Molecular and phenotypic analyses reveal the non-identity of the *Phaeobacter gallaeciensis* type strain deposits CIP 105210^T and DSM 17395

Nora Buddruhs,[1]† Silke Pradella,[1]† Markus Göker,[1] Orsola Päuker,[1] Rüdiger Pukall,[1] Cathrin Spröer,[1] Peter Schumann,[1] Jörn Petersen[1] and Thorsten Brinkhoff[2]

Correspondence
Jörn Petersen
joern.petersen@dsmz.de

[1]Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

[2]Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Oldenburg, Germany

The marine genus *Phaeobacter* currently comprises six species, some of which were intensively studied mainly due to their ability to produce secondary metabolites. The type strain of the type species, *Phaeobacter gallaeciensis* BS107^T, has been deposited at several public culture collections worldwide. Based on differences in plasmid profiles, we detected that the alleged *P. gallaeciensis* type strains deposited at the Collection Institute Pasteur (CIP; Paris, France) as CIP 105210 and at the German Collection of Microorganisms and Cell Cultures (DSMZ; Braunschweig, Germany) as DSM 17395 are not identical. To determine the identity of these strains, we conducted DNA–DNA hybridization, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), 16S rRNA gene and internal transcribed spacer (ITS) sequence analyses, as well as physiological experiments. Based on the detailed 16S rRNA gene reanalysis we showed that strain CIP 105210 most likely corresponds to the original *P. gallaeciensis* type strain BS107^T. In contrast, the *Phaeobacter* strain DSM 17395 exhibits a much closer affiliation to *Phaeobacter inhibens* DSM 16374^T (=T5^T) and should thus be allocated to this species. The detection of the dissimilarity of strains CIP 105210^T and DSM 17395 will influence future comparative studies within the genus *Phaeobacter*.

INTRODUCTION

The genus *Phaeobacter,* currently comprising the species *Phaeobacter gallaeciensis*, *P. inhibens*, *P. daeponensis, P. caeruleus*, *P. arcticus* and *P. leonis* (Gaboyer *et al.*, 2013), belongs to the marine *Roseobacter* clade. It was established by Martens *et al.* (2006) after reclassification of *Roseobacter gallaeciensis* (Ruiz-Ponte *et al.*, 1998) as *P. gallaeciensis*, which is the type species of the genus, and description of *P. inhibens* as a new species. During recent years, *Phaeobacter* strains have received a lot of interest due to the production of various secondary metabolites (e.g. Berger *et al.*, 2011, 2012; Brinkhoff *et al.*, 2004; Bruhn *et al.*, 2007; Geng *et al.*, 2008; Martens *et al.*, 2007; Seyedsayamdost *et al.*, 2011a, b).

Recently the genomes of three *Phaeobacter* strains have been published, *P. gallaeciensis* DSM 17395, *P. gallaeciensis* 2.10 (Thole *et al.*, 2012) and *P. gallaeciensis* ANG1 (Collins & Nyholm, 2011). However, evidence already indicated that strain ANG1 is more similar to *P. daeponensis* DSM 23529^T (=TF-218^T) than to either DSM 17395 or DSM 24588 (=2.10) (unpublished results). Strain 2.10 was previously used in competition experiments of bacterial biofilms on the thalloid green alga *Ulva australis* (Rao *et al.*, 2005). Various physiological and genetic aspects of *P. gallaeciensis* DSM 17395 have also been studied, such as the pathway and substrate specificity of the algal metabolite

†These authors contributed equally to this work.

Abbreviations: ccc, covalently closed circular; DDH, DNA–DNA hybridization; ITS, internal transcribed spacer; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; ML, maximum-likelihood; MP, maximum-parsimony; PFGE, pulsed-field gel electrophoresis; PM, Phenotype MicroArray.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the *Phaeobacter* strains CIP 105210^T, DSM 16374^T, DSM 17395 and DSM 24564^T are KC176239, KC176240, KC176241 and KC176242, respectively. The GenBank/EMBL/DDBJ accession numbers for the 16S–23S rRNA gene internal transcribed spacer of the *Phaeobacter* strains CIP 105210^T, DSM 16374^T, DSM 17395, DSM 23529^T, DSM 23566^T, DSM 24564^T and DSM 25627^T are KC176233, KC176234, KC176235, KC176236, KC176237, KC176238 and KC907729, respectively.

Four supplementary figures and four supplementary tables are available with the online version of this paper.

dimethylsulfoniopropionate (DMSP) catabolism (Dickschat *et al.*, 2010), the compatibility of the plasmids (Petersen, 2011), and the primary metabolism by proteome analyses (Zech *et al.*, 2009).

With the description of the species *P. gallaeciensis* (basonym *R. gallaeciensis*) in 1998, the type strain BS107^T was primarily deposited at the Collection Institute Pasteur (CIP; Paris, France) as CIP 105210 (Ruiz-Ponte *et al.*, 1998). According to the strain history (http://www.straininfo.net/strains/620650), the CIP referred the strain to the Colección Española de Cultivos Tipo (CECT; Burjassot, Spain) and to the American Type Culture Collection (ATCC; Manassas, USA), which in turn referred it to the Japan Collection of Micro-organisms at the RIKEN Bioscience Center (Tsikiba, Japan) followed by a transfer to the NITE (National Institute of Technology and Evaluation) Biological Resource Center (Kisarazu, Japan). At these culture collections the derivatives of strain CIP 105210 were designated CECT 7277^T, ATCC 700781^T, JCM 21319^T and NBRC 16654^T, respectively. The Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (DSMZ; Braunschweig, Germany) independently requested *P. gallaeciensis* BS107^T from the laboratory of the original depositor in 2005 and included it as DSM 17395 in the strain collection. Strain DSM 17395 was subsequently collected by the Laboratorium voor Microbiologie (LMG; Gent, Belgium) and deposited as LMG 24391^T. When investigating plasmid profiles of various *Phaeobacter* strains, we observed differences between the strains CIP 105210 and DSM 17395 even though both were considered identical with the type strain BS107^T. This is critical, as due to the broad scientific interest in the *P. gallaeciensis* type strain, it was either obtained from the public culture collections or retrieved from other sources several times. For example, Seyedsayamdost *et al.* (2011b) allegedly used strain BS107^T to investigate the mutualistic or pathogenic symbioses between *P. gallaeciensis* and the unicellular haptophycean alga *Emiliania huxleyi*. It was indicated that these authors received the strain BS107^T from a collaborating laboratory; hence the biological identity of the strain used is ultimately unclear.

In this study, we consequently reassessed the biological identity of these strains. We compared in detail the characteristics of the strains CIP 105210 and DSM 17395 with those of the description of BS107^T given by Ruiz-Ponte *et al.* (1998) and with those of other closely related *Phaeobacter* strains, i.e. *P. gallaeciensis* DSM 24588 (=2.10; Thole *et al.*, 2012) and *P. inhibens* DSM 16374^T (=T5^T; Martens *et al.*, 2006). Based on our results, according reclassifications are proposed.

