Description of *Labrenzia alexandrii* gen. nov., sp. nov., a novel alphaproteobacterium containing bacteriochlorophyll *a*, and a proposal for reclassification of *Stappia aggregata* as *Labrenzia aggregata* comb. nov., of *Stappia marina* as *Labrenzia marina* comb. nov. and of *Stappia alba* as *Labrenzia alba* comb. nov., and emended descriptions of the genera *Pannonibacter*, *Stappia* and *Roseibium*, and of the species *Roseibium denhamense* and *Roseibium hamelinense*

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A slightly pink-coloured strain, strain DFL-11^T, was isolated from single cells of the marine dinoflagellate *Alexandrium lusitanicum* and was found to contain the genes encoding two proteins of the photosynthetic reaction centre, *pufL* and *pufM*. 16S rRNA gene sequence analysis revealed that the novel strain belonged to the α -2 subgroup of the *Proteobacteria* and was most closely related to *Stappia aggregata* (97.7 % similarity), *Stappia alba* (98.0 %) and *Stappia marina* (98.0 %). Dark-grown cells of strain DFL-11^T contained small amounts of bacteriochlorophyll *a* (bchl *a*) and a carotenoid. Cells of strain DFL-11^T were rods, 0.5–0.7 $\times$ 0.9–3.0 μ m in size and motile by means of a single, subpolarly inserted flagellum. The novel strain was strictly aerobic and utilized a wide range of organic carbon sources, including fatty acids, tricarboxylic acid cycle intermediates and sugars. Biotin and thiamine were required as growth factors. Growth was obtained at sea salt concentrations of between 1 and 10 % (w/v), at a pH between 6 and 9.2 and at a temperature of up to 33 °C (optimum, 26 °C). Nitrate was not reduced and indole was not produced from tryptophan. Strain DFL11^T was resistant to potassium tellurite and transformed it to elemental tellurium. The major respiratory lipoquinone was ubiquinone 10 (Q10). The polar lipids comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylcholine, an unidentified aminolipid and the glycolipid sulphoquinovosyldiacylglyceride. The fatty acids comprised 16 : 1 ω 7c, 16 : 0, 18 : 1 ω 7c, 18 : 0, 11-methyl 18 : 1 ω 6t, 11-methyl 20 : 1 ω 6t, 20 : 1 ω 7c, 22 : 0, 22 : 1 and the hydroxy fatty acids

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Abbreviations: AL, unidentified aminolipid; bchl *a*, bacteriochlorophyll *a*; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PMME, phosphatidylmonomethylethanolamine; SQDG, sulphoquinovosyldiacylglyceride.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DFL-11^T is AJ582083.

An electron micrograph showing flagellar insertion in cells of strain DFL-11^T and a table featuring a similarity matrix of 16S rRNA gene sequences for strain DFL-11^T and related taxa are available as supplementary material in IJSEM Online.

3-OH 14 : 0, 3-OH 16 : 0 (ester-linked), 3-OH 18 : 0, 3-OH 20 : 1 and 3-OH 20 : 0, all of which are amide-linked. The DNA G + C value was 56 mol%. Comparative analysis of α -2 subgroup 16S rRNA gene sequences showed that the type species of the genus *Stappia*, *Stappia stellulata*, is only distantly related to *S. aggregata* (95.3% sequence similarity). Based on the combination of the 16S rRNA gene sequence data, a detailed chemotaxonomic study and the biochemical and physiological properties of members of the genera *Stappia*, *Pannonibacter* and *Roseibium*, it is proposed that *S. aggregata*, *S. alba*, *S. marina* are transferred to a new genus, *Labrenzia* gen. nov., as *Labrenzia aggregata* comb. nov., *Labrenzia alba* comb. nov. and *Labrenzia marina* comb. nov. The type species of the new genus is *Labrenzia alexandrii* sp. nov., with strain DFL-11^T (= DSM 17067^T = NCIMB 14079^T) as the type strain. The *pufLM* genes of the photosynthesis reaction centre were shown to be present in some, but not all, species of the new genus *Labrenzia* and they were identified for the first time in *S. stellulata*. In accordance with the new data collected in this study, emended descriptions are provided for the genera *Pannonibacter*, *Roseibium* and *Stappia*.

