Hellea balneolensis gen. nov., sp. nov., a prosthecate alphaproteobacterium from the Mediterranean Sea

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Footnote: The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 26III/A02/215^T is AY576758.

The graph showing the effect of temperature on the maximum growth rate (µmax) of strain 26III/A02/215^T (Fig. S1) is available in IJSEM online.

A novel aerobic, heterotrophic, prosthecate bacterium designated 26III/A02/215^T, was isolated from surface waters of the north-western Mediterranean sea. Cells were Gram-negative, straight to slightly curved rods, forming red colonies on agar plates. The strain grew at 15-37°C inclusive (optimum:

30°C), and optimally at seawater salinity. Growth on organic acids, amino-acids and complex organic substrates was observed. The fatty acids (> 5%) detected in strain 26III/A02/215^T were $C_{17:1}\omega6c$, $C_{18:1}\omega7c$, and $C_{17:0}$. The lipid pattern indicated the presence of phosphatidylglycerol, glucuronopyranosyldiglyceride, monoglycosyldiglyceride, an unidentified glycolipid and three unidentified phospholipids. Phosphatidylethanolamine and diphosphatidylglycerol were absent. Ubiquinone Q10 was the only respiratory lipoquinone. The G+C content of the genomic DNA was 46.8 mol%.

Comparative 16S rRNA gene sequence analysis indicated that strain 26III/A02/215^T belonged to the *Hyphomonas-Hirschia-Robiginitomaculum* branch of the order *Caulobacterales.* This affiliation was consistent with the results of polar lipid analyses. Among this group, the novel isolate was most closely related to *Robiginitomaculum antarcticum* (93.9% 16S rDNA sequence similarity). On the basis of genotypic, chemotaxonomic and phenotypic distinctness, we propose a novel genus, *Hellea* gen. nov., with *Hellea balneolensis* sp. nov. as the type species. The type strain is 26III/A02/215^T (= DSM 19091^T = CIP 109500^T = OOB 269^T).

The phylum *Proteobacteria* is one of the 24 phyla of the domain *Bacteria*, described in *Bergey's Manual of Systematic Bacteriology*, 2nd edn (Garrity & Holt, 2001). To date, more than 200 genera have been described, making this phylum one of the largest bacterial phyla. Representative members of this group are widely distributed in nature and are physiologically and metabolically diverse. The phylum *Proteobacteria* is currently divided into 5 classes, called the *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria* and *Epsilonproteobacteria*, all of which have been defined exclusively on the basis of 16S rRNA gene sequence analysis (Garrity & Holt, 2001). At present, the class *Alphaproteobacteria* Garrity *et al*. 2006 (Validation List 107, Garrity *et al*., 2005a) is composed of seven orders: *Caulobacterales* Henrici & Johnson, 1935 (Henrici & Johnson, 1935), *Kordiimonadales* Kwon *et al*. 2005 (Kwon *et al*., 2005), *Rhodobacterales* Garrity *et al*. 2006 (Validation List 107, Garrity *et al*., 2005b) *Rhodospirillales* Pfenning & Trüper, 1971 (Pfenning & Trüper, 1971), *Rickettsiales* Gieszczykiewicz, 1939 (Gieszczykiewicz, 1939), *Rhizobiales* Kuykendall 2006 (Validation List 107, Kuykendall, 2005) and

Sphingomonadales Yabuuchi & Kosako, 2006 (Validation List 107, Yabuuchi & Kosako, 2005). Marine species make up more than half of the *Alphaproteobacteria* described to date.

Some confusion is being caused at present by the different taxonomic placement of the "stalked" bacteria. While Lee *et al.* (2005) place the members of the genera *Hyphomonas*, *Oceanicaulis*, *Hirschia*, and *Maricaulis* in a new family, the *Hyphomonadaceae*, within the order *Caulobacterales* (which includes members of the family *Rhodobacteraceae*), Garrity *et al*. (2005a) have placed members of these genera within the family *Rhodobacteraceae*, within the order *Rhodobacterales*, leaving the members of the family *Caulobacteraceae* within the order *Caulobacterales*. This situation is particularly unsatisfactory since use of the name of the order *Caulobacterales* alone does not give unambiguous information on which taxa are to be included within it. Furthermore, Lee *et al.* (2005) dealt with the taxonomy of the family *Rhodobacteraceae* before the name was validly published (Validation List 107). Paradoxically Lee *et al*. (2005) created a new family, the family *Hyphomonadaceae*, with the type defined as the genus *Hyphomonas*, a taxon specifically included in the taxon proposed by Garrity *et al.* (2005b) as the family *Rhodobacteraceae*. Based on the principle of priority, the family proposed by Garrity *et al.* (2005b) must be named after the earliest validly published family name, which is the family *Hyphomonadaceae*. The family name *Rhodobacteraceae* Garrity *et al.* 2006 may only be used if specifically defined to exclude the type genus of the family *Hyphomonadaceae*. It should be noted that members of the genera *Woodsholea* (Abraham *et al*., 2004) and *Robiginitomaculum* (Lee *et al.*, 2007) should be included in the family *Hyphomonadaceae* Lee *et al*. 2007.