METHODS

Source of bacteria and culturing. The *Phaeobacter* strains DSM 16374^T (=T5^T), DSM 17395, DSM 23529^T (=TF-218^T), DSM 23566^T (=20188^T), DSM 24564^T, DSM 24588 (=2.10) and DSM 25627^T (=306^T) as well as *Roseobacter litoralis* DSM 6996^T, *Marinovum algicola* DSM 10251^T and *Roseobacter denitrificans* DSM 7001^T were obtained from the DSMZ. The *Phaeobacter* strain CIP 105210 was obtained from the CIP. Unless otherwise stated, cells were grown in marine broth 2216 (MB; Difco) or on MB agar at 28 °C (and at 25 °C in case of *P. leonis* DSM 25627^T).

Profiles of the extrachromosomal elements. To analyse the plasmid content of the *Phaeobacter* strains, their high-molecular-mass total genomic DNA was prepared within agarose plugs as previously described and subjected to pulsed-field gel electrophoresis (PFGE; Pradella *et al.*, 2010). PFGE was performed in a contour-clamped homogeneous electric field (CHEF) system on a CHEF-DR III device (Bio-Rad) with 1 % or 1.2 % agarose gels and modified 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 0.1 mM EDTA) at 14 °C. PFGE parameters, namely pulse time ramps and run times, were varied both to resolve chromosomal and extrachromosomal DNA and to identify different plasmid conformations (Römling *et al.*, 1996). Two PFGE parameter sets were applied to assess plasmid topology: (i) set A, 1 % (w/v) agarose gel with pulse times of 1 to 48 s for 24 h at 200 V (6 V cm^{-1}) and (ii) set B, 1 % (w/v) agarose gel with pulse times of 1 to 20 s for 22 h at 200 V (6 V cm^{-1}). At least two PFGE gels were evaluated to determine plasmid sizes. The resulting plasmid profiles were interpreted as described by Pradella *et al.* (2010). Conventional unidirectional gel electrophoresis of DNA was in 0.8 % agarose gels and 1× TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) at 10 °C and 70 mA for 8.5 h. The BAC Tracker supercoiled DNA ladder (from 38 to 120 kb; Epicentre) was used to size plasmids with covalently closed circular (ccc) DNA topology.

16S rRNA gene and 16S–23S rRNA gene internal transcribed spacer (ITS) analysis. The PCR amplification of 16S rRNA genes from the genomic DNA of the *Phaeobacter* strains was done as described by Rainey *et al.* (1996). For the PCR amplification of the ITS region, the primer pair 16S_1401f 5′-GRGCCTTGYACACACCG-3′ (Lane, 1991) and 23S_130r 5′-GGTTBCCCCATTCRG-3′ (Gürtler & Stanisich, 1996) was used. Resulting PCR products were cycle sequenced with the primers mentioned above in 'Extended Hot Shot' reactions as offered by the Seqlab company, Germany. The sequence analysis tool BioEdit 7.0.1 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) was utilized for 16S rRNA gene and ITS sequence editing. The accession numbers of retrieved 16S rRNA gene sequences from *P. gallaeciensis* BS107^T (Ruiz-Ponte *et al.*, 1998), *P. inhibens* T5^T (Martens *et al.*, 2006), *P. daeponensis* TF-218^T, *P. arcticus* 20188^T, *P. gallaeciensis* LSS9 and *P. leonis* 306^T were Y13244, AY177712, NR_044026, NR_043888, GQ906799 and HE661585, respectively. Further 16S rRNA gene or ITS sequences used in this study were extracted from the genome sequences of *Phaeobacter* strains DSM 17395 (ABIF01000000), DSM 24588 (=2.10; CP002972–CP002975) and ANG1 (AFCF01000000) using the Integrated Microbial Genomes (IMG) system (http://img.jgi.doe.gov/cgi-bin/w/main.cgi; Markowitz *et al.*, 2012).

Sequences were aligned with MAFFT version 6.850b, using the '*–genafpair*' option but default settings otherwise (Katoh *et al.*, 2005). Phylogenetic analysis under the maximum-likelihood (ML) criterion (Felsenstein, 1981) was conducted with RAxML version 7.2.8, using its novel rapid bootstrap option combined with the autoMRE bootstrapping criterion (Pattengale *et al.*, 2010) with subsequent search for the best tree under the GTRMIX approach (Stamatakis *et al.*, 2008). Branch-and-bound search for the best trees under the maximum-parsimony (MP) criterion (Fitch, 1971) was done with PAUP* version 4.0b10 (Swofford, 2002), treating gaps as missing data and collapsing branches of zero minimum length; 1000 bootstrap replicates were conducted in the same manner. The resulting best trees were rooted using the midpoint-rooting method (Farris, 1972; Hess & De Moraes Russo, 2007).

MALDI-TOF MS protein analysis. Whole-cell protein extracts of the *Phaeobacter* strains CIP 105210, DSM 17395, DSM 24588, DSM 16374^T, DSM 23529^T, DSM 23566^T, DSM 24564^T and DSM 25627^T were analysed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Microflex L20 mass spectrometer (Bruker Daltonics) equipped with a N_2 laser. Sample preparation for MALDI-TOF MS protein analysis was carried out according to the ethanol/formic acid extraction protocol recommended by Bruker Daltonics as described in detail by Tóth *et al.* (2008). The MALDI-TOF mass spectra were analysed with the BioTyper software (version 3.0; Bruker Daltonics).

DNA–DNA hybridization (DDH). Cells of the *Phaeobacter* strains CIP 105210, DSM 17395, DSM 16374^T and DSM 24588 were disrupted by using a Constant Systems TS 0.75 kW (IUL Instruments) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) and modified by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multi-cell changer and a temperature controller with *in situ* temperature probe (Varian). Each strain was measured in two technical replicates and the mean result was taken.

Growth and hydrolysis experiments. To determine the substrate spectrum of the *Phaeobacter* strains CIP 105210 and DSM 17395, cells were grown in artificial seawater (ASW; solution of Sigma sea salts: S9883) supplemented with 10 mg caseine hydrolysate l^{-1} (CAS: 65072-00-6; Merck) to avoid auxotrophy and with different carbon sources at a concentration of 0.1 % each, including 2-ketoglutarate, acetate, L-arabinose, butyrate, cellobiose, citrate, D-fucose, glucosamine, glycerol, L-leucine, maltose, L-rhamnose, L-serine, D-sorbitol, succinate, sucrose, trehalose, Tween 20, Tween 40 and Tween 80. The tests were performed in the 24-well microtitre-plate format. In detail, cells were grown on MB agar for 2 days at 28 °C, then harvested with a sterile swab and inoculated in ASW medium. The cell suspension was thereby adjusted to a turbidity of 80 % transmittance using a turbidimeter (AES Chemunex BLG 3531). Afterwards, 2 ml of each suspension was pipetted into a 24-well microtitre plate (Greiner). A sample lacking any carbon source was included as negative control. Cells were incubated in a microtitre plate reader (Infinite F200 pro; Tecan) at 23 °C and with shaking at 88 r.p.m. for 7 days. Growth was automatically measured every 15 min with the Infinite F200 system as increase of the OD_{600}. Growth at temperatures of 4 °C and 37 °C was determined in 200 ml MB within Erlenmeyer flasks for one month and measured photometrically as increase of the OD_{600} using an Ultrospec II spectrophotometer (LKB-Biochrom).

