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Roseimaritima ulvae gen. nov., sp. nov. and *Rubripirellula obstinata* gen. nov., sp. nov. two novel planctomycetes isolated from the epiphytic community of macroalgae

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Running title: *Roseimaritima ulvae* gen. nov., sp. nov and *Rubripirellula obstinata* gen. nov., sp. nov.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains UC8^T, UF3, UF42 and LF1^T are HQ845508, HQ845532, HQ845534 and DQ986201, respectively.

Abstract

Four isolates, belonging to the deep-branching phylum *Planctomycetes*, were recovered from the biofilm of two marine macroalgae, *Ulva* sp. and *Laminaria* sp., from the Northern coast of Portugal. These strains were light pink- or red-pigmented; the cells were variable in shape and usually organized in rosettes. They had a dimorphic cell cycle with budding reproduction. The organisms were chemoheterotrophic, strictly aerobic and mesophilic. The 16S rRNA gene sequence analysis showed that the strains belong to the family *Planctomycetaceae* with *Rhodopirellula* as the closest genus. The isolates form two separate branches (strain LF1^T forms one branch and the strains UC8^T, UF3 and UF42 form a second branch) clearly separated from *Rhodopirellula baltica* with 94.2% and 93.8% 16S rRNA gene sequence similarity, respectively.

Based on differential characteristics that distinguish the novel genera from *R. baltica*, such as cell size and shape, ultrastructure, enzymatic activities, substrate utilization pattern, fatty acid composition, phospholipid profiles and phylogeny we propose that the isolates represent two novel genera of the order *Planctomycetales*, *Roseimaritima ulvae* gen. nov., sp. nov. (type strain is UC8^T =DSM 25454^T=LMG 27778^T) and *Rubripirellula obstinata* gen. nov., sp. nov. (type strain is LF1^T = LMG 27779^T=CECT 8602^T).

Keywords: New taxa, *Planctomycetes*, macroalgae, biofilm

Scope of the paper: Taxonomic paper

The *Planctomycetes* represent a deep-branching group of *Bacteria* phylogenetically related to the phyla *Verrucomicrobia*, *Chlamydiae* and *Lentisphaerae* [25]. Several characteristics of the planctomycetes are unique in prokaryotic organisms; these include absence of peptidoglycan, a proteinaceous cell wall and compartmentalized cell structure [8]. In general, they are metabolically diverse and widespread. The *Planctomycetes* comprise the order *Planctomycetales,* with eleven genera that comprise fifteen species described [2, 11, 12, 26], the order *Phycisphaerales* comprising one genus with only one species [9] and the '*Candidatus* Brocadiales' with five candidate genera [10].

In recent years, several studies reported the association of planctomycetes with marine macroalgae [7, 13]. The kelp *Laminaria hyperborean* possess a biofilm community dominated by these bacteria [1]. A new order of *Planctomycetes,* the *Phycisphaerales*, was proposed to include a novel isolate obtained from the surface of a *Porphyra* sp. [9]. Isolation of epiphytic planctomycetes of macroalgae reveals a great phylogenetic diversity [13]. Here, we describe two novel species based on four isolates designated UC8^T, UF3, UF42 and LF1^T from the epiphytic bacterial community of *Ulva* sp. and *Laminaria* sp..

The strains described in this study were isolated by Lage and Bondoso [13] at Carreço (UC8^T from *Ulva* sp.) and Porto (UF3 and UF42 from an *Ulva* sp. and LF1^T from a *Laminaria* sp.). Strain UC8^T was isolated from the aqueous extract of *Ulva* sp. on modified M629 agar (5 g l^{-1} peptone, 0.5 g l^{-1} yeast extract, 20 ml l^{-1} Hutner's basal salts, 10 ml l^{-1} vitamin solution in 90% natural seawater – NSW). Strains UF3 and UF42 were isolated on modified M629 broth inoculated with pieces of *Ulva* sp. and strain LF1^T was isolated in modified M13 broth (0.25 g l^{-1} peptone, 0.25 g l^{-1} yeast extract, 0.25 g l^{-1} glucose, 20 ml l^{-1} Hutner's basal salts, 10 ml l^{-1} vitamin solution in 90% natural seawater – NSW buffered with 5 mM Tris-HCl, pH 7.5) inoculated with portions of *Laminaria* sp. Strains were routinely maintained in modified M13 at 26 ºC (LF1^T) or 30 ºC (UC8^T, UF3 and UF42), in the dark. For long term storage, isolates were stored at -80 ºC in sterile natural seawater with 20% (w/v) glycerol. Unless stated otherwise, morphological, biochemical and physiological tests were performed at 26 ºC for LF1^T and 30 ºC for UC8^T, UF3 and UF42 in M13 modified media with 1.6% agar or in liquid cultures (200 rpm). The

type strain of *Rhodopirellula baltica* SH1^T(DSM 10527^T), obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, was used for comparative purposes.

Cell morphology and motility were observed by optical microscopy during the exponential phase of growth. For scanning electron microscopy (SEM), exponentially growing cells were fixed in 2.5% glutaraldehyde in Marine buffer, pH 7.0 [27] for 2.5 h, dehydrated through a graded ethanol series, critical point dried and observed with a HITACHI S-570. For transmission electron microscopy (TEM), cells were harvested from 5 day old plates and cryopreserved as described before [2].

