Celeribacter neptunius gen. nov., sp. nov., a new member of the class *Alphaproteobacteria*

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A whitish Gram-negative, motile, aerobic bacterium, designated strain H 14^T, was isolated from seawater collected at St Kilda beach in Port Phillip Bay, Melbourne, Australia. Analysis of 16S rRNA gene sequences revealed that the organism belonged to the *Roseobacter* lineage of the class *Alphaproteobacteria*, forming a distinct evolutionary lineage at the genus level. Strain H 14^T was distantly related to the genera *Nautella*, *Ruegeria* and *Pseudoruegeria* (family *Rhodobacteraceae*). Strain H 14^T was unable to degrade gelatin, casein, chitin, agar and starch, did not produce any carotenoids, did not possess bacteriochlorophyll *a* and had a limited ability to utilize carbon sources. Strain H 14^T grew with concentrations of 1–8% (w/v) NaCl and over a temperature range of 5–35 °C. Phosphatidylglycerol was the major phospholipid (90%); phosphatidylcholine (7.9%) and phosphatidylethanolamine (2.0%) were present in minor quantities. The predominant fatty acids were C_{18:1}ω7c (82.4%), C_{18:1}ω9c (5.1%) and C_{18:0} (3.8%). The DNA G + C composition for strain H 14^T was 59.1 mol%. Based on the results of physiological, biochemical, chemotaxonomic and phylogenetic investigations, a new genus, *Celeribacter* gen. nov., with the type species *Celeribacter neptunius* sp. nov. is proposed. The type strain of the type species is H 14^T (=KMM 6012^T=CIP 109922^T).

The phylogenetically diverse class *Alphaproteobacteria* has undergone intensive reclassifications (Uchino *et al.*, 1998; Soller *et al.*, 2000; Martens *et al.*, 2006) and the list of new members is constantly growing (e.g. Yoon *et al.*, 2008). This study aims to describe another novel representative of the class *Alphaproteobacteria*.

Strain H 14^T was isolated from seawater collected from Port Phillip Bay, Melbourne, Australia, at St Kilda beach on 12 December 2007. A seawater sample was taken from the first metre below the surface. The sample handling and isolation procedure used was as previously described

(Ivanova *et al.*, 1996, 2004; Webb *et al.*, 2009). Determination of the phenotypic properties, used for the characterization of new isolates, was performed by using standard procedures (Smibert & Krieg, 1994) and as described by Ivanova *et al.* (1996, 1998).

Cell size and morphology (see Supplementary Fig. S1 in IJSEM Online) were examined using electron microscopy as previously described (Ivanova *et al.*, 2005). To study physiological properties, bacteria were grown on marine agar (MA) 2216 (BD) at 22–24 °C. Motility was studied using hanging drop preparations. The following physiological and biochemical properties were examined: oxidation/fermentation of glucose (Hugh & Leifson, 1953), Gram staining, reduction of nitrate and nitrite, catalase [with 5% (v/v) H₂O₂] and oxidase (Kóvacs, 1956) activities, gelatin liquefaction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase activities, poly-β-hydroxybutyrate and acetoin production (Voges–Proskauer test), sodium requirement [0, 1, 3, 6, 8, 10, 12, 15% (w/v) NaCl], indole and H₂S production, and the

Abbreviation: BChl *a*, bacteriochlorophyll *a*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of H 14^T is FJ535354.

Images of cells of strain H 14, polar lipid patterns and two additional phylogenetic trees constructed using parsimony and maximum likelihood algorithms are available as supplementary figures with the online version of this paper. An additional table detailing the fatty acid contents of the novel strain is also available.

ability to hydrolyse starch, Tween 80, casein, DNA and agar. The temperature range for growth was examined on MA incubated at 2, 5, 10, 30, 35, 37 and 42 °C. Other physiological and biochemical tests were performed with the API 20E, API 50 and API ZYM systems (BioMérieux).

Susceptibility to antibiotics was tested by the conventional diffusion plate technique, using MA and discs impregnated with following antibiotics (µg per disc unless otherwise stated): chloramphenicol (30), benzylpenicillin (10 U), carbenicillin (100), nalidixic acid (30), lincomycin (15), rifampicin (5), ofloxacin (5), ampicillin (10), cephalixin (30), oleandomycin (15), cephazolin (30), tetracycline (30), kanamycin (30), oxacillin (10), streptomycin (30), gentamicin (10), erythromycin (15), polymyxin (300 U) and neomycin (30). The morphological, growth, physiological and biochemical characteristics of the novel strain are given in Table 1 and in the genus and species descriptions.

