Nereida ignava gen. nov., sp. nov., a novel aerobic marine α -proteobacterium that is closely related to uncultured *Prionitis* (alga) gall symbionts

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A Gram-negative, slightly halophilic, non-pigmented, strictly aerobic, chemo-organotrophic bacterium was isolated from Mediterranean sea water off the Spanish coast near Valencia. This strain was poorly reactive, being unable to grow in most carbon sources analysed in minimal medium. However, good growth was observed when more complex media and longer incubation times were used. Phylogenetic analysis based on an almost complete 16S rRNA gene sequence placed strain 2SM4^T within the *Roseobacter* group, in the vicinity of uncultured bacteria described as gall symbionts of several species of the red alga *Prionitis*. Sequence similarity values between strain 2SM4^T and the closest neighbouring species were below 95.0%. The cellular fatty acid composition of the Mediterranean strain confirmed its position within the 'Alphaproteobacteria', sharing 18:1 ω 7c as the major cellular fatty acid. The phylogenetic distance from any taxon with a validly published name and also a number of distinguishing features support the designation of strain 2SM4^T as representing a novel genus and species, for which the name *Nereida ignava* gen. nov., sp. nov. is proposed. The type strain is 2SM4^T (=CECT 5292^T=DSM 16309^T=CIP 108404^T=CCUG 49433^T).

The *Roseobacter* group classified within the order 'Rhodobacterales' in the last release of the *Taxonomic Outline of the Prokaryotes* (Garrity *et al.*, 2003) includes bacteria from a large number of marine environments (a Mediterranean coastal lagoon, an Atlantic Ocean central gyre, the Black Sea, the North Sea and Antarctic sea ice, among others) that have diverse types of metabolism (phototrophy, aerobic sulfite oxidation, organic sulfur compound degradation and lignin degradation) (Benlloch *et al.*, 1995; Bowman *et al.*, 1997; Gonzalez *et al.*, 1997; Gosink & Staley, 1995; Wagner-Döbler *et al.*, 2004). Thus it is clear that these bacteria play an important role in carbon, sulfur and nitrogen cycling

(Gonzalez *et al.*, 1999, 2000). The *Roseobacter* group also includes a number of environmental clones of uncertain biological significance. Several of these clones have been found consistently in geographically distant sites around the oceans of the world; to date, most remain uncultured and without cultured neighbours. The use of innovative culturing methods has allowed the cultivation of various α -proteobacteria, such as '*Pelagibacter ubique*' of the SAR11 clade (Rappé *et al.*, 2002).

Several bacteria belonging to the *Roseobacter* group have been described as epiphytes or symbionts of marine organisms such as bryozoans and algae (micro- and macroalgae). Nevertheless, the impact of such associations remains to be determined. In the red algal genus *Prionitis*, gall formation is known to be induced by bacteria phylogenetically related to the *Roseobacter* group (Ashen & Goff, 1996, 1998). It is well known that bacterial gall formation is species specific, and coevolution between the red algal hosts and their associated bacteria has been reported (Ashen & Goff, 2000). However, the physiological

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Nereida ignava* CECT 5292^T is AJ748748.

Electron micrographs of strain 2SM4^T and a table of differential oxidation profiles on Biolog GN plates are available as supplementary material in IJSEM Online.

function of these galls has not been determined, and the bacteria have not been cultivated as pure cultures.

The present study reports on one bacterium, strain 2SM4^T, isolated from Mediterranean sea water, that is phylogenetically related, in terms of 16S rRNA gene sequences, to the gall symbionts. Strain 2SM4^T was obtained from sea water surrounding cultivated oysters 2–3 miles off the Mediterranean coast near Valencia (Spain). In a previous phenotypic study, it clustered in phenon 40 (Ortigosa *et al.*, 1994) together with other unreactive marine bacteria, and remained unidentified.