Growth temperature range was examined from 5 to 45 ºC in 5 ºC intervals and NaCl tolerance was determined in artificial sea water based media ASW; [15] supplemented with NaCl up to concentrations ranging from 3.5 to 10%. Minimal and maximum salinity requirement for growth was examined in ASW-based media with increasing proportions of ASW ranging from 0-50% and 90-300% (100% ASW corresponds to 34.5‰ salinity). The requirement for seawater salts was performed by replacing the ASW by 3.3% of NaCl in distilled water. The pH range for growth was determined in liquid media using 10 mM of each of the following buffers: MES for pH 4.5, 5.0, 5.5, 6.0 and 6.5, Tris-HCl for pH 7.5 and 8.5, CHES for pH 9.0, 10.0, 10.5 and CAPS for pH 11.0. Vitamin requirement was determined in M20c medium [20] prepared with 90% natural seawater to which different vitamin solutions (1% [v/v] omitting one vitamin at a time) were added. Results were considered positive after two transfers in the same medium.

Hydrolysis of starch, casein, elastin, alginate and carboxymethyl-cellulose, oxidase and catalase activities were determined using standard methods [21, 24]. Cellulase activity was detected by the liquefaction of carboxymethyl-cellulose based medium around the colonies. Other enzymatic activities were evaluated with the API ZYM and API 20NE systems (bioMérieux) according to manufacturer's instructions with the exception that cell suspensions were prepared in 20‰ marine salts [16] and results were recorded after 10 days for API 20 NE and 48 h for API ZYM. Carbon metabolism was studied by oxidation (BIOLOG GN2 MicroPlate), acidification (API 50 CH) and assimilation of carbon sources by conventional methods (basal medium supplemented with each carbon source). Single-carbon source assimilation tests were determined for the type strains UC8^T and LF1^T in 20 ml screw capped tubes

containing 7 ml of liquid medium composed of 90% NSW buffered with 5 mM Tris-HCl, pH 7.5, to which filter-sterilized Hutner's basal salts (20 ml l^{-1}), $NH_4(SO4)_2$ (0.5 g l^{-1}), Na_2HPO_4 (0.05 g l^{-1}), vitamin solution (10 ml l^{-1}) and the filter-sterilized carbon source (1.0 g l^{-1}) were added. Growth was evaluated by measuring the turbidity of the cultures at 600 nm. To inoculate GN2 MicroPlate test strips, liquid cultures were grown for 48h, centrifuged and resuspended in sterile 0.75% ASW without $CaCl_2$ [17] and results were examined after 10 days. API 50 CH system was inoculated with 48h-liquid cultures resuspended in glucose-free medium M13 prepared with 50% ASW and supplemented with 0.01 g l^{-1} phenol red. Results were recorded after 60 h of incubation. Nitrogen sources utilization was determined in medium containing 90% NSW, glucose (0.5 g l^{-1}), Na_2HPO_4 (0.05 g l^{-1}), Hutner's basal salts (20 ml l^{-1}) and vitamin solution (10 ml l^{-1}), buffered with 5 mM Tris-HCl pH 7.5 and supplemented with 1.0 g l^{-1} of each of the twenty natural amino acids, as well as peptone, yeast extract, casamino acids, N-acetyl-glucosamine (NAG), urea, ammonium, nitrate or nitrite. Turbidity of the cultures was measured at 600 nm. Anaerobic growth was tested in anaerobic chambers (GENbox anaer; bioMérieux) and results were recorded after three weeks.

Cultures for chemotaxonomic analysis were grown in liquid modified M13 medium until late exponential phase at 26 ºC for all the strains. Cells were harvested by centrifugation and washed in Tris-HCl 0.1 M, pH 7.5 before subsequent analyses. Polar lipids were extracted from freeze-dried cells as described previously [6]. Individual polar lipids were separated by two-dimensional thin-layer chromatography (TLC) and visualized as described previously [6]. Respiratory quinones were extracted from freeze-dried cells, purified by TLC and separated by HPLC as described by da Costa et al. [4]. Fatty acid methyl esters (FAMEs) were obtained from fresh wet biomass, separated, identified and quantified with the standard MIS Library Generation Software (Microbial ID Inc.) as described previously [5].

For G+C content, DNA was isolated as described before [19] and the content was determined by HPLC [18]. The almost complete 16S rRNA gene of $UC8^T$, UF3, UF42 and $LF1^T$ was amplified and analysed as described by Bondoso et al. [13]. Sequences were assembled with Vector NTI (Invitrogen), manually examined and aligned with closely related sequences from NCBI database with ClustalW [23]. Phylogenetic trees were generated in MEGA version 5.03 [22] using different

calculation methods including neighbour joining, maximum parsimony and maximum likelihood to test for the stability of the tree.

BOX-PCR and ERIC-PCR of strains UC8^T, UF3 and UF42 were performed as described, respectively, by Winkelman et al. [28] and by Lage et al. [14] using 100 ng of genomic DNA per 25 µl PCR reaction. Fingerprinting profiles were visualised after separation by electrophoresis in a 2% agarose gel at 60V for 90 min in Tris–acetate–EDTA buffer. The gel was post-stained with ethidium bromide for 45 min and gel images were acquired in a GE Typhoon gel scanner.

Insert Figure 1

Colonies of strains UC8^T, UF3, UF42 and LF1^T were circular, small, convex, translucent and light pink or almost red in LF1^T on M13 agar. Strains UC8^T, UF3 and UF42 attached to surfaces when grown in liquid media as is seen with other planctomycetes [2]. Cells of strains UC8^T, UF3 and UF42 were circular to ovoid and never pear-shaped like *R. baltica*, and organized in rosettes of large numbers of cells (Fig 1a). Cell size was also smaller than that of *R. baltica*. LF1^T cells were ovoid to pear-shaped, usually organized in rosettes of 3 up to 10 cells (Fig 1c). All isolates reproduced by budding (Fig. 1).