INTRODUCTION

To date, more than 40 aerobic bacterial species have been described that synthesize bacteriochlorophyll *a* and these species are usually referred to as aerobic anoxygenic phototrophs (Yurkov & Beatty, 1998; Rathgeber *et al.*, 2004; Wagner-Döbler & Biebl, 2006). The majority of them belong to the α -3 and α -4 subgroups of the *Proteobacteria*, including members of the typical marine genera *Roseobacter* (α -3) and *Erythrobacter* (α -4), although early reports also centred on members of the genus *Methylobacterium* (see Harashima *et al.*, 1989 for a review). A smaller group of four more or less extremophilic genera were assigned to the α -1 subgroup. Within the α -2 group, aerobic anoxygenic phototrophs were, until recently, represented only by members of the genus *Roseibium*, strains of which were isolated from biological material from Shark Bay in West Australia (Shiba *et al.*, 1991; Nishimura *et al.*, 1994; Suzuki *et al.*, 2000).

During our search for aerobic marine bacteria that contain the genes encoding the photosynthetic reaction centre, *pufL* and *pufM*, we obtained two groups of aerobic anoxygenic phototrophs that belonged to the α -2 subgroup of the *Proteobacteria* according to their 16S rRNA gene sequences (Allgaier *et al.*, 2003). One group was found to be closely related to members of the genera *Ahrensia* and *Hoeflea* and has already been described (Biebl *et al.*, 2006). The other group, presently consisting of only one strain, was found to be related to the genera *Roseibium* and *Stappia* and is characterized in the present study.

METHODS

Cultivation. Two media were used for culturing the strains, both prepared with 20 g sea salts (Sigma) l⁻¹. A complex medium containing (l⁻¹) 3 g Bacto peptone (Difco) and 0.5 g yeast extract (Difco) was prepared for general use. For substrate tests, a mineral medium was used that contained (l⁻¹) 0.3 g (NH₄)₂SO₄, 0.1 g KH₂PO₄, 1 ml trace element solution SL12 (Pfennig & Trüper, 1992), a suitable carbon source and 0.1 g yeast extract or a vitamin solution (Biebl *et al.*, 2005). The pH was adjusted to 7.5 using 0.5 M H₂SO₄. Cultures were incubated at 30 °C in the dark unless otherwise indicated. Growth was measured turbidometrically at 650 nm.

The influence of light and oxygen was tested in soft agar tubes (1% agar) using the mineral medium with either acetate or glucose as substrates. Incubation was under aerobic and anaerobic conditions in the dark as well as in the light. A purple sulfur bacterium, *Rhodobacter veldkampii* DSM 11550^T, was used for comparison.

The following strains were used for reference: *Stappia stellulata* DSM 5886^T, *Stappia aggregata* DSM 13394^T, *Stappia marina* DSM 17023^T, *Stappia alba* DSM 18320^T, *Roseibium denhamense* JCM 10543^T, *Roseibium hamelinense* JCM 10544^T and *Pannonibacter phragmitetus* DSM 14782^T.

Electron microscopy. Cells in the mid-exponential growth phase were adsorbed onto carbon-Formvar foils for 1 min. Cells were washed once with water, blotted and air-dried. They were shadow-cast at 15° elevation with platinum-carbon and analysed with an energy-filtered transmission electron microscope (CEM902; Zeiss) as described by Golyshina *et al.* (2000). Cells were also embedded and processed for sectioning as described by Yakimov *et al.* (1998).

Physiological and biochemical tests. Tests that required liquid cultures were performed in 22.5 ml metal-capped test tubes containing 5 ml medium. Sigma sea salts were used for determination of the salt requirement in the complex medium in concentrations up to 10.5% (w/v). The temperature range for growth was determined using a gradient shaking incubator (Toyo Kogaku Sangyo Co. Ltd.) that allowed the temperature to be adjusted between 15 and 45 °C at intervals of 3 °C. The pH range was tested at intervals of 0.5 units between pH 5.0 and 9.5 (initial pH). Growth was measured at an early stage of the growth phase before the pH was appreciably changed by growth. Carbon sources for substrate tests were supplied at a concentration of 1 g l⁻¹. Requirement for vitamins was determined in mixtures of seven vitamins (Biebl *et al.*, 2005) in which one was omitted. The precultures were grown in a medium without any growth factors. Degradation of polymers was tested on agar plates using the complex medium. Starch was added at a concentration of 2 g l⁻¹, alginate at 7.5 g l⁻¹ and Tween 80 (lipase test) and gelatin at 4 g l⁻¹. Starch degradation was demonstrated with Lugol's solution, alginate degradation by clear zones around the colonies, degradation of Tween 80 by the formation of insoluble calcium salts and gelatin liquefaction was confirmed by precipitation of undigested gelatin with saturated ammonium sulfate solution. Nitrate reduction capacity was checked in the 22.5 ml tubes with 10 ml of mineral medium supplemented with 0.4 g NaNO₃ and 0.5 g yeast extract l⁻¹. Nitrogen formation was demonstrated using Durham tubes and formation of nitrite and consumption of nitrate were tested with Merckoquant test sticks (Merck). The presence of catalase and oxidase and indole formation were determined according to Gerhardt

