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NOTE

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Mariniblastus fucicola gen. nov., sp. nov. a novel planctomycete associated with macroalgae

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Abstract

One strain of a novel genus and species of the order *Planctomycetes*, designated FC18^T, was isolated from the epiphytic community of *Fucusspiralis*. This strain was non-pigmented in medium M13 but was slightly pink pigmented on medium M14, containing four-fold the levels of glucose, peptone and yeast extract of M13. The organism was primarily spherical, with unicellular non-motile forms and rosettes. The optimal temperature for growth was about 25 °C and the optimal pH was 7.5. FC18^T was chemoorganotrophic and aerobic. Several sugars, polyols and amino acids were assimilated. The major fatty acids were $C_{18:1}\omega9c$, $C_{14:0}$ and $C_{16:0}$. The major polar lipids were phosphatidylglycerol (PG) and two unknown lipids. Menaquinone 5 (MK-5) was the main respiratory quinone, but MK-6 was also present. The results of the 16S rRNA gene sequence analysis confirmed the affiliation of this organism to the order *Planctomycetales*, family *Planctomycetaceae*, with *Blastopirellula marina* as the closest relative with only 86 % sequence similarity. On the basis of physiological, biochemical and chemotaxonomic characteristics we propose that FC18^T(=LMG 29748^T=DSM 26290^T) represents a novel species of a novel genus of the family *Planctomycetaceae* for which we propose the name *Mariniblastusfucicola* gen. nov., sp. nov.

Results of recent studies have indicated that planctomycetes are commonly associated with macroalgae [1–3]. These organisms can reach up to 51 % of the total bacterial community in the kelp *Laminaria hyperborea* [4] and seem to be widespread in other macroalgae [5]. Macroalgal surfaces are rich habitats for the planctomycetes that possess a large number of sulfatase genes [6], which give them the potential for the degradation of the sulphated polymers produced by the algae. The planctomycete characterized herein was isolated from the epiphytic community of *Fucus spiralis* from the North coast of Portugal, being the only cultured representative from this algal species. However, close relatives have been found in high abundance in 16S rRNA gene clone libraries from *Laminaria hyperborea* (72–97.8 %) [4] and *Fucus vesiculosus* [7], in denaturing gradient gel electrophoresis (DGGE) analysis of *Chondrus crispus*, *Fucus spiralis*, *Porphyra dioica*, *Sargassum muticum* and a species of the genus *Ulva* [3] and in the phycosphere of *Enteromorpha prolifera* Chu *et al.*, (unpublished).

An organism, designated FC18^T, was isolated using HEPPSO-buffered M13 medium, from the microbial biofilm of the marine macroalga *Fucus spiralis* sampled in Carreço (41°44′ N 81°52′ W) along the northern coast of Portugal [5]. Cultures were routinely maintained in M13 at 26 °C in the dark. The type strains of *Blastopirellula marina* (DSM 3645^T) and *Rhodopirellula baltica* (DSM 10527^T) were used for comparison.

Morphology was observed by optical microscopy and transmission electron microscopy (TEM) during the exponential phase of growth in M13 medium. For negative staining, 1 or 4 % uranyl acetate was used. For TEM, cells were fixed in 2.5 % (w/v) glutaraldehyde in marine buffer (pH 7.2) for 2 h and post-fixed in 1 % (v/v) osmium tetroxide in the same buffer for 4 h followed by 1 % uranyl acetate for 1 h. Cells were dehydrated through a graded ethanol series, followed by propylene oxide and embedded in Epon resin. Ultrathin sections were stained for 10 min in 1 % (v/v) uranyl acetate and for 10 min in Reynolds lead citrate. The sections were examined using a 100CXII transmission electron microscope (JEOL).