SEM observation of UC8^T and LF1^T revealed the presence of fimbriae (Fig. 1b, d) and a flagellum on the reproductive pole of the cells. TEM of the strains UC8^T and LF1^T confirmed the distinct morphological differences between the new strains and the closest species, *R. baltica*, although the characteristic *Planctomycetes* cell structure was present (Fig. 2). Cells had fimbriae in the apical and reproductive pole and the holdfast was present on the opposite pole. Crateriform pits on the cell surface, typical of *Planctomycetes,* were distributed in the reproductive pole. Compartimentalization was seen in the paryphoplasm and the pirellulosome, the latter with ribosomes, storage inclusions and condensed DNA forming a visible nucleoid. UC8^T cells had an extensive paryphoplasm with granular appearance and a small pirellulosome while the paryphoplasm is fibrillar and the pirellulosome is more prominent in LF1^T. The pirellulosome was normally close to the apical pole. Strain LF1^T possessed a robust holdfast and the cells were surrounded by a thick cell wall and a glycocalyx, characteristics that differentiate it from *R. baltica*. Some LF1^T cells had hump-like protrusions similar to those seen in *Pirellula staleyi* ATCC 35122 [3].

Insert Figure 2

Insert Table 1

Optimum growth temperature was about 30 ºC for the isolates represented by strain UC8^T and 25 ºC for strain LF1^T. All strains required seawater for growth. The temperature, salinity tolerance and pH ranges are shown in Table 1. Differential characteristics between strains, including the type strain of *R. baltica* with API 50CH, Biolog GN2 and API ZYM are shown in Table 2. Members of the UC8^T group oxidized twenty (of 28) carbohydrates in the BIOLOG GN2 and produced acid from thirty-four of 49 carbohydrates in the API 50 CH. Strain LF1^T oxidized only 10 substrates in the Biolog GN2 and produced acid from only 20 carbohydrates. *R. baltica* SH1^T was the strain that oxidized more carbohydrates (22 in the BIOLOG GN and 40 in the API 50CH). All strains examined used carbohydrates as single carbon and energy sources but did not assimilate the amino acids or organic acids.

Strains UC8^T, UF3 and UF42 were cytochrome oxidase and catalase positive and LF1^T was cytochrome oxidase positive and catalase negative. Strains UC8^T, UF3 and UF42 were able to hydrolyse esculin, starch but not urea, gelatine, alginate, casein and elastin.Strain LF1^T only hydrolysed starch. Nitrate was reduced to nitrite by all isolates.

The strains utilised several amino acids and proteinaceous supplements as sources of nitrogen. Nitrate and ammonium were also utilized but urea was not. Nitrite was only utilised by strain UC8^T. In contrast to the majority of the other planctomycetes described, LF1^T was unable to utilize NAG at 1 % (w/v) concentration (Table 2). Of the vitamins tested, all strains required vitamin B12. Anaerobic growth was not observed.

Insert Table 2

The polar lipid composition of strains UC8^T, LF1^T and *R. baltica* SH1^T by TLC indicated that diphosphatidylglycerol (DPG) was one of the major polar lipids of UC8^T but was absent from LF1^T (Fig. S1). An aminophospholipid and unknown lipids 3 and 4 were only present in *R. baltica* SH1^T and phospholipid 2 was only present in LF1^T. The major respiratory lipoquinone of all planctomyces examined was menaquinone 6 (MK-6) as is usual in this phylum. The fatty acid composition of strains UC8^T, UF3 and UF42 showed a predominance of $C_{18:1}\omega 9c$ and $C_{16:0}$ which accounted for about 43-48% and 33-35% of the total fatty acids, respectively (Table S1). The major fatty acid of strain LF1^T was $C_{18:1}\omega 9c$ (42%) with smaller amounts of $C_{16:0}$ (17%) and $C_{17:1}\omega 8c$ (12%). These profiles were distinct from that of *R. baltica* SH1^T, which had predominantly $C_{18:1}\omega 9c$ (43%), $C_{16:0}$ (27%) and summed feature 3

($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) (18%). All new strains had $C_{18:0}$ 3-OH and $C_{20:1}\omega9c$ fatty acids which were absent in *R. baltica* SH1^T.

Insert Figure 3

Phylogenetic analysis of the nearly complete 16S rRNA gene showed the affiliation of UC8^T, UF3, UF42 and LF1^T to the *Planctomycetes*, with *R. baltica* SH1^T as the most closely related organism (Fig. 3). The phylogenetic distance of the 16S rRNA gene between members of the UC8^T group and LF1^T was around 4 %. The 16S rRNA gene sequence similarity was 93.6% between the UC8^T group and *R. baltica* SH1^T and 94.2% between strain LF1^T and *R. baltica* SH1^T. Strains belonging to the UC8^T group shared 100% pairwise sequence similarity among isolates and were most closely related (99%) to an uncultured bacterial clone SBS-FW-053 isolated from the biofilm of seawater reverse osmosis (SWRO) membranes. The closest cultured planctomycete to this group was *Pirellula* sp. 158, isolated from the Kiel Fjord, with 97.0% sequence similarity. Strain LF1^T had no cultured relatives; the closest being an uncultured clone from marine sediments and from the sponge *Astrosclera willeyana* with 95.0% 16S rRNA gene sequence similarity. Phylogenetic analysis using the neighbour-joining, maximum-likelihood and parsimony methods showed that both groups form two branches in a separate cluster from *Rhodopirellula baltica* (Fig. 3).

Fingerprinting profiles with ERIC and BOX primers revealed that isolates UC8^T and UF42 were genetically identical (Fig. S2), even though they were isolated from different sites but from the same species of macroalga. Although similar, strain UF3 had a different profile from UC8^T and UF42.

