NOTE

***Erythrobacter citreus* sp. nov., a yellow-pigmented bacterium that lacks bacteriochlorophyll *a*, isolated from the western Mediterranean Sea**

¹ Institut für Mikrobiologie und Genetik, Universität Wien, A-1030 Wien, Austria

² Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08901, USA

³ Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

⁴ Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

⁵ Institut für Medizinische Biologie, AG Allgemeine Mikrobiologie, Universität Wien, A-1090 Wien, Austria

Ewald B. M. Denner,¹ Dietmar Vybiral,¹ Michal Koblížek,² Peter Kämpfer,³ Hans-Jürgen Busse^{1,4} and Branko Velimirov⁵

Author for correspondence: Ewald B. M. Denner. Tel: +43 1 4277 54676 or 54677. Fax: +43 1 4277 9546. e-mail: ewald.denner@univie.ac.at

Two facultatively oligotrophic, intensely yellow-pigmented bacterial strains, RE35F/1^T and RE10F/45, have been previously isolated from the western Mediterranean Sea (Bay of Calvi, Corsica, France) by 0.2 µm membrane filtration. The organisms were Gram-negative, catalase- and oxidase-positive, strictly aerobic, rod-shaped and non-motile. Their respiratory lipoquinone profiles consisted exclusively of ubiquinone-10 (Q-10) and the G+C contents of their DNAs were 62.0 and 62.4 mol %, respectively. Among the cellular fatty acids, octadecenoic acid (18:1 ω 7c) was the major component. Both isolates also contained hydroxy fatty acids (14:0 2-OH, 18:1 2-OH and 16:0 iso 3-OH) and branched fatty acids (15:0 anteiso, 16:0 anteiso and 17:0 anteiso). Polar lipid fingerprints were characterized by the presence of a sphingoglycolipid. Comparative analyses of their 16S rRNA gene sequences indicated that both isolates were phylogenetically closely related (sequence similarity of 99.9%) and formed a coherent cluster with aerobic bacteriochlorophyll *a*-containing species of the *Erythrobacter*/*Porphyrobacter*/*Erythromicrobium* cluster within the family *Sphingomonadaceae*. The closest relative was *Erythrobacter litoralis* DSM 8509^T (97.4 and 97.5 % 16S rRNA gene sequence similarity between this strain and RE35F/1^T and RE10F/45, respectively). DNA–DNA reassociation studies confirmed that strains RE35F/1^T and RE10F/45 represent a single species (79.6 % DNA homology), but also demonstrated that they do not belong to the species *Erythrobacter litoralis* (25.2 and 34.2 % DNA homology, respectively). Notably, both RE35F/1^T and RE10F/45 lacked bacteriochlorophyll *a*. Based upon phenotypic and molecular evidence, a novel species of the genus *Erythrobacter*, *Erythrobacter citreus* sp. nov., is proposed. Strain RE35F/1^T (= CIP 107092^T = DSM 14432^T) is the type strain.

Keywords: 0.2 µm-filterable bacteria, western Mediterranean Sea, α -*Proteobacteria*, strain RE35F/1^T, *Erythrobacter citreus* sp. nov.

The family *Sphingomonadaceae* (α -4 subclass of the *Proteobacteria*) harbours a number of species that belong to nine different genera (Kosako *et al.*, 2000;

Published online ahead of print on 18 February 2002 as DOI 10.1099/ijse.0.01885-0.

Abbreviations: BChl *a*, bacteriochlorophyll *a*; pNA, *para*-nitroanilide; pNP, *para*-nitrophenyl.

The EMBL accession number for the 16S rDNA sequence of strain RE10F/45 is AF227259.

Takeuchi *et al.*, 2001). With the exception of the recently proposed species *Sphingomonas alaskensis* (Vancanneyt *et al.*, 2001), species that have been isolated from marine environments have, to date, only been found in the genus *Erythrobacter* (Shiba & Simidu, 1982; Yurkov *et al.*, 1994). *Erythrobacters* are red/orange-pigmented, obligately aerobic bacteria containing bacteriochlorophyll *a* (BChl *a*) and are known as aerobic anoxygenic phototrophs (Yurkov & Beatty, 1998). The genus represents a separate branch