Unless otherwise stated, all physiological tests were performed in triplicate, in M13 medium at 25 °C and with shaking (200 r.p.m.). Medium M14 was occasionally used

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Abbreviations: DGGE, denaturing gradient gel electrophoresis; TEM, transmission electron microscopy.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain FC18^T is HQ845450.
One supplementary figure is available with the online Supplementary Material.

which, compared with M13, contains a four-fold concentration of glucose, peptone and yeast extract. Results were recorded after seven days visually or by measuring the OD_{600}. The growth temperature was tested in 5 °C increments ranging from 5 to 45 °C. The pH range for growth was determined in Falcon tubes using 20 mM MES for pH 5.5 and 6.5, Tris-HCl for pH 7.5, HEPES for 8.5, CHES for pH 9.5 and CAPS for pH 10.5. Growth was tested in natural seawater (34.5 % salinity) without dilution or with dilutions of 7, 14, 21, 28, 35 and 90 %. Growth in medium M13 with NaCl was performed by replacing natural seawater with distilled water and adding increasing amounts of NaCl ranging from 0 to 3.15 % (90 % seawater salinity). Growth at higher salinities was assessed in M13 medium prepared with artificial sea water [8] supplemented with different concentrations of NaCl ranging from 0 to 9 %. Vitamin requirement was assessed by testing FC18^T on M20c medium [9] prepared with 0.1 % casamino acids and 0.25 % glucose and supplemented or not with different vitamin solutions. Results were observed after two transfers. The effects of antibiotics [tetracycline and streptomycin (1, 10, 100 and 1000 µg ml^{-1}) and gentamicin sulfate salt hydrate and rifampicin (1, 10 and 100 µg ml^{-1})] were tested by their incorporation in M13 agar medium and incubation with 10 µl of strain suspension.

Assimilation of single-carbon sources was examined in liquid medium composed of 90 % natural sea water (NSW) buffered with 5 mM Tris–HCl, pH 7.5, 0.05 % $NH_4(SO4)_2$ and 0.05 % Na_2HPO_4 supplemented with filter-sterilized Hutner's basal salts (DSMZ medium 603) (20 ml l^{-1}), vitamin solution (DSMZ medium 603) (10 ml l^{-1}) and 0.1 % (w/v) of the carbon source. For the API ZYM and API 20NE systems, inoculation of the test strips was done as described by the manufacturer except that the cells were resuspended in 2 % marine salts (Sigma) [10] and results were observed after 10 days for API 20NE and 48 h for API ZYM. Hydrolysis of gelatin, aesculin, arbutin, starch, xylan, Tween 20 and 80 were determined using standard methods [11, 12].

Nitrogen source utilization by the isolate was determined in 20 ml screw capped tubes with medium containing 90 % NSW buffered with 5 mM Tris–HCl, pH 7.5, 0.05 % glucose, 0.005 % Na_2HPO_4, supplemented with Hutner's basal salts (20 ml l^{-1}), vitamin solution (10 ml l^{-1}), and 0.1 % of each nitrogen source.

Cultures for chemotaxonomic analysis were grown in M13 medium at 26 °C until late-exponential phase. Cells for polar lipid analysis were harvested by centrifugation, washed in Tris–HCl 0.1 M, pH 7.5 and resuspended in the same buffer. Lipid extraction and two dimensional TLC were performed as described previously [13]. Lipoquinones were extracted from lyophilised cells, purified by TLC and separated by HPLC [14]. Fatty acid methyl esters (FAMEs) were obtained from fresh wet biomass, separated, identified and quantified using the standard MIS Library Generation Software (Microbial ID) as described previously [15].

Determination of the DNA G+C content was performed by HPLC [16] after DNA extraction [17].

The phylogenetic position of the novel isolate was assessed by means of 16S rRNA gene sequencing. The phylogenetic tree was reconstructed using the sequences retrieved from GenBank database. The sequences were aligned using CLUSTALW, and a maximum likelihood (ML) phylogenetic tree was generated applying the general time-reversible model with gamma distributed with invariant sites (G+I) rates and a neighbour-joining phylogenetic tree was generated applying the maximum composite likelihood model in MEGA5.1.