The mole G+C content of the DNA of group UC8^T and LF1^T was 57-59% and 56.1%, respectively, which were higher than the mole G+C content of *R. baltica* SH1^T (54.1%).

Group UC8^T forms a cluster phylogenetically apart from *Rhodopirellula baltica* and strain LF1^T with more than 6% dissimilarity in the 16S rRNA gene sequence, which is indicative of a novel genus within the *Planctomycetes*. Furthermore, it possesses morphological and chemotaxonomic characteristics that clearly distinguish it from the most closely related genera. UC8^T forms small spherical-shape cells with a different ultrastructure compared to the pear-shaped cells characteristics of the PRB group. The phospholipid profile of UC8^T is similar to that of *R. baltica* SH1^T, but diphosphatidylglycerol (DPG) is one of the major phospholipids of UC8^T which is a minor component in *R. baltica* SH1^T. Furthermore, there are two additional major

unknown lipids in the type strain of *R. baltica* that are not present in UC8^T. UC8^T possesses higher amounts of C16:0 fatty acid (35%) and lower amounts of C16:1 ω7c and/or C16:1 ω6c (2.4%) compared to *R. baltica* SH1^T (26.8% and 17.9%). Besides, UC8^T possesses C18:0 3-OH and C 20:1 ω9c fatty acids that are absent in *R. baltica* SH1^T.

Isolate LF1^T shows 6% difference in the 16S rRNA gene to *R. baltica* SH1^T, which once again is indicative of a novel genus. Other characteristic that distinguishes strain LF1^T from *Rhodopirellula* includes a distinct phospholipid profile from that of *R. baltica* SH1^T with undetectable diphosphatidylglycerol as well as other lipids present in the new organism. The fatty acid profile of both strains are also distinct; there are substantial difference in the levels of C16:0 (17%) in LF1^T and (28%) in *R. baltica* SH1^T; cyclo-C19:0 ω10c/19 ω6, C20:1 ω9c and C20:0 are absent in *Rhodopirellula* and UC8^T but are present in strain LF1^T. Other striking difference in LF1^T is very restricted carbon sources utilization when compared to *Rhodopirellula* and UC8^T. Also, LF1^T is not able to utilize NAG as carbon and nitrogen sources, a common feature among planctomycetes.

Based on the distinctive morphological, metabolic and chemotaxonomic characteristics that discriminate the two novel isolates from *Rhodopirellula*, we are of the opinion that the organism represent two new species of two novel genera for which we propose the name *Roseimaritima ulvae* gen. nov., sp. nov. (UC8^T) and *Rubripirellula obstinata* gen. nov., sp. nov. (LF1^T).

Description of *Roseimaritima* gen. nov.

Roseimaritima (*ro.se.i.ma.ri'ti.ma*. L. adj. roseus, rose-coloured; L. adj. *maritimus -a -um*, of the sea, marine; N.L. fem. n. *Roseimaritima*, the marine rose-coloured bacterium.)

Cells have peptidoglycan-less cell walls, form primarily spherical but some cells are ovoid, form rosettes of variable number of cells. Colonies are light pink. Crateriform pits at the reproductive pole and holdfast in the opposite pole. Stalks are absent. Reproduce by budding. Chemoheterotrophic, strictly aerobic, catalase- and cytochrome oxidase- positive. The major respiratory quinone is MK-6. The major fatty acids are $C_{18:1}$ω9c and $C_{16:0}$. The predominant polar lipids are phosphatidylcholine, phosphatidylglycerol and diphosphatidylglycerol. This genus is

a member of the family *Planctomycetaceae*. The type species is *Roseimaritima ulvae.*

Description of *Roseimaritima ulvae* sp. nov.

Roseimaritima ulvae (ul'va.e. N.L. gen. n. *ulvae*, of *Ulva*, the generic name of the host alga, *Ulva* sp. - the source of isolation). Cells are spherical to ovoid with 1.1-1.8 x 0.9-1.5 µm in diameter, possess a dimorphic life cycle with a motile phase. Colonies in M13 medium are translucent and smooth. Optimum growth temperature is about 30 ºC (temperature range is between 15 ºC and 35 ºC), pH optimum is about 7.5 (pH range is from 6.5 to 10). Requires sea salts for growth with a minimum of 20%-25% of ASW salinity. The maximum salinity for growth is 175% ASW. Vitamin B12 is required for growth. Major fatty acids are $C_{18:1}\omega 9c$ (43-48%) and $C_{16:0}$ (33-35%). Starch, esculin and carboxymethyl cellulose are degraded; urea, casein, elastin and alginate are not. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamilase, valine arylamidase, cysteine arylamidase, acid phosphatase, α-glucosidase and α-galactosidase are positive in the API ZYM; other activities are negative. Indole is not produced. Arginine dihydrolase is absent. Nitrate is reduced to nitrite. N-acetylglucosamine, D-galactose, L-rhamnose, D-arabinose, D-glucose, xylose, sucrose, maltose, lactose, D-cellobiose, D-trehalose, D-mannitol, lactulose, dextran are assimilated. Amino acids, casamino acids, D-fructose, L-fucose, D-ribose, L-sorbose, D-raffinose, ribitol, sorbitol, *myo*-inositol, erythritol, D-arabitol, glycerol, succinate, α-ketoglutarate, malate, citrate, benzoate, fumarate, formate, acetate, pyruvate and inulin are not assimilated. Acid is produced from D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-mannitol, methyl-α-D-mannopyranoside, methyl-αD-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, D-raffinose, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose and potassium 5-ketogluconate in the API 50 CH. Oxidizes N-acetylglucosamine, N-acetylgalactosamine, cellobiose, D-fructose, L-fucose, D-galactose, gentibiose, D-glucose, α-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melobiose, β-methylglucoside, D-raffinose, L-rhamnose, sucrose, D-trehalose, turanose, acetic acid, gluconic acid, D-glucuronic acid, D,L-lactic acid, succinic acid, glycerol, glucoronamide, glucose-1-phosphate in the

BIOLOG GN2 System. Aspartate, arginine, glutamine, threonine, peptone, yeast extract, casamino acids, NAG, ammonia, nitrate and nitrite serve as sources of nitrogen. Urea is not utilized.