Fig. 1. Phylogenetic dendrogram based on 16S rRNA gene sequence data indicating the phylogenetic position of strains RE35F/1^T and RE10F/45 within the *Erythrobacter*/*Porphyrobacter*/*Erythromicrobium* cluster of the *Sphingomonadaceae* (Kosako *et al.*, 2000). Percentages of the sequence similarities were calculated and corrected for substitutions using the Jukes & Cantor (1969) parameters. The dendrogram was generated by the neighbour-joining method (Saitou & Nei, 1987) with the program NEIGHBOR contained in the PHYLIP software package version 3.57c (Felsenstein, 1995). Percentages of bootstrap support (> 50%) from 1000 resamplings are indicated at the branching points. Bar, estimated evolutionary distance (i.e. a mean of 1 substitution, at any nucleotide position, per 100 nucleotide positions) which has occurred from the point of divergence of the 16S rRNA gene sequences. *Agrobacterium tumefaciens* was used as the outgroup; tree construction was performed with TREEVIEW version 1.5.2 (Page, 1996).

within the family *Sphingomonadaceae*, clustering together with the genera *Erythromicrobium* (Yurkov *et al.*, 1994) and *Porphyrobacter* (Fuerst *et al.*, 1993; Hanada *et al.*, 1997), which are likewise aerobic anoxygenic phototrophs containing BChl *a*, but have been isolated from freshwater. Here, another species of the genus *Erythrobacter* is reported which has been isolated from seawater and is yellow-pigmented, but lacks BChl *a*.

In a study on the occurrence and taxonomy of 0.2 µm-filterable bacteria in the western Mediterranean Sea, several strains were isolated in May 1994 which were shown to belong to different taxa (Vybiral *et al.*, 1999). The strains were from seawater collected from a depth

of 35 m in the Bay of Calvi (western coast of Corsica, France). The water column of this site has all the features of an oligotrophic environment and the benthos is characterized by an extensive seagrass system that extends down to 40 m depth within the Bay of Calvi (Velimirov & Walenta-Simon, 1992). Among these strains, an intensely yellow-pigmented bacterium, designated RE35F/1^T, was isolated. A distinctive feature of this organism was its ability to grow on both complex-peptone-based media such as Bacto marine agar 2216 (Difco) and on low-nutrient media, e.g. seawater agar or SWPY-75 agar (Vybiral *et al.*, 1999). 16S rRNA analysis indicated that RE35F/1^T is most closely related to the genus *Erythrobacter*. As mentioned before, one of the main defining

characteristics of *Erythrobacter* species, and of all aerobic anoxygenic phototrophic bacteria, is the presence of BChl *a*, an essential component of the light-harvesting complexes of these organisms. In spite of solid evidence for a relationship at the generic level to this group of bacteria, BChl *a* could not be detected in strain RE35F/1^T. Another remarkable defining characteristic of erythrobacters is their intense red/orange pigmentation, which is due to their extremely complex carotenoid composition (Takaichi *et al.*, 1988, 1990, 1991; Yurkov *et al.*, 1994). The yellow pigmentation of RE35F/1^T observed when it was cultivated under the same conditions, e.g. aerobic or semi-aerobic incubation in the dark (Shiba & Simidu, 1982; Yurkov *et al.*, 1994), differed clearly from that seen in *Erythrobacter* species. In June 1996, another 0.2 µm-filterable, yellow-pigmented bacterial strain (RE10F/45) was isolated from the same water column (10 m depth) which was phenotypically almost identical to strain RE35F/1^T. Here, the description of a novel species of the genus *Erythrobacter* which is yellow-pigmented and lacks BChl *a* is reported.