Results of phylogenetic analysis of the nearly complete 16S rRNA gene (1439 bp) indicated the affiliation of FC18^T to the family *Planctomycetaceae* (Fig. 1). The closest phylogenetic relatives were *Blastopirellula marina* DSM 3645^T (86 % sequence similarity). *Rhodopirellula baltica* SH1^T and *Pirellula staleyi* DSM 6068^T (both 85 % sequence similarity) with FC18^T. The closest uncultured relatives were retrieved from the phycoshere of *Enteromorpha prolifera* [JF769591 and JF769713 (98 % sequence similarity)] and from the surfaces of the kelp *Laminaria hyperborea* [4] with 98 % sequence similarity (HM369086). In a previous DGGE study of the planctomycete community of macroalgae, close relatives to FC18^T were observed to be associated with *Sargassum muticum*, *Chondrus crispus*, a species of the genus *Ulva*, *Fucus spiralis* and *Porphyra dioica* from the two sites on the northern coast of Portugal [3]. Similar results were also obtained for *Chondrus crispus*, a member of the genus *Ulva* and *Fucus spiralis* in a clone library study [18].

When grown in M13 agar medium, FC18^T formed small, circular, translucent, non-pigmented colonies (Fig. 2a), but the colonies were light-pink on M14 agar medium (Fig. 2b). When cultivated in liquid medium the cells tended to form aggregates, which were also formed by *B. marina*. Strain FC18^T had a variable cell shape, being mostly spherical (Fig. 2c–d) but some cells were ovoid to *Pirellula*-like (pear-shaped). The organism could also form rosettes (Fig. 2e). Their variable size was around 1.2–1.6 µm but smaller (<1 µm) and larger cells (>2.0 µm) were also seen. They reproduced by budding (Figs 2c and 3). Motility could not be observed in early exponential-phase cultures.

Negatively stained cells showed the presence of crateriform pits typical of members of the order *Planctomycetes* distributed on the reproductive pole of the cells; fimbriae, holdfast and budding were also observed (Fig. 3a, b). Ultrathin sections of FC18^T cells revealed the presence of ribosomes, condensed DNA and several inclusions of an unknown nature (Fig. 3c). Crateriform pits typical of members of the order *Planctomycetes* were clearly distributed on the reproductive pole of the cell.

FC18^T had a doubling time of about 13 h in M13 liquid medium. Minimal temperature for growth was 10 °C and the strain grew at up to 30 °C. Seawater was essential for growth. The organism was aerobic. Growth was observed