The members of the families *Hyphomonadaceae* and *Caulobacteraceae* contain organisms that share the particular feature of being appendaged (Poindexter, 1981; Abraham *et al*., 1999; Weiner *et al*., 2000; Strömpl *et al*., 2003). As indicated by their vernacular name (in Latin, *caulis* means stalk), these 'caulobacteria' bear one or several stalks, so-called prosthecae. These stalks are cytoplasm extrusions that undoubtedly play a role in attachment. As a result, they increase significantly the surface to volume ratio of the cells. Consequently, they have often been interpreted as an evolutionary adaptation to life in oligotrophic waters. Most genera of the families *Hyphomonadaceae* and *Caulobacteraceae* (i.e. members of the genera *Hyphomonas, Caulobacter, Asticcacaulis, Phenylobacterium, Hirschia, Robiginitomaculum, Woodsholea, Maricaulis, Oceanicaulis* and *Brevundimonas*) are widely distributed in marine environments (Anast &

Smit, 1988), and especially (but not exclusively) in oligotrophic waters. They are believed to play an important role in the mineralization of dissolved organic matter (Abraham *et al*., 1999).

In this study, a novel marine caulobacterium is described. Based on the results of a polyphasic taxonomic analysis, the strain 26III/A02/215^T represents a novel species and genus, *Hellea balneolensis* gen. nov., sp. nov.

In September 2001, coastal waters were collected in the bay of Banyuls-sur-mer (42°29'N, 3°08'E), in the Mediterranean Sea, France. A sea sample from the surface microlayer was spread on marine agar 2216 (MA; Difco) plate, and then incubated at 25°C. After 2 weeks, a red-coloured colony was picked, purified by repeated streaking on MA plates, and referenced as strain 26III/A02/215^T (Agogué *et al*., 2005). Stock cultures were stored at −80°C in marine broth 2216 (MB; Difco) supplemented with 5% (v/v) DMSO or 35% (v/v) glycerol, until characterization.

Both strands of the almost complete 16S rRNA gene (1412 bp) of the strain were sequenced from one single colony, as described elsewhere (Agogué *et al*., 2005). This sequence was compared to those in available databases by use of the BLAST program (Altschul *et al*., 1990) and then aligned to its nearest neighbours using the CLUSTALX program (Thompson *et al*., 1997). Alignments were refined manually using the SEAVIEW program (Galtier *et al*., 1996). Phylogenetic trees were constructed by the PHYLIP (PHYlogeny Inference Package) version 3.63 software (http://evolution.genetics.washington.edu/phylip/getme.html) on the basis of evolutionary distance (neighbour-joining method with Jukes and Cantor corrections) (Saitou and Nei, 1987) and maximum likelihood (Felsenstein, 1981). The robustness of the inferred topologies was assessed by bootstrap analyses based on 1000 bootstrap resamplings for the neighbour-joining and 100 replications for the maximum likelihood method (Felsenstein, 1985). The 16S rRNA gene-based analysis located the strain 26III/A02/215^T within the class *Alphaproteobacteria*, in the bacterial domain. The results of different phylogenetic reconstructions performed with different treeing algorithms located the novel isolate within the *Hyphomonas-Hirschia-Robiginitomaculum* branch, amongst the marine caulobacteria of family *Hyphomonadaceae* (Lee *et al*., 2005), order *Caulobacterales* (Fig. 1). Within this branch, the novel

isolate clustered with the recently described genus *Robiginitomaculum* (Lee *et al*., 2007) sharing 93.9% 16S rDNA sequence similarity with the only species of this genus. The level of 16S rRNA gene sequence similarity between strain 26III/A02/215^T and representative of the genera *Hyphomonas* and *Hirschia* ranged from 89 to 92%.

The DNA G+C content was determined, by the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig Germany), by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al*. (1989). The G+C content of strain 26III/A02/215^T was 46.8 mol%. Thus, it differed by more than 10 mol% from the DNA G+C content of its closest relative *Robiginitomaculum antarcticum* (60.3 mol%). Clearly, this large difference in the DNA base ratio, together with the 16S rDNA level of similarity, suggest that strain 26III/A02/215^T belongs to a novel genus (Rosselló-Mora and Amann, 2000).

Colonies on MA were circular, smooth, brilliant, convex, with an entire edge and intensely pigmented brick-red. After 1 week incubation, colonies were about 1 mm in diameter. Morphological characteristics of the cells were determined by light microscopy (Olympus AX70) and by transmission electron microscopy (Hitachi H-7500) after negative staining with uranyl acetate (Raguénès *et al*., 1997). Briefly, cells of strain 26III/A02/215^T were Gram-negative, thin straight to curved rods bearing one polar stalk (Fig. 2). Cells bearing several lateral stalks were occasionally observed. Mid-exponential phase cells were 2.70-5.60 μm in length (mean 3.58 ± 0.88 μm, n=15), 0.28-0.48 μm in width (mean 0.42 ± 0.06 μm, n=15), some of which produced a stalk(s). When present, the stalk was more generally cylindrical and extended centrally along the cell axis from one pole. This stalk showed constriction sites distributed equally all along the tube, but which not corresponded to a compartmentalization. This type of constriction has been observed previously in *Oceanicaulis alexandrii* (Strömpl *et al*., 2003). Stalked-cells were non-motile, while non-stalked cells were motile by means of a polar flagellum. Cells divided by budding.