Exoenzyme activities (hydrolysis of gelatin, starch and Tween 80) were analysed using MB solidified with 4 % (w/v) gelatin or 1.5 % (w/v) agarose and supplemented with 0.2 % (w/v) starch and 1 % (v/v) Tween 80, respectively, as described by Smibert & Krieg (1981). As a positive control, *R. litoralis* DSM 6996^T was used for gelatin and Tween 80 hydrolysis and *M. algicola* DSM 10251^T for starch hydrolysis. Reduction of nitrate was tested according to Smibert & Krieg (1981) in MB supplemented with 0.1 % (w/v) potassium nitrate; *R. denitrificans* DSM 7001^T served as a negative control. The assays were incubated for 7 days at 28 °C, except for the hydrolysis of starch, conducted at 20 °C. The growth and hydrolysis tests described above were all performed in three technical replicates.

Phenotype MicroArray (PM) experiments. To determine the metabolic properties of the *Phaeobacter* strains CIP 105210, DSM 17395, DSM 24588 and DSM 16374^T we used the PM technology (Biolog; Bochner, 2009). The *Phaeobacter* strains were grown on MB agar for 48 h and subsequently analysed using the Phenotype MicroArray MicroPlate PM01 and PM02-A (AES Chemunex BLG 12111, BLG 12112) over 70 h; thus 190 different carbon sources were tested. Each strain was measured in three biological replicates. The inoculation medium was modified according to the requirements of marine bacteria, i.e. 10 ml of the inoculation fluid IF-0a (AES Chemunex BLG 72268) was supplemented with 1200 µl artificial seawater stock solution, 120 µl vitamin stock solution, 12 µl trace element stock solution, 120 µl $NaHCO_3$ buffer, 428 µl ultrapure H_2O and 120 µl DyeD (AES Chemunex BLG 74224). The stock solutions had the following composition (l^{-1}): (i) artificial seawater stock solution: 200 g NaCl, 40 g Na_2SO_4, 30 g $MgCl_2 . 6H_2O$, 5 g KCl, 2.5 g NH_4Cl, 2 g KH_2PO_4, 1.5 g $CaCl_2 . 2H_2O$; (ii) trace element stock solution: 2.1 g $FeSO_4 . 7H_2O$, 13 ml 25 % HCl, 5.2 g Titriplex III (Na_2EDTA; adjust pH to 6.0–6.5 to resolve), 190 mg $CoCl_2 . 6H_2O$, 144 mg $ZnSO_4 . 7H_2O$, 100 mg $MnCl_2 . 4H_2O$, 36 mg $Na_2MoO_4 . 2H_2O$, 30 mg H_3BO_3, 24 mg $NiCl_2 . 6H_2O$, 2 mg $CuCl_2 . 2H_2O$; (iii) vitamin stock solution: 100 mg thiamine, 20 mg niacin, 8 mg 4-aminobenzoic acid, 2 mg biotin; and (iii) buffer stock solution: 19 g $NaHCO_3$.

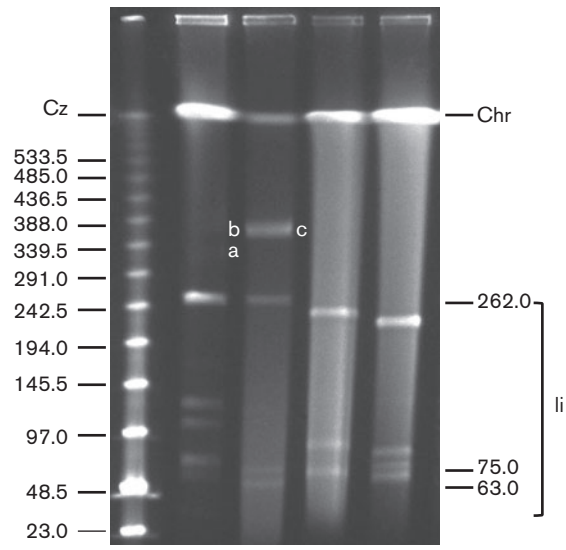
The cells were suspended in the modified inoculation medium using a sterile swab. The turbidity was adjusted to a cell density of 85 % transmittance using a turbidimeter (AES Chemunex BLG 3531) and 100 µl of the cell suspension were pipetted in each of the wells. The MicroPlates were sealed with Parafilm, incubated at 28 °C and measured in the Omnilog unit (Biolog). The results were analysed using the R package '*opm*' (Vaas *et al.*, 2012). The curve parameter maximum height (A) was estimated for each substrate, differences were visualized using heat maps, and the data were discretized into negative, ambiguous and positive reactions using the built-in functions of '*opm*' under default settings.

RESULTS

Profiles of the extrachromosomal elements

The high-molecular-mass genomic DNA of different *Phaeobacter* strains was separated with PFGE. A representative PFGE gel resolving linear DNA molecules in the size range from 23 to 533 kb is shown in Fig. 1(a). In addition to the chromosomes (Fig. 1a, Chr) a distinct number of extrachromosomal bands was revealed for each of the strains CIP 105210, DSM 17395, DSM 24588 and DSM 16374^T. To determine the conformation of the detected extrachromosomal DNA (ccc versus linear; Pradella *et al.*, 2010; Römling *et al.*, 1996), we varied the PFGE conditions (PFGE parameter set A and B) in different gel runs. Using PFGE parameter set A, the fuzzy, faint bands within the lanes of strains CIP 105210 and DSM 17395 (Fig. 1a, marked a, b and c, respectively) ran at approximately 319 (a) and 380 (b, c) kb (Fig. 1a). With PFGE parameter set B (data not shown) band (a) ran at 184 kb and bands (b) and (c) ran at approximately 210 kb indicating that the respective bands migrated independently of the PFGE parameters applied. From this anomalous migration behaviour we concluded that the inherent DNA had a circular conformation. The sizes of the detected ccc DNA were estimated as 66 (a) and 79 (b, c) kb by conventional electrophoresis using the BAC Tracker as ccc size marker (data not shown). As these sizes were close to those estimated for the linearized plasmids of 64 and 77 kb in *Phaeobacter* strain CIP 105210 and 75 kb in strain DSM 17395 (see below), it is most likely that they represent the same plasmids in different conformations.

