Porphyrobacter cryptus sp. nov., a novel slightly thermophilic, aerobic, bacteriochlorophyll *a*-containing species

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Two strains of a novel aerobic, bacteriochlorophyll *a*-containing species of the α -4 subclass of the *Proteobacteria* were isolated from the hot spring at Alcafache in central Portugal. 16S rRNA gene sequence-based phylogenetic analyses showed the two novel isolates to be phylogenetically related to members of the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*. The strains produce reddish-orange-pigmented colonies, have an optimum growth temperature of about 50 °C and could be distinguished from the species *Porphyrobacter tepidarius*, which also has a high growth temperature, primarily on the basis of the fatty acid composition. The novel species does not grow anaerobically in the presence or absence of a light source. The strains of the novel species utilize several single carbon sources for growth, most of which are also used by *P. tepidarius*. The species status of strains ALC-2^T and ALC-3 was confirmed by low reassociation values of the DNA with species of the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*. Phenotypic characteristics and 16S rRNA gene sequence analyses also show that strains ALC-2^T (= DSM 12079^T = ATCC BAA-386^T) and ALC-3 (= DSM 12080) represent a novel species, for which the name *Porphyrobacter cryptus* sp. nov. is proposed.

INTRODUCTION

Many species within the α -subclass of the *Proteobacteria* synthesize bacteriochlorophyll *a* (Bchl *a*) under aerobic conditions. Photoautotrophic growth under anaerobic or aerobic conditions has not been shown in these organisms, although some organisms may be photoheterotrophic under a limited range of cultivation conditions (Takamiya *et al.*, 1987). Many of these organisms are exclusively chemo-organotrophic, leading to the view that the organisms are photoheterotrophic under unknown growth conditions. The α -4 subclass of the *Proteobacteria* comprises the Bchl *a*-containing species of the genera *Erythrobacter* (Shiba &

Simidu, 1982; Yurkov *et al.*, 1994), *Erythromicrobium* (Yurkov *et al.*, 1994) and *Porphyrobacter* (Fuerst *et al.*, 1993; Hanada *et al.*, 1997), *Erythromonas* (Yurkov *et al.*, 1997) and *Sandaracinobacter* (Yurkov *et al.*, 1997). The species *Erythrobacter longus* and *Erythrobacter litoralis* originate from marine samples, are slightly halophilic and multiply by binary fission. The species *Erythromicrobium ramosum* also grows by binary fission, but, as the name implies, this organism can form slightly branching cells. The species *Porphyrobacter neustonensis*, unlike the other Bchl *a*-synthesizing bacteria of the α -4 subclass of the *Proteobacteria*, produces unusual pleomorphic cells, some of which have stalk-like structures and reproduce by budding. The other species of this genus, *Porphyrobacter tepidarius*, produces short, ovoid, rod-shaped cells, divides by binary fission and has an optimum growth temperature of about 45–50 °C. Another species, invalidly described as '*Citromicrobium bathyomarinum*', was isolated from an abyssal hot-spring plume, is mesophilic, yellow-pigmented, contains Bchl *a* under aerobic growth and forms pleomorphic cells that resemble those of *Erythromicrobium ramosum* (Yurkov *et al.*, 1999). Other strains closely related to the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*

Abbreviation: Bchl *a*, bacteriochlorophyll *a*.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain ALC-2^T, *Erythrobacter longus* DSM 6997^T, *Erythrobacter litoralis* DSM 8509^T, *Erythromicrobium ramosum* DSM 8510^T, *Porphyrobacter neustonensis* DSM 9434^T and *Porphyrobacter tepidarius* DSM 10594^T are respectively AF465834–AF465839.

TLC profiles of pigments of the novel isolates and reference species are available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>).

have been isolated, but these have not been characterized, although 16S rRNA gene sequences have been placed in public databases and can be used for phylogenetic analysis.