The G+C mole content of the DNA of the type strain is 57.0 ± 0.6% (HPLC method).

The type strain is UC8^T (=DSM 25454=LMG 27778) isolated from the epiphytic community of *Ulva* sp.

Description of *Rubripirellula* gen. nov.

Rubripirellula (Ru.bri.pi.rel'lul.a. L. adj. *ruber -bra -brum*, red; N.L. fem. n. *Pirellula* name of a bacterial genus; N.L. fem. n. *Rubripirellula*, red-colored *Pirellula*).

Colonies are red colored and cells are pear-shaped to ovoid forming rosettes; cells do not possess peptidoglycan in their cell wall. Crateriform pits in the reproductive pole and holfast in the opposite pole. Reproduce by budding. Chemoheterotrophic with restricted carbohydrate utilization, strictly aerobic, catalase positive and cytochrome oxidase negative. The major respiratory quinone is MK-6. The major fatty acid is $C_{18:1}\omega$9c. The predominant polar lipids are phosphatidylcholine and phosphatidylglycerol. This genus is a member of family *Planctomycetaceae*. The type species is *Rubripirellula obstinata.*

Description of *Rubripirellula obstinata* sp. nov.

Rubripirellula obstinata (ob.sti.na'ta; L. fem. adj. *obstinata*, stubborn, obstinate due to an inconstant growth)

Cells are pear-shaped to ovoid with 1.5-2.0 x 1.3-1.7 µm, possess a dimorphic life cycle with a motile phase. Cells are surrounded by a glycocalyx. Colonies in M13 are translucent and smooth. Optimum growth temperature is about 25 ºC (temperature range for growth is between 10 ºC and 30 ºC); the optimum pH for growth is about 7.5 (pH range for growth is from 6.5 to 10). Requires sea salts for growth and a minimum salinity (ASW) of 50%. Maximum salinity for growth is 125% ASW. Vitamin B12 is required. Major fatty acids are $C_{18:1}\omega$9c (42%), $C_{16:0}$ (17%) and $C_{17:1}$ ω8*c* (12%). Starch is degraded; esculin, cellulose, urea, casein, elastin and alginate are not degraded. Indole is not produced. Arginine dihydrolase is absent.

Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamilase, valine arylamidase, cystine arylamidase, acid phosphatase are positive in API ZYM;

other activities are negative. Nitrate is reduced to nitrite. D-fructose, D-galactose, L-rhamnose, D-glucose and xylose are assimilated. Amino acids, NAG, casamino acids, L-fucose, D-ribose, D-arabinose, L-sorbose, sucrose, maltose, lactose, D-cellobiose, D-trehalose, lactulose, D-raffinose, D-mannitol, dextran, ribitol, sorbitol, *myo*-inositol, erythritol, D-arabitol, glycerol, succinate, α-ketoglutarate, malate, citrate, benzoate, fumarate, formate, acetate, pyruvate and inulin are not assimilated. Acid is produced from D-xylose, L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, methyl-αD-glucopyranoside, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, gentiobiose, D-lyxose and potassium 5-ketogluconate in the API 50 CH.

The organism oxidizes D-cellobiose, D- fructose, D-galactose, gentibiose, D-glucose, α-lactose, lactulose, maltose, D-mannose, L-rhamnose, acetic acid, D-glucuronic acid, D,L-lactic acid and inosine in BIOLOG GN2 System. Aspartate, arginine, peptone, yeast extract, casamino acids, ammonia, nitrate serve as sources of nitrogen. Urea, NAG and nitrite are not utilized.

The G+C mole content of the DNA is 56.1 ± 0.2% (HPLC method).

The type strain is LF1^T (=LMG 27779=CECT 8602) isolated from the epiphytic community of *Laminaria* sp.