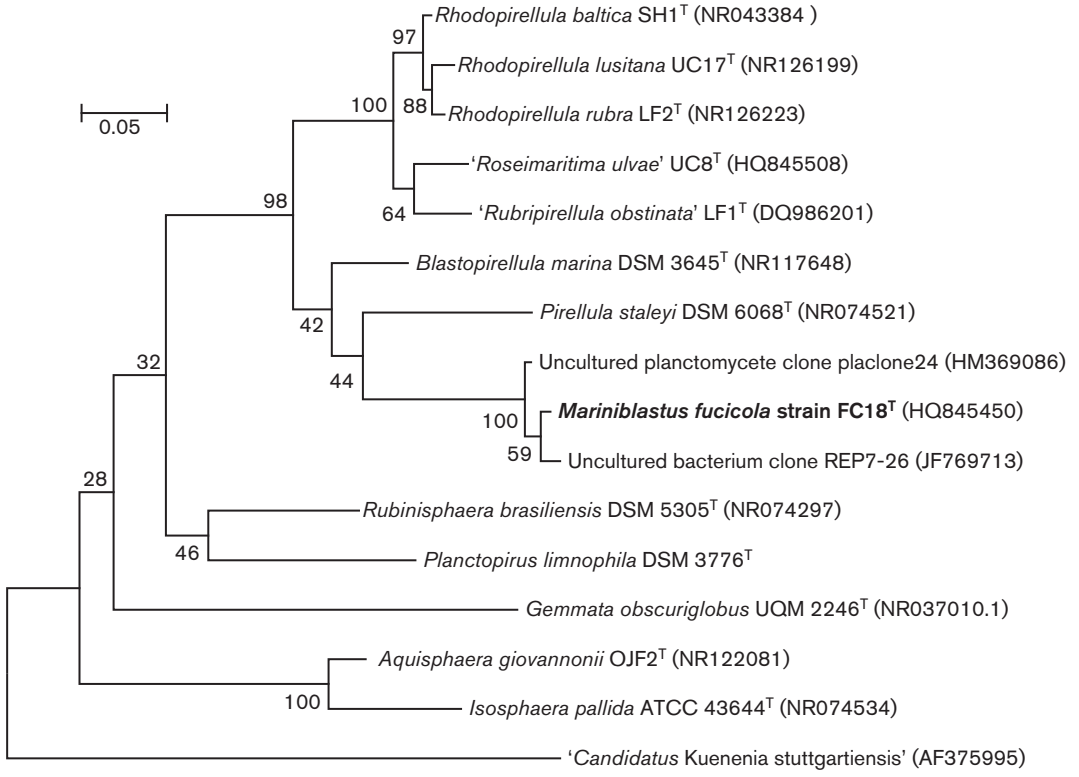

Fig. 1. Optimal maximum-likelihood 16S rRNA gene phylogenetic tree showing the relationships between FC18^T and other representatives of the phylum *Planctomycetes* (GenBank accession numbers are shown in parentheses). The tree was based on the general time reversible model with gamma distributed with invariant sites (G+I) rates. Numbers on the tree refer to bootstrap values based on 1000 replicates. '*Candidatus* Kuenenia stuttgartiensis' (AF375995) was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

from 35 % up to 90 %of the optimal seawater concentration (100 % seawater is 3.45 %salinity). When NaCl concentrations were tested in ASW, FC18^T grew with between 0.5 % and 5 % with an optimum at 2.5 %. The strain grew at pH 6.5 to 8.5 with a maximum at 7.5. Nitrate was reduced to nitrite. No vitamin requirement was observed for FC18^T. Other characteristics of the novel organism are listed in Table 1.

Major polar lipids of FC18^T as well as of *B. marina* as detected by TLC were phosphatidylglycerol (PG) and two unknown lipids (Fig. S1, available in the online Supplementary Material).

The major respiratory lipoquinone was menaquinone 5 (MK-5), but MK-6 was also present. The fatty acid composition of FC18^T showed a predominance of $C_{18:1}\omega 9c$, $C_{14:0}$ and $C_{16:0}$ (Table 2). The G+C content of the DNA of FC18^T was 54.2±0.3 mol%.

Phylogenetic analysis of FC18^T, assessed by 16S rRNA gene sequence similarities, confirmed the distinctness of FC18^T from closely related organisms, moreover the fatty acid composition of this organism is distinct from that of its closest phylogenetic relative. Other phenotypic results, namely the carbon source assimilation results, reinforce the opinion that FC18^T represents an undescribed species of a novel genus for which we propose the name *Mariniblastus fucicola* gen. nov., sp. nov.

DESCRIPTION OF *MARINOBLASTUS* GEN. NOV.

Mariniblastus gen. nov. (Ma.ri.ni.blas′tus. L. adj. *marinus*, marine; Gr. masc. n. *blastos*, shoot, bud; N.L: masc. n. *Mariniblastus*, a marine bud-forming bacterium).

Cells are generally sphaerical to ovoid, forming rosettes. Crateriform pits in the reproductive pole. Reproduce by budding. Chemoheterotrophic, aerobic, catalase- and cytochrome oxidase-positive. The major respiratory quinone is menaquinone 5 (MK-5), menaquinone 6 (MK-6) is also detected. The major polar lipids are phosphatidylglycerol (PG) and two unknown lipids; diphosphatidylglycerol (DPG) is also detected. This genus is a member of the family *Planctomycetaceae*. The type species is *Mariniblastus fucicola*.

DESCRIPTION OF *MARINOBLASTUS FUCICOLA* SP. NOV.

Mariniblastus fucicola sp. nov. (fu.ci′co.la. N. L. n. *Fucus*, a genus of brown algae; L. suff. *-cola*, inhabitant, dweller; N.