We recently isolated several slightly thermophilic, reddish-orange-pigmented organisms from a hot spring, in central Portugal, with a vent temperature of 50 °C. These organisms produced Bchl *a* under aerobic conditions, and phylogenetic analysis indicated that strains ALC-2^T and ALC-3 were closely related to the species of the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*. Phylogenetic, physiological, biochemical and chemotaxonomic characteristics of the novel slightly thermophilic strains showed that these isolates belonged to a novel species. However, our study also indicated that the species *Erythrobacter longus* and *Erythrobacter litoralis*, *Erythromicrobium ramosum* and *P. neustonensis* and *P. tepidarius* possess few, if any, known characteristics that distinguish these organisms at the genus level. It is necessary to re-examine the taxonomic relationships of the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*, but it cannot be done at this stage. We propose, therefore, that strains ALC-2^T and ALC-3 should be included in the genus *Porphyrobacter* under the name *Porphyrobacter cryptus* sp. nov. because this organism has phenotypic characteristics that most resemble those of *P. tepidarius*.

METHODS

Isolation and bacterial strains. Strains ALC-2^T and ALC-3 were isolated from the run-off of the hot spring located at Alcafache in central Portugal. Water samples were transported without temperature control and filtered, the same day, through membrane filters (Gelman type GN-6, pore size 0.45 µm, diameter 47 mm). The filters were placed on the surface of *Thermus* and R₃A media solidified with agar (2%, w/v) (Reasoner & Geldreich, 1985; Williams & da Costa, 1992). The plates containing the filters were wrapped in plastic bags and incubated at 45 or 50 °C for up to 3 days. Cultures were purified by subculturing on *Thermus* medium and were maintained at -70 °C in the same medium with 15% glycerol. The type strains of *Erythrobacter longus* (DSM 6997^T), *Erythrobacter litoralis* (DSM 8509^T), *Erythromicrobium ramosum* (DSM 8510^T), *P. neustonensis* (DSM 9434^T) and *P. tepidarius* (DSM 10594^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, and used for comparative purposes.

Morphological, biochemical and tolerance characteristics. Cultures were grown routinely in PE medium, described by Hanada *et al.* (1997), containing the following (l⁻¹): sodium glutamate (0.5 g), sodium succinate (0.5 g), sodium acetate (0.5 g), yeast extract (0.5 g), Casamino acids (0.5 g), Na₂S₂O₃ · 5H₂O (0.5 g), KH₂PO₄ (0.38 g), K₂HPO₄ (0.39 g), (NH₄)₂SO₄ (0.5 g), basal salts solution (5 ml) and a vitamin solution (1 ml). The basal salts medium contained the following (l⁻¹): FeSO₄·7H₂O (1.1 g), MgSO₄·7H₂O (24.6 g), CaCl₂·2H₂O (2.9 g), NaCl (23.4 g), MnSO₄·4H₂O (146.5 mg), ZnSO₄·7H₂O (28.8 mg), Co(NO₃)₂·6H₂O (29.2 mg), CuSO₄·5H₂O (25.2 mg), Na₂MoO₄·2H₂O (24.2 mg), H₃BO₃ (31.0 mg) and EDTA (4.5 g). The vitamin solution contained the following (per 100 ml): nicotinic acid (100 mg), thiamine hydrochloride (100 mg), biotin (5 mg), *p*-aminobenzoic acid (50 mg), vitamin B₁₂ (1 mg), pyridoxine

hydrochloride (50 mg), pantothenic acid (50 mg) and folic acid (50 mg); it was sterilized by filtration and added to the autoclaved medium after cooling.

Electron microscopy was performed on exponential-phase cells fixed with aqueous 4% paraformaldehyde, 1.25% glutaraldehyde and 10 mM calcium chloride at room temperature. The cells were washed by centrifugation after 4 h with 50 mM cacodylate buffer supplemented with 10 mM calcium chloride (pH 6.4) and fixed at room temperature for 2 h with osmium tetroxide in veronal-acetate buffer containing 10 mM calcium chloride (Silva & Macedo, 1983, 1987). Samples were embedded in Epon resin after dehydration in ethanol. The sections were contrasted with uranyl acetate followed by lead citrate. Cells were visualized and photographed with a Zeiss EM 10 C electron microscope. Cell dimensions and motility were determined by phase-contrast microscopy; the number and position of flagella were visualized by light microscopy after staining of the cells with the Ryu stain (Heimbrook *et al.*, 1989).

The growth temperature range of strain ALC-2^T was examined by measuring the turbidity (610 nm) of cultures incubated in 300 ml metal-capped Erlenmeyer flasks, containing 100 ml PE medium, in a reciprocal water-bath shaker. The pH range for growth was examined at 50 °C in the same medium as described previously by Moreira *et al.* (2000).