et al. (1981). The reaction to tellurite was tested either in complex medium or in the mineral medium using sodium acetate (1.37 g l^{-1}) as a carbon source. Analytical grade K_2TeO_4 was added aseptically to the autoclaved media from a sterile stock solution. Due to its limited solubility in seawater medium, in particular in acetate medium, K_2TeO_4 was added only up to a final concentration of 1 g l^{-1} . The media were then adjusted to pH 7.6 with sterile 1 M NaOH and dispensed aseptically into sterile metal-capped test tubes in aliquots of 5 ml. Cultures were incubated at 30°C on a rotary shaker. Tellurium formation was recognized by black–brown to jet-black colouration of the culture.

Photosynthetic pigments. Photosynthetic pigments were extracted from 30 ml culture grown in complex medium in the dark. Bacterial cells were harvested by centrifugation and pigments were extracted with acetone/methanol (7:2). The absorption spectrum was recorded in a Shimadzu UV-3000 double beam spectrophotometer. Bacteriochlorophyll *a* (bchl *a*) absorption was measured at 772 nm after 1 h of incubation at room temperature in absolute darkness. An extinction coefficient of 75 mmol cm^{-1} (Clayton, 1963) was used for the calculation of bchl *a* concentration.

Fatty acids, respiratory lipoquinones and polar lipids.

Lipoquinones and polar lipids were extracted and separated according to the methods described by Tindall (1990). The fatty acid content was determined by the method described by Labrenz *et al.* (1999). The unusual fatty acids 11-methyl 18:1 ω 6t and 13-methyl 20:1 ω 6t were characterized by performing several microderivatizations on the methyl ester extracts obtained by acidic methanolysis and GC/MS analysis. Hydrogenation with Pd/C furnished saturated methyl esters carrying a methyl group at positions 11 or 13, respectively (Francke *et al.*, 1989). Double bond positions were determined by the formation of dimethyl disulfide adducts, showing double bonds at C-12 or C-14 (Scribe *et al.*, 1988). Alternatively, formation of 3-pyridinemethanol was performed, but the resulting mass spectra proved to be disappointing. They did not allow for the unequivocal determination of the localization of the double bonds in the methyl-branched compounds (Harvey, 1982). The configuration of the double bonds of 11-methyl 18:1 ω 6t and the bishomologue (*E*)-13-methyleicosa-14-enoic acid (13-methyl 20:1 ω 6t) were determined by comparison of GC retention behaviour with published values of synthesized (*E*)- and (*Z*)-diastereomers of 11-methyl 18:1 ω 6 (Carballeira *et al.*, 1998). While the (*E*)-diastereomer elutes slightly after 18:0 on an apolar phase, the (*Z*)-diastereomer elutes before this acid. The 13-methyl 20:1 ω 6 present in the strains eluted slightly after 20:0, indicating the (*E*)-configuration of the double bond.

16S rRNA gene sequences and phylogenetic inferences.

DNA extraction, amplification and sequencing of the 16S rRNA gene has been described (Allgaier *et al.*, 2003). The sequence was manually aligned and compared with published sequences from the DSMZ 16S rRNA gene sequence database, including sequences available from the Ribosomal Data Project (Maidak *et al.*, 2001) and EMBL. The manual alignment was constructed with the BioEdit program (Hall, 1999) and used for calculating the distance matrix with DNAdist. A phylogenetic dendrogram was inferred using the Neighbour-Joining method contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analysis was based on 1000 resamplings.

Determination of the DNA G+C content and amplification of the *pufLM* genes. The G+C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989). Amplification of the *pufLM* genes was performed as described by Allgaier *et al.* (2003).

RESULTS AND DISCUSSION

Morphological and physiological features

Strain DFL-11^T was isolated from single, washed cells of the dinoflagellate *Alexandrium lusitanicum* using culture ME207 of the Biological Institute of Helgoland (Germany). The cells were plated on agar prepared with tenfold diluted Marine Broth 2216 (Difco). After three weeks of incubation at room temperature under a natural dark–light regime, slightly pink colonies appeared that were transferred to undiluted Marine Agar 2216 and purified. One isolate, strain DFL-11^T, was maintained and further investigated.