Cultures were maintained on marine agar (MA; Difco) slants at room temperature and as suspensions in marine broth 2216 (MB; Difco) plus 10 % (v/v) glycerol at –80 °C. They were routinely grown at 24–26 °C on MA or MB. Most of the phenotypic characterization methods were performed as previously described (Macián *et al.*, 2001). The organism was unable to ferment sugars under anaerobic conditions, as determined on anaerobic Hugh & Leifson O/F medium (Difco) with half-strength artificial sea water (ASW) (400 mM NaCl, 100 mM MgSO₄·7H₂O, 20 mM KCl and 20 mM CaCl₂·H₂O; Baumann & Baumann, 1981). Strain 2SM4^T exhibited catalase and oxidase activities. It did not reduce nitrate to nitrite in nitrate broth and it was also unable to grow in the denitrification medium of Baumann (Baumann & Baumann, 1981). Thus, it is a strict aerobe. Strain 2SM4^T was mesophilic, growing from 13 to 28 °C, but not at 4 or 37 °C on solid medium (MA).

Cells grew on MA as unpigmented, regular, opaque colonies. Colonies were pinpoints less than 1 mm in diameter after 48 h incubation. The cells neither swarmed nor luminesced. Strain 2SM4^T required sea water-based media for growth, being unable to grow in salt tolerance agar (1 %, w/v, tryptone; 0.3 %, w/v, yeast extract; 1.5 %, w/v, agar) with the addition of Na⁺, K⁺, Mg²⁺ and Ca²⁺ ions either as chlorides or with the substitution of MgCl₂ by MgSO₄, but showed good growth on MB and MA. The salinity range supporting growth on MA, diluted or supplemented with NaCl as reported in Macián *et al.* (2005), was between 1.36 and 8 % (w/v) total salts. Thus, strain 2SM4^T is a slight halophile with complex ionic requirements similar to those of the recently described species *Thalassobacter stenotrophicus* (Macián *et al.*, 2005).

Isolate 2SM4^T was negative for the following enzymic activities: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, H₂S production from thiosulfate, indole production from tryptophan and sulfite oxidation (Gonzalez *et al.*, 1999). No hydrolytic activities were detected on the following substrates: casein, gelatin, starch, alginate, agar, DNA, Tween 80 and lecithin. These tests were performed in media supplemented with marine salts (MA or half-strength ASW). DNase agar and Tween 80 agar, supplemented with half-strength ASW, and MB plus 12 % (w/v) gelatin did not support growth of strain 2SM4^T.

Utilization of several compounds as sole carbon and energy sources was analysed on basal medium agar [BMA: 50 mM Tris/HCl, pH 7.5; 19 mM NH₄Cl; 0.33 mM K₂HPO₄·3H₂O; 0.1 mM FeSO₄·7H₂O and 1.3 % (w/v) purified agar (Oxoid) on half-strength ASW; Baumann & Baumann, 1981]. The BMA was supplemented with 0.1 g yeast extract l⁻¹ since the organism was originally reported as being unable to grow on minimal medium (Ortigosa *et al.*, 1994). Carbohydrates were added at 2 g l⁻¹ whereas the remaining compounds were added at 1 g l⁻¹. Cells were incubated for 30 days. Positive control plates were prepared with 5 g yeast extract l⁻¹ whereas negative control plates consisted of BMA plus 0.1 g yeast extract l⁻¹. Growth was scored as negative when growth was equal to, or less than, that in the negative control plates. In general, strain 2SM4^T utilized few substrates and was slowly reactive, since all positive results were recorded after 14 days incubation; even then, the amount of cell mass obtained was scarce in many instances. Detailed results of the nutritional tests are listed in the species description.

The substrate-oxidation profile was obtained by using the Biolog system according to the recommendations of the manufacturer, except for the preparation of the inoculum. Strain 2SM4^T was grown for 2–3 days on MA, since it did not grow on Biolog Universal growth medium plus blood, and the inoculating fluid was half-strength ASW. The plate was incubated at 28 °C for 2 days. Positive oxidation was obtained only with maltose; borderline reactions were observed with adonitol, lactulose, D-mannitol, D-raffinose, turanose, γ -hydroxybutyric acid, α -ketovaleric acid, L-alaninamide, DL-carnitine, inosine and 2-aminoethanol. This behaviour is in agreement with the slow growth and narrow utilization pattern of strain 2SM4^T with regard to sole carbon and energy sources in BMA.