All biochemical and tolerance tests were performed, as described previously (Santos *et al.*, 1989), in PE liquid medium or PE medium solidified with agar at the optimum growth temperature for up to 5 days. Single-carbon-source assimilation tests were performed in a minimal medium composed of PE salts, vitamin solution and filter-sterilized carbon sources (2.0 g l⁻¹), but without the other organic components. Growth was examined daily by measuring the turbidity of cultures incubated in 20 ml screw-capped tubes containing 10 ml medium for a total of 5 days. Positive and negative control cultures were grown in PE medium and in minimal medium without a carbon source. Anaerobic growth was assessed in cultures grown in the basal salts of PE medium incubated in anaerobic chambers with an H₂/CO₂ atmosphere.

Polar lipid, lipoquinone and fatty acid composition. Cultures used for polar lipid and lipoquinone analyses were grown at the optimum growth temperature until the exponential phase of growth. The cells were recovered by centrifugation, polar lipids were extracted and the individual polar lipids were separated by one-dimensional TLC as described previously (Prado *et al.*, 1988).

Lipoquinones were extracted from freeze-dried cells, purified by TLC and separated with a Gilson HPLC apparatus by using a reverse-phase column (RP18, Spherisorb, S5 ODS2) with methanol/heptane (10:2, v/v) as the mobile phase and were detected at 269 nm (Tindall, 1989).

Cultures for fatty acid analysis were grown on agar plates, in sealed plastic bags submerged in a water bath, at their optimum growth temperatures for 24 h. Fatty acid methyl esters were obtained from fresh wet biomass and analysed as described previously (Moreira *et al.*, 2000) using the MIS Library Generation software (Microbial ID).

Analysis of bacteriochlorophyll and carotenoids. Cultures in the exponential phase of growth were washed twice by centrifugation using a MOPS buffer (MOPS-NaOH, 0.01 M; KCl, 0.1 M; MgCl₂, 0.001 M; pH 7.5) and disrupted by sonication with a Vibra Cell-100w sonicator (Sonics & Materials). The disrupted suspension was centrifuged to sediment cells and debris and the absorption spectrum of the supernatant was examined on a Jasco spectrophotometer (model 7800). Bacteriochlorophylls were also extracted with acetone/methanol (7:2, v/v).

Carotenoids were extracted with chloroform/methanol, as described by Liaaen-Jensen & Jensen (1971), in the dark to avoid photo-oxidation of the pigments. TLC was performed on silica gel G plates (no. 5626; Merck) using a solvent system composed of chloroform/methanol (95:12.5, v/v).

Determination of G+C content of DNA, DNA-DNA reassociation studies and 16S rDNA sequence determination and phylogenetic analyses. The DNA was isolated as described by Cashion *et al.* (1977). The G+C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989). DNA for DNA-DNA reassociation studies was extracted and purified by the procedure of Marmur (1961). The degree of DNA reassociation was determined spectrophotometrically from the initial renaturation rates, according to De Ley (1970). Renaturation rates were measured in $1 \times$ SSC (0.15 M NaCl and 0.015 M trisodium citrate at pH 7.0) using a Uvikon 940 spectrophotometer (Kontron) equipped with a thermostat-controlled cuvette chamber. The optimal renaturation temperature used in each case was calculated from the G+C content (De Ley, 1970). Each hybridization experiment was performed at least twice.

Extraction of genomic DNA for 16S rRNA gene sequence determination, PCR amplification of the 16S rRNA gene and sequencing of purified PCR products were carried out as described previously (Rainey *et al.*, 1996). Purified reactions were electrophoresed using a model 310 Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequences determined in this study were aligned against representative reference sequences of members of the *Proteobacteria* by using the ae2 editor (Maidak *et al.*, 1999). The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms were generated using various treeing algorithms contained in the PHYLIP package (Felsenstein, 1993).