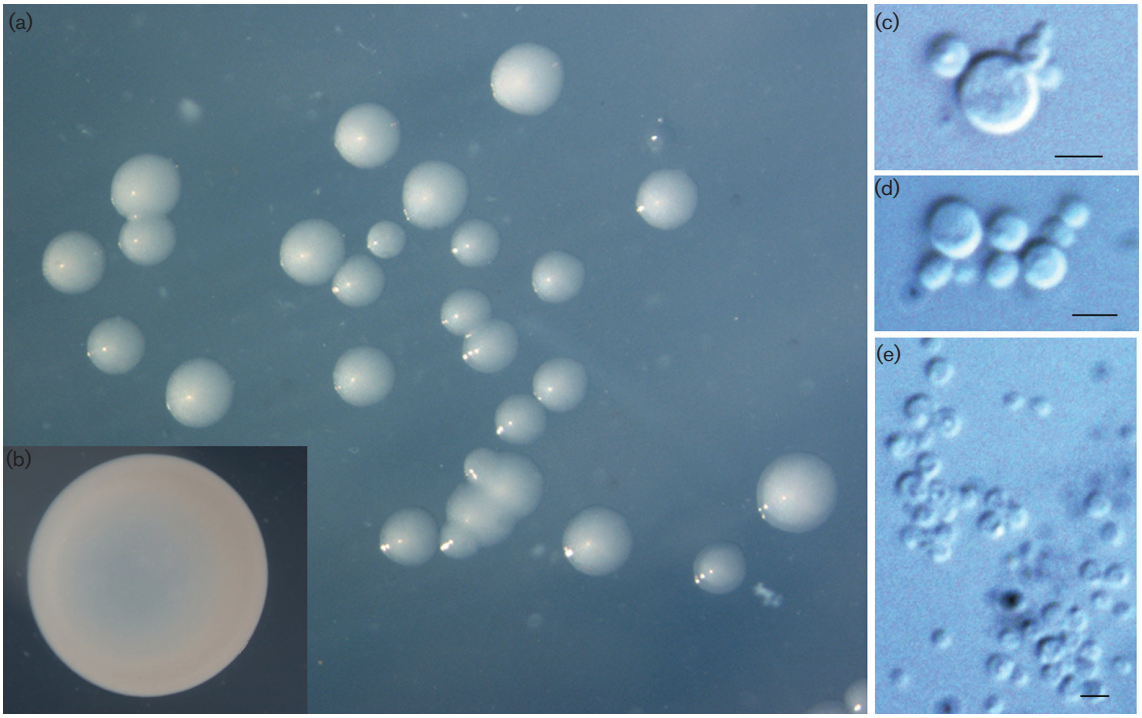

Fig. 2. Morphological characteristics of strain FC18^T. Colonies grown in M13 medium (a) and in M14 medium (b) showing the difference in colouration. (c–e) Cell morphology under phase-contrast microscopy showing the shape of the cells, the budding reproduction and aggregates. Bars, 2 µm.