(a)



(b)

Plasmid sizes (kb)	*Phaeobacter* strain			
	CIP 105210T	DSM 17395	DSM 24588	DSM 16374T
262		+(*)		
253	+(*)			
238			+	
227				+
127	+			
108	+			
94			+	
86				
77	++			
75		+(†)		
74				+
70			+	
67				+
64	+			
63		+(‡)		
36	+(§)			

Fig. 1. (a) PFGE plasmid profiles of the *Phaeobacter* strains CIP 105210^T, DSM 17395, DSM 24588 and DSM 16374^T based on uncut high-molecular-mass genomic DNA. (b) Calculated plasmid sizes as mean values taken from at least two different gel runs. The PFGE conditions were: 1 % (w/v) agarose gel with pulse times of 1 to 48 s for 24 h at 200 V (6 V cm^{-1}). Chr, chromosomal DNA; λ, lambda phage concatemers as molecular-mass PFGE markers (New England Biolabs); li, linear. (*), The two largest plasmids (linearized forms) of *Phaeobacter* strains CIP 105210^T and DSM 17395 migrated about the same distance in the gel and thus seemed to have an identical size. In contrast, both bands could be clearly distinguished by their size in other PFGE runs (data not shown) using different DNA sample preparations. DNA mobility is largely influenced by the DNA concentration of the sample. The observed discrepancy can thus be explained by the relatively high DNA concentration in CIP 105210^T (compared to DSM 17395^T), which retards band migration (Römling *et al.*, 1996). (†) (‡), The PFGE-based plasmid size estimations of 75 and 63 kb of DSM 17395 correspond to the plasmid sizes of 78 and 65 kb, respectively, determined by genome sequencing (Thole *et al.*, 2012; NC_018287.1, NC_018288.1). (§), The 36 kb plasmid of *P. gallaeciensis* CIP 105210^T had a very low fluorescence intensity and is thus hardly visible on the gel image. + +, The 77 kb band of strain CIP 105210^T showed increased fluorescence intensity and presumably represents a double band (plasmid duplet).

By contrast, the sharp bands between 23 kb and 262 kb were separated strictly in accordance with their size when PFGE parameter sets A and B were used. They were thus assumed linear (li, Fig. 1a), most possibly originating from randomly linearized ccc plasmids (Pradella *et al.*, 2010).

Regarding the linearized plasmid fraction of the *Phaeobacter* strains, which was very well suited to determine the plasmid complement of the strains and their sizes (Pradella *et al.*, 2010), seven extrachromosomal replicons were evident in *P. gallaeciensis* CIP 105210, four in *P. inhibens* DSM 16374^T and three in the strains DSM 17395 and DSM 24588. The estimated sizes of the detected plasmids (17 altogether) ranged from 36 to 262 kb (Fig. 1b). They were all different, but their size distribution in the individual strains showed some similarity, i.e. all *Phaeobacter* strains have one large plasmid (262, 253, 239 and 227 kb in strains DSM 17395, CIP 105210, DSM 24588 and DSM 16374^T, respectively) and two or three smaller ones in the size range between 63 and 77 kb. Our PFGE analysis thus indicated that the *Phaeobacter* strains CIP 105210 and DSM 17395 – both deposited as type strain of *Phaeobacter gallaeciensis* – are not identical.

Classification of the *Phaeobacter* strains using 16S rRNA gene sequence, MALDI-TOF MS protein and 16S–23S rRNA gene ITS analyses

16S rRNA gene sequence analysis. We re-evaluated the phylogenetic relationships of the *Phaeobacter* strains and therefore resequenced the PCR-amplified 16S rRNA genes of strains DSM 17395, DSM 16374^T and CIP 105210. In the phylogenetic tree inferred from 16S rRNA gene sequences of representative members of the genus *Phaeobacter*, as well as strains DSM 24588 (=2.10), ANG1 and LSS9, for which finished or draft genome sequences exist (Collins &

Nyholm, 2011; Fernandes *et al.*, 2011; Thole *et al.*, 2012; Fig. 2), the *Phaeobacter* strains DSM 17395, DSM 24588 (=2.10), DSM 16374^T and CIP 105210 clustered together (*P. gallaeciensis*/*P. inhibens* cluster) and were well separated from the *P. arcticus*/*P. leonis* lineage and the branch formed by *P. caeruleus*, *Phaeobacter* sp. ANG1 and *P. daeponensis* (16S rRNA gene identity $\geqslant$97.8 %). Within the *P. gallaeciensis*/*P. inhibens* cluster, the 16S rRNA gene of strain CIP 105210 (KC176239) grouped together with the originally deposited BS107^T sequence (Y13244), exhibiting 72 % and 91 % support from MP and ML bootstrapping, respectively. The 16S rRNA gene sequences of the *Phaeobacter* strains DSM 17395, DSM 24588 and DSM 16374^T (KC176240) were identical and differed by four bases from the *P. gallaeciensis* CIP 105210 sequence KC176239 (16S rRNA gene identity of 99.7 %; see below).

Neither the 16S rRNA gene sequence of *P. gallaeciensis* CIP 105210 (KC176239) nor the sequence of *P. inhibens* DSM 16374^T (KC176240) was exactly identical to that of the original deposit, *P. gallaeciensis* BS107^T (Y13244) or *P. inhibens* T5^T (AY177712), respectively (Fig. 2, Fig. S1 available in IJSEM Online). More precisely, the 16S rRNA gene sequences of the alleged *P. gallaeciensis* type strains differed at the base positions (*Escherichia coli* numbering; Gutell *et al.*, 1994) 47, 260, 777, 928, 930, 1030, 1210 and 1387 (Fig. S1; Table S1); and those of the alleged *P. inhibens* type strains at the positions 29, 1210, 1387, 1436, 1459, 1466 and 1480 (Fig. S1; Table S2). We assessed in detail whether these discrepancies could be caused by sequencing errors, as is already indicated by the long-terminal branches leading to BS107^T and T5^T (Fig. 2). We thus compared the respective sequences with the bacterial 16S rRNA variability map (Baker *et al.*, 2003) and/or the 16S rRNA secondary structure model (Gutell *et al.*, 1994) and showed that the 16S rRNA gene sequences provided in this study were all in accordance with bases categorized as conserved by Baker *et al.* (2003) or the proposed rRNA secondary structure (Tables S1 and S2), whereas the previously determined 16S rRNA gene sequences Y13244 and AY177712 were flawed.