RESULTS AND DISCUSSION

Morphological, physiological and biochemical characteristics

The hot spring at Alcafache in central Portugal has a vent temperature of about 50.6 °C and a pH of 8.3. Strains ALC-2^T and ALC-3 formed reddish-orange-pigmented colonies, similar to those of the species of *Erythrobacter*, *Porphyrobacter* and *Erythromicrobium*. The two novel isolates had optimum growth temperatures between 50 and 55 °C, which were slightly higher than that of the type strain of *P. tepidarius* (Fig. 1). The higher growth temperature range of strains ALC-2^T and ALC-3 was particularly noticeable at supraoptimal temperatures, since the novel organism grew at 55 °C while *P. tepidarius* did not. Strains ALC-2^T and ALC-3 had a pH range for growth between 6.0 and 9.0, with an optimum pH for growth between 7.5 and 8.0.

The strains formed short, rod-shaped cells of variable width (0.7–1.4 µm) and length (1.0–2.8 µm) during exponential phase in liquid cultures. The cells were motile by means of one polar flagellum. The ultrastructure of strain ALC-2^T was typical of a bacterium with a Gram-negative cell envelope (Fig. 2). The organism has an envelope consisting of a cytoplasmic membrane without invaginations or other internal membrane systems, a thin electron-dense peptidoglycan layer and an outer, convoluted outer membrane. Lipid

Fig. 1. Effect of temperature on the growth rates of the type strain of *P. tepidarius* (■), strain ALC-2^T (●) and strain ALC-3 (○).

Fig. 2. Transmission electron micrograph of a section of strain ALC-2^T fixed with aldehyde/osmium tetroxide/uranyl acetate. OM, Outer membrane of the cell wall; PG, peptidoglycan layer of the cell wall; CM, cytoplasmic membrane. Bar, 0.3 µm.

(poly-β-hydroxyalkanoates) and polyphosphate inclusions were also present. The type strain of *P. tepidarius* and strains ALC-2^T and ALC-3 were cytochrome oxidase- and catalase-positive, growth was not observed under anaerobic conditions, nitrate was not reduced and they had almost identical phenotypic characteristics (Table 1).

Polar lipid, quinone, fatty acid and Bchl composition

Strains ALC-2^T and ALC-3, as well as the type strains of the species of *Erythrobacter*, *Porphyrobacter* and *Erythromicrobium*,

Table 1. Phenotypic characteristics that distinguish *P. tepidarius* from strain ALC-2^T in minimal PE medium at their optimum growth temperatures

Results for strain ALC-3 were identical to those shown for strain ALC-2^T. Both taxa degraded gelatin, arbutin and Tweens 20, 40 and 60; both taxa assimilated glucose, maltose, sucrose, cellobiose, pyruvate, glutamate and arginine; neither taxon assimilated fructose, galactose, mannose, L-sorbose, lactose, trehalose, D-xylose, D- or L-arabinose, L-sorbose, L-fucose, L-rhamnose, raffinose, melibiose, melezitose, ribose, lyxose, D- or L-arabitol, xylitol, i-erythritol, glycerol, sorbitol, *myo*-inositol, ribitol, galactitol, malate, acetate, citrate, succinate, α -ketoglutarate, *N*-acetylglucosamine, salicin, serine, asparagine, proline or glutamine.

Characteristic	<i>P. tepidarius</i>	ALC-2 ^T
Degradation of:		
Xylan	—	+
Starch	—	+
Aesculin	—	+
Assimilation of:		
Turanose	+	—
L-Xylose	—	+
Acetate	+	—

had identical polar lipid patterns, dominated by phosphatidylethanolamine (results not shown). The major respiratory quinone was ubiquinone 10. The major fatty acids of strains

ALC-2^T and ALC-3, *P. neustonensis*, *P. tepidarius*, *Erythrobacter litoralis*, *Erythrobacter longus* and *Erythromicrobium ramosum* were unsaturated; however, there were large differences in the fatty acid compositions of the organisms (Table 2). In all strains, 18:1 ω 7c was the major fatty acid, varying between 33.5% in *Erythromicrobium ramosum* and 72% of the total fatty acids in *Erythrobacter litoralis*. The hydroxy fatty acid 14:0 2-OH was present in all strains, while 15:0 2-OH was detected in the two ALC isolates, *Erythrobacter longus* and *Erythromicrobium ramosum*. An unidentified fatty acid, with an equivalent chain-length of 17.607, was detected only in the type strains of *P. neustonensis* and *Erythrobacter longus*.