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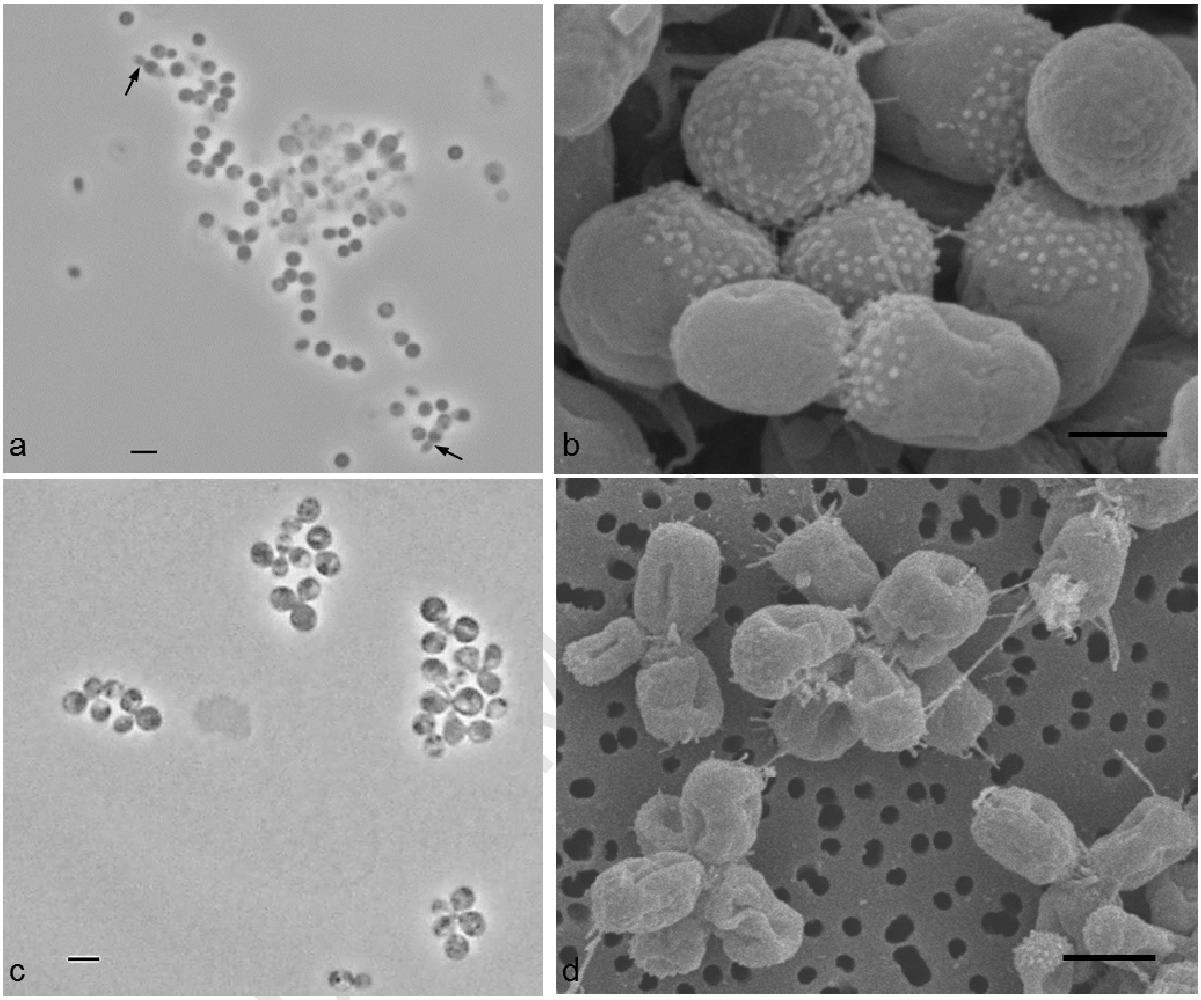

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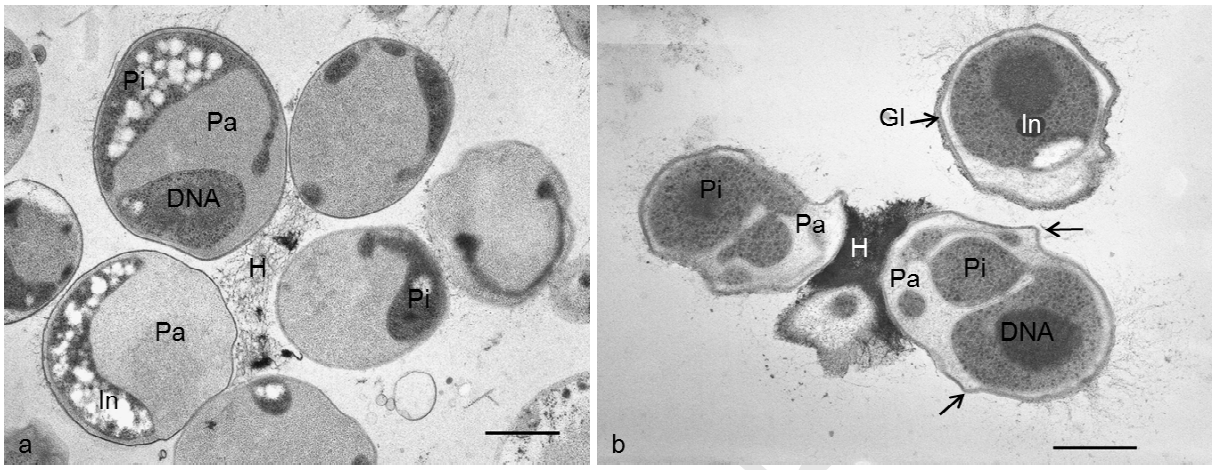