16S rRNA gene sequence and phylogenetic analysis

The 16S rDNA of strain RE10F/45 amplified by PCR was determined (1359 nt) as described previously (Denner *et al.*, 2001). Sequence comparison revealed that isolate RE10F/45 shared a sequence similarity of 99.9% with the previously isolated strain RE35F/1^T (16S rDNA sequence accession no. AF118020; Vybiral *et al.*, 1999). Database searching using FASTA3 (Pearson & Lipman, 1988; Pearson, 1990) showed further that the sequence of RE10F/45 is most similar to that of *Erythrobacter* species. On the basis of this data, nucleotide sequences of next closest relatives were retrieved from the EMBL database, aligned manually using the program PILEUP (Devereux *et al.*, 1984) and edited to remove nucleotide positions of ambiguous alignment and gaps. The comparative evolutionary distance analyses (PHYLIP software package, version 3.57c; Felsenstein, 1995) using a continuous stretch of 1290 nt, demonstrated that strains RE35F/1^T and RE10F/45 formed a coherent cluster with the genera *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*, branching from the *Erythrobacter* lineage (Fig. 1). The topography of the branching order of the *Erythrobacter*/*Erythromicrobium*/*Porphyrobacter* cluster within the dendrogram was supported by identical branching orders generated by maximum-likelihood and maximum-parsimony analyses (data not shown). The highest sequence similarities of RE35F/1^T and RE10F/45 were found with *Erythrobacter litoralis* (97.4 and 97.5%, respectively), *Erythrobacter longus* (97.2 and 97.3%, respectively) and *Erythromicrobium ramosum* (97.0%). Similarities of the 16S rRNA gene sequences of RE35F/1^T and RE10F/45 with those of other validly described taxa were 96.3 and 96.6% (with *Porphyrobacter tepidarius*) and 95.0 and 95.6% (with *Porphyrobacter neustonensis*).

Comparison of whole-cell protein patterns by SDS-PAGE (Denner *et al.*, 2001) indicated a relatedness of RE35F/1^T and RE10F/45 at the species level (data not shown). To substantiate that both isolates are members of a single species, DNA–DNA hybridizations were carried out. Although, physiological and biochemical traits clearly distinguished RE35F/1^T and RE10F/45 from species of the genera *Erythrobacter* and *Erythromicrobium* (Table 1), DNA relatedness was also determined with one species from each of these two genera displaying highest 16S rDNA sequence similarities. For the hybridization analysis, DNA was isolated from lyophilized cell material by hydroxyapatite chromatography (Cashion *et al.*, 1977). Levels of DNA–DNA hybridization were determined spectrophotometrically (De Ley *et al.*, 1970; Huß *et al.*, 1983; Escara & Hutton, 1980) using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermo-programmer and plotter. Renaturation rates were computed with the program TRANSFER.BAS (Jahnke, 1992). The DNA–DNA hybridization analysis showed a high level of relatedness (79.6%) between RE35F/1^T and RE10F/45. The levels of DNA relatedness with *Erythrobacter litoralis* DSM 8509^T (25.2 and 34.2%, respectively) and *Erythromicrobium ramosum* DSM 8510^T (20.3 and 34.4%, respectively) unequivocally demonstrated that RE35F/1^T and RE10F/45 do not belong to either of these two species.

Chemotaxonomy

Analyses of the chemical composition of cell constituents of RE10F/45 and RE35F/1^T revealed that both isolates possess chemotaxonomic features that are congruent with those defined for members of the family *Sphingomonadaceae* (Kosako *et al.*, 2000). The respiratory lipoquinone profiles analysed by HPLC (Tindall, 1990) consisted exclusively of ubiquinone-10 (Q-10). The DNA G+C compositions of RE35F/1^T and RE10F/45, as determined by HPLC (Cashion *et al.*, 1977; Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984; Visuvanathan *et al.*, 1989), were 62.0 and 62.4 mol%, respectively. The polyamine pattern of strain RE35F/1^T contained predominantly spermidine and lacked *sym*-homospermidine (Vybiral *et al.*, 1999), which supports its close relationship with *Erythrobacter* and *Erythromicrobium* (Hamana & Takeuchi, 1998). Fatty acid analysis by GLC, according to the standard protocol of the Microbial Identification system (MIDI) as described by Kämpfer & Kroppenstedt (1996), showed that the major fatty acid of the cellular lipids of RE35F/1^T and RE10F/45 is octadecenoic acid (18:1 *ω*7c); 2-hydroxymyristic acid (14:0 2-OH) was also present in both strains (Table 1). Furthermore, the polar lipid fingerprints of RE35F/1^T and RE10F/45 obtained by two-dimensional TLC (Vybiral *et al.*, 1999) were essentially identical and, again, highly similar to those of *Erythrobacter litoralis* DSM 8509^T and *Erythrobacter longus* LMG 3982^T (E. B. M. Denner, unpublished

Table 1. Comparative phenotypic characteristics of *Erythrobacter citreus* strains and reference type strains of most closely related bacteria