Cell morphology and motility were examined by using optical, scanning electron and transmission electron microscopy. Cells grown on MA for 2–3 days were suspended in half-strength ASW and observed on wet mounts by using phase-contrast microscopy (using Leica MBRD apparatus). In parallel, cell suspension was adsorbed onto Isopore (Millipore) membrane filters, dehydrated with a graded series of ethanol [50, 80 and 100 % (v/v) ethanol], critical-point dried with CO₂ (Autosamdri-814; Tousimis) and sputter-coated (using Bio-Rad apparatus) with a gold-palladium film to a thickness of approximately 10 nm. Samples were examined in a Hitachi S-4100 field emission scanning microscope with 7–15 mm working distance and at an acceleration voltage of 10 kV. Pictures were stored digitally and processed using EMIP 3.0. A drop of cell suspension was deposited on a grid and examined in a JEOL JEM-1010 transmission electron microscope at 60 kV after negative staining with 2 % (w/v) phosphotungstic acid. Young (48 h) pure cultures of strain 2SM4^T showed a variety of cell morphologies, ranging from coccoid cells to regular rods, tear-shaped cells with polar buds and even very elongated (filamentous) cells (Fig. 1). Cell

Fig. 1. Scanning electron micrographs showing the characteristic variations in cell morphology of strain 2SM4^T. Modes of cell division can also be discerned. Additional pictures are available as supplementary figures in IJSEM Online. Bars, 2.5 µm (a) and 1 µm (b, c).

division by budding was observed in several cells, yielding almost coccoid forms, but binary fission could also be detected (Fig. 1). On wet mounts, cells were non-motile; flagella were not observed after cell staining with the method of Heimbrogue *et al.* (1989) or by electron microscopy (see supplementary figure available in IJSEM Online). Bright granules were never observed in the cells, suggesting that poly-β-hydroxybutyrate and gas vesicles are not formed by strain 2SM4^T.

The cellular fatty acid composition of strain 2SM4^T, determined by GLC at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) by using a previously described method (Kämpfer & Kroppenstedt, 1996), confirmed the affiliation of the Mediterranean isolate to the *Roseobacter* group. In total, only seven fatty acids were detected. The major fatty acid was 18:1ω7c (81.4%), followed by 18:0 (6%), 16:0 (5%), 11-methyl 18:1ω7c (3.3%), 10:0 3-OH (1.9%) and 20:1ω7c (0.8%). In addition, a fatty acid (1.5%) was present that could be identified only as either 19:1ω6c or 19:0 cyclo.

The G+C content (mol%) was determined by HPLC at the DSMZ, according to the procedure of Mesbah *et al.* (1989). The value for strain 2SM4^T was 56 mol%.

The 16S rRNA gene phylogeny of strain 2SM4^T was investigated by using procedures that have been reported previously (Macián *et al.*, 2001). Almost full-length 16S

Fig. 2. Maximum-likelihood tree, as implemented in the ARB program package (Ludwig *et al.*, 2004), based on the 16S rRNA gene sequences of *N. ignava* gen. nov., sp. nov. 2SM4^T and its closest relatives. For reconstruction of the tree, more than 100 almost complete sequences from type strains of species within the order 'Rhodobacterales' were included and later removed to simplify the view. Bootstrap values above 50% are shown at the nodes; percentages are based on 1000 resamplings. Bar, 5% sequence divergence.