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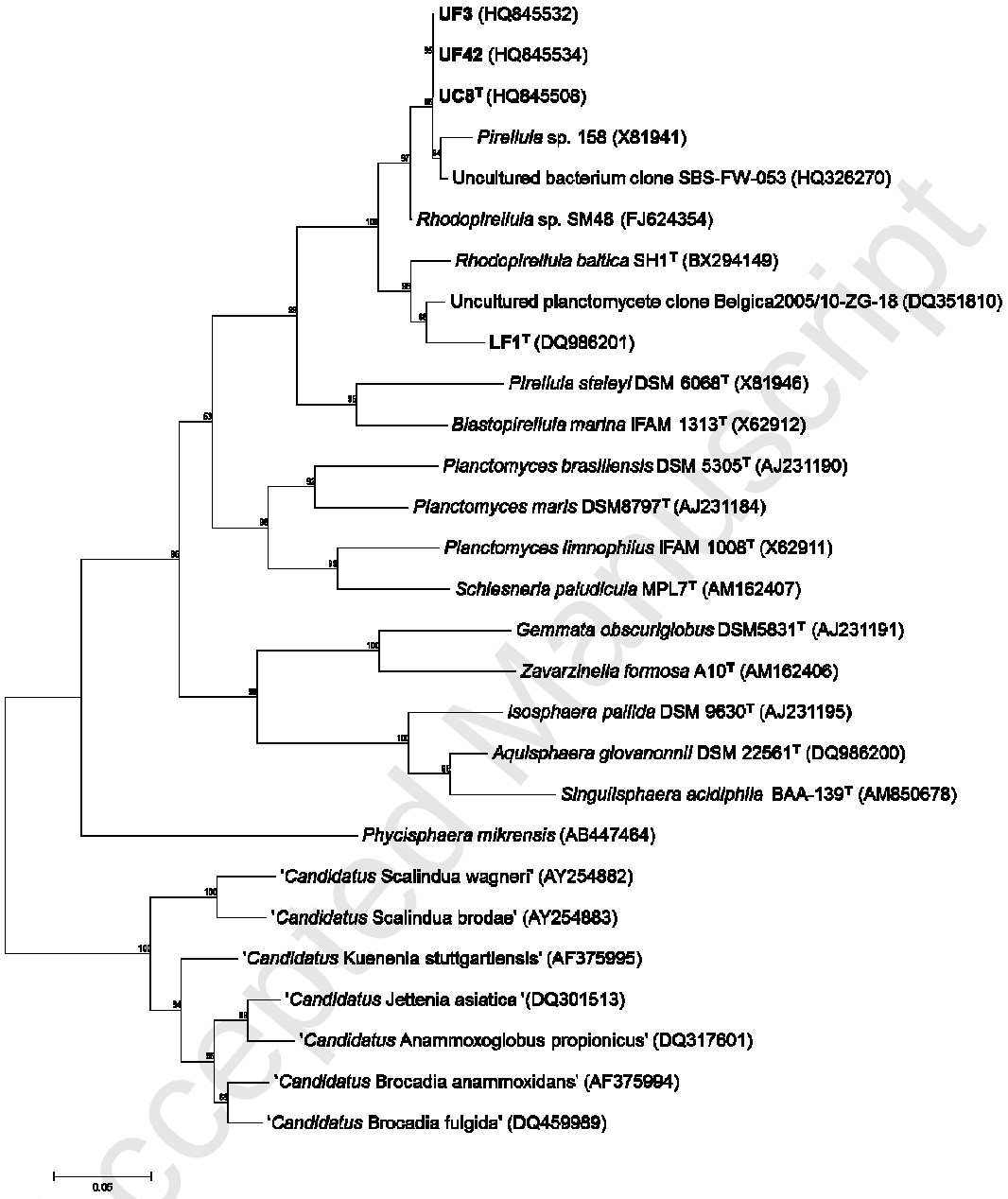

Figure 3.

Legends

Figure 1. Morphological characteristics of strains UC8^T and LF1^T; (a and c) show phase-contrast and (b and d) show electron microscopy. Cells of strain UC8^T are spherical to ovoid, with budding (arrows) and the insertion of fimbrae in the reproductive pole (b) are observed. Cells of strain LF1^T are ovoid to pear shaped, usually organized in rosettes. Cells are attached by the holdfast (d). Bars - a) and c) 2 µm; b) 0.5 µm; d) 1 µm.

Figure 2. Transmission electron microscopy of strains UC8^T (a) and LF1^T (b). Typical planctomycete cell plan divided into the paryphoplasm (Pa) and the pirellulosome (Pi) is observed. Cells are bound by the holdfast (H) and LF1^T is surrounded by a glycocalyx (Gl). Inclusions (In) and condensed DNA can be seen. LF1^T presents hump-like protusions (arrows). Bars – 0.5 µm.

Figure 3. Optimal maximum likelihood 16S rRNA gene phylogenetic tree showing the relationships between strains UC8^T, UF3, UF42 and LF1^T and other representatives of the phylum *Planctomycetes* (accession numbers are shown in parenthesis). The tree was based on the Jukes-Cantor model. Numbers on the tree refer to bootstrap values based on 1000 replicates. Only values above 50% are shown. *"Candidatus"* genera Anammox were used as outgroup. Bar - 0.05 substitutions per nucleotide position.

Table 1. Differential characteristics of the two novel genera *Roseimaritima*, *Rubripirellula* and the closest genus *Rhodopirellula*.

	Roseimaritima	***Rubripirellula***	***Rhodopirellula***
Cell size (µm)	1.1-1.8 x 0.9-1.5	1.5-2 x 1.3-1.7	1.0–2.5x1-2–2-3[a]
Cell shape	Spherical to ovoid	Pear-shaped to ovoid	Pear-shaped to ovoid[a]
Cell arrangement	Rosettes 20-40 cells	Rosettes 2-10 cells	Rosettes of variable number of cells
Pigmentation	Light Pink	Red	Pink to Red[a]
Salinity tolerance range:			
ASW range	20-175%	50-125%	10-175%
Maximum NaCl (% w/v)	5%	4%	5%
Temperature for growth (ºC):			
Range	15-35	10-30	5-30
Optimum	30	25	28[a]
pH range	6.5-10	7.5-10.5	5.5-10.5
Carbon sources			
NAG	+	-	+
Ribose	-	-	+
Raffinose	+	-	-
Arabinose	+	-	-
Sucrose	+	-	+
Maltose	+	-	+

Lactose	+	-	+
Trehalose	+	-	+
Mannitol	+	-	-
Lactulose	+	-	-
Dextran	+	-	-
Nitrogen sources			
Alanine	-	-	+
Asparagine	-	-	+
Phenylalanine	-	-	+
Threonine	+	-	-
Proline	+	-	+
NAG	+	-	+
Nitrite	+	-	+
Catalase	+	-	+
FAMEs			
Summed feature 3[b]	2.4	8.4	17.9
$C_{16:0}$	35.1	17.3	26.8
$C_{17:1}\omega 8c$	2.0	12.0	5.1
Polar lipids			
Diphosphatidylglycerol	++	-	+

543 +, positive; -, negative; ++, strongly positive

544 All the strains assimilated galactose, rhamnose, glucose and xylose. None of the strains utilize
545 casamino acids, fucose, sorbose, raffinose, ribitol, sorbitol, m-inositol, erithritol, arabitol, glycerol,
546 succinate, ketoglutarate, malate, pyruvate, citrate, acetate, benzoate, fumarate, formate and inulin.
547 All the strains utilized aspartate, arginine, glutamine, casamino acids, yeast extract, peptone and
548 ammonium as nitrogen sources. The following were not utilized: gluconate, glutamate, cysteine,
549 cystine, guanine, histidine, lysine, methionine, ornithine, serine, tyrosine, tryptophan, valine and
550 urea.