In order to analyse respiratory quinones and polar lipids, strain 26III/A02/215^T was grown for 3 days on MB medium at 30°C, and checked for purity. Initial analyses of the polar lipids and respiratory quinones were carried out by the Identification Service, DSMZ, Braunschweig, Germany. Ubiquinone (Q10) was

determined to be the sole respiratory quinone. The thin layer chromatogram obtained with cell extracts from the novel isolate was very characteristic (Fig. 3). The polar lipid pattern showed the presence of phosphatidylglycerol (PG), monoglycosyldiglyceride (MGDG), glucuronopyranosyldiglyceride (GUDG), one unidentified glycolipid (Gl) and three phospholipids (PL1, PL2, PL3). The presence of the polar lipids monoglycosyldiglyceride (MGDG) and glucuronopyranosyldiglyceride (GUDG) appears to be a characteristic signature for other members of the families *Hyphomonadaceae* and *Caulobacteraceae*, together with the absence of phosphatidylethanolamine, phosphatidylcholine and diphosphatidylglycerol. The presence of two unidentified phospholipids PL2 and PL3, together with an unidentified glycolipid appeared to be a characteristic feature of the lipid pattern of this taxon. The determination of the whole-cell fatty acid composition was performed on cultures grown at 30°C for 72h on marine agar 2216. The analysis was carried out at the DSMZ according to the standard protocol of the Microbial Identification System (MIDI Inc., Del. USA, 2001). Extracts were analysed using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described by Kämpfer & Kroppenstedt (1996). Results are summarized in Table 1. The fatty acids in strain 26III/A02/215^T comprised $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{19:0}$, $C_{17:1}\omega8c$, $C_{17:1}\omega6c$, $C_{18:1}\omega9c$, $C_{18:1}\omega7c$, $C_{20:1}\omega7c$, 3-OH $C_{10:0}$, 3-OH $C_{11:0}$, 3-OH $C_{12:1}$, 2-OH $C_{18:1}$, TBSA 10-methyl $C_{18:0}$, Summed feature 3, Summed feature 7. The presence of $C_{18:1}\omega7c$, together with Q10 is typical of the vast majority of taxa within the *Alphaproteobacteria*. Although the polar lipid composition of the recently described *Robiginitomaculum antarcticum* was not reported, there were clear differences in the fatty acid patterns, in particular the distribution of the 3-hydroxy fatty acids, which are probably derived from lipopolysaccharide. A number of recent publications are also not complete with regard to the chemotaxonomic data. In papers on the genera *Oceanicaulis* (Strömpl *et al.*, 2003), *Woodsholea* (Abraham *et al*., 2004) and some *Maricaulis* (Abraham *et al*., 2002) species, the quinone composition has not been reported. In the case of *Oceanicaulis*, 3-OH fatty acids are not reported, probably because only fatty acids from extracted lipids have been reported. Reports on the polar lipid composition may be incomplete because emphasis has been placed on the presence of phosphate and sulfonic acid containing lipids (Strömpl *et al*., 2003, see also Abraham *et al*., 1997). The glycolipids that are otherwise characteristic for this evolutionary group are not mentioned.

Unless stated otherwise, physiological characterization was carried out aerobically in marine broth medium (MB 2216; Difco), in triplicate, and cell suspension incubated with agitation in the dark. Growth was routinely monitored by measuring the increase in optical density at 600 nm using a spectrophotometer. Cell numbers were determined by flow cytometry (Marie *et al*., 2000) in order to calculate calibration curves 'Cell numbers = f(OD_{600})'. Growth rates were calculated using linear regression analysis from five to nine points along the logarithmic portions of the resulting growth curves. Growth temperature was tested over the range 9-44°C (i.e. 9, 15, 20, 25, 30, 33, 37, 44°C). The novel isolate was found to be mesophilic, growing at 15-37°C; optimal growth yields occurred at 30°C (see Supplementary Fig. S1 in IJSEM online). The optimum pH for growth was tested at 30°C in buffered MB medium and was found to be around pH 6.0-8.0. Salt tolerance was tested at 30°C in MB medium prepared with various concentrations of NaCl (0.02, 0.5, 1, 2, 3, 4, 5, 6, 7 and 9% w/v). Results indicated that the strain was a general typical marine-type halophile. Growth was observed in media containing 0.02% (w/v) to 5% (w/v) NaCl, but it was better in media containing half- to full-strength seawater salinity. The optimal NaCl concentration for growth was around 3% (w/v) NaCl.

