INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Fidalgo *et al.*, *Int J Syst Evol Microbiol* 2017;67:2026–2030
DOI 10.1099/ijsem.0.001914

MICROBIOLOGY SOCIETY

Saccharospirillum correiae sp. nov., an endophytic bacterium isolated from the halophyte *Halimione portulacoides*

Cátia Fidalgo,[1] Jaqueline Rocha,[1] Diogo Neves Proença,[2] Paula Vasconcelos Morais,[2,3] Artur Alves[1,*] and Isabel Henriques[1]

Abstract

A Gram-stain negative, oxidase- and catalase- positive, motile, aerobic, non-pigmented spirillum, designated CPA1^T, was isolated from the surface-sterilized tissues of a halophyte, *Halimione portulacoides*, collected from a salt marsh in Aveiro, Portugal. The isolate was mesophilic, facultatively alkaliphilic and halophilic, and grew between 18 and 42.5 °C (optimum 30 °C), from pH 5.0 to 11.5 (optimum 7.0–7.5), from 0.5 to 5 % NaCl (w/v, optimum 2 %). Analysis of the 16S rRNA gene sequence showed that this strain belongs to the genus *Saccharospirillum*, as the highest sequence similarities were observed with *Saccharospirillum impatiens* EL-105^T (96.46 %), *Saccharospirillum salsuginis* YIM-Y25^T (96.32 %) and *Saccharospirillum aestuarii* IMCC 4453^T (95.17 %). The next closest matches were with other genera and below 95.0 %. Phylogenetic analyses revealed that the strain forms a robust clade with other species of the genus *Saccharospirillum*. The main respiratory quinone was Q-8 and the major fatty acids were $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The DNA G+C content was 55.2 mol%. Molecular, physiological and biochemical differences between strain CPA1^T and other type strains of species of the genus *Saccharospirillum* support the addition of this novel species to the genus, and the name *Saccharospirillum correiae* sp. nov. is proposed, with CPA1^T (=CECT 9131^T =LMG 29516^T) as the type strain.

The genus *Saccharospirillum*, established by Labrenz *et al.* [1], belongs to the family *Saccharospirillaceae* and, at the time of writing, it comprises three species with validly published names, all of which have been isolated from saline environments: *Saccharospirillum impatiens* from a hypersaline lake [1], *Saccharospirillum salsuginis* from subterranean brine [2], and *Saccharospirillum aestuarii* from mudflats [3]. The genus has been described as Gram-negative spirilla, non-spore forming, containing poly-β-hydroxybutyrate, which may form coccoid bodies in older cultures, and positive for peroxidase, catalase and cytochrome oxidase activities, with Q-8 as the prevalent respiratory quinone, and $C_{16:1}\omega7c$, $C_{16:0}$ and $C_{18:1}\omega7c$ as the predominant fatty acids [1]. This genus has also been described as comprising obligate aerobes, microaerophiles or facultative anaerobes. The motility and presence of flagella has been determined to be species-dependent [3].

In a study of the bacterial diversity associated with the internal tissues of the halophyte *Halimione portulacoides*, 665 isolates were obtained and characterized [4]. Briefly, healthy specimens of the halophyte were collected in Ria de Aveiro, Portugal, and above- and below-ground tissues were separated and surface sterilized. Dilutions of macerated tissues were plated in Tryptic Soy Agar (TSA, Merck), R2A (Merck) and Marine Agar (MA, Difco). The present study focuses on strain CPA1^T, an isolate obtained from surface sterilized above-ground tissues of *H. portulacoides*. Strain CPA1^T was routinely streaked on MA medium and incubated under aerobic conditions at 28 °C.