To enable bacteriochlorophyll *a* (BChl *a*) isolation and characterization, strain H 14^T was grown at 28 °C on MA over 48 h and the bacterial mass was collected from five Petri plates as described earlier by Ivanova *et al.* (2005). The bacterial cells were disrupted using a homogenizer (MPW-324; Mechanika Preczyjna) and the entire homogenate was extracted twice using methanol. The combined organic extract phases were evaporated to dryness. The resulting extract was used to determine the presence of BChl *a* as previously described (Ivanova *et al.*, 2005).

For the analysis of phospholipids and fatty acids, the novel strain was grown at 28 °C on MA and cells were harvested after an incubation period of 48 h. The lipids were extracted by a method modified from that of Bligh & Dyer (1959). The detection and identification of lipids and fatty acids was performed according to a previously described method (Ivanova *et al.*, 2005). Polar lipids were separated using two-dimensional micro-thin layer chromatography with the solvent systems described by Vaskovsky & Terekhova (1979). Phosphatidylglycerol was found to be the major phospholipid (90 %) and phosphatidylcholine (7.9 %) and phosphatidylethanolamine (2 %) were found to be present in minor quantities. An unidentified aminolipid and traces of lysophosphatidylethanolamine were also detected (see Supplementary Fig. S2 in IJSEM Online). The predominant fatty acids were found to be C_{18:1}ω7c (82.4 %), C_{18:1}ω9c (5.1 %) and C_{18:0} (3.8 %). The presence of fatty acid C_{18:1}ω9c in the novel strain, a feature that is not found in the fatty acid profiles of members of the genera *Nautella* and *Pseudoruegeria* (Yoon *et al.*, 2007; Vandecandelaere *et al.*, 2009), and a higher proportion of the principal fatty acids, i.e. C_{18:1}ω7c, were found to be characteristic features of the fatty acid profile of the new isolate. The polar lipids and the major cellular fatty acids for strain H 14^T are presented in Supplementary Table S1 (available in IJSEM Online).

DNA was isolated from the novel strain according to the method described by Marmur (1961). The G + C content

of the DNA was found to be 59.1 mol% as determined by the thermal denaturation method (Marmur & Doty, 1962).

The 16S rRNA gene sequence was amplified and sequenced by the Australian Genome Research Facility (AGRF) Laboratories (Brisbane, Australia). The 16S rRNA gene sequence of strain H 14^T was compared against a database of cultured species via BLAST analysis (<http://bioinfo.unice.fr/blast>), the 16S rRNA gene sequence database at DDBJ (<http://blast.ddbj.nig.ac.jp/top-e.html>) and the EzTaxon database of type strains (Chun *et al.*, 2007) in order to retrieve most similar sequences of recognized bacteria. The 150 sequences retrieved were aligned with CLUSTAL X 2.0 (Larkin *et al.*, 2007). Alignments were then manually checked with SeaView (Galtier *et al.*, 1996) and domains common to all sequences were used to derive a first phylogenetic tree using the most recent version of SeaView. A BIONJ analysis allowed the selection of the 37 sequences of the most closely related type strains. Alignments were checked again and a full phylogenetic analysis was undertaken using the BIONJ, parsimony (DNAPARS) and maximum-likelihood algorithms, excluding positions containing indels, to confirm the tree topology (see Supplementary Figs S3 and S4 in IJSEM Online). For the neighbour-joining (NJ) analysis, a matrix distance was calculated using a Kimura two-parameter correction. Bootstraps were performed using 1000 replications. BIONJ was used according to the method described by Gascuel (1997) and maximum-likelihood was determined using PhyML (Guindon & Gascuel, 2003). The phylogenetic trees were drawn using TreeDyn (Chevenet *et al.*, 2006) or SeaView. Among type strains, *Nautella italica* LMG 24365^T, *Pseudoruegeria aquimaris* SW-255^T and *Roseovarius crassostreae* CV919-312^T showed gene sequence similarity values between 96.7 % and 96 % (55, 46 and 56 differences in 1421 positions, respectively) with strain H 14^T clustered with *Nautella italica* LMG 24365^T, with only 77 % bootstrap support. Notably, strain H 14^T did not cluster with any other recognized species, clearly indicating that the new isolate belongs to a separate genus.

