5–37
Lipase	–	–	–	–	+	–	w
Carbon source utilization							
Galactose	–	+	–	+	–	+	+
Sucrose	+	+	+	+	–	+	+
Glycine	+	+	–	–	–†	–	+
L-Tyrosine	–	–	+	–	–†	–	–
m-Hydroxybenzoate	+	–	–	–	–	–	–

*Data from Espinosa *et al.* (2010).

†Data from the present study.

Table 2. Cellular fatty acid composition of strains IVIA-Po-14b^T, IVIA-Po-145^T, IVIA-Po-155^T, IVIA-Po-181^T, IVIA-Po-159^T and type strains of closely related species of the genus *Marinomonas*

Strains: 1, *M. pontica* 46-16^T; 2, IVIA-Po-14b^T; 3, IVIA-Po-145^T; 4, IVIA-Po-155^T; 5, *M. dokdonensis* DSW10-10^T; 6, IVIA-Po-181^T; 7, IVIA-Po-159^T. All data are from this study, unless indicated otherwise. Values are percentages of total fatty acids. —, Not detected.

Fatty acid	1*	2	3	4	5	6	7
C _{10:0} 3-OH	6.54	9.72	7.84	8.25	7.61	12.64	15.70
Unknown 11.799	—	4.01	0.64	0.39	8.43	5.01	—
C _{12:0}	—	1.36	—	1.21	3.65	—	—
C _{12:1} 3-OH	8.40	3.65	5.92	7.70	—	6.71	14.26
C _{14:0}	1.60	1.02	1.02	1.12	0.69	1.05	1.17
C _{16:0}	11.73	11.81	13.30	22.20	8.92	15.15	12.95
C _{16:1} ω7c	34.26	22.73	24.05	27.25	20.48	13.10	15.96
C _{17:0} cyclo	—	—	—	—	—	—	—
C _{18:0}	2.27	4.35	4.07	3.08	2.46	3.69	3.92
C _{18:1} ω7c	22.90	43.52	43.60	37.50	40.05	32.83	33.16

*Data from Espinosa *et al.* (2010).

IVIA-Po-159^T, represent two novel species, for which the names *M. posidonica* sp. nov. and *M. aquiplantarum* sp. nov. are proposed.

This study has revealed that the seagrass *Posidonia oceanica* harbours a diverse group of bacterial species belonging to the genus *Marinomonas*, within which the creation of five novel species is proposed.

Description of *Marinomonas alcarazii* sp. nov.

Marinomonas alcarazii (al.ca.ra'zi.i. N.L. masc. gen. n. *alcarazii* of Alcaraz, named after Miquel Alcaraz, Spanish scientist).

Cells are helical, motile with a single polar flagellum, Gram-reaction-negative, strictly aerobic, catalase-positive and oxidase-negative. Organic growth factors are not required. Requires Na⁺ for growth. Grows in 0.5–10% (w/v) NaCl and at 5–37 °C. Negative for amylase, agarase, lipase and protease (gelatin) activities. Glucose, galactose, maltose, sucrose, sorbitol, glycine, glutamate, α-ketoglutarate, fructose, malate and acetate are used as carbon and energy sources but lactose, butyrate, valerate, L-tyrosine and *m*-hydroxybenzoate are not. The major fatty acids (>10% of total fatty acids) are C_{16:0}, C_{16:1}ω7c and C_{18:1}ω7c.

The type strain is IVIA-Po-14b^T (=CECT 7730^T =NCIMB 14671^T). The DNA G+C content of the type strain is 46.2 mol%.

Description of *Marinomonas rhizomae* sp. nov.

Marinomonas rhizomae (rhi.zo'ma.e. N.L. n. *rhizoma* (from Gr. n. *rhizôma*) a rhizome; N.L. gen. n. *rhizomae* of a

rhizome, the part of the plant *Posidonia oceanica* from which the type strain was isolated).

Cells are curved rods, motile with a single polar flagellum, Gram-reaction-negative, strictly aerobic, catalase-positive and oxidase-negative. Organic growth factors are not required. Requires Na⁺ for growth. Grows in 0.5–10% (w/v) NaCl and at 5–25 °C. Negative for amylase, agarase, lipase and protease (gelatin) activities. Glucose, maltose, sucrose, sorbitol, L-tyrosine, glutamate, α-ketoglutarate, fructose, malate and acetate are used as carbon and energy sources but galactose, lactose, butyrate, valerate, glycine and *m*-hydroxybenzoate are not. The major fatty acids (>10% of total fatty acids) are C_{16:0}, C_{16:1}ω7c and C_{18:1}ω7c.