Genomic DNA of the strain was extracted using a Genomic DNA Purification kit #0513 (Thermo Scientific), following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR with universal primers 27F and 1492R, as described elsewhere [4], and sequenced with primers 27F [5] and 704F [6]. Analysis of similarity using a near full length 16S rRNA gene sequence (1418 nt) also gave evidence that strain CPA1^T belongs to the genus *Saccharospirillum*, as highest similarities were seen with this genus: *S. impatiens* EL-105^T (96.46 %), *S. salsuginis* YIM-Y25^T (96.32 %) and *S. aestuarii* IMCC 4453^T (95.17 %). The next

Author affiliations: [1]Departamento de Biologia, CESAM, Universidade de Aveiro, Aveiro, Portugal; [2]CEMUC, University of Coimbra, 3030-788 Coimbra, Portugal; [3]Department of Life Sciences, FCTUC, University of Coimbra, 3000-456 Coimbra, Portugal.
***Correspondence:** Artur Alves, artur.alves@ua.pt
Keywords: *Saccharospirillaceae*; endophytic; salt marsh; halophytes; taxonomy.
Abbreviations: ML, Maximum likelihood; MP, Maximum parsimony; NJ, Neighbour joining.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CPA1^T is KY310592.
One supplementary figure is available with the online Supplementary Material.

closest matches occurred with type strains of the genus *Reinekea*, with less than 95.0 % 16S rRNA gene sequence similarities. Similarities with type strains belonging to other genera were below 92.0 %.

For phylogenetic analyses, the sequences of closely related taxa were obtained from the EzTaxon database [7]. Sequence alignments were carried out with CLUSTAL Omega [8], and BioEdit version 7.2.5 [9] was used to edit the aligned sequences. Phylogenetic analyses were performed using MEGA version 6.0 [10] by using Kimura's two-parameter model [11] in the reconstruction of neighbour-joining (NJ), [12], maximum-likelihood (ML), [13] and maximum parsimony (MP), [14] trees. Bootstrap values based on 1000 replications were obtained in these phylogenetic analyses. The topology of the ML tree was the same as the MP tree. Fig. 1 shows that strain CPA1^T forms a robust clade with the three species of the genus *Saccharospirillum* with validly published names. High bootstrap values were observed, ranging from 72 to 96 %, depending on the tree building method, confirming CPA1^T as a potential novel member of this genus.

Optimal growth conditions for strain CPA1^T were determined using MA medium. The temperature range for growth and the optimum were tested by incubating at 4, 18, 26, 30, 37, 42.5 and 50 °C. At the optimum growth temperature, the pH range and optimum were assayed using pH values from 4 to 12, at intervals of 0.5 . At the optimum growth temperature and pH, the strain's growth requirements for, and tolerance to salt was assessed using the following NaCl concentrations: 0.5 (TSA without additional NaCl), 2 (MA without additional NaCl), 3 [MA supplemented with 1 % NaCl (w/v)] , 5, 10, 12, 15 and 20 % NaCl (w/v).

Growth in biochemical and phenotypic tests was determined on MA, and incubation was conducted at 30 °C for 48 h, unless otherwise stated. A Gram staining kit (Merck) was used according to the manufacturer's instructions. Determination of cell size, as well as morphology and motility was carried out using light microscopy (Nikon 80i). The hanging-drop method [15] was used to assess gliding motility, after growing the strain in half strength MA for 72 h, and using cavity slides. Catalase and oxidase activities were determined using H_2O_2-reagent (Liofilchem) and oxidase strips (Liofilchem), respectively. Growth in thioglycollate medium (Merck) was used to assess oxygen metabolism, and incubation was followed for 7 days. Production of H_2S was assessed using Kligler's Iron Agar (Merck). Ability to hydrolyse starch, casein, cellulose and xylan was assessed by methods described in Fidalgo *et al.* [4].

Biochemical tests were carried out using API 20NE, API ZYM and API 50CH strips (bioMérieux) according to the manufacturer's instructions, except for using 0.9 % (w/v) NaCl solution for preparing cell suspensions. *Saccharospirillum impatiens* DSM 12546^T was tested under the same conditions for comparison. These results are given in the species description and Table 1. Some results of the API 50CH tests of *S. impatiens* DSM 12546^{T}differed from the original description by Labrenz *et al.* [1]. The differences observed may result from the use of different suspension medium and different incubation temperatures.