Strain 26III/A02/215^T was found to be aerobic. Conventional phenotypic tests including those for oxidase, catalase, tween esterase and nitrate reductase were performed according to standard methods (Smibert & Krieg, 1994). The results are given in Table 2. Biochemical tests were performed at 30°C using api®ZYM (bioMérieux) and Biolog GN2 microplates (Oxoid). These tests were inoculated with cells grown on MA plates, swabbed from the surface of the agar plates and then suspended in ASW ½ (diluted artificial seawater) to the density specified by the manufacturer. Supplementary biochemical tests were also performed using api®20NE strips (bioMérieux), following the manufacturer's instructions. The data obtained are given in Table 2. Testing for oxidation of carbon sources with Biolog GN2 plates indicated that the strain was able to oxidize a wide range of organic acids and amino acids. To confirm these results and to test for the capability of the strain to catabolize different substrates as sole carbon and energy source, with oxygen as a terminal electron acceptor, the strain was grown aerobically, in the dark, on a mineral medium supplemented with one substrate. The defined medium (modified from Widdel *et al.* 2004) had the following composition (l^{-1}): phosphate buffer, 30 mM; NaCl 20 g, $MgCl_2.6H_2O$ 3 g, $CaCl_2.2H_2O$ 1.0 g,

NH_4Cl 0.3 g, KCl 0.5 g, Na_2SO_4 3 g, $NaNO_3$ 1 g; trace element solution, 1 ml; selenite-tungstate solution, 1 ml; vitamin solution, 1 ml. The strain was found to grow heterotrophically on a wide range of substrates. It catabolized organic acids, amino acids, and complex substrates for energy and growth (Table 2). The carbohydrates tested were unable to support growth when provided alone in the medium.

Antibiotic sensitivity tests were performed by using susceptibility discs (Biorad) or filter-paper discs impregnated with different antibiotics. Discs were placed on MA plates spread with a culture of the isolate and were then incubated at 30°C for one week. Susceptibility was scored as positive at zone diameters above 10 mm. The results are summarized in Table 2.

During the course of this work, we also have had cause to re-examine the taxonomy of members of the families *Hyphomonadaceae* and *Caulobacteraceae*. The placement of members of the genera *Hyphomonas*, *Hirschia*, *Maricaulis*, and *Oceanicaulis*, in the family *Rhodobacteraceae* (Garrity *et al*. 2005a) has been called into question by Lee *et al*. (2005). Independent work on the genome of *Hyphomonas neptunium* has indicated that the 16S rDNA sequence based interpretation may be prone to error (Badger *et al*., 2005; Badger *et al*., 2006). This conclusion is also in accord with the chemical composition reported for members of these genera, which share a number of distinctive similarities with members of the genera *Caulobacter*, *Brevundimonas*, *Asticcacaulis* and *Phenylobacterium*. Similarly, extensive chemotaxonomic work on additional taxa within the family *Rhodobacteraceae*, as defined by Garrity *et al.* (2005a) would also indicate inconsistencies (Biebl *et al*., 2005a, 2005b, 2006, 2007; Martens *et al*., 2006; Labrenz *et al.*, 1999, 2000) with the proposal of Lee *et al*. (2005) to unite members of the families *Rhodobacteraceae* (as defined by Lee *et al.* (2005)), *Hyphomonadaceae* and *Caulobacteraceae* (as defined by Lee *et al*., 2005 and Garrity *et al*., 2005a). Clearly the family *Hyphomonadaceae* should comprise the genera *Hirschia*, *Hyphomonas*, *Maricaulis*, *Oceanicaulis*, *Woodsholea*, *Robiginitomaculum*, and the new taxon proposed here. It is interesting to note that this family may be subdivided into two groups, one with cells that divide by budding, the other by binary fission. In addition, there is some evidence that there may also be a correlation between the two groups and the polar lipid patterns, although additional work is needed to test this hypothesis. When such work is completed it would be appropriate to emend the description and circumscription of the family *Hyphomonadaceae* (Lee *et al*. 2005) in the light of chemotaxonomic data, bringing it into line with

recommendations dating back to the *ad hoc* committee reports of Wayne *et al*. (1987) and Murray *et al*. (1990). A similar treatment of the family *Caulobacteraceae* would be appropriate, which comprises the genera *Caulobacter*, *Brevundimonas*, *Asticcacaulis* and *Phenylobacterium*. The order *Caulobacterales* should also be restricted to include only the members of the families *Caulobacteraceae* and *Hyphomonadaceae* and emended accordingly. A further consequence would be that the members of the family *Rhodobacteraceae* as defined by Lee *et al*. (2005), should be formally assigned to a family that excludes the type of the family *Hyphomonadaceae*. Based on published chemotaxonomic data it would also be prudent to test whether members of that taxon should be further divided into several families and all included in the order *Rhodobacterales*.

Briefly, the results of our genotypic, chemotaxonomic, morphological and physiological investigations, together with the phylogenetic analyses, revealed that strain 26III/A02/215^T is distinct from other members of the family *Hyphomonadaceae*. The main characteristics differentiating the novel isolate from its closest phylogenetic neighbours are summarized in Table 2. In brief, the novel taxon can be distinguished from all its closest relatives, with the exception of members of the genus *Hirschia*, by its significantly lower G+C content. The fatty acid composition and polar lipid composition represent other distinctive criteria between the new taxon and other members of the family *Hyphomonadaceae*. Although much emphasis is put on the "major fatty acids" in the majority of recent taxonomic papers we emphasise here, the fact that the large amounts of 18:1ω7c (together with the presence of Q10) only indicate that this genus is a member of the *Alphaproteobacteria* and cannot be described as "characteristic" of this, or any other genus. On the contrary the sum of chemotaxonomic data, not only clearly place it within the family *Hyphomonadaceae*, order *Caulobacterales*, but also provides a unique signature for with taxon within these higher taxa. In terms of other phenotypic features, differences in morphological characteristics such as the fine structure of the stalk, its position, the flagellation of the cells, the colonial pigmentation and the mode of division of the cells can also be use to distinguish the novel isolate from members of the genera *Robiginitomaculum*, *Hyphomonas*, *Hirschia*, *Woodsholea*, *Oceanicaulis* and *Maricaulis* (Table 2).