All of the strains examined produced Bchl *a* aerobically in the dark and had identical absorption spectra, with a small peak at about 800 nm and a larger peak at about 870 nm, characteristic of Bchl *a* (Fig. 3). Acetone/methanol extracts had an absorption peak centred at about 770 nm, indicative of the presence of Mg-Bchl *a* (results not shown). The carotenoids were not identified, but TLC of the extracts showed some variability in the pigments of the *Erythrobacter*/*Porphyrobacter*/*Erythromicrobium* group, although two predominant polar carotenoids were present in all strains (TLC profiles of pigments of the novel isolates are available as supplementary material in IJSEM Online at <http://ijs.sgmjournals.org/>).

Table 2. Mean fatty acid composition of the type strains of the species of the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* group and strain ALC-2^T

Fatty acid compositions were determined at 37 °C as well as at the optimum growth temperature for each strain.

Fatty acid	<i>P. tepidarius</i>		<i>P. neustonensis</i>		ALC-2 ^T		<i>Erythrobacter litoralis</i>		<i>Erythrobacter longus</i>		<i>Erythromicrobium ramosum</i>	
	37 °C	45 °C	30 °C	37 °C	37 °C	50 °C	30 °C	37 °C	30 °C	37 °C	30 °C	37 °C
12:0	—	—	—	—	—	—	—	1.1	—	—	—	0.4
14:0	0.4	0.2	0.3	0.8	—	—	0.7	1.1	0.2	—	0.2	0.6
15:0	0.5	—	0.3	—	0.8	1.3	0.3	—	1.2	1.2	3.4	2.9
14:0 2-OH	5.1	6.6	7.6	9.1	4.7	1.9	4.6	5.3	2.8	1.9	3.2	5.4
16:1 ω 7c	4.3	1.4	1.7	3.9	1.2	0.5	3.0	7.5	1.0	0.2	1.3	2.7
16:0	16.2	18.4	4.3	12.6	5.0	5.7	4.3	6.5	12.5	12.8	7.4	10.3
15:0 2-OH	—	0.4	—	—	7.4	4.9	—	—	3.9	3.5	8.2	9.7
17:0 iso	—	—	—	—	—	—	—	—	0.6	0.7	0.8	0.5
17:1 ω 8c	—	—	—	—	2.9	2.7	—	—	4.0	4.6	4.7	2.6
17:1 ω 7c	—	0.6	0.6	—	—	—	1.7	1.5	—	—	—	—
17:1 ω 6c	2.4	1.8	0.9	—	21.1	27.3	1.8	1.6	2.3	1.9	36.4	25.1
17:0	—	0.4	—	—	7.5	16.5	—	—	5.9	8.1	6.4	5.7
16:0 iso3-OH	—	0.2	2.5	1.1	—	—	0.9	—	—	—	0.2	—
16:0 2-OH	0.3	0.6	1.7	1.9	—	—	1.4	1.5	0.8	0.5	0.2	—
Unknown*	—	—	7.8	4.2	—	—	—	—	11.1	12.2	1.4	—
18:1 ω 7c	67.7	64.8	67.0	63.9	45.5	35.3	77.2	71.9	50.6	49.1	24.1	33.5
18:1 ω 5c	1.9	2.2	2.6	2.4	1.2	1.0	2.7	2.1	—	—	0.6	0.6
18:0	0.6	1.7	0.3	—	1.1	1.4	—	—	1.1	1.2	0.2	—

*Unknown fatty acid with equivalent chain length of 17.607.

Fig. 3. Absorption spectra of cells of *Erythromicrobium ramosum* DSM 8510^T (1), *Erythrobacter litoralis* DSM 8509^T (2), strain ALC-2^T (3) and *P. tepidarius* DSM 10594^T (4) disrupted ultrasonically in MOPS buffer.