Determination of G+C content was performed by HPLC [16]. The result obtained for strain CPA1^T was 55.2 %, which is close to the values determined for other species of the genus *Saccharospirillum*, which are 54.5 to 54.8 mol% for *S. impatiens* EL-105^T [1], 58.5 mol% for *S. salsuginis* YIM-Y25^T [2], and 56.5 mol% for *S. aestuarii* IMCC 4453^T [3].

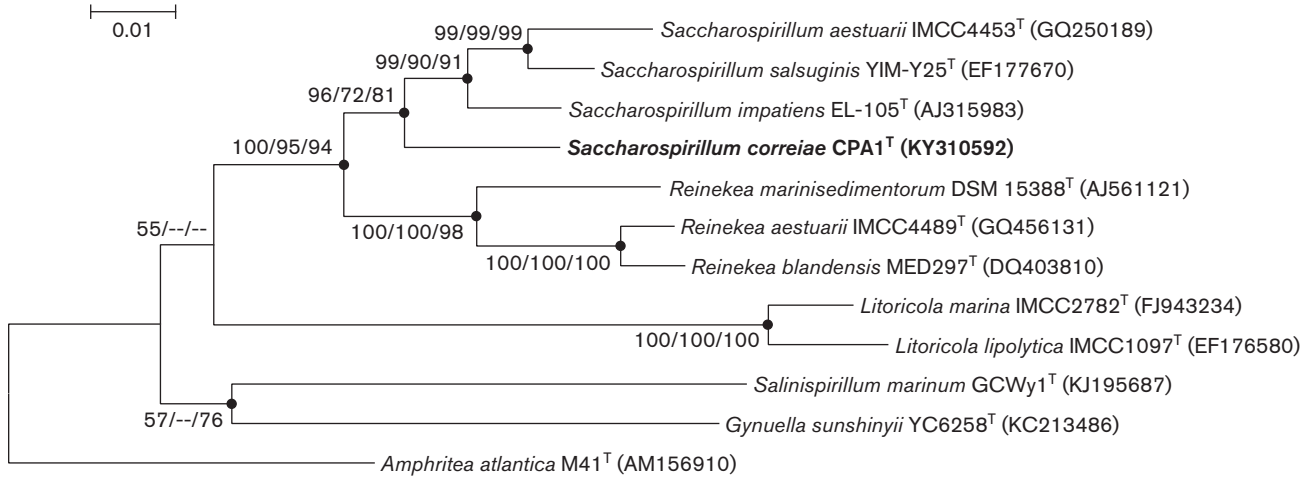

Fig. 1. Neighbour-joining (NJ) tree showing the phylogenetic positions of strain CPA1^T and representatives of other related taxa, based on 16S rRNA gene sequences. Filled circles indicate nodes that were also recovered in the maximum-parsimony (MP) tree and the maximum-likelihood (ML) tree, based on the same sequences. Bootstrap values (expressed as percentages of 1000 replications) ≥50 % are shown at branching points for NJ/ML/MP trees. *Amphritea atlantica* M41^T was used as an outgroup. Accession numbers for the type strains are shown in parenthesis. Bar, 0.01 nt substitution rate (K_{nuc}) units.

Table 1. Differential characteristics of strain CPA1^T and species of the genus *Saccharospirillum* with validly published names

Strains: 1, CPA1^T; 2, *S. impatiens* DSM 12546^T (data from this study unless otherwise stated); 3, *S. salsuginis* YIM-Y25^T [2]; 4, *S. aestuarii* IMCC 4453^T [3]. +, Positive; w, weakly positive; −, negative; ND, not determined;