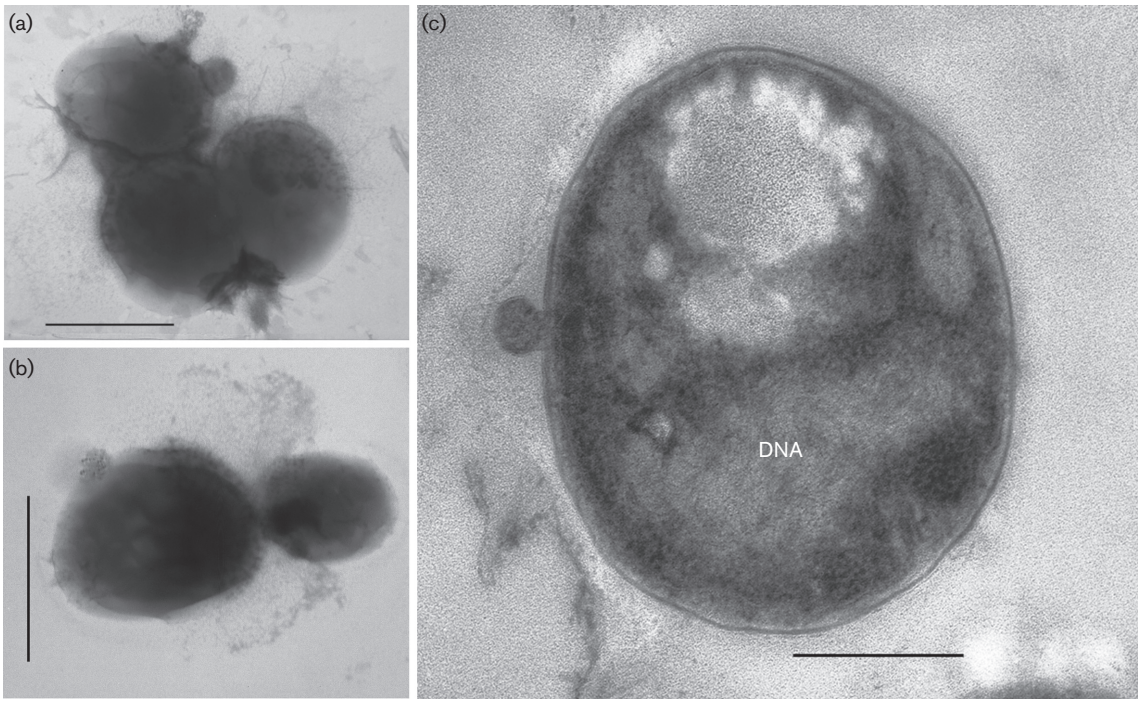

Fig. 3. Cells negatively stained with uranyl acetate (a, b). Transmission electron microscopy of strain FC18^T (c). Bars, 0.5 µm.

Table 1. Differential characteristics between FC18^T and *Blastopirellula marina* DSM 3645^T

+, Positive; –, negative; ND, not determined. Both strains were oxidase- and catalase-positive, reduced nitrate to nitrite and hydrolysed gelatin and aesculin, Neither strain hydrolysed starch, Tween 20, Tween 80 and xylan. Both strains assimilated the following carbon sources D-glucose, D-fructose, D-galactose, sucrose, maltose, lactose, cellobiose, trehalose, *N*-acetyl-glucosamine and D-mannitol, but did not assimilate L-fucose, L-sorbose, D-ribose, D-arabinose, L-arabinose, inulin, glycerol, ribitol, sorbitol, *myo*-inositol, erythritol, D-arabitol, α-ketoglutarate, acetate, succinate, malate, citrate, benzoate, fumarate and formate. Both strains assimilated the following nitrogen sources *N*-acetyl-glucosamine, casamino acids, ammonium, peptone, nitrate, aspartate, L-glutamate, L-alanine, L-proline and L-glutamine, but did not assimilate urea, nitrite, L-histidine, L-lysine, L-arginine, L-valine, L-phenylalanine, L-methionine, L-threonine, cysteine, cystine, tyrosine and tryptophan.

Characteristics	FC18^T	*Blastopirellula marina* DSM 3645^T*
Cell size (μm)	1.0–2.0 in diameter	0.7–1.5×1.0–2.0*
Cell arrangement	Mainly spherical	Ovoid to pear shaped*
Salinity tolerance (% ASW)	35–150	12.5–175*
NaCl for growth (%)		
Optimum	2.5	ND
Range	0.5–5.0	ND
Temperature for growth (°C)		
Optimum	25	27–33†
Range	10–30	ND-38†
pH for growth		
Optimum	7.5	6.5–7.5‡
Range	6.5–8.5	5.5–8.5‡
Hydrolysis of		
Arbutin	–	+‡
Assimilation of carbon sources		
L-Rhamnose	+	–‡
D-Xylose	+	–‡
Raffinose	+	–‡
Casamino acids	+	–‡
Pyruvate	+	–‡
Assimilation of nitrogen sources		
L-Aspargine	–	+‡
L-Serine	–	+‡
L-Ornithine	–	+‡
Major lipoquinone	MK-5	MK-6
DNA G+C content (mol%)	54.2	57.0*

*Data from Schlesner *et al.* [9].

†Data from Schlesner [19].

‡Data from this study.