551 [a]Data from Schlesner et al. [19].

552 [b]summed feature 3 comprises $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c.

553 **Table 2.** Differential characteristics of strains UC8^T, UF3, UF42, LF1^T and *R. baltica*
554 SH1^T in API 50CH, API ZYM and Biolog GN2.

	UC8^T	**UF3**	**UF42**	**LF1^T**	***R. baltica* SH1^T**
API 50CH					
D-arabinose	+	+	+	-	+
L-arabinose	+	+	+	-	+
D-ribose	+	+	+	-	+
L-rhamnose	+	+	-	-	+
Methyl-ßD-xylopyranoside	-	-	-	-	+
D-mannitol	+	+	+	-	+
Methyl-ßD-Mannopyranoside	w	+	w	-	+
Amygdalin	w	+	+	-	+
D-melibiose	+	+	+	-	+
D-saccharose (sucrose)	+	+	+	-	+
D-trehalose	+	+	+	-	+
D-melezitose	-	+	-	-	+
D-raffinose	w	+	w	-	+
xylitol	-	+	-	-	+
D-turanose	+	+	+	-	+
D-tagatose	+	+	+	-	+
D-fucose	+	+	+	-	+
L-fucose	+	+	+	-	+
D-arabitol	-	w	-	-	-
L-arabitol	-	w	-	-	+

Potassium 2-ketogluconate	w	-	-	-	+
API ZYM					
α-galactosidase	+	+	+	-	+
ß -galactosidase	-	-	+	-	+
α -glucosidase	+	+	+	-	+
ß-glucosidase	-	-	-	-	+
N-acetyl-ß-glucosaminidase	-	-	-	-	+
Trypsin	-	-	+	-	+
Biolog GN					
Dextrin	-	-	-	-	+
Glycogen	-	+	-	-	-
N-Acetyl-DGalactosamine	+	+	+	-	+
N-Acetyl-DGlucosamine	+	+	+	-	+
L-Arabinose	-	-	+	-	+
D-Arabitol	-	-	+	-	-
L-Fucose	+	+	+	-	+
D-Mannitol	+	+	+	-	+
D-Melibiose	+	+	+	-	+
β-Methyl-D-Glucoside	+	+	+	-	+
D-Psicose	+	+	-	-	-
L-Rhamnose	-	+	-	+	+
D-Sorbitol	-	+	-	-	-
Sucrose	+	+	+	-	+
D-Trehalose	+	+	+	-	+
Turanose	+	+	+	-	+

Pyruvic Acid Methyl Ester	-	-	-	-	+
Succinic Acid Mono-Methyl-Ester	-	-	-	-	+
Acetic Acid	+	-	-	+	+
D-Galacturonic acid	-	+	-	-	-
D-Gluconic acid	+	+	+	-	+
D-Glucosaminic acid	+	-	-	-	-
Succinic Acid	+	-	+	-	-
Propionic Acid	-	-	+	-	-
Glucuronamide	+	+	+	-	+
L-Glutamic Acid	-	+	+	-	-
Inosine	-	-	-	+	-
Glycerol	+	+	+	-	+
D,L-α-Glycerol Phosphate	-	+	+	-	-
α-D-Glucose-Phosphate	+	+	+	-	-

555 +, positive; -, negative; w, weakly positive.

556 All the strains produced acid from D-xylose, L-xylose, D-galactose, D-glucose, D-frutose, D-mannose, D-
557 sorbose, D-rhamnose, methyl-αD-glucopyranoside, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose,
558 gentiobiose, D-lyxose and potassium 5-ketogluconate. Acid was not produced from glycerol, erythritol, D-
559 adonitol, dulcitol, inositol, inulin, starch, glycogen and potassium gluconate.
560 All the strains were positive for alkaline phosphatase, esterase C4, esterase lipase C8, leucine, valine and
561 cystine arylamidase, acid phosphatase. All the strains were negative for lípase C14, α-chymotrypsin, naphthol-
562 AS-BI-phosphohydrolase, ß-glucuronidase, α-mannosidase, α-fucosidase.
563 All the strains oxidized D-cellobiose, D-frutose, D-galactose, gentiobiose, α-D-Glucose, α-D-lactose, lactulose,
564 maltose, D-mannose, D-glucuronic acid, D,L-lactic acid. None of the strains oxidized tween 40 and 80, adonitol,
565 erythritol, m-inositol, xylitol, Cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, α-hydroxybutyric
566 acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxy-phenylacetic acid, itaconic acid, α-keto butyric
567 acid, α-keto glutaric acid, α-keto valeric acid, malonic acid, quinic acid, D-saccharic acid, sebacic Acid,
568 succinamic ccid, L-alaninamide, D-alanine, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, glycyl-L-
569 aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-
570 proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, D,L-carnitine, uridine, thymidine, phenyethylamine,
571 putrescine, 2-aminoethanol, 2,3-butanediol, D-glucose-6-phosphate.
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