Characteristic	1	2	3	4
Cell morphology	Spirilla	Spirilla*	Spirilla	Curved rods
Motility	Motile	Motile*	Motile	Non motile
Relation to oxygen	Obligately aerobic	Aerobic to microaerophilic*	Obligately aerobic	Facultatively anaerobic
Temperature range	18–42.5 °C	<2.5–43 °C*	15–50 °C	10–42 °C
pH range	5–11.5	5.5–9.5*	6–10	5–12
NaCl (w/v) range	0.5–5 %	<1–13 %*	1–15 %	0.5–10 %
H_2S production	−	+*	−	−
Hydrolysis of:				
Casein	+	ND	−	+
Starch	−	variable*	−	−
Carboxymethyl cellulose	−	ND	ND	+
API 20NE				
Hydrolysis of gelatin	+	−†	−	+
Reduction of nitrates to nitrites	−	−†	+	+
Indole production	−	−	+	−
Assimilation of D-mannose and D-maltose	−	−	+	ND
Assimilation of malic acid	−	−†	+	ND
API ZYM				
Alkaline phosphatase, esterase (C4), esterase lipase (C8), α-glucosidase	+	w	+	ND
Naphtlol-AS-BI-phosphohydrolase, β-galactosidase	w	w	+	ND
Leucine arylamidase	+	+†	+	ND
Acid phosphatase	+	−	+	ND
N-acetyl-β-glucosaminidase	+	−	ND	ND
α-Fucosidase	w	−	ND	ND
API 50CH				
D-Ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-cellobiose, D-maltose, D-trehalose, glycogen, L-rhamnose, D-lactose (bovine), D-saccharose (sucrose)	−	−†	+	ND
Inositol, D-manitol, D-sorbitol, L-arabinose, D-raffinose	−	−	+	ND
Glycerol, salicin	−	−†	−	ND
Aesculin ferric citrate, D-melibiose, starch, D-turanose, D-fucose, gentibiose	−	−†	ND	ND
DNA G+C content (mol%)	55.2	54.5–54.8*	58.5	56.5

*Data from [1].

†Result differed from that published in [1].

Assessment of fatty acid profiles, polar lipids and quinones was conducted as described by Proença *et al.* [17] and performed in parallel with similar assessments of *S. impatiens* DSM 12546^T. To obtain cells for fatty acid profile determination, growth was obtained on MA at 30 °C for 48 h. The main fatty acids observed for strain CPA1^T were $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), which add up to over 78 % of the total (complete profile in Table 2). Summed features arise when the equivalent chain length value obtained corresponds to a fatty acid that cannot be separated from another fatty acid. Consequently, the relative concentration of the possible two or more fatty acids is given as a singular value [18]. The results are in accordance with what has previously been described for the genus. Growth for determination of quinones and polar lipids was obtained using Marine Broth (MB) and incubating at 30 °C for 72 h. The main quinone detected was Q-8, which is in accordance with data for the genus. Q-9 was also detected, but in minor amounts. The polar lipid profiles obtained for strain CPA1^T and *S. impatiens* DSM 12546^T were very similar, suggesting that both strains belong to the same genus (Fig. S1, available in the online Supplementary Material). The lipids phosphatidylethanolamine, monomethylphosphatidylethanolamine and phosphatidylcholine were identified in the profile of strain CPA1^T, but diphosphatidylglycerol and phosphatidylglycerol were not detected.

Table 2. Fatty acid composition of CPA1^T and type strains of species of the genus *Saccharospirillum*

Strains: 1, CPA1^T; 2, *S. impatiens* DSM 12546^T (data from this study); 3, *S. impatiens* EL-105^T (data from [1]); 4, *S. salsuginis* YIM-Y25^T [2]; 5, *S. aestuarii* IMCC 4453^T [3]. Values represent percentage of total fatty acids. ECL, equivalent chain length; —, not detected; TR, trace amounts (<1 %). Summed features represent groups of two or more fatty acids that could not be separated. Summed feature 2 contains $C_{12:0}$ aldehyde, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ and/or unknown ECL 10.927; summed feature 3 contains $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 8 contains $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