L. n. *fucicola*, inhabitant of *Fucus*, a genus of brown algae from which this strain was isolated).

Table 2. Fatty acid profiles of strains FC18^T, *Blastopirellula marina* DSM 3645^T and *Rhodopirellula baltica* DSM 10527^T grown at 25 °C in M13 medium

Strains: 1, FC18^T;2, *B. marina* DSM 3645^T; 3, *R. baltica* DSM 10527^T Results are percentages of the total fatty acids (mean±SD of two to four analyses); values for fatty acids present at levels of less than 0.5 % are not shown; TR, trace amount (<0.5 %); –, not detected. ECL, Equivalent chain length. A summed feature represents groups of two or three fatty acids that could not be separated by GLC with the MIDI System: summed feature 3 comprises $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 8 comprises $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$.

Fatty acids	ECL	1	2	3
Unknown 11.970	11.970	0.5±0.1	1.3±0.2	0.7±0.1
$C_{14:0}$	14.000	21.7±0.5	1.2±0.2	TR
Iso-$C_{15:0}$	14.623	1.2±0.2	–	–
$C_{15:0}$	15.000	–	1.9±0.2	TR
Summed feature 3	15.822	5.8±0.3	11.0±0.5	12.8±0.4
$C_{16:0}$	16.000	21.1±0.5	36.0±0.8	26.2±0.6
$C_{17:1}\omega8c$	16.792	–	4.6±0.2	3.7±0.3
$C_{17:0}$	17.000	–	1.4±0.3	0.9±0.2
$C_{18:1}\omega9c$	17.769	36.7±0.8	33.9±0.7	49.5±0.8
Summed feature 8	17.823	2.1±0.2	3.8±0.3	3.0±0.2
$C_{18:0}$	18.000	8.9±0.4	1.6±0.2	1.4±0.2
Unknown 18.790	18.790	–	0.6±0.1	0.6±0.1
Unknown 19.600	19.600	0.8±0.1	–	–
$C_{20:1}\omega9c$	19.770	–	1.6±0.2	–

Cells are mainly sphaerical to ovoid with dimensions of 1.0×2.0 μm, mon-motile. Crateriform structures are restricted to the reproductive cell pole. Colonies are small, circular, translucent and non-pigmented on M13, but light-pink on M14 agar. Optimum growth temperature is 25 °C; temperature range is between 10 and 30 °C, pH range is between 6.5 and 8.5, optimum pH is 7.5. Requires sea salts to grow and a minimum of salinity (ASW) of 0.5 %. Maximum salinity for growth is 5 %. Vitamin B_{12} is not required. Major fatty acids are $C_{18:1}\omega9c$, $C_{14:0}$ and $C_{16:0}$. Gelatin and aesculin are hydrolysed; arbutin, starch, xylan, Tween 20 and 80 are not degraded. Alkaline phosphatase, leucine arylamilase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase are positive in API ZYM; other activities are negative. Arginine dihydrolase and nitrate reduction to nitrite are positive on the API 20NE. Assimilates D-glucose, D-fructose, D-galactose, L-rhamnose, D-xylose, raffinose, sucrose, maltose, lactose, cellobiose, trehalose, *N*-acetyl-glucosamine, D-mannitol, pyruvate and casamino acids; L-fucose, L-sorbose, D-ribose, D-arabinose, L-arabinose, inulin, glycerol, ribitol, sorbitol, *myo*-inositol, erythritol, D-arabitol, α-ketoglutarate, acetate, succinate, malate, citrate, benzoate, fumarate and formate are not assimilated. Utilizes *N*-acetyl-glucosamine, casamino acids, ammonium, peptone, nitrate, aspartate, L-glutamate, L-alanine, L-proline and L-glutamine as nitrogen sources; but not utilize urea, nitrite, L-asparagine, L-histidine, L-lysine, L-arginine, L-valine, L-phenylalanine, L-

methionine, L-threonine, cysteine, cystine, tyrosine and tryptophan.

The type strain is FC18^T (LMG 29748^T=DSM 26290^T) isolated from the surface of *Fucus spiralis*. The DNA G+C content of the type strain is 54.2±0.3 mol% (HPLC method).

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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