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

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A novel aerobic, heterotrophic, prosthecate bacterium, designated 26III/A02/215^T, was isolated from surface water of the north-western Mediterranean Sea. Cells stained Gram-negative and were straight to slightly curved rods, forming red colonies on agar plates. The strain grew at 15–37 °C inclusive (optimum 30 °C) and grew optimally at seawater salinity. Growth was observed on organic acids, amino acids and complex organic substrates. The fatty acids (>5 %) detected in strain 26III/A02/215^T were C_{17:1}ω6c, C_{18:1}ω7c 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 Q-10 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*–*Robiginotomaculum* 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 *Robiginotomaculum antarcticum* (93.9 % 16S rRNA gene sequence similarity to the type strain). 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 of *Hellea balneolensis* 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 one of the largest bacterial phyla. Members of this group are widely distributed in nature and are physiologically and metabolically diverse. The phylum *Proteobacteria* is currently divided into five classes, 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 (Garrity *et al.*, 2005a) is composed of seven orders: *Caulobacterales* Henrici and Johnson 1935, *Kordiimonadales* Kwon *et al.* 2005, *Rhodobacterales* Garrity *et al.* 2006 (Garrity *et al.*, 2005b), *Rhodospirillales* Pfenning and Trüper 1971, *Rickettsiales* Gieszczykiewicz 1939, *Rhizobiales* Kuykendall 2006 (Kuykendall, 2005) and *Sphingomonadales* Yabuuchi and Kosako 2006 (Yabuuchi & Kosako, 2005). Marine species make up more than half of the species 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

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Abbreviation: TBSA, tuberculostearic acid.

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

A graph showing the effect of temperature on the maximum growth rate of strain 26III/A02/215^T is available as supplementary material with the online version of this paper.

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 (Garrity *et al.*, 2006). 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 be used only 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* include 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, strain 26III/A02/215^T represents a novel species and genus.

In September 2001, a coastal water sample was collected in the bay of Banyuls-sur-mer (42° 29' N 3° 08' E), on the Mediterranean coast of France. A sample from the surface microlayer was spread on a 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 a 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 CLUSTAL_X program (Thompson *et al.*, 1997). Alignments were refined manually using the SEAVIEW program (Galtier *et al.*, 1996). Phylogenetic trees were constructed by using the PHYLIP software version 3.63 (<http://evolution.genetics.washington.edu/phylip/getme.html>) on the basis of evolutionary distance (neighbour-joining method with Jukes and Cantor corrections) (Saitou & 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 the 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 rRNA gene sequence similarity with the type strain of the only species of this genus. The 16S rRNA gene sequence similarity between strain 26III/A02/215^T and representatives 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 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 level of 16S rRNA gene sequence similarity, suggest that strain 26III/A02/215^T belongs to a novel genus (Rosselló-Mora & Amann, 2001).

Colonies on MA were circular, smooth, brilliant and convex with an entire edge and were intensely pigmented brick-red. After 1 week of 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 stained Gram-negative and were 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 long (mean 3.58 ± 0.88 µm, *n*=15) and 0.28–0.48 µm wide (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 did not correspond to compartment-

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 rRNA gene sequences of related species. Sequence data for 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 branch nodes. The positioning of the novel isolate was confirmed by the maximum-likelihood method. Bar, 1 substitution per 100 nucleotide positions.

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 constricted regularly on its length (open arrows). Bar, 0.5 μ m.

lization. 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 in 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 of the DSMZ. Ubiquinone (Q-10) was determined to be the sole respiratory quinone. The TLC plate obtained with cell extracts from the novel isolate was very characteristic (Fig. 3). The polar lipid pattern showed the presence of phosphatidylglycerol, monoglycosyldiglyceride, glucuronopyranosyldiglyceride, an unidentified glycolipid (GL) and three phospholipids (PL1, PL2, PL3). The presence of the polar lipids monoglycosyldiglyceride and glucuronopyranosyldiglyceride 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 72 h on MA. The analysis was carried out at the DSMZ according to the standard

Fig. 3. Polar lipids of strain 26III/A02/215^T following separation by two-dimensional TLC. PG, Phosphatidylglycerol; PL1, PL2, PL3, phospholipids; GL, unidentified glycolipid; MGDG, monoglycosyldiglyceride; GUDG, glucuronopyranosyldiglyceride.

protocol of the Microbial Identification System (MIDI, 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}ω8*c*, C_{17:1}ω6*c*, C_{18:1}ω9*c*, C_{18:1}ω7*c*, C_{20:1}ω7*c*, 3-OH C_{10:0}, 3-OH C_{11:0}, 3-OH C_{12:1}, 2-OH C_{18:1}, tuberculostearic acid (TBSA; 10-methyl C_{18:0}), summed feature 3 and summed feature 7. The presence of C_{18:1}ω7*c* together with Q-10 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-OH fatty acids, which are probably derived from lipopolysaccharide. A number of recent publications are also not complete with regard to chemotaxonomic data. In papers on the genera *Oceanicaulis* (Strömpl *et al.*, 2003) and *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.