The type strain is IVIA-Po-145^T (=CECT 7377^T =NCIMB 14431^T). The DNA G+C content of the type strain is 45.5 mol%.

Description of *Marinomonas foliarum* sp. nov.

Marinomonas foliarum (fo.li.a'rum. L. gen. pl. n. *foliarum* of leaves, the part of the plant *Posidonia oceanica* from which the type strain was isolated).

Cells are curved rods, motile with a single polar flagellum, Gram-reaction-negative, strictly aerobic, catalase-positive and oxidase-negative. Organic growth factors are not required. Requires Na⁺ for growth. Grows in 0.5–10% (w/v) NaCl and at 5–25 °C. Negative for amylase, agarase, lipase and protease (gelatin) activities. Glucose, maltose, galactose, sucrose, sorbitol, glutamate, α-ketoglutarate, fructose, malate and acetate are used as carbon and energy sources but lactose, butyrate, valerate, tyrosine, glycine and *m*-hydroxybenzoate are not. The major fatty acids (>10% of total fatty acids) are C_{16:0}, C_{16:1}ω7c and C_{18:1}ω7c.

The type strain is IVIA-Po-155^T (=CECT 7731^T =NCIMB 14672^T). The DNA G+C content of the type strain is 46.2 mol%.

Description of *Marinomonas posidonica* sp. nov.

Marinomonas posidonica (po.si.do'ni.ca. N.L. fem. adj. *posidonica* relating to the genus name *Posidonia*, of *Posidonia oceanica*, the plant from which the type strain was isolated).

Cells are helical, motile with a single polar flagellum, Gram-reaction-negative, strictly aerobic, catalase-positive and oxidase-negative. Organic growth factors are not required. Requires Na⁺ for growth. Grows in 0.5–10% NaCl and at 5–25 °C. Negative for amylase, agarase, lipase and protease (gelatin) activities. Glucose, maltose, galactose, sucrose, sorbitol, glutamate, α-ketoglutarate, fructose, malate and acetate are used as carbon and energy sources but lactose, butyrate, valerate, tyrosine and *m*-hydroxybenzoate are not. The major fatty acids (>10% of total fatty acids) are C_{10:0} 3-OH, C_{16:0}, C_{16:1}ω7c and C_{18:1}ω7c.

The type strain is IVIA-Po-181^T (=CECT 7376^T =NCIMB 14433^T). The DNA G+C content of the type strain is 44.7 mol%.

Description of *Marinomonas aquiplantarum* sp. nov.

Marinomonas aquiplantarum (a.qui.plan.ta'rum. L. fem. n. aqua water; L. fem. n. planta plant; N.L. gen. pl. n. aquiplantarum of aquatic plants, referring to *Posidonia oceanica*).

Cells are helical, motile with polar flagella at one or both poles of the cells, Gram-reaction-negative, strictly aerobic, catalase-positive and oxidase-negative. Organic growth factors are not required. Requires Na⁺ for growth. Grows in 0.5–10% (w/v) NaCl and at 5–37 °C. Negative for amylase, agarase and protease (gelatin) activities. Lipase (Tween 80) activity is variable with some strains showing a low activity. Glucose, maltose, galactose, sucrose, sorbitol, glutamate, α -ketoglutarate, fructose, malate and acetate are used as carbon and energy sources but lactose, butyrate, valerate, tyrosine and *m*-hydroxybenzoate are not. The major fatty acids (>10% of total fatty acids) are C_{10:0} 3-OH, C_{12:1} 3-OH, C_{16:0}, C_{16:1} ω 7c and C_{18:1} ω 7c.

The type strain is IVIA-Po-159^T (=CECT 7732^T =NCIMB 14673^T). The DNA G+C content of the type strain is 47.8 mol%.

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References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Chang, H. W., Roh, S. W., Kim, K. H., Nam, Y. D., Yoon, J. H., Oh, H. M. & Bae, J. W. (2008). *Marinomonas basaltis* sp. nov., a marine bacterium isolated from black sand. *Int J Syst Evol Microbiol* **58**, 2743–2747.