rRNA gene sequences were obtained using a LiCor automated sequencer (MWG Biotech) and subsequently analysed using the ARB software package (Ludwig *et al.*, 2004). Automated sequence alignments were inspected by eye and corrected manually using the sequence editor ARB_EDIT. According to the recommendations of Ludwig *et al.* (1998), alternative treeing methods (maximum parsimony, maximum likelihood and distance matrix) and data subsets were employed, using the appropriate ARB tools to test the robustness of local topologies. Comparative analyses of the sequence obtained in this study established that strain 2SM4^T was phylogenetically most closely related to uncultured gall symbionts of the algal genus *Prionitis*, which clustered together as shown in Fig. 2. The 16S rRNA gene sequence relatedness between strain 2SM4^T and the *Prionitis* gall symbionts ranged from 94.9 to 95.5%. With respect to established species, the most similar 16S rRNA gene sequence was that of *Sulfitobacter pontiacus* (95.0%

sequence similarity). In addition to the large evolutionary distance that can be inferred from this value, this organism clustered in all trees examined separately from strain 2SM4^T (Fig. 2). Other species belonging to the 'Alphaproteobacteria' showed even lower levels of relatedness.

Phenotypic and genetic traits that distinguish isolate 2SM4^T from its phylogenetic neighbours within the *Roseobacter* clade are summarized in Table 1. Differential oxidation profiles on Biolog GN plates are available in a supplementary table in IJSEM Online. All these results support the description of a novel genus and species in the 'Alphaproteobacteria', for which we propose the name *Nereida ignava* gen. nov., sp. nov.

Description of *Nereida* gen. nov.

Nereida [Ne.re.i'da. L. fem. n. *Nereida* (= L. fem. n. *Nereis*) a Nereid, a sea nymph, referring to the habitat of the bacteria].

Table 1. Phenotypic characteristics that differentiate *N. ignava* gen. nov., sp. nov. from related species

Species: 1, *N. ignava*; 2, *Oceanibulbus indolifex*; 3, *Roseobacter denitrificans*; 4, *Roseobacter litoralis*; 5, *Staleyia guttiformis*; 6, *Sulfitobacter brevis*; 7, *Sulfitobacter delicatus*; 8, *Sulfitobacter dubius*; 9, *Sulfitobacter mediterraneus*; 10, *Sulfitobacter pontiacus*. Symbols: +, only positive results reported; –, only negative results reported; W, weak; ND, not determined; NG, no growth. Data are from Ivanova *et al.* (2004), Labrenz *et al.* (2000), Pukall *et al.* (1999), Wagner-Döbler *et al.* (2004) and this study.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell morphology	Coccus to club-shaped rod	Irregular rod	Ovoid cells to rods	Ovoid cells to rods	Rod	Rod	Rod	Rod	Rod	Rod
Pigment*	N	N	P	P	P†	N	N	N	N	N
Motility	–	–	+	+	+	+	–	+	+	+
Flagella	–	–	+	+	+	–	–	+	+	+
NO ₃ →NO ₂	–	–	+	–	+	–	W	+	–	+
Salinity range (% w/v)	1.4–8	1–7	ND	ND	<1–4	<1–8	1–8	1–12	0.2–8	0.5–8
Growth with only Na ⁺ added	–	+	–	–	+	+	+	+	+	+
Growth with Na ⁺ , K ⁺ , Mg ²⁺ and Ca ²⁺	–	+	+	+	+	+	+	+	+	+
Growth at:										
4 °C	–	–	+	V	–	+	–	–	+	+
37 °C	–	–	–	–	–	–	+	–	–	–
Amylase	–	–	–	–	–	–	–	–	+	+
Gelatinase	NG	–	+	+	–	–	+	+	+	–
Alginase	–	ND	–	–	–	–	+	–	–	–
Tween 80 hydrolysis	NG	–	+	+	+	+	+	–	+	+
Sulfite oxidation	–	ND	ND	ND	–	–	+	+	+	+
Utilization of:										
Melibiose	–	–	ND	ND	W	+	ND	ND	–	W
Glycerol	–	+	ND	ND	+	ND	ND	ND	+	ND
L-Glutamate	–	+	+	+	+	+	ND	ND	+	ND
L-α-Alanine	–	+	ND	ND	+	ND	ND	ND	+	+
L-Aspartate	–	+	ND	ND	+	ND	ND	ND	W	ND
Fatty acids (%):										
18:0	6	–	1.3	1.2	<1	<1	–	–	<1	<1
18:1ω7c 11-methyl	3.3	–	–	–	–	–	–	–	–	–
G + C content (mol%)	56	60.1	59.6	56.3–58.1	55–56.3	58	60	63.7	59	62.1

*P, Pink; N, none (colonies may appear cream, whitish, beige or brown in colour).