Strains: 1, *Erythrobacter citreus* ($n = 2$); 2, *Erythrobacter litoralis* DSM 8509^T; 3, *Erythrobacter longus* LMG 3982^T; 4, *Erythromicrobium ramosum* DSM 8510^T. Tests positive for all strains: catalase and oxidase; assimilation of acetate; hydrolysis of 2-deoxythymidine-5'-*p*NP-phosphate, L-alanine-*p*NA, L-glutamate- γ -3-carboxy-*p*NA and L-proline-*p*NA. Tests negative for all strains: assimilation of *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-mannose, α -D-melibiose, L-rhamnose, D-ribose, salicin, D-trehalose, adonitol, iso-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, 4-aminobutyrate, fumarate, itaconate, mesaconate, L-alanine, β -alanine, L-histidine, L-leucine, L-ornithine, L-serine, L-tryptophan and phenylacetate; hydrolysis of aesculin, *p*NP- β -D-galactopyranoside, *p*NP- β -D-glucuronide, *p*NP- α -D-glucopyranoside, *p*NP- β -D-glucopyranoside, *p*NP- β -D-xylopyranoside, bis-*p*NP-phosphate, *p*NP-phenyl phosphate and *p*NP-phosphoryl choline. +, Positive; (+) weak reaction; v+, type strain is positive; v(+), type strain is weak positive; -, negative. Data for *Erythrobacter litoralis*, *Erythrobacter longus* and *Erythromicrobium ramosum* were compiled from Yurkov *et al.* (1994), Shiba & Simidu (1982), Shiba (1991) and this study. As reported previously (Vybiral *et al.*, 1999), the oxidase test for *Erythrobacter citreus* RE35F/1^T is negative with commercially available test strips (Merck).

Characteristic	1	2	3	4
Cell shape	Rod	Rod	Rod	Rod, branched
Cell size (μ m)	0.3–0.7 $\times$ 1.0–1.5	0.2–0.3 $\times$ 1.0–1.3	0.3–0.4 $\times$ 2.0–5.0	0.6–1.0 $\times$ 1.3–2.5
Motility	—	+	+	+
Pigmentation	Yellow	Red-orange	Red-orange	Red-orange
BChl <i>a</i>	—	+	+	+
Nitrate reduction	+	—	+	—
Assimilation of:				
D-Glucose	v(+)	+*	+†	+*
D-Maltose	—	+	+	+
Sucrose	—	+	+	—
D-Xylose	—	+	+	—
Propionate	v+	+	—	—
<i>cis</i> -Aconitate	v+	—	—	—
<i>trans</i> -Aconitate	+	+	—	—
Adipate	+	+	—	—
Azelate	v+	—	—	—
Citrate	v(+)	+‡	—†	—‡
Glutarate	+	+	—	—
DL-3-Hydroxybutyrate	+	+	—	—
DL-Lactate	—	+†	—†	—‡
L-Malate	—	+‡	—†	+*
Oxoglutarate	—	+	—	—
Pyruvate	—	+*	+†	+*
Suberate	+	+	—	+
L-Aspartate	+	+	—	—
L-Phenylalanine	—	+	—	—
L-Proline	+	+	—	+
3-Hydroxybenzoate	—	+	—	—
4-Hydroxybenzoate	—	+	—	—
Hydrolysis of <i>p</i> NP- α -D-glucopyranoside	—	—	—	+

* Data in agreement with that published by Yurkov *et al.* (1994).

† Data in agreement with that published by Shiba & Simidu (1982).

‡ Data not in agreement with that published by Yurkov *et al.* (1994).

results). Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine were readily identified by their chromatographic behaviour and staining characteristics in strains RE35F1^T, RE10F/45, DSM 8509^T and LMG 3982^T. Finally, a sphingoglycolipid characteristic of all taxa of the family *Sphingomonadaceae* examined to

date (Busse *et al.*, 1999; Kosako *et al.*, 2000) was also present in the cellular lipids of RE35F/1^T and RE10F/45.