Furthermore, we examined whether the four differences in the 16S rRNA gene sequences of *P. gallaeciensis* CIP 105210 (KC176239) and *P. inhibens* DSM 16374^T (KC176240) were genuine. They were localized at the base positions 614 (*P. gallaeciensis*: G; *P. inhibens*: A) and 626 (*P. gallaeciensis*: C; *P. inhibens*: U) within the 16S rRNA variable region V4 (Baker *et al.*, 2003) and at the positions 835 (*P. gallaeciensis*: G; *P. inhibens*: A) and 851 (*P. gallaeciensis*: C; *P. inhibens*: U) within the variable V5 region, respectively (*E. coli* numbering; Fig. S1; Table S3). Comparison with the secondary 16S rRNA structure model (Gutell *et al.*, 1994) and a simulation of the rRNA folding using the Mfold web server (Zuker, 2003) indicated that bases 614 and 626 paired in the variable region V4 stem–loop (Fig. 3); similarly, bases 835 and 851 matched in the V5 stem–loop (Fig. S2). We thus assumed that the present transitions of G and C in *P. gallaeciensis* to A and U in *P. inhibens*, respectively, reflect genuine and characteristic mutations in the 16S rRNA genes of these species.

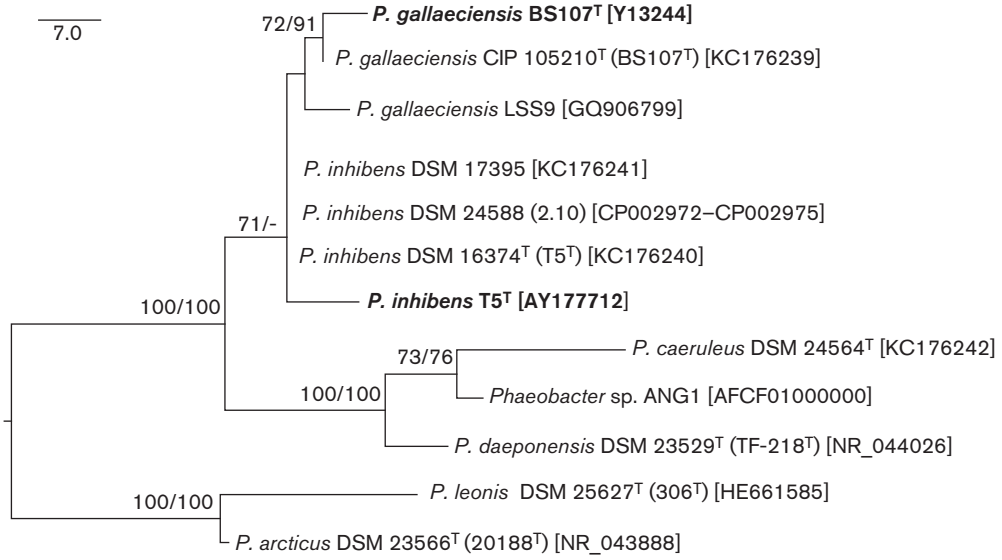


Fig. 2. Midpoint-rooted MP phylogeny inferred from 16S rRNA gene sequences of *Phaeobacter* strains closely related to *P. inhibens* and *P. gallaeciensis*. Branches are scaled in terms of the minimum number of substitutions (using DELTRAN optimization; Stamatakis *et al.*, 2008). Numbers above branches are support values from MP (left) and ML (right) bootstrapping. Original designation of strains that are deposited at culture collections is indicated in parentheses; square brackets give the respective accession number.

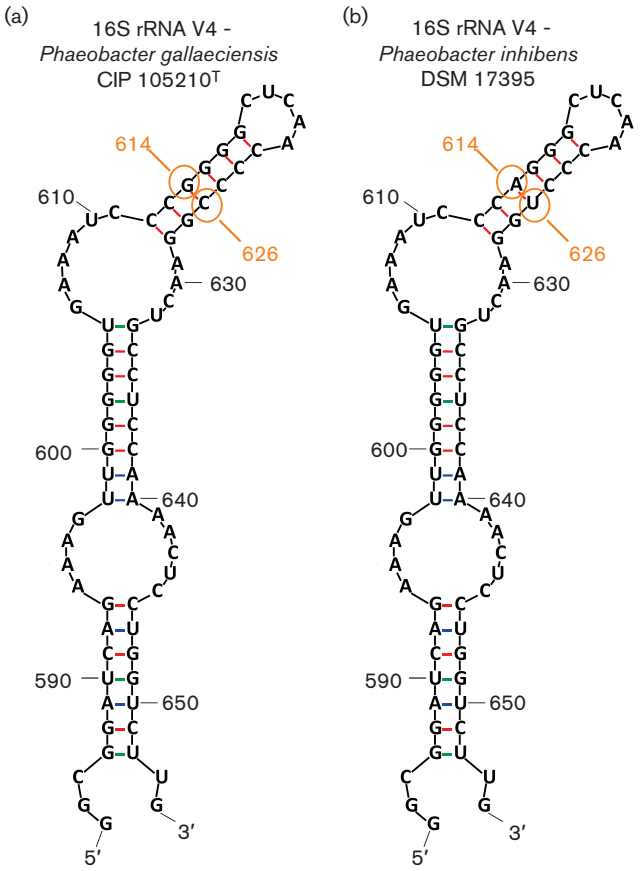


Fig. 3. Secondary structure of the 16S rRNA variable region V4 of *P. gallaeciensis* CIP 105210^T (a) and *P. inhibens* DSM 17395 (b) demonstrating transition of bases 614 and 626 (*E. coli* numbering; bases 529 and 541 according to the CIP 105210^T numbering). RNA folding was simulated using the Mfold web server for nucleic acid folding and hybridization prediction (Zuker, 2003; http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form).

Considering these bases, the 16S rRNA gene sequence of *Phaeobacter* strain CIP 105210 resembled the original one of BS107^T (Y13244), which would indicate that strain CIP 105210 is the type strain of *P. gallaeciensis*.

MALDI-TOF MS analysis. In the MALDI-TOF MS dendrogram (Fig. 4), the *Phaeobacter* strains DSM 16374^T, DSM 24588 and DSM 17395 not only formed a cluster but were virtually indistinguishable from each other. Strain CIP 105210 appeared as the sister group of those three strains, whereas *P. daeponensis* and *P. caeruleus* as well as *P. arcticus* and *P. leonis* were well set apart.

ITS analysis. A comparable picture was observed in the ITS analysis (Fig. 5). *Phaeobacter* strain DSM 17395 appeared as sister strain of *P. inhibens* DSM 16374^T with 93 % support under ML and 99 % support under MP. The sister-group relationship of these and strain DSM 24588 was supported with 70 % and 88 % bootstrap values, respectively, to the exclusion of *P. gallaeciensis* CIP 105210. *Phaeobacter sp.* ANG1 was placed in a distinct cluster together with the type strains of *P. daeponensis* and *P. caeruleus* (100 % support).

DNA–DNA hybridization.

In contrast to the highly similar genomic DNA between the strains DSM 17395 and DSM 16374^T (82 %) as well as between the strains DSM 16374^T and DSM 24588 (83 %), strain CIP 105210 shared only 62 % and 63 % DNA–DNA relatedness to the strains DSM 17395 and DSM 16374^T, respectively (Table 1). This is below the threshold of 70 % recommended by Wayne *et al.* (1987) hence indicating the status of strain CIP 105210 in a separate species. Conversely, the values clearly above 70 % indicate that strains DSM 17395, DSM 16374^T and DSM 24588 belong to the same species.