Table 1. Whole-cell fatty acid profile of strain 26III/A02/215^T cultivated on MA

Values are percentages of the fatty acids that were assigned to fatty acids in the peak-naming table of the MIDI database (MIDI, 2001); only 62 % of the fatty acid peaks could be assigned, and unknown components displaying ECL (equivalent chain-length) values of 16.760, 17.608, 18.116, 18.585, 18.797 and 19.347 were detected. The nomenclature is as follows: the first number indicates the number of carbon atoms in the molecule; 'iso', 'OH' and 'cyclo' indicate iso-branched, 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. *c*, *cis* isomer. Major fatty acids are indicated in bold.

Fatty acid	Proportion (%)
Saturated	
C _{16:0}	0.98
C_{17:0}	5.63
C _{18:0}	1.81
C _{19:0}	0.79
Monounsaturated	
C _{17:1} ω8 <i>c</i>	2.18
C_{17:1}ω6<i>c</i>	6.60
C _{18:1} ω9 <i>c</i>	1.06
C_{18:1}ω7<i>c</i>	67.22
C _{20:1} ω7 <i>c</i>	0.68
Hydroxy	
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	
TBSA	1.39
Summed features	
Summed feature 3*	0.66
Summed feature 7*	4.11

*Summed feature 3 contains C_{16:1}ω7*c* and/or 2-OH iso-C_{15:0} and summed feature 7 contains one or more of C_{19:0} cyclo ω10*c*, C_{19:1}ω6*c* and/or unknown ECL 18.846.

Unless stated otherwise, physiological characterization was carried out aerobically in MB in triplicate and the cell suspension was incubated with agitation in the dark. Growth was monitored routinely 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 number=*f*(OD₆₀₀)'. Growth rates were calculated using linear regression analysis from five to nine points along the logarithmic portions of the resulting growth curves. Growth was tested at 9, 15, 20, 25, 30, 33, 37 and 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 available in IJSEM Online). The optimum pH for growth was tested at 30 °C in buffered

MB and was found to be around pH 6.0–8.0. Salt tolerance was tested at 30 °C in MB 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 typical marine-type halophile. Growth was observed in media containing 0.02–5 % (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). 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 ASW¹/₂ (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. 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 capacity of the strain to catabolize different substrates as sole carbon and energy sources 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.*, 2006) had the following composition (l⁻¹): phosphate buffer, 30 mM; NaCl, 20 g; MgCl₂ · 6H₂O, 3 g; CaCl₂ · 2H₂O, 1.0 g; NH₄Cl, 0.3 g; KCl, 0.5 g; Na₂SO₄, 3 g; NaNO₃, 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. The carbohydrates tested were unable to support growth when provided alone in the medium. The results of the phenotypic characterization are given in the species description and Table 2.

Antibiotic sensitivity tests were performed by using susceptibility discs (Bio-Rad) 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 1 week. Susceptibility was scored as positive at zone diameters above 10 mm (Table 2).

During the course of this work, we have also 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 rRNA gene sequence-based interpretation may be prone to error (Badger *et al.*,