Despite their clear phylogenetic affiliation within the radiation of aerobic anoxygenic phototrophic bacteria, no BChl *a* was detected *in vivo* in cells of RE35F/1^T

Fig. 2. Comparison of the pigment HPLC elution profile for *Erythrobacter citreus* RE35F/1^T and *Erythrobacter longus* LMG 3982^T. Pigments were detected by an absorption detector at 440 nm (lower curve) and a fluorescence detector at 780 nm (upper curves). The record of the fluorescence detector demonstrates the absence of BChl *a* in strain RE35F/1^T.

and RE10F/45 within the sensitivity of the instrument (~ 10 pM BChl *a*) using the infrared fast repetition rate fluorometer method (Kolber *et al.*, 2000). The absence of BChl *a* in both isolates was also confirmed by HPLC analysis (Fig. 2) using a Shimadzu class VP HPLC system composed of an SCL-10A controller, SIL-10A autoinjector, two LC-10AT pumps and equipped with a Microsorb MV C8 3 μ m 100-A column. Spun bacterial cell pellets were extracted using an acetone:methanol mixture (7:2), and were immediately analysed (50 μ l). Pigments were separated by a linear gradient using a modified Mantoura & Llewellyn (1983) method: 0.0' (100% A), 9.0' (100% B), 17.5' (100% B), 18.0' (100% A) and 20.0' (100% A), where solvent A comprised 80% methanol in water containing 1.5 g tetrabutylammonium acetate l⁻¹ and 7.7 g ammonium acetate l⁻¹, and solvent B was 100% methanol. The flow rate was set to 0.75 ml min⁻¹. Pigments were detected with the SPD-10AV absorption detector at 440 nm and the RF-10AXL fluorescence detector using a 370 nm excitation and 780 nm emission wavelength. RE35F/1^T and RE10F/45 displayed the same pigment composition with one major and several minor carotenoids clearly distinct from that of *Erythrobacter longus* LMG 3982^T. The major unidentified polar carotenoid accounted for about 80% of total pigments.

Standard bacteriological characterization and differentiation

In terms of their physiological and biochemical properties, isolates RE35F/1^T and RE10F/45 were highly similar. Cells of both strains were strictly aerobic, Gram-negative, non-spore-forming, non-motile and rod-shaped (0.3–0.7 $\times$ 1.0–1.5 μ m). Although both strains were isolated by 0.2 μ m mem-

brane filtration, microscopic (Leitz, Diaplan) examinations revealed that their mean cell dimensions after cultivation on complex-peptone-based media were $\sim 0.4 \times 1.2$ μ m. When cultivated on SWPY-2000 agar (Vybiral *et al.*, 1999) or Bacto marine agar 2216, RE35F/1^T and RE10F/45 formed intensely yellow-pigmented circular, entire, low-convex and smooth colonies. On sea water agar, the colonies were also yellow, circular, entire, low-convex and smooth, but translucent. Both strains were able to grow between 4 and 37 °C; optimal growth occurred between 25 and 30 °C. Neither of the strains required Na⁺ ions for growth. The crude acetone extract of the yellow pigment of the RE10F/45 strain showed an absorbance spectrum identical to that of strain RE35F/1^T, showing peaks at 453 and 481 nm and a slight inflexion at 427 nm. Antimicrobial susceptibility testing using the disc diffusion method as described by Vybiral *et al.* (1999) indicated that strain RE10F/45 is likewise sensitive to 30 μ g chloramphenicol, 15 μ g oleandomycin and 10 μ g vancomycin, but is resistant to 30 μ g ampicillin, 10 μ g gentamicin, 15 μ g lincomycin, 30 μ g neomycin, 30 μ g kanamycin, 10 IU penicillin G, 300 IU polymyxin B, 10 μ g streptomycin, 30 μ g tetracycline and 10 μ g 2,4-diamino-6,7-diisopropylpteridine (vibriostatic agent O/129). In contrast to RE35F/1^T, strain RE10F/45 was sensitive to 10 μ g streptomycin and 10 IU penicillin G, but resistant to 150 μ g vibriostatic agent O/129.

Expanded metabolic profiling by means of a miniaturized assay method (Kämpfer *et al.*, 1991) combined with standard bacteriological tests (Vybiral *et al.*, 1999) clearly demonstrated that RE35F/1^T and RE10F/45 are distinguishable from *Erythrobacter* species, as well as from *Erythromicrobium ramosum* (Table 1). Moreover, their cellular fatty acid profiles were also unique and suitable for differentiation (Table 2). Finally, both isolates were unequivocally distant at the genomic level to their closest phylogenetic neighbour, *Erythrobacter litoralis*. Based upon these findings combined with previously published data (Vybiral *et al.*, 1999), it is concluded that the two strains, RE35F/1^T and RE10F/45, represent a novel species of the genus *Erythrobacter* which is yellow-pigmented and does not possess BChl *a*. In conclusion, the proposal to assign a non-BChl *a*-containing species to the genus *Erythrobacter* means that this trait has to be removed from the genus description as a general characteristic of its members. This idea is supported by recent isolation of a similar yellow-pigmented marine Mn(II)-oxidizing bacterium, designated strain SD-21, which also lacks BChl *a*. Database searches demonstrated that the 16S RNA gene sequence of SD-21 is most similar (> 98% identity) to that of RE35F/1^T and to *Erythrobacter* species (> 97% identity) (Francis *et al.*, 2001).