Cells of strain DFL-11^T were rods of $0.5\text{--}0.7 \times 0.9\text{--}3.0 \text{ }\mu\text{m}$. Unequal ends in the longer cells were often observed (Fig. 1). Aggregates, often star-shaped, occurred frequently and have previously been found in many taxa within the *Alphaproteobacteria*. Cells were motile by means of a single, subpolarly inserted flagellum (Fig. 2 and see Supplementary Fig. S1 in IJSEM Online). Ultrathin-sections showed a typical Gram-negative cell wall (Fig. 2, inset). Colonies on Marine Agar 2216 appeared beige to slightly pink, almost transparent and smooth with an entire margin.

When grown in peptone/yeast extract medium, at least 1 % (w/v) of artificial sea salts (Sigma) was required. No growth occurred at 0.5 % (w/v) sea salts. The cultures had a broad growth optimum of between 1 and 7 % (w/v) sea salts. At 10 % (w/v) sea salts, the onset of growth was delayed. Growth was observed at temperatures between 15 and 34°C , with an optimum at 26°C . Below 20°C , growth was very slow and no growth occurred above 35°C . The pH optimum was between pH 7.0 and 8.5; slow growth was observed down to pH 6.0 and up to pH 9.2. In mineral medium supplemented with 0.1 g l^{-1} yeast extract, strain DFL-11^T utilized all organic carbon sources tested (see species

Fig. 1. Phase-contrast photomicrograph of cells of strain DFL-11^T mounted on agar. The culture was grown in marine peptone medium. Unequal ends are highlighted by arrows. Bar, $10 \text{ }\mu\text{m}$.

Fig. 2. Electron micrograph of cells of strain DFL-11^T. The shadow-cast preparation shows the bacterium to be subpolarly flagellated as is obvious from the flattened cell body, which clearly shows the insertion (ins; a detailed view is shown in the lower inset) of an individual flagellum (fl) in a dividing cell (s, septum). The arrowhead at the bottom indicates the shadowing direction. The sectioned cell wall (upper inset) shows Gram-negative architecture, as indicated by the outer membrane (om), murein (m) and cytoplasmic membrane (cm). Bars, 1 μ m (main panel), 100 nm (upper inset).

description) except for methanol, ethanol and glycerol. Yeast extract could be replaced by the addition of biotin and thiamine (10 and 50 μ g l⁻¹, respectively). Gelatin was hydrolysed by the novel strain, but not Tween 80 (lipase), starch or alginate. Nitrate was not reduced. No growth occurred under anaerobic conditions in the light when acetate or glucose was the substrate. Glucose was not fermented. The strain was positive for catalase and oxidase activities and did not form indole from tryptophan.

Addition of 1 g l⁻¹ sodium thiosulfate resulted in a higher yield of cell mass (22 % increase in a mineral medium with 1 g l⁻¹ acetate and 15 % in a peptone-based medium) suggesting that oxidation of this sulfur compound provides additional energy for growth. In contrast to the majority of Gram-negative bacteria, strain DFL-11^T is only moderately sensitive to the rare earth salt potassium tellurite. At concentrations of 0.05 g l⁻¹ tellurite, 65 % growth inhibition was observed in peptone medium and 35 % growth inhibition in mineral medium; this increased to 75 % growth inhibition at 0.5 g l⁻¹ tellurite in peptone medium (60 % growth inhibition in mineral medium). Elemental tellurium was formed in small amounts in mineral medium, but not in peptone medium. This mode of reaction to

tellurite is similar to that described for *Roseococcus thiosulfatophilus* by Yurkov *et al.* (1996).

Photosynthetic pigments in strain DFL-11^T

Using specific primers, it has been shown previously that strain DFL-11^T contained the photosynthetic reaction centre genes *pufL* and *pufM*, suggesting that the complete photosynthetic apparatus might be present. However *bchl a* could initially not be detected (Allgaier *et al.*, 2003). Meanwhile, using higher amounts of cell mass, *bchl a* was clearly demonstrated after extraction with acetone/methanol (7 : 2). The *bchl a* content per cell mass was low in peptone-based medium [0.3 nmol (mg protein)⁻¹], but in the same range as found for other weakly pigmented aerobic bacteriochlorophyll-producing bacteria (Sato, 1978; Yurkov *et al.*, 1993). The absorption spectrum of the acetone/methanol extract showed the typical infra-red peak of *bchl a* at 772 nm (Fig. 3). Absorption between 420 and 550 nm is due to a carotenoid, probably spheroidenone as inferred from comparison with the absorption spectrum of *Dinoroseobacter shibae*, where this carotenoid has been identified (Biebl *et al.*, 2005). Due to the low *bchl a* content, it was not possible to obtain an adequate *in vivo* absorption spectrum with the

Fig. 3. Absorption spectrum of an acetone/methanol extract (7:2) of strain DFL-11^T. A pellet from 165 ml dark-grown culture was extracted with 1.5 ml solvent.

existing methods used to reduce light scattering of the cells. However, infrared maxima were seen at about 800 and 865 nm.