In conclusion, on the basis of the phylogenetic position and of genotypic, chemotaxonomic and physiological, biochemical and morphological differences, we propose that the isolate 26III/A02/215^T

should be assigned as the type strain of a novel genus and species, for which the name *Hellea balneolensis* gen. nov., sp. nov. is proposed.

Description of *Hellea* gen. nov.

Hellea (He.lle'a. L. fem. n. *Helle* a sea goddess in Greek mythology; N. L. fem. n. *Hellea*, named after *Helle* in reference to the marine origin of the strain). Cells are Gram-negative, non-spore forming, rod-shaped to vibrioid, and dimorphic: usually, they possess one polar stalk (prostheca) and are non-motile or / they are non-stalked and motile by means of a polar flagellum. Aerobic and heterotrophic. Mesophilic. Neutrophilic. Grows best at salt concentrations close to marine salinity. The predominant quinone is Q10. Polar lipids comprise glucuronopyranosyldiglyceride, monoglycosyldiglyceride, phosphatidylglycerol, and unidentified glycolipid and phospholipids. Fatty acids comprise $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{19:0}$, $C_{17:1}\omega8c$, $C_{17:1}\omega6c$, $C_{18:1}\omega9c$, $C_{18:1}\omega7c$, $C_{20:1}\omega7c$, 3-OH $C_{10:0}$, 3-OH $C_{11:0}$, 3-OH $C_{12:1}$, 2-OH $C_{18:1}$, TBSA 10-methyl $C_{18:0}$, Summed feature 3, Summed feature 7 (percentage compositions are given in Table 1). The G+C content of the DNA is close to 47 mol%. The genus *Hellea* belongs to the class *Alphaproteobacteria*, order *Caulobacterales*, family *Hyphomonadaceae*, showing a distant relatedness to prosthecate bacteria of marine origin, namely members of the genera *Hyphomonas*, *Robiginitomaculum*, *Hirschia*, *Woodsholea*, *Maricaulis* and *Oceanicaulis*. The type species is *Hellea balneolensis*. This is the only species within the genus.

Description of *Hellea balneolensis* sp. nov.

Hellea balneolensis (bal.ne'o.len.sis. M. L. n. *Balneola*, the ancient name of Banyuls-sur-mer; N. L. fem. adj. *balneolensis*, pertaining to *Balneola* from where the strain was isolated). In addition to the characters described for the genus, the species is characterised by the following properties. Colonies on MA medium are round, convex, brilliant and pigmented a brick red colour. Optimal growth occurs at 30°C, with a growth range from 15 to 37°C. pH optimum is close to neutrality. Grows at NaCl concentrations from 0.02% to 5% (w/v), with a clear optimum at 3% (w/v) NaCl. Growth occurs on acetate, citrate, propionate, pyruvate, succinate, aspartate, glutamate, L-alanine, L-asparagine, L-histidine, L-proline, casamino acids, peptone, tryptone, yeast extract and D-mannitol. Substrates positive in Biolog GN2 plates are all the substrates cited above and as well as *cis*-aconitic acid, D-glucuronic acid, β-hydroxybutyric acid, γ-hydroxy butyric acid, α-ketoglutaric acid, methyl-pyruvate, quinic acid, urocanic acid, L-pyroglutamic acid, hydroxyl-L-proline, putrescine, *n*-acetyl glucosamine, D-arabitol, *m*-inositol and xylitol. Does not reduce NO_3^-. Catalase positive, oxidase negative. Tween 40 and tween 80 hydrolysis activities are positive.

The G+C content is 46.8 mol%.

The type strain, 26III/A02/215^T (DSM 19091^T, CIP 109500^T = OOB 269^T), was isolated from the surface microlayer of coastal waters, in the bay of Banyuls-sur-mer, north-western Mediterranean sea, France (42°29'N, 3°08'E).

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TABLES and FIGURES

Table 1. **Whole cell fatty acid profile of strain 26III/A02/215 cultivated on marine agar.**

Values are percentages of total fatty acids. The nomenclature is as follows: the first number indicates the number of carbon atoms in the molecule. The prefixes 'iso', 'OH' and 'cyclo' indicate isobranched, hydroxy or cyclic fatty acids. The second number following the colon indicates the number of double bonds present. The position of the double bond is indicated by the carbon atom position starting from the methyl (ω) end of the molecule. The suffix *c* indicates the *cis* isomer. Summed feature contain one or more of each fatty acid. Summed features: **3**, $C_{16:1}\omega7c$ and/or 2-OH iso-$C_{15:0}$; **7**, $C_{19:0}$ cyclo $\omega10c/C_{19:1}$ $\omega6c$ and/or unknown ECL 18.846. ECL, equivalent chain length. TBSA, tuberculostearic acid (10-methyloctadecanoic acid). Major fatty acids are indicated by bold values. Only 62% of the fatty acid peaks could be assigned to the fatty acids listed in the peak naming table of the MIS database (MIS, Microbial identification System; MIDI, Del. USA). Unknown ECL that were detected are: 16.760, 17.608, 18.116, 18.585, 18.797 and 19.347.