Description of *Erythrobacter citreus* sp. nov.

Erythrobacter citreus (cit.re.us. M.L. adj. *citreus* describing the lemon-yellow pigmentation).

Table 2. Cellular fatty acid composition (%) of *Erythrobacter citreus*, *Erythrobacter litoralis*, *Erythrobacter longus* and *Erythromicrobium ramosum*

Strains: 1, *Erythrobacter citreus* RE35F/1^T; 2, *Erythrobacter citreus* RE10F/45; 3, *Erythrobacter litoralis* DSM 8509^T; 4, *Erythrobacter longus* LMG 3982^T; 5, *Erythromicrobium ramosum* DSM 8510^T. Strains RE35F/1^T and RE10F/45 were cultivated on Merck I-agar (pH 8.4) at 28 °C; strains DSM 8509^T and DSM 8510^T were grown on Bacto marine agar 2216 (Difco) at 28 °C; and strain LMG 3982^T was grown on R2A agar (Oxoid) at 30 °C for 48 h prior to fatty acid analysis. Fatty acid nomenclature: unsaturated fatty acids, the position of the double bond can be located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*.

Compound*	1	2	3	4	5
Saturated fatty acids:					
14:0	0.4	—	—	—	—
15:0	0.3	0.9	2.3	1.5	—
16:0	8.2	4.7	6.7	8.3	3.0
17:0	—	2.6	1.9	7.9	—
18:0	—	—	—	1.3	—
Unsaturated fatty acids:					
16:1 ω 5 <i>c</i>	3.6	2.8	—	—	—
17:1 ω 6 <i>c</i>	5.6	24.5	30.2	3.7	39.7
17:1 ω 8 <i>c</i>	0.7	3.0	3.3	5.0	5.0
18:1 ω 5 <i>c</i>	4.2	3.7	—	—	—
18:1 ω 7 <i>c</i>	49.3	35.4	38.3	60.8	45.9
11-methyl 18:1 ω 7 <i>c</i>	4.4	1.6	—	—	—
Hydroxy fatty acids:					
14:0 2-OH	0.9	0.6	6.0	3.8	—
15:0 2-OH	—	1.0	5.5	7.8	2.7
16:0 2-OH	—	1.6	—	—	—
18:1 2-OH	2.0	0.9	—	—	—
16:0 iso 3-OH	1.0	1.3	—	—	—
18:1 iso 3-OH	—	0.7	—	—	—
Branched fatty acids:					
17:0 iso	—	0.8	3.1	—	—
14:0 anteiso	—	0.5	—	—	—
15:0 anteiso	0.6	0.4	—	—	—
16:0 anteiso	0.7	0.5	—	—	—
17:0 anteiso	1.2	0.8	—	—	—
Summed features: *					
3	14.9	8.9	2.6	—	3.5
5	1.3	1.0	—	—	—
7	—	0.5	—	—	—

* Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained one or more of the following fatty acids: 16:1 ω 7*c*/15:0 iso 2-OH. Summed feature 5 contained one or more of the following fatty acids: 18:2 ω 6,9*c*/18:0 anteiso/18:2 ω 6,9*c*. Summed feature 7 contained one or more of the following fatty acids: 19:1 ω 6*c*/19:0 cyclo ω 10*c*/19:0 ω 6.