Growth, hydrolysis and PM experiments

The growth and hydrolysis experiments for *Phaeobacter* strains CIP 105210 and DSM 17395 could only partially reproduce those conducted by Ruiz-Ponte *et al.* (1998) (Table S4). The results for strain CIP 105210 differed from all other series of measurements by growth of this strain on L-arabinose and hydrolysis of Tween 80. Strain DSM 17395 showed no specific characteristics, but it – as well as CIP 105210 – differed from strain BS107^T (Ruiz-Ponte *et al.*, 1998) as they grew on serine (like T5^T; Martens *et al.*, 2006) and showed slow growth on L-rhamnose and 2-ketoglutarate (Table S4). The overall number of specific differences of all other strains to T5^T (Martens *et al.*, 2006) was four (growth on citrate, glucosamine and on MB at 4 °C or 37 °C).

In contrast, the PM experiments, which are more sensitive than bacterial growth tests because they monitor substrate respiration (Bochner *et al.*, 2001), yielded significant physiological differences between all four tested *Phaeobacter* strains, DSM 24588, DSM 16374^T, DSM 17395 and CIP 105210 (Figs S3 and S4). The physiological similarity between strains CIP 105210 and DSM 17395 was high, but the differences between the two were clearly reproducible. According to the discretization approach implemented in '*opm*' (Vaas *et al.*, 2012), respiration on tyramine (PM01-H04; blue box Fig. S3) was positive in DSM 17395 and DSM 16374, weak in DSM 24588 but negative in CIP 105210. Respiration on butyrate (PM02A-D12; Fig. S4) was positive in CIP 105210 and DSM 24588, weak in DSM 16374^T, but negative in DSM 17395.

Regarding the common subset of growth or hydrolysis experiments on the one hand and PM experiments on the other hand, the results were identical with a few exceptions. Expectedly, no substrate was detected on which growth (or hydrolysis) was measurable but respiration was not observed, whereas on some substrates respiration was detected by PM analysis even though these substrates sustained no growth.

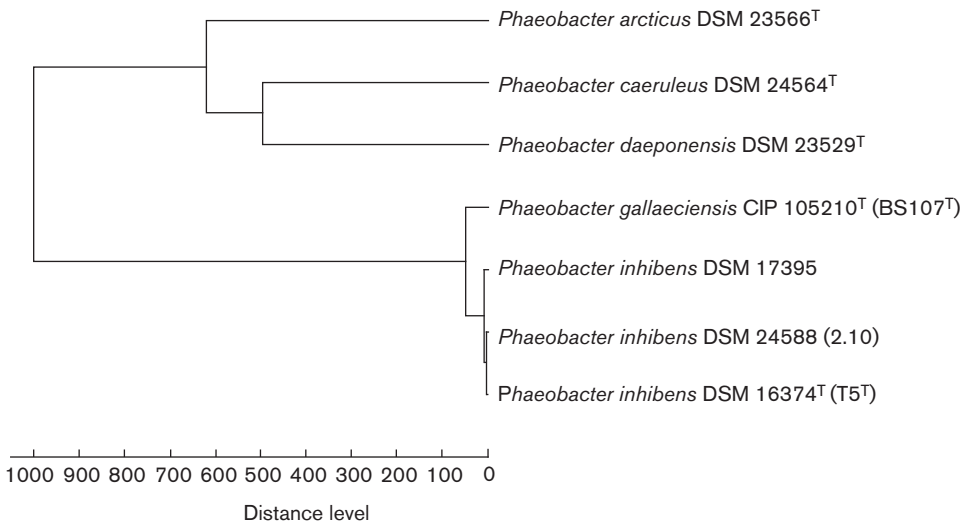


Fig. 4. Score-oriented dendrogram showing the similarity of MALDI-TOF mass spectra from cell extracts of selected *Phaeobacter* strains. The dendrogram was generated by the BioTyper software (version 3.0; Bruker Daltonics).

Accordingly, a weak PM reaction on L-arabinose (PM01-A02) and a positive PM reaction on citrate (PM01-F02) were observed for all four tested strains. A positive PM reaction to Tween 80 (PM01-E05) was observed for strains DSM 24588 and CIP 105210, whereas strains DSM 17395 and DSM 16374^T showed a weak reaction (compare red boxes in Fig. S3 with Table S4).

DISCUSSION

According to the PFGE profiles of the extrachromosomal elements – which are largely supported by the complete genome sequences of the *Phaeobacter* strains DSM 17395, DSM 24588 (Thole *et al.*, 2012), DSM 16374^T (Dogs, M. and others, unpublished) and CIP 105210 (Frank, O. and

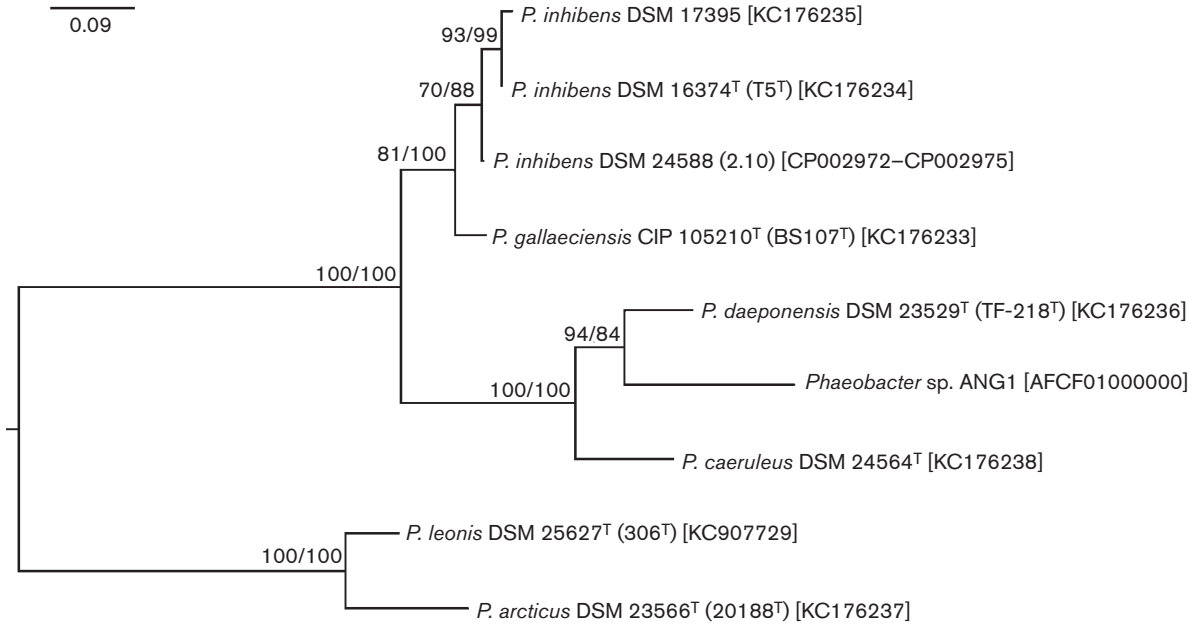


Fig. 5. Midpoint-rooted ML phylogeny inferred from ITS sequences of *Phaeobacter* strains closely related to *P. inhibens* and *P. gallaeciensis*. Branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from ML (left) and MP (right) bootstrapping. Original designation of strains that are deposited at culture collections is indicated in parentheses; square brackets give the respective accession number.