Table 2. Phenotypic and genotypic characteristics of strain 26III/A02/215^T

+, 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	Result
API ZYM/API 20NE tests	
Alkaline phosphatase	+
Esterase	+
Esterase lipase	+
Naphthol-AS-BI-phosphohydrolase	+
β-Galactosidase	+
Urease	–
Nitrate reductase	–
Hydrolysis of aesculin (β-glucosidase)	+
Hydrolysis of gelatin	–
Glucose fermentation	–
Oxidation (Biolog)/utilization as sole carbon and energy source (minimal mineral medium) of:	
Acetic acid, citric acid	+ / +
cis-Aconitic acid	+ / ND
D-Glucuronic acid	+ / ND
β- and γ-Hydroxybutyric acids	+ / ND
α-Ketoglutaric acid	+ / ND
Propionic acid, pyruvic acid, succinic acid	+ / +
Methyl pyruvate	+ / ND
Quinic acid	+ / ND
Urocanic acid	+ / ND
L-Aspartic acid, L-glutamic acid, L-alanine, L-asparagine, L-histidine, L-proline	+ / +
L-Pyroglutamic acid, hydroxy-L-proline	+ / ND
Putrescine	+ / ND
Casamino acids	ND / +
Peptone, tryptone, yeast extract	ND / +
N-Acetylglucosamine	+ / ND
D-Arabitol, myo-inositol	+ / ND
D-Mannitol	+ / +
Xylitol	+ / ND
Dextrin, D-mannose, cellobiose	w / ND
α-D-Glucose, starch	w / w
Susceptibility to (μg per disc):	
Ciprofloxacin (100), rifampicin (100), vancomycin (100)	vs
Oxacillin (5), penicillin (6), tetracycline (100)	s

2005, 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, b, 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* and *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 and 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*, which comprises the genera *Caulobacter*, *Brevundimonas*, *Asticcacaulis* and *Phenylobacterium*, would be appropriate. The order *Caulobacterales* should also be restricted to include only the members of the families *Caulobacteraceae* and *Hyphomonadaceae* and its description 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 that differentiate the novel isolate from its closest phylogenetic neighbours are summarized in Table 3. 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. Although much emphasis is put on the 'major fatty acids' in the majority of recent taxonomic papers, we emphasize here the fact that the large amounts of 18:1 ω 7c (together with the presence of Q-10) indicate only that this genus is a member of the *Alphaproteobacteria* and cannot be described as 'characteristic' of this or any other genus. In contrast, the sum of chemotaxonomic data not only places it clearly within the family *Hyphomonadaceae*, order *Caulobacterales*, but also provides a unique signature for this 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 used to distinguish the novel isolate from members of the genera *Robiginitomaculum*, *Hyphomonas*, *Hirschia*, *Woodsholea*, *Oceanicaulis* and *Maricaulis* (Table 3).

In conclusion, on the basis of the phylogenetic position and of genotypic, chemotaxonomic, physiological, biochemical and morphological differences, we propose that isolate 26III/A02/215^T should be assigned as the type strain of a novel species within a new genus, for which the name *Hellea balneolensis* gen. nov., sp. nov. is proposed.

Description of *Hellea* gen. nov.

Hellea (Hel.le'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 first strain).

Cells are Gram-negative, non-spore-forming, rod-shaped to vibrioid and dimorphic; they usually 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. Grow best at salt concentrations close to marine salinity. The predominant quinone is Q-10. Polar lipids comprise glucuronopyranosyldiglyceride, monoglycosyldiglyceride, phosphatidylglycerol and an unidentified glycolipid and phospholipids. Fatty acids comprise C_{16:0}, C_{17:0}, C_{18:0}, C_{19:0}, C_{17:1} ω 8c, C_{17:1} ω 6c, C_{18:1} ω 9c, C_{18:1} ω 7c, C_{20:1} ω 7c, 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 and summed feature 7 (percentage composition for the type strain of the type species is given in Table 1). The G + C content of the DNA of the type strain of the type species is 46.8 mol%. The genus 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*, currently 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 type strain was isolated).

In addition to the characters described for the genus, the species is characterized 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 growth at 15–37 °C. The pH optimum is close to neutrality. Grows at NaCl concentrations of 0.02–5 % (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 as well as *cis*-

Table 3. Characteristics that differentiate *Hellea* gen. nov. from related genera of the family *Hyphomonadaceae*