On the basis of the physiological, biochemical, chemotaxonomic and phylogenetic data (Fig. 1), strain H 14^T represents a novel genus and species within the class *Alphaproteobacteria*. A summary of the phenotypic differential characteristics of strain H 14^T and its closest phylogenetic neighbours is presented in Table 1. Comparative analysis of phenotypic traits indicated that strain H 14^T exhibited a characteristic phenotypic and chemotaxonomic profile. For example, strain H 14^T could be distinguished from other related species identified by the phylogenetic analysis by the negative oxidase reaction and by different utilization of D-glucose, citrate, D-xylose and L-arabinose. The proportion of three major fatty acids, C_{18:0}, C_{18:1}ω9c and C_{18:1}ω7c, and three major phospholipids, phosphatidylglycerol, phosphatidylcholine and phosphatidylethanolamine, can be regarded as taxonomic markers for the new genus. Based on these results, a new

Table 1. Characteristics that differentiate strain H 14^T from other phylogenetically related species

Taxa: 1, H 14^T (data from this study); 2, *Nautella italica* LMG 24365^T (Vandecandelaere *et al.*, 2009); 3, *Jannaschia rubra* 4SM3^T (Macian *et al.*, 2005); 4, *Ketogulonicigenium* (Urbance *et al.*, 2001); 5, *Loktanelia* (Ivanova *et al.*, 2004); 6, *Nereida ignava* 2SM4^T (Pujalte *et al.*, 2005); 7, *Oceanicola marinus* AZO-C^T (Cho & Giovannoni, 2004); 8, *Octadecabacter* (Gosink *et al.*, 1997); 9, *Pseudoruegeria aquimaris* SW-255^T (Yoon *et al.*, 2007); 10, *Roseisalinus antarcticus* EL-88^T (Labrenz *et al.*, 2005); 11, *Roseovarius* (Labrenz *et al.*, 1999; Boettcher *et al.*, 2005; Yoon *et al.*, 2008); 12, *Ruegeria* (Uchino *et al.*, 1998; Arahal *et al.*, 2005; Martens *et al.*, 2006); 13, *Shimia marina* CL-TA03^T (Choi & Cho, 2006); 14, *Thalassobacter arenae* GA2-M15^T (Kim *et al.*, 2009); 15, *Thalassobius* (Arahal *et al.*, 2005). All species are catalase-positive. +, Positive; −, negative; w, weakly positive reaction; v, variable reaction depending on strain; ND, no data available; NG, no growth; AL, unidentified aminolipid; LPE, lysophosphatidylethanolamine; DP, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DNA G + C content (mol%)	59	61	59–65	52–54	62–69	56	64–72	57	67	67	58–66	54–64	57	59	57–61
Bacteriochlorophyll <i>a</i> production	−	ND	−	ND	ND	ND	−	−	ND	−	v	−	−	+	ND
Motility	+	+	v	v	v	−	−	−	+	−	v	v	+	+	v
Pigment	−	Beige	v	Brown	v, pink	−	Yellowish	−	Yellow greyish	Red	Pink beige	v	Beige	Deep brown	−
Oxidase	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Growth at 37 °C	−	+	−	v	v	−	+	+	+	−	−	−	−	+	+
Temperature range for growth (°C)	5–35	4–45	4–32	27–31	4–37	13–28	4–40	5–37	15–49	<3–33.5	4–40	5–37	15–35	13–37	13–37
Growth range with NaCl (% w/v)	1–8	1–5	0.34–9	0–4	0–14	1.4–8	0–10	0–8	0–9	0.1–13	0–15	0.6–12	3–7	0.85–7	1–8
Nitrate reduction	+	−	−	ND	v	−	w	ND	+	+	−	+	+	−	v
Production of:															
Lipase (Tween 80)	−	+	v	ND	v	ND	+	ND	−	+	v	−	+	−	v
Amylase	−	−	−	ND	v	−	−	ND	−	−	−	v	w	−	−
Gelatinase	v	−	−	ND	−	ND	−	−	−	−	v	v	+	−	v
Utilization of:															
D-Glucose	+	−	v	+	−	w	v	−	+	+	−	+	−	−	+
Citrate	−	−	+	ND	−	w	−	−	+	v	v	+	+	−	+
D-Xylose	−	+	+	ND	−	−	−	−	+	+	−	v	−	−	v
L-Arabinose	−	+	−	+	−	−	v	+	−	+	−	−	−	−	v
Major polar lipids	PE, PG, PC, LPE, AL	ND	PG, DPG, PC, PE, AL	ND	ND	ND	ND	ND	PG, DPG, PE, PL, GL	DPG, PC	ND	PC, PG, DPG, PE, PL	ND	PG, DPG, PC, AL	ND