Table 1. Mean DNA–DNA similarity values ($n=2$) between the *Phaeobacter* strains CIP 105210^T, DSM 17395, DSM 16374^T and DSM 24588

Phaeobacter strain	CIP 105210^T	DSM 17395	DSM 16374^T	DSM 24588
CIP 105210^T	×			
DSM 17395	61.7±2.7 %	×		
DSM 16374^T	62.7±0.6 %	82.2±0.2 %	×	
DSM 24588	ND	ND	82.8±1.0 %	×

ND, Not determined.

others, unpublished) – DDH similarities, 16S rRNA gene sequence analysis, 16S–23S rRNA gene ITS sequence analysis, MALDI-TOF MS protein analysis, and high-throughput phenotyping using the PM technology, the *Phaeobacter* strains CIP 105210 and DSM 17395, both supposed to be deposits of the type strain of *P. gallaeciensis* BS107^T, are biologically clearly distinct. ITS sequence and MALDI-TOF analysis additionally showed that DSM 17395 (and DSM 24588) group together with *P. inhibens* DSM 16374^T to the exclusion of CIP 105210. As confirmed by DDH (⩾76 % similarity), DSM 16374^T, DSM 17395 and DSM 24588 are conspecific, i.e. all belong to the species *P. inhibens*. Analysis of 16S rRNA gene sequences was in accordance with this finding, too, because the sequences of these strains were identical (if the resequenced 16S rRNA gene sequence of DSM 16374^T was considered). Our sequence analyses confirmed the finding of Thole *et al.* (2012) that the *Phaeobacter* sp. ANG1 does not belong to the species *P. gallaeciensis*.

Because DSM 17395 must hence be excluded from the species *P. gallaeciensis*, the question arises whether the alternative type strain deposit, CIP 105210, represents *P. gallaeciensis* BS107^T. DDH analysis (<70 % similarity) indicates that CIP 105210 is not conspecific with *P. inhibens*. Analysis of growth behaviours and enzymic activities could not fully reproduce the findings of Ruiz-Ponte *et al.* (1998), but given the overall low number of characters tested, the low number of known differences to the type strain of the sister species, *P. inhibens*, and the well-known difficulties in reproducing physiological tests in distinct laboratories in general, the significance of these discrepancies is unclear. Essentially, based on the newly generated CIP 105210 16S rRNA gene sequence that is identical to the one from BS107^T, except for deviations that were likely to be sequencing errors, we could clearly document the type strain status of *P. gallaeciensis* CIP 105210^T. As the strains CIP 105210^T and DSM 17395 have been independently deposited at the CIP and the DSMZ, respectively, it is the most probable explanation that the later strain has been mixed-up prior to deposition.

Research laboratories are usually not equipped with sufficient resources to verify the biological identity of their cultures. Moreover, culture collections have to cope with the deposition of interchanged or contaminated strains and the quality of incoming material will presumably even deteriorate due to the decline of basic microbiological methodology in the era of molecular biology. Problems are expected particularly if confusion with closely related strains has occurred, as in the case of DSM 17395, which apparently belongs to the sister species of the correct strain. Hence, it is advisable that researchers working on a certain strain exactly denote the source from which it was received. Providing the accession numbers of culture-collection deposits (such as 'CIP 105210^T' or 'DSM 17395') should thus be preferred over just stating the original strain designator (such as 'BS107^T') irrespective of the source from which the strain has been received. In any case, with respect to cultivatable microbes, only strains with a demonstrable history should be considered in serious research.

The three homologous plasmids of the completely sequenced *P. inhibens* strains DSM 17395 and DSM 24588 exhibit a long-range synteny (Thole *et al.*, 2012), but several indels (insertions/deletions) are responsible for the deviating plasmid sizes [262 versus 238 kb (DnaA-like replicon; Petersen, 2011), 75(78) versus 94 kb (RepB-I), 65(63) versus 70 kb (RepA-I); Fig. 1]. Homologues of these replicons may also be present in the sister species *P. gallaeciensis* CIP 105210^T e.g. represented by the 253, 77 and 64 kb replicons. However, the conspicuously different plasmid profiles in *P. gallaeciensis* and *P. inhibens* (Fig. 1) may reflect horizontal recruitment of four additional replicons in *P. gallaeciensis* CIP 105210^T. The same explanation is supported by the presence of a type IV secretion system on the fourth 86 kb plasmid of the *P. inhibens* type strain DSM 16374^T (Dogs, M. and others, unpublished), which may be responsible for plasmid mobilization via conjugation (Petersen *et al.*, 2013). In the near future, genome sequencing and comparative genomics of more distantly related strains, such as *Phaeobacter arcticus*, will help to reveal the extent of horizontal exchange and vertical evolution within the *Roseobacter* clade.

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REFERENCES

Baker, G. C., Smith, J. J. & Cowan, D. A. (2003). Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods* **55**, 541–555.

Berger, M., Neumann, A., Schulz, S., Simon, M. & Brinkhoff, T. (2011). Tropodithietic acid production in *Phaeobacter gallaeciensis* is regulated by *N*-acyl homoserine lactone-mediated quorum sensing. *J Bacteriol* **193**, 6576–6585.

Berger, M., Brock, N. L., Liesegang, H., Dogs, M., Preuth, I., Simon, M., Dickschat, J. S. & Brinkhoff, T. (2012). Genetic analysis of the upper phenylacetate catabolic pathway in the production of tropodithietic acid by *Phaeobacter gallaeciensis*. *Appl Environ Microbiol* **78**, 3539–3551.

Bochner, B. R. (2009). Global phenotypic characterization of bacteria. *FEMS Microbiol Rev* **33**, 191–205.

Bochner, B. R., Gadzinski, P. & Panomitros, E. (2001). Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. *Genome Res* **11**, 1246–1255.

Brinkhoff, T., Bach, G., Heidorn, T., Liang, L., Schlingloff, A. & Simon, M. (2004). Antibiotic production by a *Roseobacter* clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. *Appl Environ Microbiol* **70**, 2560–2565.