Genera: 1, *Hellea* gen. nov.; 2, *Robiginitomaculum* (Lee *et al.*, 2007); 3, *Hyphomonas* (Weiner *et al.*, 1985, 2000; Moore *et al.*, 1984); 4, *Hirschia* (Schlesner *et al.*, 1990); 5, *Oceanicaulis* (Strömpl *et al.*, 2003); 6, *Maricaulis* (Abraham *et al.*, 2002; Sittig & Hirsch, 1992); 7, *Woodsholea* (Abraham *et al.*, 2004). Hydroxy fatty acids (which probably originate from the lipopolysaccharide) are shown in bold. Fatty acids indicated in parentheses are found in some strains. v, Variable; (+), some strains grow under these conditions; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Colony colour	Brick red	Rusty-orange	Grey or colourless*	Yellow	Colourless	Colourless	Colourless
Protheca(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 lipids†	PG, MGDG, GUDG, GL, PL ₁ , PL ₂ , PL ₃	ND	MGDG, GUDG, PG, Tau	MGDG, GUDG, PG, GL‡	PG, SQDG [MGDG, GUDG]§	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 _{17:1ω6c} , C _{17:1ω8c} , C _{18:1ω7c} , C _{17:0}	3-OH C_{9:0} , 3-OH C_{10:0} , 3-OH C_{11:0} , C _{15:1ω8c} , C _{15:1ω6c} , C _{15:0} , C _{16:0} , C _{16:1ω9c} , C _{16:1ω7c} , C _{17:0} , C _{17:1ω8c} , C _{17:0} , C _{17:1ω6c} , C _{18:0} , C _{18:1ω7c} , C _{18:1ω9c}	3-OH C_{12:0} , 3-OH C_{12:1} , (C _{15:0}), C _{16:0} , C _{17:1ω8ll} , (C _{17:1ω6}), C _{17:0} , C _{18:1ω7} , 11-Me C _{18:1ω6} , C _{19:1ω8}	3-OH C_{12:0} , 3-OH C_{14:1} , C _{16:1ω11c} , C _{16:1ω7c} , C _{16:0} , C _{18:0} , C _{18:1ω7c} , C _{18:2ω7}	3-OH FAME¶# , C _{16:0} , C _{17:1ω6} , C _{17:0} , C _{18:1ω7} , C _{18:0} , 7-Me C _{18:1ω6} , C _{19:0} **	3-OH iso-C_{11:0} , C _{16:0} , C _{17:0} , C _{16:1ω7c#} , iso C _{17:0} , iso-C _{17:1ω9c} , C _{17:1ω6c} , C _{17:1ω8c} , C _{18:1ω7c#} , C _{18:1ω9c}	3-OH C_{12:0} , C _{16:0} , C _{17:0} , C _{18:0} , C _{18:1ω7c}
Quinone(s)	Q-10	ND	Q-10 or Q-11	Q-10	ND	Q-10	ND
DNA G + C content (mol%)	47	60	57–64	45–47	61–62	62–65	65

*May produce a water-soluble brown/red–brown pigment.

†PG, Phosphatidylglycerol; MGDG, monoglycosyldiglyceride; SQDG, 1,2-diacyl-3-*O*-sulfoquinovosylglycerol; GUDG, glucuronopyranosyldiglyceride; Tau, 1,2-diacyl-3- α -D-glucuropyranosyl-*sn*-glycerol taurineamide; PL, unidentified phospholipid; GL, unidentified glycolipids.

‡B. J. Tindall, unpublished.

§The lipids MGDG and GUDG are not mentioned specifically, but the lipid fraction containing them was not investigated.

||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 were not specified.

¶Hydroxylated fatty acids not mentioned, but only the fatty acids from a polar lipid fraction were examined.

#Data from Abraham *et al.* (2004).

***cis*- or *trans*-isomers not specified.

aconitic acid, D-glucuronic acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, α -ketoglutaric acid, methyl pyruvate, quinic acid, urocanic acid, L-pyrogutamic acid, hydroxy-L-proline, putrescine, N-acetylglucosamine, D-arabitol, myo-inositol and xylitol. Does not reduce nitrate. Catalase-positive and oxidase-negative. Tween 40 and Tween 80 hydrolysis activities are positive.

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

Acknowledgements

We thank Marie-Line Escande for assistance with the transmission electron microscopy and Hélène Agogué for her contribution to the initial sequencing work. We acknowledge Dr J. P. Euzéby for support in the Latin etymologies of the genus and species names. This work was supported financially by the Equipe Mixte de Recherche linking the Université Pierre et Marie Curie and the Centre National de la Recherche Scientifique to the Pierre Fabre Laboratories. The project was also carried out in the framework of the MarBEF Network of Excellence 'Marine Biodiversity and Ecosystem Functioning' which is funded by the Sustainable Development, Global Change and Ecosystems Program of the European Community's Sixth Framework Program (contract no. GOCE-CT-2003-505446). This publication is contribution number MPS-07059 of MarBEF. It was also partly funded by the French program 'Bio-diversité et Changement Global – project: development of a coastal microbial observatory' from the Institut Français de la Biodiversité.

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