Fatty acid	**Strain 26III/A02/215^T grown on marine agar**
Saturated fatty acids	
$C_{16:0}$	0.98
$C_{17:0}$	**5.63**
$C_{18:0}$	1.81
$C_{19:0}$	0.79
Monounsaturated fatty acids	
$C_{17:1}\omega8c$	2.18
$C_{17:1}\omega6c$	**6.60**
$C_{18:1}\omega9c$	1.06
$C_{18:1}\omega7c$	**67.22**
$C_{20:1}\omega7c$	0.68
Hydroxy fatty acids	
2-OH $C_{18:1}$	2.51
3-OH $C_{10:0}$	2.23
3-OH $C_{11:0}$	0.92
3-OH $C_{12:1}$	1.23
Methyl substituted fatty acid	
TBSA 10-methyl $C_{18:0}$	1.39
Summed features	
Summed feature 3	0.66
Summed feature 7	4.11

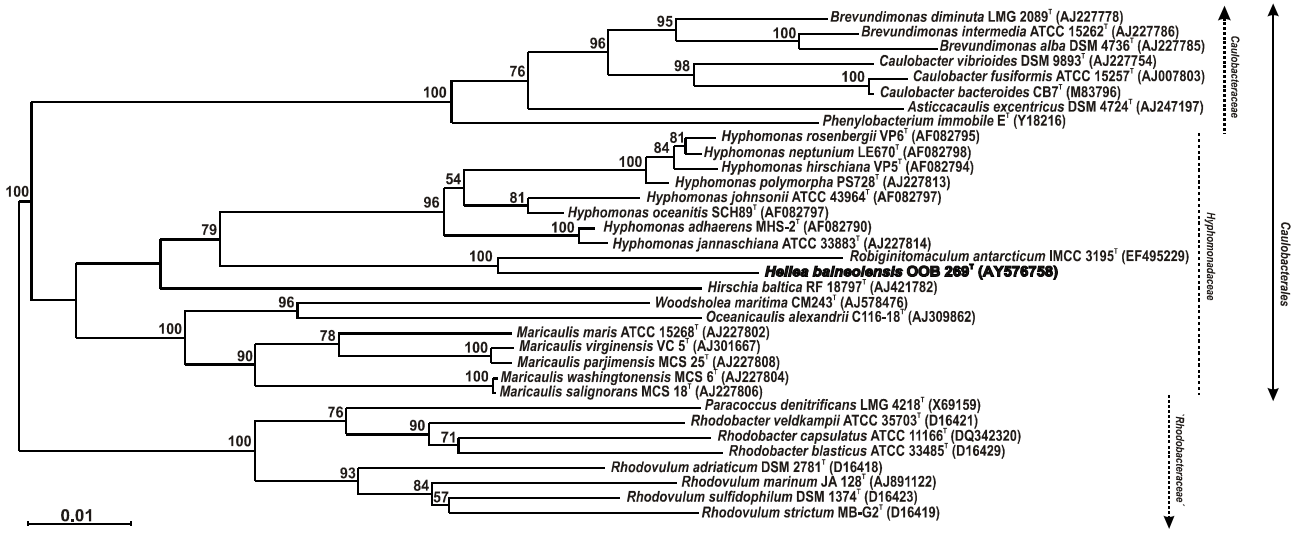

Fig. 1. **Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain 26III/A02/215^T within the order** *Caulobacterales* **(as outlined in this article), class *Alphaproteobacteria***. The alignment was performed with 16S rDNA sequences of related species. Sequence data of reference strains were obtained from the GenBank/EMBL and/or RDP databases. Accession numbers are indicated in parentheses. The topology shown corresponds to an unrooted tree obtained by the neighbour-joining algorithm, established using the PHYLIP package. Bootstrap values (from 1000 replicates) are indicated at the branch nodes. The positioning of the novel isolate was confirmed by the maximum likelihood method. The scale bar indicates 1.0 nt substitutions per 100 nt.

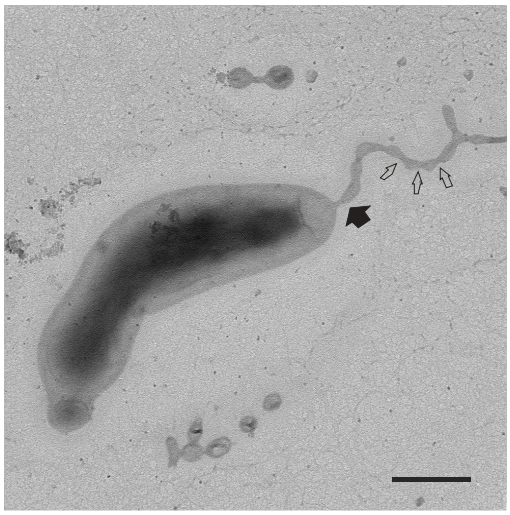

Fig. 2. Transmission electron micrograph of a budding cell of strain 26III/A02/215^T negatively stained with uranyl acetate. It can be observed that the products of the cell division are unequal (simple budding). The cell bears a polar stalk (black arrow) which is an open ring regularly constricted on its length (open arrows). Bar, 0.5 μm.