Fig. 1. Phylogenetic position of strain H 14^T according to the 16S rRNA gene sequence analysis. Sequences in this tree were chosen from a larger tree obtained after a distance analysis was performed on a larger dataset. Percentages of bootstraps (1000 replications) are indicated for branches when higher than 50%. Bar, 0.01 nucleotide substitutions per site.

genus and a novel species, *Celeribacter neptunius* gen. nov., sp. nov., is proposed.

Description of *Celeribacter* gen. nov.

Celeribacter (Ce.le.ri.bac'ter. L. adj. *celer* -eris -e quick, rapid; N.L. masc. n. *bacter* rod; N.L. masc. n. *Celeribacter* rapidly growing rod).

Rod-shaped cells. Gram-negative. Do not form endospores or resting stages. Aerobic. Anaerobic growth occurs by fermentation of D-glucose by anaerobic respiration of nitrate. Chemoorganotroph. Cytochrome oxidase-negative, catalase-positive. Reduce nitrate to nitrite. Three major fatty acids, C_{18:0}, C_{18:1}ω_{9c} and C_{18:1}ω_{7c}, and three major phospholipids, phosphatidylglycerol, phosphatidylcholine and phosphatidylethanolamine, are characteristic of the genus. The type species of the genus is *Celeribacter neptunius*.

Description of *Celeribacter neptunius*

Celeribacter neptunius [nep.tu'ni.us. L. masc. adj. *neptunius* pertaining to Neptunius (Roman god of the sea), referring to the habitat of the bacteria].

Short rod-shaped cells with slightly irregular ends, approx. 0.8–1.8 μm long and 0.4–0.9 μm wide. Single polar or

subpolar flagellum. Gram-negative. Chemoorganotroph with respiratory metabolism. Does not accumulate poly-β-hydroxybutyrate as an intracellular reserve product and does not have an arginine dihydrolase system. The colonies are uniformly round, 1–3 mm in diameter, regular, convex and smooth after incubation for 48–74 h on MA. No diffusible pigment, carotenoids or BChl *a* produced in the medium. Does not form endospores. Requires Na⁺ or seawater for growth. Growth occurs in media with 1–8 % (w/v) NaCl. Mesophilic. Temperature for growth ranges from 5 to 35 °C, with an optimum at 25 °C. No growth is detected at 37 °C. The pH for growth ranges from 4.0 to 10.0, with optimum growth at pH 7.5–8.0. Does not decompose gelatin, agar, starch, casein, laminarin, chitin or DNA. Tween 80 is weakly utilized. Urease-positive. Negative for indole, H₂S and acetoin production and for arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-glucosidase and β-glucosidase are produced. With the API 50 system, D-xylose, L-rhamnose, methyl α-D-glucopyranoside, D-lyxose, D-arabitol, D-glucose, D-fructose, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, D-lactose (bovine origin), sucrose and trehalose are utilized. Susceptible to (μg per disc unless otherwise stated): chloramphenicol (30), benzylpenicillin (10 U), carbenicillin (100), nalidixic

acid (30), rifampicin (5), ofloxacin (5), ampicillin (10), cephalixin (30), cephazolin (30), tetracycline (30), kanamycin (30), oxacillin (10), streptomycin (30), gentamicin (10), erythromycin (15) and neomycin (30). Resistant to: lincomycin (15), polymyxin (300 U) and oleandomycin (15). Phosphatidylglycerol, phosphatidylcholine and phosphatidylethanolamine are the major phospholipids. The main cellular fatty acid is C_{18:1}ω7 (82.4 %).

The type strain, H 14^T (=KMM 6012^T=CIP 109922^T), was isolated from seawater collected in the Port Phillip Bay of the Tasman Sea of the Pacific Ocean. The DNA G+C content of the type strain is 59.1 mol%.

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