†Delayed.

Gram-negative, strictly aerobic, chemo-organotrophic, slightly halophilic bacteria. Oxidase- and catalase-positive. Cells are coccoid to elongated rods, most of them being tear-shaped. Usually 0.2–0.3 µm in width by 1–3 µm in length; non-motile. Cells show polar budding. Slightly halophilic; no growth can be obtained without sea water or the addition of combined marine salts to the medium. Mesophilic. Neither gas vesicles nor poly-β-hydroxybutyrate accumulation is observed. Does not ferment carbohydrates and is unable to reduce nitrate to nitrite or N₂. The main cellular fatty acids include 18:1ω7c, 18:0 and 16:0. The genus is affiliated to the ‘*Alphaproteobacteria*’, order ‘*Rhodobacterales*’, and so far contains only one species, *Nereida ignava*, which is the type species. The DNA G + C content of the type species is 56 mol%.

Description of *Nereida ignava* sp. nov.

Nereida ignava (ig.na'va. L. fem. adj. *ignava* lazy).

In addition to the characteristics that define the genus, the species has the characteristics described below. It does not swarm or luminesce. Requires at least 1.36 % (w/v) marine salts and tolerates up to 8 % (w/v) salts, failing to grow at 9 % (w/v). Positive growth at 13 and 28 °C. No growth detected at 4, 37 or 40 °C. Does not hydrolyse casein, starch, lecithin, alginate or agar. Does not grow in gelatin, DNA or Tween 80. Produces negative results in tests for arginine dihydrolase, lysine and ornithine decarboxylase, H₂S production from thiosulfate, indole production from tryptophan and sulfite oxidation. Utilizes the following compounds as carbon and energy sources after 14 days incubation, provided that the medium is supplemented with 0.01 % (w/v) yeast extract (an indication that undetermined growth factors are required): maltose, succinate, fumarate, malate, lactate and sarcosine. In the same conditions, it grows weakly on D-glucose, D-mannose, D-mannitol, pyruvate, propionate, acetate, citrate, α-ketoglutarate, L-serine, L-ornithine, γ-aminobutyric acid and citrulline. The following substrates are not used: D-ribose, D-fructose, L-arabinose, D-xylose, D-galactose, D-trehalose, L-rhamnose, cellobiose, sucrose, lactose, D-melibiose, salicin, amygdalin, D-gluconate, D-glucuronate, D-galacturonate, N-acetylglucosamine, glycerol, D-sorbitol, meso-inositol, glycerate, cis-aconitate, DL-β-hydroxybutyrate, p-hydroxybenzoate, D-saccharic acid, glycine, L-leucine, L-arginine, L-alanine, L-glutamate, L-threonine, L-aspartate, L-lysine, L-tyrosine, L-histidine and putrescine.

The type strain, 2SM4^T (= CECT 5292^T = DSM 16309^T = CIP 108404^T = CCUG 49433^T), was isolated from Mediterranean sea water off Valencia (Spain).

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Ciencia y Tecnología. D. R. A. has a contract with the Universitat de València under the Ramón y Cajal program (Ministerio de Ciencia y Tecnología).