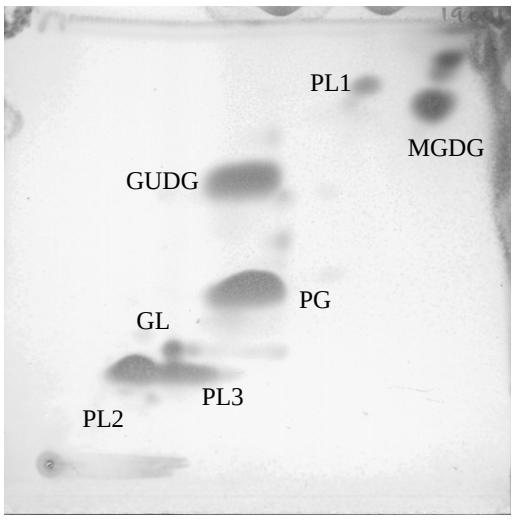

Fig. 3. Polar lipids of strain 26III/A02/215^{T}. Legend: PG, phosphatidylglycerol; PL1, PL2, PL3, phospholipids; GL, unidentified glycolipid; MGDG, monoglycosyldiglyceride; GUDG, glucuronopyranosyldiglyceride.

Table 1. Phenotypic and genotypic characteristics of strain 26III/A02/215^{T}. Legend: +, positive; ─, negative; W, weakly positive; ND, not determined; VS, very susceptible (diameter of inhibition zone > 20 mm); S, susceptible (diameter of inhibition zone: 10-20 mm).

Characteristic	**Strain 26III/A02/215^{T}**
Temperature range for growth (°C) – [Optimum]	15-37 [30]
NaCl range for growth (%) – [Optimum]	0.02-5 [3]
Catalase activity	+
Oxidase activity	─
Hydrolysis of tween 40	+
Hydrolysis of tween 80	+
API ZYM / API 20NE	
Alkaline phosphatase	+
Esterase	+
Esterase lipase	+
Naphtol-AS-BI-phosphohydrolase	+
β-galactosidase	+
Urease activity	─
Nitrate reductase activity	─
Hydrolysis of aesculin (β-glucosidase)	+
Hydrolysis of gelatin	─
Glucose fermentation	─
Oxidation of (Biolog) / *Utilization as sole carbon and energy source* (minimal mineral medium)	
Acetic acid	+ / +
cis-aconitic acid	+ / ND
Citric acid	+ / +
D-Glucuronic acid	+ / ND
β-Hydroxy butyric acid	+ / ND
γ-Hydroxy butyric acid	+ / ND
α-keto glutaric acid	+ / ND
Propionic acid	+ / +
Pyruvic acid	+ / +
Methyl-pyruvate	+ / ND
Quinic acid	+ / ND
Succinic acid	+ / +
Urocanic acid	+ / ND
L-aspartic acid	+ / +
L-glutamic acid	+ / +
L-pyroglutamic acid	+ / ND

L-alanine	+ / +
L-asparagine	+ / +
L-histidine	+ / +
Hydroxy-L-proline	+ / ND
L-proline	+ / +
Putrescine	+ / ND
Casamino acids	ND / +
Peptone	ND / +
Tryptone	ND / +
Yeast extract	ND / +
n-acetyl glucosamine	+ / ND
D-arabitol	+ / ND
m-inositol	+ / ND
D-mannitol	+ / +
xylitol	+ / ND
Dextrin	w / ND
D-mannose	w / ND
D-cellobiose	w / ND
α-D-glucose	w / w
Starch	w / w
Susceptibility to :	
Ciprofloxacin (100 µg per disc)	VS
Oxacillin (5 µg per disc)	S
Penicillin (6 µg per disc)	S
Rifampicin (100 µg per disc)	VS
Tetracyclin (100 µg per disc)	S
Vancomycin (100 µg per disc)	VS
DNA G+C content (mol%)	46.8

478

479 **Table 2. Characteristics differentiating *Hellea* from the related genera of the family *Hyphomonadaceae*.**