Fatty acid	1	2	3	4	5
Unknown ECL					
15.272	TR	2.2	—	—	—
17.314	1.7	—	—	—	—
Saturated					
$C_{12:0}$ aldehyde	—	—	—	—	4.2
$C_{14:0}$	1.5	TR	—	TR	TR
$C_{16:0}$	31.4	27.4	19.0	11.4	24.3
$C_{17:0}$	TR	TR	—	1.2	TR
$C_{18:0}$	TR	TR	—	—	1.1
Unsaturated					
$C_{16:1}\omega7c$	—	—	21.8	—	—
$C_{17:1}\omega6c$	—	TR	—	1.2	TR
$C_{17:1}\omega8c$	TR	TR	—	2.3	TR
$C_{18:1}\omega7c$	—	—	51.2	53.4	—
$C_{19:1}$	—	—	1.9	—	—
Branched-chain					
iso-$C_{14:0}$ 3-OH	—	—	—	1.4	—
iso-$C_{16:0}$	TR	TR	—	13.3	7.9
iso-$C_{18:0}$	—	—	—	TR	1.5
iso-$C_{18:1}$ H	—	—	—	1.2	—
Hydroxyl					
$C_{14:0}$ 3-OH	—	—	1.6	—	—
$C_{14:1}$ 3-OH	—	—	2.3	—	—
Cyclic					
cyclo-$C_{17:0}$	4.0	TR	—	—	TR
cyclo-$C_{19:0}\omega8c$	2.1	—	—	1.4	TR
Summed features					
2	5.4	2.8	—	3.8	—
3	3.3	15.9	—	—	—
8	46.8	44.5	—	—	46.6
3*	—	—	—	5.2	6.7
7†	—	—	—	—	1.4

*Summed feature 3 (from [2]) contains $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$.

†Summed feature 7 (from [3]) contains $C_{19:1}\omega6c$ and/or $C_{19:1}\omega7c$.

The physiological and biochemical test results for strain CPA1^T are given in the species description. The phylogenetic and 16S rRNA gene sequencing data, as well as the similarities in biochemical and physiological characteristics, indicate that strain CPA1^T belongs to the genus *Saccharospirillum*. This novel strain is distinguishable from other species in the genus with validly published names, and these differentiating characteristics are listed in Table 1. As the threshold for genomic delineation of a novel species (97 % 16S rRNA gene sequence similarity) [19, 20] was not surpassed, there was no need for DNA–DNA relatedness tests to be performed. So, CPA1^T is suggested to represent a novel species of the genus *Saccharospirillum*, and the name *Saccharospirillum correiae* sp. nov. is proposed.

DESCRIPTION OF *SACCHAROSPIRILLUM CORREIAE* SP. NOV.

Saccharospirillum correiae (*cor.rei'ae*. N.L. gen. masc. n. *correiae* of Correia, in honour of Portuguese microbiologist António Correia).

Cells are Gram-stain-negative, oxidase and catalase positive, motile (not by gliding) obligately aerobic, non-pigmented spirilla (0.47–0.93 µm×2.33–7.6 µm). Colonies are whitish, opaque in the centre and less so around the regular smooth edges and 0.5–1.5 mm in diameter after incubation in MA for 48 h at 30 °C. Moderately halophilic, mesophilic, and facultatively alkaliphilic, growing at salinities of 0.5 to 5 % NaCl (w/v) with the optimum at 2 % NaCl (w/v). Grows from 18 °C to 42.5 °C (optimum 30 °C) and from pH 5.0 to 11.5 (optimum pH 7.0–7.5). Does not produce H_2S. Hydrolyses casein and xylan, does not hydrolyse starch and cellulose. In API 20NE strips, it is positive for β-glucosidase, protease (hydrolysis of gelatine) and β-galactosidase (hydrolysis of para-nitrophenyl-β-D-galactopyranose); and negative for the reduction of nitrates to nitrites, nitrates to nitrogen, indole production, fermentation (D- glucose), arginine dihydrolase, urease, assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In API ZYM strips, it is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase (weakly), β-galactosidase (weakly), α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase (weakly); and negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase and α-mannosidase. In API 50CH strips it does not produce acid from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-manitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, D-celiobiose, D-maltose, D-lactose (bovine), D-melibiose, D-saccharose (sucrose), D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentibiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The main fatty acids are $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), and the main respiratory quinone is Q-8. The main polar lipids comprise phosphatidylethanolamine,

monomethylphosphatidylethanolamine and unidentified polar lipids.