The description is based on data compiled from Vybiral *et al.* (1999) and data generated in the present study. Cells are strictly aerobic, Gram-negative, rod-shaped (0.3–0.7 $\times$ 1.0–1.5 μ m), non-spore-forming and

non-motile. Catalase- and oxidase-positive. Yellow intracellular pigments (carotenoids) are produced. The acetone-extracted pigment shows two peaks at 453 and 481 nm, as well as a slight inflexion at 427 nm. Cells do not contain BChl *a*. Colonies on complex media such as SWPY-2000 agar or Bacto marine agar 2216 are opaque, intensely yellow, circular, entire, low-convex and smooth. Colonies on sea water agar are translucent, yellow, circular, entire, low-convex and smooth. Na⁺ ions are not required for growth. Nitrate is reduced to nitrite. The following compounds are assimilated: acetate, *trans*-aconitate, glutarate, DL-3-hydroxybutyrate, suberate, L-aspartate and L-proline. The type strain RE35F/1^T also utilizes propionate, *cis*-aconitate, adipate and azelate. The following compounds are hydrolysed: 2-deoxythymidine-5'-*para*-nitrophenyl (*p*NP) phosphate, L-alanine-*para*-nitroanilide (*p*NA), L-glutamate- γ -3-carboxy-*p*NA and L-proline-*p*NA. The compounds which are not assimilated or hydrolysed are listed in Table 1. Susceptible to bacitracin, 30 μ g chloramphenicol, 15 μ g oleandomycin and 10 μ g vancomycin. Resistant to 30 μ g ampicillin, 10 μ g gentamicin, 15 μ g lincomycin, 30 μ g neomycin, 30 μ g kanamycin, 10 IU penicillin G, 300 IU polymyxin B, 10 μ g streptomycin, 30 μ g tetracycline and 10 μ g vibriostatic agent O/129. Strain RE10F/45 is, in contrast to strain RE35F/1^T, sensitive to streptomycin and penicillin G, but resistant to 150 μ g vibriostatic agent O/129. Ubiquinone Q-10 is the respiratory lipoquinone system. The main component in the polyamine pattern is spermidine. Phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol are the major polar lipids; phosphatidylcholine and a sphingoglycolipid are present in minor concentrations. The major cellular fatty acids are 18:1 ω 7*c*, 16:0, 17:1 ω 6*c* and 16:1 ω 7 and/or 15:0 iso 2-OH; hydroxylated fatty acids are 14:0 2-OH, 16:0 2-OH, 18:1 2-OH and 16:0 iso 3-OH. The DNA G+C content is 62.0–62.4 mol% (HPLC). The type strain is RE35F/1^T (= CIP 107092^T = DSM 14432^T). Isolated from the western Mediterranean Sea (Bay of Calvi, Corsica, France) by 0.2 μ m membrane filtration.

Acknowledgements

The study was supported by the FWF (Projekt Nr P11936-MOB) granted to B.V. We thank the staff of the research station STARESO sa-Pointe Revellata, Calvi, Corsica (France) for providing research facilities. Analyses of the G+C content of DNAs and DNA–DNA hybridization studies were performed by the identification service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig, Germany.

References

- Busse, H.-J., Kämpfer, P. & Denner, E. B. M. (1999). Chemotaxonomic characteristics of *Sphingomonas*. *J Ind Microbiol Biotechnol* **23**, 242–251.
- 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.

- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 143–153.
- Denner, E. B. M., Mark, B., Busse, H.-J., Turkiewicz, M. & Lubitz, W. (2001). *Psychrobacter proteolyticus* sp. nov., a new psychrotrophic, halotolerant bacterium excreting a cold-adapted metalloprotease isolated from the Antarctic krill *Euphausia superba*. *Syst Appl Microbiol* **24**, 44–53.
- Devereux, J., Haeberli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethylsulphoxide solutions: acceleration of renaturation rate. *Biopolymers* **19**, 1315–1327.
- Felsenstein, J. (1995). PHYLIP (phylogenetic inference package), version 3.57c. Department of Genetics, University of Washington, Seattle, WA, USA.
- Francis, C. A., Co, E. M. & Tebo, B. M. (2001). Enzymatic manganese (II) oxidation by a marine α -proteobacterium. *Appl Environ Microbiol* **67**, 4024–4029.
- Fuerst, J. A., Hawkins, J. A., Holmes, A., Sly, L. I., Moore, C. J. & Stackebrandt, E. (1993). *Porphyrobacter neustonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from fresh water. *Int J Syst Bacteriol* **43**, 125–134.
- Hamana, K. & Takeuchi, M. (1998). Polyamine profiles as chemotaxonomic marker within alpha, beta, gamma, delta and epsilon subclasses of class *Proteobacteria*: distribution of 2-hydroxyputrescine and homospermidine. *Microbiol Cult Coll* **14**, 1–14.
- Hanada, S., Kawase, Y., Hiriashi, A., Takaichi, S., Matsuura, K., Shimada, K. & Nagashima, K. V. P. (1997). *Porphyrobacter tepidarius* sp. nov., a moderately thermophilic aerobic photosynthetic bacterium isolated from a hot spring. *Int J Syst Bacteriol* **47**, 408–413.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kolber, Z. S., Van Dover, C. L., Niederman, R. A. & Falkowski, P. G. (2000). Bacterial photosynthesis in surface waters of the open ocean. *Nature* **407**, 177–179.
- Kosako, Y., Yabuuchi, E., Naka, T., Fujiwara, N. & Kobayashi, K. (2000). Proposal of *Sphingomonadaceae* fam. nov., consisting of *Sphingomonas* Yabuuchi et al. 1990, *Erythrobacter* Shiba and Shimidu 1982, *Erythromicrobium* Yurkov et al. 1994, *Porphyrobacter* Fuerst et al. 1993, *Zymomonas* Kluver and van Niel 1936, and *Sandaracinobacter* Yurkov et al. 1997, with the type genus *Sphingomonas* Yabuuchi et al. 1990. *Microbiol Immunol* **44**, 563–575.
- Mantoura, R. F. C. & Llewellyn, C. A. (1983). The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Anal Chim Acta* **151**, 297–314.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Pearson, W. R. (1990). Rapid and sensitive sequence comparison with FASTP and FASTA. *Methods Enzymol* **183**, 63–98.
- Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA* **85**, 2444–2448.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shiba, T. (1991). *Roseobacter litoralis* gen. nov., sp. nov., and *Roseobacter denitrificans* sp. nov., aerobic pink-pigmented bacteria which contain bacteriochlorophyll a. *Syst Appl Microbiol* **14**, 140–145.
- Shiba, T. & Simidu, U. (1982). *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll a. *Int J Syst Bacteriol* **32**, 211–217.
- Takaichi, S., Shimada, K. & Ishidsu, J. I. (1988). Monocyclic cross-conjugated carotenal from an aerobic photosynthetic bacterium, *Erythrobacter longus*. *Phytochem* **27**, 3605–3609.
- Takaichi, S., Shimada, K. & Ishidsu, J. I. (1990). Carotenoids from the aerobic photosynthetic bacterium, *Erythrobacter longus*: β -carotene and its hydroxyl derivatives. *Arch Microbiol* **153**, 118–122.
- Takaichi, S., Furihata, K., Ishidsu, J. I. & Shimada, K. (1991). Carotenoid sulphates from the aerobic photosynthetic bacterium, *Erythrobacter longus*. *Phytochem* **30**, 3411–3415.
- Takeuchi, M., Hamana, K. & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405–1417.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Vancanneyt, M., Schut, F., Snauwaert, C., Goris, J., Swings, J. & Gottschal, J. C. (2001). *Sphingomonas alaskensis* sp. nov., a dominant bacterium from a marine oligotrophic environment. *Int J Syst Evol Microbiol* **51**, 73–79.
- Velimirov, B. & Walenta-Simon, M. (1992). Seasonal changes in specific growth rates, production and biomass of a bacterial community in the water column above a Mediterranean Seagrass system. *Mar Ecol Prog Ser* **80**, 237–248.
- Visuvanathan, S., Moss, V. S., Stanford, J. L., Hermon-Taylor, J. & McFadden, J. J. (1989). Simple enzymatic method for isolation of DNA from diverse bacteria. *J Microbiol Methods* **10**, 59–64.
- Vybiral, D., Denner, E. B. M., Haller, C. M., Busse, H.-J., Witte, A., Höfle, M. G. & Velimirov, B. (1999). Polyphasic classification of 0.2 μ m filterable bacteria from the western Mediterranean Sea. *Syst Appl Microbiol* **22**, 635–646.
- Yurkov, V. V. & Beatty, J. T. (1998). Aerobic anoxygenic phototrophic bacteria. *Microbiol Mol Biol Rev* **62**, 695–724.
- Yurkov, V. V., Stackebrandt, E., Holmes, A. & 7 other authors (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll a-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* **44**, 427–434.