480

Characteristic	Genus						
	Hellea	***Robiginitomaculum***[¶]	***Hyphomonas****	***Hirschia***†	***Oceanicaulis***‡	***Maricaulis***§	***Woodsholea***[#]
Colony colour	Brick red	Rusty-orange	Grey or colourless may produce a water soluble brown/red-brown pigment	Yellow	colourless	colourless	colourless
Prostheca(e)	One, polar	One, polar	One to two, polar	One to two, polar	One, polar	One, polar	One, polar
Stalk cross wall	+	–	–	–	+	–	+
Mode of division	Budding	Binary fission	Budding	Budding	Binary fission	Binary fission	Binary fission
Flagellation	Monotrichous, polar	Absent	One to three, polar	Monotrichous, polar	Monotrichous, polar	Monotrichous, polar	Monotrichous, polar
Nitrate reduction	–	+	+	–	+	±	–
Growth at 6% NaCl	–	–	V	ND	+	±	+
Polar lipid(s)[¶#]	PG, MGDG, GUDG, GL, PL_1, PL_2, PL_3	ND	MGDG, GUDG, PG, Tau	MGDG, GUDG, PG, GL[5]	(PG), SQDG [MGDG, GUDG[1]]	PG, SQDG, Tau MGDG, GUDG	SQDG, Tau, MGDG, GUDG
Major fatty acids	3-OH $C_{10:0}$, 3-OH $C_{12:1}$, 3-OH $C_{11:0}$, $C_{17:1}\omega6c$, $C_{17:1}\omega8c$, $C_{18:1}\omega7c$, $C_{17:0}$	3-OH $C_{9:0}$, 3-OH $C_{10:0}$, 3-OH $C_{11:0}$, $C_{15:1}\omega8c$, $C_{15:1}\omega6c$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}\omega9c$, $C_{16:1}\omega7c$, $C_{17:1}\omega8c$, $C_{17:0}$, $C_{17:1}\omega6c$, $C_{18:0}$, $C_{18:1}\omega7c$, $C_{18:1}\omega9c$	3-OH $C_{12:0}$, 3-OH $C_{12:1}$, ($C_{15:0}$) $C_{16:0}$, $C_{17:1}\omega8$[3], ($C_{17:1}\omega6$), $C_{17:0}$, $C_{18:1}\omega7$, 11-Me-$C_{18:1}\omega6$, $C_{19:1}\omega8$	3-OH $C_{12:0}$, 3-OH $C_{14:1}$ $C_{16:1}\omega11c$, $C_{16:1}\omega7c$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}\omega7c$, $C_{18:2}\omega7$	3-OH FAME[2]*, $C_{16:0}$, $C_{17:1}\omega6$[4], $C_{17:0}$, $C_{18:1}\omega7$, $C_{18:0}$, 7-Me-$C_{18:1}\omega6$, $C_{19:0}$	3-OH iso $C_{11:0}$, $C_{16:0}$, $C_{17:0}$, $C_{16:1}\omega7c$*, $C_{17:0}$, iso $C_{17:0}$, iso $C_{17:1}\omega9c$, $C_{17:1}\omega6c$, $C_{17:1}\omega8c$, $C_{18:1}\omega7c$*, $C_{18:1}\omega9c$	3-OH $C_{12:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{18:1}\omega7c$
Quinones	Q10	ND	Q10 or Q11	Q10	ND	Q10	ND
DNA G+C content (mol%)	47	60	57-64	45-47	61-62	62-65	65

¶Data from Lee *et al*. (2007)
*Data from Weiner *et al*. 1985, 2000; Moore *et al*., 1984
†Data from Schlesner *et al*. (1990)
‡Data from Strömpl *et al*. (2000)
§Data from Abraham *et al*. (2002), Sittig and Hirsch (1992)
#Data from Abraham *et al*. (2004)
[1] The lipids MGDG and GUDG are not specifically mentioned, but the lipid fraction containing them was not investigated.
[2] Hydroxylated fatty acids not mentioned, but only the fatty acids from a polar lipid fraction was examined
[3] Fatty acid nomenclature used in the original paper was the Δ nomenclature – this has been converted to the ω nomenclature in this table. Cis- or trans- isomers not specified
[4] Cis- or trans- isomers not specified
[5] Tindall, unpublished
Hydoxy fatty acids (which probably originate for the lipopolysaccharide are underlined
¦ PG, Phosphatidylglycerol; MGDG, monoglycosyldiglyceride; SQDG, 1,2-diacyl-3-*O*-sulfoquinovosylglycerol; GUDG, glucuronopyranosyldiglyceride; SQDG, sulfo-quinovosyl diacylglycerol; Tau, 1,2-diacyl-3-α-D-glucuropyranosyl-*sn*-glycerol taurineamide; PL, unidentified phospholipid; GL, unidentified glycolipids.
Legend: V, variable; ND Not determined

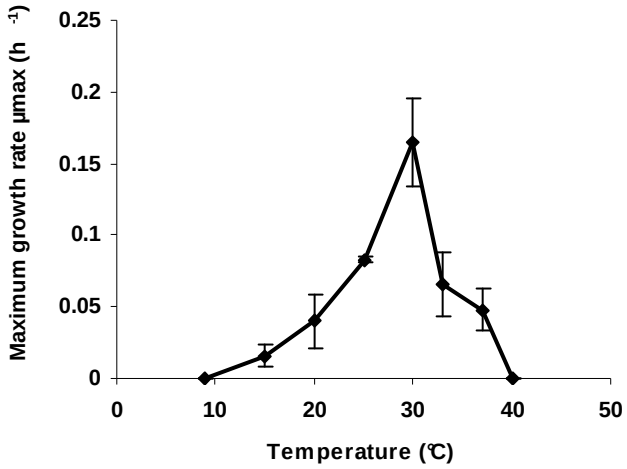

Fig. S1. **Effect of temperature on the maximum growth rate of strain 26III/A02/215^{T}.** The strain was grown in MB medium. Growth rates were calculated by performing linear regression analysis along the logarithmic part of the growth curves.