Bruhn, J. B., Gram, L. & Belas, R. (2007). Production of antibacterial compounds and biofilm formation by *Roseobacter* species are influenced by culture conditions. *Appl Environ Microbiol* **73**, 442–450.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Collins, A. J. & Nyholm, S. V. (2011). Draft genome of *Phaeobacter gallaeciensis* ANG1, a dominant member of the accessory nidamental gland of *Euprymna scolopes*. *J Bacteriol* **193**, 3397–3398.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Dickschat, J. S., Zell, C. & Brock, N. L. (2010). Pathways and substrate specificity of DMSP catabolism in marine bacteria of the *Roseobacter* clade. *ChemBioChem* **11**, 417–425.

Farris, J. S. (1972). Estimating phylogenetic trees from distance matrices. *Am Nat* **106**, 645–668.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Fernandes, N., Case, R. J., Longford, S. R., Seyedsayamdost, M. R., Steinberg, P. D., Kjelleberg, S. & Thomas, T. (2011). Genomes and virulence factors of novel bacterial pathogens causing bleaching disease in the marine red alga *Delisea pulchra*. *PLoS ONE* **6**, e27387.

Fitch, W. M. (1971). Towards defining the course of evolution: minimal change for a specified tree topology. *Syst Zool* **20**, 406–416.

Gaboyer, F., Tindall, B. J., Ciobanu, M.-C., Duthoit, F., Le Romancer, M. & Alain, K. (2013). *Phaeobacter leonis* sp. nov., an alphaproteobacterium from Mediterranean Sea sediments. *Int J Syst Evol Microbiol* **63**, 3301–3306.

Geng, H., Bruhn, J. B., Nielsen, K. F., Gram, L. & Belas, R. (2008). Genetic dissection of tropodithietic acid biosynthesis by marine roseobacters. *Appl Environ Microbiol* **74**, 1535–1545.

Gürtler, V. & Stanisich, V. A. (1996). New approaches to typing and identification of bacteria using the 16S–23S rDNA spacer region. *Microbiology* **142**, 3–16.

Gutell, R. R., Larsen, N. & Woese, C. R. (1994). Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. *Microbiol Rev* **58**, 10–26.

Hess, P. N. & De Moraes Russo, C. A. (2007). An empirical test of the midpoint rooting method. *Biol J Linn Soc Lond* **92**, 669–674.

Huß, V. A., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* **33**, 511–518.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Markowitz, V. M., Chen, I.-M. A., Palaniappan, K., Chu, K., Szeto, E., Grechkin, Y., Ratner, A., Jacob, B., Huang, J. & other authors (2012). IMG: the Integrated Microbial Genomes database and comparative analysis system. *Nucleic Acids Res* **40** (Database issue), D115–D122.

Martens, T., Heidorn, T., Pukall, R., Simon, M., Tindall, B. J. & Brinkhoff, T. (2006). Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte *et al.* 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay *et al.* 1995) Uchino *et al.* 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*. *Int J Syst Evol Microbiol* **56**, 1293–1304.

Martens, T., Gram, L., Grossart, H.-P., Kessler, D., Müller, R., Simon, M., Wenzel, S. C. & Brinkhoff, T. (2007). Bacteria of the *Roseobacter* clade show potential for secondary metabolite production. *Microb Ecol* **54**, 31–42.

Pattengale, N. D., Alipour, M., Bininda-Emonds, O. R. P., Moret, B. M. E. & Stamatakis, A. (2010). How many bootstrap replicates are necessary? *J Comput Biol* **17**, 337–354.

Petersen, J. (2011). Phylogeny and compatibility: plasmid classification in the genomics era. *Arch Microbiol* **193**, 313–321.

Petersen, J., Frank, O., Göker, M. & Pradella, S. (2013). Extrachromosomal, extraordinary and essential — the plasmids of the Roseobacter clade. *Appl Microbiol Biotechnol* **97**, 2805–2815.

Pradella, S., Päuker, O. & Petersen, J. (2010). Genome organisation of the marine *Roseobacter* clade member *Marinovum algicola*. *Arch Microbiol* **192**, 115–126.

Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.

Rao, D., Webb, J. S. & Kjelleberg, S. (2005). Competitive interactions in mixed-species biofilms containing the marine bacterium *Pseudoalteromonas tunicata*. *Appl Environ Microbiol* **71**, 1729–1736.

Römling, U., Fislage, R. & Tümmler, B. (1996). Macrorestriction mapping an analysis of bacterial genomes. In *Nonmammalian Genomic Analysis: A Practical Guide*, pp. 165–195. Edited by B. Birren & E. Lai. San Diego, CA: Academic Press.

Ruiz-Ponte, C., Cilia, V., Lambert, C. & Nicolas, J. L. (1998). *Roseobacter gallaeciensis* sp. nov., a new marine bacterium isolated from rearings and collectors of the scallop *Pecten maximus*. *Int J Syst Bacteriol* **48**, 537–542.

Seyedsayamdost, M. R., Carr, G., Kolter, R. & Clardy, J. (2011a). Roseobacticides: small molecule modulators of an algal-bacterial symbiosis. *J Am Chem Soc* **133**, 18343–18349.

Seyedsayamdost, M. R., Case, R. J., Kolter, R. & Clardy, J. (2011b). The Jekyll-and-Hyde chemistry of *Phaeobacter gallaeciensis*. *Nat Chem* **3**, 331–335.

Smibert, R. M. & Krieg, N. R. (1981). Phenotypic characterization. In *Manual of Methods for General Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol* **57**, 758–771.

Swofford, D. L. (2002). PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4.0b10. Sunderland: Sinauer Associates.

Thole, S., Kalhoefer, D., Voget, S., Berger, M., Engelhardt, T., Liesegang, H., Wollherr, A., Kjelleberg, S., Daniel, R. & other authors (2012). *Phaeobacter gallaeciensis* genomes from globally opposite locations reveal high similarity of adaptation to surface life. *ISME J* **6**, 2229–2244.

Tóth, E. M., Schumann, P., Borsodi, A. K., Kéki, Z., Kovács, A. L. & Márialigeti, K. (2008). *Wohlfahrtiimonas chitiniclastica* gen. nov., sp. nov., a new gammaproteobacterium isolated from *Wohlfahrtia magnifica* (Diptera: Sarcophagidae). *Int J Syst Evol Microbiol* **58**, 976–981.

Vaas, L. A. I., Sikorski, J., Michael, V., Göker, M. & Klenk, H.-P. (2012). Visualization and curve-parameter estimation strategies for efficient exploration of phenotype microarray kinetics. *PLoS ONE* **7**, e34846.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Zech, H., Thole, S., Schreiber, K., Kalhöfer, D., Voget, S., Brinkhoff, T., Simon, M., Schomburg, D. & Rabus, R. (2009). Growth phase-dependent global protein and metabolite profiles of *Phaeobacter gallaeciensis* strain DSM 17395, a member of the marine *Roseobacter*-clade. *Proteomics* **9**, 3677–3697.

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* **31**, 3406–3415.