References

- Ashen, J. B. & Goff, L. J. (1996). Molecular identification of a bacterium associated with gall formation in the marine red alga *Prionitis lanceolata*. *J Phycol* **32**, 286–297.
- Ashen, J. B. & Goff, L. J. (1998). Galls on the marine red alga *Prionitis lanceolata* (Halymeniaceae): specific induction and subsequent development of an algal-bacterial symbiosis. *Am J Bot* **85**, 1710–1721.
- Ashen, J. B. & Goff, L. J. (2000). Molecular and ecological evidence for species specificity and coevolution in a group of marine algal-bacterial symbioses. *Appl Environ Microbiol* **66**, 3024–3030.
- Baumann, P. & Baumann, L. (1981). The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In *The Prokaryotes*, vol. II, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. Schlegel. Berlin: Springer.
- Benlloch, S., Rodríguez-Valera, F. & Martínez-Murcia, A. J. (1995). Bacterial diversity in two coastal lagoons deduced from 16S rDNA PCR amplification and partial sequencing. *FEMS Microbiol Ecol* **18**, 267–279.
- Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.
- Garrity, G. M., Bell, J. A. & Lilburn, T. G. (2003). Taxonomic outline of the procaryotes. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, release 4.0. New York: Springer. doi:10.1007/bergesoutline200310
- Gonzalez, 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.
- Gonzalez, J. M., Kiene, R. P. & Moran, M. A. (1999). Transformation of sulfur compounds by an abundant lineage of marine bacteria in the α-subclass of the class *Proteobacteria*. *Appl Environ Microbiol* **65**, 3810–3819.
- Gonzalez, J. M., Simo, R., Massana, R., Covert, J. S., Casamayor, E. O., Pedros-Alio, C. & Moran, M. A. (2000). Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. *Appl Environ Microbiol* **66**, 4237–4246.
- Gosink, J. J. & Staley, J. T. (1995). Biodiversity of gas vacuolate bacteria from Antarctic Sea ice and water. *Appl Environ Microbiol* **61**, 3486–3489.
- Heimbrook, M. E., Wang, W. L. L. & Campbell, G. (1989). Staining bacterial flagella easily. *J Clin Microbiol* **27**, 2612–2615.
- Ivanova, E. P., Gorshkova, N. M., Sawabe, T. & 8 other authors (2004). *Sulfitobacter delicatus* sp. nov. and *Sulfitobacter dubius* sp. nov., respectively from a starfish (*Stellaster equestris*) and sea grass (*Zostera marina*). *Int J Syst Evol Microbiol* **54**, 475–480.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Labrenz, M., Tindall, B. J., Lawson, P. A., Collins, M. D., Schumann, P. & Hirsch, P. (2000). *Staleyella 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.

Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M. & Schleifer, K.-H. (1998). Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis* **19**, 554–568.

Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Macián, M. C., Ludwig, W., Schleifer, K. H., Garay, E. & Pujalte, M. J. (2001). *Thalassomonas viridans* gen. nov., sp. nov., a novel marine γ -proteobacterium. *Int J Syst Evol Microbiol* **51**, 1283–1289.

Macián, M. C., Arahál, D. R., Garay, E., Ludwig, W., Schleifer, K. H. & Pujalte, M. J. (2005). *Thalassobacter stenotrophicus* gen. nov., sp. nov., a novel marine α -proteobacterium isolated from Mediterranean sea water. *Int J Syst Evol Microbiol* **55**, 105–110.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Ortigosa, M., Garay, E. & Pujalte, M. J. (1994). Numerical taxonomy of aerobic, Gram-negative bacteria associated with oysters and surrounding seawater of the Mediterranean coast. *Syst Appl Microbiol* **17**, 589–600.

Pukall, R., Buntetuß, 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.

Rappé, M. S., Connon, S. A., Vergin, K. L. & Giovannoni, S. J. (2002). Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418**, 630–633.

Wagner-Döbler, I., Rheims, H., Felske, A., El-Ghezal, A., Flade-Schröder, D., Laatsch, H., Lang, S., Pukall, R. & Tindall, B. J. (2004). *Oceanibulbus indolifex* gen. nov., sp. nov., a North Sea alpha-proteobacterium that produces bioactive metabolites. *Int J Syst Evol Microbiol* **54**, 1177–1184.