Mean base composition of DNA, DNA–DNA reassociation studies and 16S rRNA gene sequence comparison

The G + C content of the DNA of the two *Porphyrobacter* species, *Erythrobacter longus*, *Erythromicrobium ramosum* and isolates ALC-2^T and ALC-3 ranged between 60 and 67 mol%. The G + C content of strains ALC-2^T and ALC-3 was 66.2 mol%. Whole-genome DNA–DNA reassociation values among the species mentioned above were very low, ranging between 21 and 40 % (Table 3). Partial 16S rRNA gene sequences comprising 1413–1414 nt were determined for strain ALC-2^T and the type strains of *Erythrobacter longus*, *Erythrobacter litoralis*, *Erythromicrobium ramosum*,

Fig. 4. 16S rRNA gene sequence-based neighbour-joining phylogenetic tree showing the position of the novel isolate within the cluster comprising the species of the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter* and their closest relatives. Bar, 5 inferred nucleotide substitutions per 100 nt (as determined by measuring the lengths of the horizontal lines joining any two organisms). Numbers at nodes refer to bootstrap values.

P. neustonensis and *P. tepidarius*. The 16S rRNA gene sequences of these type strains were redetermined in this study in order to confirm the phylogenetic positions of these strains, which do not cluster in correlation with the accepted nomenclature (e.g. *Erythrobacter litoralis* and *Erythrobacter longus*). Comparison of the 16S rRNA gene sequence of strain ALC-2^T with representative sequences of the main lineages within the *Proteobacteria* indicated that this strain fell within the α -4 subclass and more specifically, within the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* group. The highest 16S rRNA gene sequence similarity to a described taxon was to *P. tepidarius*, at 98.3 %. 16S rRNA gene sequence similarities to other members of the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* group were 95.9–98.3 %. A phylogenetic tree was reconstructed using the 16S rRNA gene sequence from each of the type strains of the described species of the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* group, seven additional strains for which 16S rRNA gene sequences are available and representative lineages within the α -4 subclass of the *Proteobacteria* (Fig. 4). Strain

Table 3. DNA G + C content and DNA–DNA reassociation between type strains of the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* group

The type strain of *Erythrobacter litoralis* was not used for comparison of DNA–DNA reassociation.

Strain/species	G + C content (mol%)*	Degree of binding (%) to:					
		1	2	3	4	5	6
1. <i>P. neustonensis</i>	65.6 ^a	100					
2. <i>P. tepidarius</i>	65.0 ^b	24	100				
3. <i>Erythrobacter longus</i>	60.0–64.0 ^b	21	22	100			
4. <i>Erythromicrobium ramosum</i>	64.6 ^c	24	24	25	100		
5. ALC-2 ^T	66.2 ^c	25	35	19	22	100	
6. ALC-3	66.2 ^c	31	40	20	21	100	100

*Data from: a, Shiba & Simidu (1982); b, Hanada *et al.* (1997); c, this study.

ALC-2^T represented a distinct lineage within the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* cluster, having an intermediate position between the *Erythrobacter* lineage and the other members of this group.

The taxon represented by strain ALC-2^T was, based on phylogenetic analysis of the 16S rRNA gene sequence, closely related to the species of the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*. Strain ALC-2^T represents an individual lineage that does not cluster with any of the previously described species of the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* group. This position is supported by the low bootstrap values within the cluster, which indicate that strain ALC-2^T is not a member of an obvious species of any of the currently described genera. These results are corroborated by the low DNA–DNA reassociation values between the strains.

Based on these results, and the current taxonomic interpretation of this group, we could consider that strains ALC-2^T and ALC-3 represent a species of a novel genus. However, the description of taxa of genus level based solely on phylogenetic inference is unacceptable because it leads to taxonomic chaos, since the novel genus would have no differentiating phenotypic characteristics. Alternatively, the ALC strains could represent a species of one of the previously described genera within the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* group. However, the phenotypic characteristics of the ALC isolates, allied to the whole-genome DNA–DNA reassociation values and the phylogenetic analyses, support the idea that these strains could be described as a novel species of any of the genera *Erythrobacter*, *Erythromicrobium* or *Porphyrobacter*, because the novel strains are not obvious members of any of the previously described genera and the genera of this group are not well defined and are phylogenetically intermixed. The possibility of combining the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter* (and the invalidly described '*Citromicrobium*') into one genus deserves consideration. In favour of this view, it should be noted that all the species of the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter* produce motile, coccoid and/or rod-shaped cells, although *P. neustonensis* also produces stalked cells and buds, while *Erythromicrobium ramosum* produces branched cells and '*C. bathyomarinum*' produces both rod-shaped and branched cells (Yurkov *et al.*, 1999). Strains ALC-2^T and ALC-3 also produce pleomorphic branched cells on solid media (results not shown), but form rod-shaped cells during exponential phase in liquid medium. The species *Erythrobacter longus*, *Erythrobacter litoralis* and '*C. bathyomarinum*' are slightly halophilic marine organisms, while the species of *Porphyrobacter* and *Erythromicrobium ramosum* originate from freshwater environments. Since '*C. bathyomarinum*' has been considered a new taxon at the generic level, and is a slightly halophilic marine organism, the argument that the species of the genus *Erythrobacter* should be considered in a separate genus from the freshwater species of the genera *Porphyrobacter* and *Erythromicrobium* cannot be