Chimetto, L. A., Cleenwerck, I., Brocchi, M., Willems, A., De Vos, P. & Thompson, F. L. (2011). *Marinomonas brasiliensis* sp. nov., isolated from the coral *Mussismilia hispida*, and reclassification of *Marinomonas basaltis* as a later heterotypic synonym of *Marinomonas communis*. *Int J Syst Evol Microbiol* **61**, 1170–1175.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Espinosa, E., Marco-Noales, E., Gómez, D., Lucas-Elío, P., Ordax, M., Garcías-Bonet, N., Duarte, C. M. & Sanchez-Amat, A. (2010). Taxonomic study of *Marinomonas* strains isolated from the seagrass *Posidonia oceanica*, with descriptions of *Marinomonas balearica* sp. nov. and *Marinomonas pollencensis* sp. nov. *Int J Syst Evol Microbiol* **60**, 93–98.

Gupta, P., Chaturvedi, P., Pradhan, S., Delille, D. & Shivaji, S. (2006). *Marinomonas polaris* sp. nov., a psychrotolerant strain isolated from coastal sea water off the subantarctic Kerguelen islands. *Int J Syst Evol Microbiol* **56**, 361–364.

HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Ivanova, E. P., Onyshchenko, O. M., Christen, R., Lysenko, A. M., Zhukova, N. V., Shevchenko, L. S. & Kiprianova, E. A. (2005). *Marinomonas pontica* sp. nov., isolated from the Black Sea. *Int J Syst Evol Microbiol* **55**, 275–279.

Johnson, J. L. (1994). Similarity analysis of DNAs. In *Methods for General and Molecular Bacteriology*, pp. 655–682. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Kumura, K., Minamishima, Y., Yamamoto, S., Ohashi, N. & Tamura, A. (1991). DNA base composition of *Rickettsia tsutsugamushi* determined by reversed-phase high-performance liquid chromatography. *Int J Syst Bacteriol* **41**, 247–248.

Lau, K. W. K., Ren, J. P., Wai, N. L. M., Lau, S. C. L., Qian, P. Y., Wong, P. K. & Wu, M. (2006). *Marinomonas ostreistagni* sp. nov., isolated from a pearl-oyster culture pond in Sanya, Hainan Province, China. *Int J Syst Evol Microbiol* **56**, 2271–2275.

Macián, M. C., Arahál, D. R., Garay, E. & Pujalte, M. J. (2005). *Marinomonas aquamarina* sp. nov., isolated from oysters and seawater. *Syst Appl Microbiol* **28**, 145–150.

Martínez-Murcia, A. J., Antón, A. I. & Rodríguez-Valera, F. (1999). Patterns of sequence variation in two regions of the 16S rRNA multigene family of *Escherichia coli*. *Int J Syst Bacteriol* **49**, 601–610.

Prabakaran, S. R., Suresh, K., Manorama, R., Delille, D. & Shivaji, S. (2005). *Marinomonas ushuaiensis* sp. nov., isolated from coastal sea water in Ushuaia, Argentina, sub-Antarctica. *Int J Syst Evol Microbiol* **55**, 309–313.

Romanenko, L. A., Uchino, M., Mikhailov, V. V., Zhukova, N. V. & Uchimura, T. (2003). *Marinomonas primoryensis* sp. nov., a novel psychrophile isolated from coastal sea-ice in the Sea of Japan. *Int J Syst Evol Microbiol* **53**, 829–832.

Romanenko, L. A., Tanaka, N. & Frolova, G. M. (2009). *Marinomonas arenicola* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **59**, 2834–2838.

Sanchez-Amat, A. & Solano, F. (2005). Genus *Marinomonas* Van Landschoot and De Ley 1984, 91 ^{VP} (Effective publication Van Landschoot and De Ley 1983, 3071). In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 284–289. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Solano, F. & Sanchez-Amat, A. (1999). Studies on the phylogenetic relationships of melanogenic marine bacteria: proposal of *Marinomonas mediterranea* sp. nov. *Int J Syst Bacteriol* **49**, 1241–1246.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Van Landschoot, A. & De Ley, J. (1983). Intra- and intergenic similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen.

nov.) and some other Gram-negative bacteria. *J Gen Microbiol* **129**, 3057–3074.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Yoon, J. H., Kang, S. J. & Oh, T. K. (2005). *Marinomonas dokdonensis* sp. nov., isolated from sea water. *Int J Syst Evol Microbiol* **55**, 2303–2307.

Zhang, D. C., Li, H. R., Xin, Y. H., Liu, H. C., Chen, B., Chi, Z. M., Zhou, P. J. & Yu, Y. (2008). *Marinomonas arctica* sp. nov., a psychrotolerant bacterium isolated from the Arctic. *Int J Syst Evol Microbiol* **58**, 1715–1718.