The type strain, CPA1^T (=CECT 9131^T=LMG 29516^T) was isolated from the surface sterilized above-ground tissues of the halophyte *Halimione portulacoides*. The G+C content of the DNA of the type strain is 55.2 mol%.

Funding information
This work was supported by European Funds (FEDER) through COMPETE and by National Funds through the Portuguese Foundation for Science and Technology (FCT) within project PhytoMarsh (PTDC/AAC-AMB/118873/2010 – FCOMP-01–0124-FEDER-019328). FCT is acknowledged for financing to CESAM (UID/AMB/50017/2013– POCI-01–0145-FEDER-007638), Artur Alves (FCT Investigator Programme – IF/00835/2013), Isabel Henriques (FCT Investigator Programme – IF/00492/2013), Diogo Neves Proença (postdoctoral grant SFRH/BPD/100721/2014) and Cátia Fidalgo (PhD grant – SFRH/BD/85423/2012).

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

References

1. Labrenz M, Lawson PA, Tindall BJ, Collins MD, Hirsch P. *Saccharospirillum impatiens* gen. nov., sp. nov., a novel γ-*Proteobacterium* isolated from hypersaline Ekho lake (East Antarctica). *Int J Syst Evol Microbiol* 2003;53:653–660.
2. Chen YG, Cui XL, Li QY, Wang YX, Tang SK *et al.* *Saccharospirillum salsuginis* sp. nov., a gammaproteobacterium from a subterranean brine. *Int J Syst Evol Microbiol* 2009;59:1382–1386.
3. Choi A, Oh HM, Cho JC. *Saccharospirillum aestuarii* sp. nov., isolated from tidal flat sediment, and an emended description of the genus *Saccharospirillum*. *Int J Syst Evol Microbiol* 2011;61:487–492.
4. Fidalgo C, Henriques I, Rocha J, Tacão M, Alves A. Culturable endophytic bacteria from the salt marsh plant *Halimione portulacoides*: phylogenetic diversity, functional characterization, and influence of metal(loid) contamination. *Environ Sci Pollut Res* 2016;23:10200–10214.
5. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E and Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. New York, NY: John Wiley and Sons; 1991. pp. 115–175.
6. Kaksonen AH, Plumb JJ, Robertson WJ, Spring S, Schumann P *et al.* Novel thermophilic sulfate-reducing bacteria from a geothermally active underground mine in Japan. *Appl Environ Microbiol* 2006;72:3759–3762.
7. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.* Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
8. McWilliam H, Li W, Uludag M, Squizzato S, Park YM *et al.* Analysis tool web services from the EMBL-EBI. *Nucleic Acids Res* 2013;41:W597–W600.
9. Hall TA. BioEdit: a user-friendly biological sequence alignment edit and analysis program for windows 95/98/NT. *Nucl Acids Symp Ser* 1999;41:95–98.
10. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
11. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
12. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
13. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
14. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 1971;20:406–416.
15. Bowman JP. Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* 2000;50:1861–1868.
16. Mesbah M, Premachandran U, Whitman WB. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39:159–167.
17. Proença DN, Nobre MF, Morais PV. *Chitinophaga costaii* sp. nov., an endophyte of *Pinus pinaster*, and emended description of *Chitinophaga niabensis*. *Int J Syst Evol Microbiol*201464:1237–1243.
18. da Costa MS, Albuquerque L, Nobre MF, Wait R. The identification of fatty acids in bacteria. In: Rainey FA, Oren A (editors). *Methods in Microbiology* (*Taxonomy of Prokaryotes*), vol. 38. Amsterdam: Elsevier; 2011. pp. 183–196.
19. Stackebrandt E, Goebel BM. Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 1994;44:846–849.
20. Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O *et al.* Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.