valid. Moreover, several other prokaryotic genera that are also composed of closely related species, notably *Thermonema* (Tenreiro *et al.*, 1997) and *Pyrobaculum* (Völkl *et al.*, 1993), contain both slightly halophilic marine and non-halotolerant freshwater species, which leads to the assumption that halophily cannot be used alone to assign the species to a particular genus. The overall fatty acid compositions of all the species (with the exception of '*C. bathyomarinum*', for which results are not available) are similar, with a predominance of unsaturated fatty acids, but there is no unique fatty acid pattern within the strains of any of the species of the genera *Erythrobacter*, *Porphyrobacter* and *Erythromicrobium* that could be viewed as indicative of generic distinctiveness. The polar lipid patterns and the quinones are identical. With the possible exception of *Erythromicrobium ramosum*, the photosynthetic apparatus of all of the species is also identical. All strains, with the exception of ALC-2^T and ALC-3, have similar carotenoid patterns on TLC.

The data we obtained and the information available from the literature lead us to believe that the genera *Erythrobacter*, *Porphyrobacter* and *Erythromicrobium* should be combined into a single genus, i.e. the genus *Erythrobacter*. However, the view that this group should be classified as one genus may not be appropriate at this time; this leads us to the second alternative, the description of a novel species of an existing genus. The novel species represented by strains ALC-2^T and ALC-3 has phenotypic characteristics that are similar to those of *P. tepidarius*; the growth temperature range of the novel species was slightly higher than that of the type strain of *P. tepidarius* and there were slight differences in the single-carbon-source assimilation patterns. However, strains ALC-2^T and ALC-3 and *P. tepidarius* had substantially different fatty acid compositions. Thus, based on the growth temperature range and the similar single-carbon-source assimilation patterns, we propose that the species represented by strains ALC-2^T and ALC-3 should be included in the genus *Porphyrobacter* as *Porphyrobacter cryptus* sp. nov.

Description of *Porphyrobacter cryptus* sp. nov. da Costa, Rainey and Nobre

Porphyrobacter cryptus (cryp'tus. M.L. masc. adj. *cryptus* from Gr. adj. *kryptos* hidden, to indicate the cryptic relationship of this species to the closely related organisms).

Forms short rod-shaped cells of variable width (0.6–1.0 µm) and length (1.0–2.8 µm). Gram stain is negative. Cells are motile by one polar flagellum. Colonies on PE medium are reddish-orange-pigmented. The optimum growth temperature is about 50 °C; growth does not occur at 60 °C. The optimum pH is between 7.5 and 8.0. Cytochrome oxidase- and catalase-positive. Cells contain Bchl *a* and carotenoids. The predominant fatty acids are monounsaturated. The major fatty acid is 18:1ω7c; 14:0 2-OH and 15:0 2-OH are present. The predominant phospholipid is phosphatidylethanolamine. Aerobic and chemo-organotrophic. Growth does not occur under anaerobic conditions in the

dark or in the light. Nitrate is not reduced to nitrite. Gelatin, starch, xylan, Tweens 20, 40 and 60 and aesculin are hydrolysed. D-Glucose, D-fructose, maltose, sucrose, cellobiose, xylose, pyruvate, arginine and glutamate serve as carbon sources. The G+C content of the DNA is 66.2 mol%. Isolated from the hot spring at Alcafache in Central Portugal. The type strain is strain ALC-2^T (=DSM 12079^T = ATCC BAA-386^T). Strain ALC-3 (=DSM 12080) is a reference strain.

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