Presence of *pufLM* genes of the photosynthetic reaction centre in *Stappia* and *Pannonibacter* species

In order to obtain a better overview of the presence of the *pufLM* genes of the photosynthetic reaction centre, we used specific primers and obtained a PCR product of the correct length for strain DFL-11^T and *S. stellulata*, while no such product was found for *S. aggregata* and *P. phragmitetus* (Fig. 4). The absence of *pufLM* genes can be interpreted as a complete lack of ability to synthesize the photosynthetic reaction centre, however their presence requires more cautious interpretation. Given the fact that the presence of bchl *a* has not been described for *S. stellulata*, we analysed acetone extracts of the cells photometrically and obtained a

Fig. 4. PCR amplification of the *pufLM* genes (1.5 kb) of the photosynthesis reaction centre for strain DFL-11^T, related strains and recognized aerobic anoxygenic phototrophs using *pufLM*-specific primers. The identity of the amplified band has been confirmed by sequencing for strain DFL-11^T, *Roseobacter litoralis* and *Roseobacter denitrificans* (Allgaier *et al.*, 2003). Lanes: M, molecular marker, GeneRuler 1 kb; 1, *P. phragmitetus* DSM 14782^T; 2, *S. aggregata* DSM 13394^T; 3, *S. stellulata* DSM 5886^T; 4, *Rsb. litoralis* DSM 6996^T; 5, *Rsb. denitrificans* DSM 7001^T; 6, strain DFL-11^T; 7, H₂O control.

very tiny peak at the expected wavelength. This shows that the cells are able to produce bchl *a*, albeit at low concentrations. Thus, their photosynthetic reaction centre can be assumed to be functional. Previous studies have shown that several strains of *Roseovarius tolerans* isolated from different depths were variable with respect to the presence of bchl *a*; hence the genus name (Labrenz *et al.*, 1999). However, PCR showed that the *pufLM* genes were present in all of these strains and some showed production of bchl *a* after several years of maintenance in culture (Labrenz *et al.*, 1999; Allgaier *et al.*, 2003). Similarly, in *Hoeflea phototrophica*, in spite of the presence of the *pufLM* genes, bchl *a* was initially not detected, but was later found in experiments at low nutrient levels and decreased salt concentrations (Biebl *et al.*, 2006). Thus, the expression of the photosynthetic reaction centre genes seems to be highly dependent on environmental parameters in these aerobic bchl *a*-producing bacteria. It is presently unclear whether the low amounts of bchl *a* seen in strain DFL-11^T under standard cultivation conditions are representative of the expression level in the natural marine environment. It has been shown in chemostat culture of this strain, in which normally only trace amounts of bchl were detected, that starvation periods in combination with illumination caused a drastic increase in bchl production after nutrient supply and darkness were resumed (Biebl & Wagner-Döbler, 2006). However, even under environmental stress, the bchl *a* level was still low in comparison with the levels found in species such as *Roseobacter denitrificans*, where bacteriochlorophyll is clearly visible during the extraction of lipid material (B. Tindall, unpublished data) and '*Erythromicrobium hydrolyticum*' (Yurkov & van Gernerden, 1993). The metabolic significance of this low bchl content is an open question. However, slight energetic advantages (i.e. the ability to harvest light energy, even if not used for photoautotrophic growth) might play an important role under the selective pressure of the open ocean environment.

The ability to synthesize the intact photosynthetic reaction centre (based on the detection of bchl *a* or of the *pufLM* genes) can vary between species that show a high degree of 16S rRNA gene sequence similarity. In the group under consideration here (see Table 1 and Fig. 4), bchl *a* is present in *S. marina* and strain DFL-11^T, but absent in *S. aggregata* and *S. alba*. Due to the high degree of similarity of these organisms with respect to fatty acids, polar lipids and morphological and physiological characteristics, together with 16S rRNA gene sequence similarity values which lie between 97.7 and 99.1%, we do not think that it is warranted to create new genera based mainly/solely on the presence or absence (of trace amounts) of bchl *a*. We encountered a similar case with two species of the genus *Hoeflea*. *H. phototrophica* contained bchl *a* and *Hoeflea marina* did not, although their 16S rRNA gene sequence similarity was 98.4% and they were virtually identical in their chemical composition (Biebl *et al.*, 2006). The *pufLM* genes encoding the photosynthetic reaction centre proteins have been found on linear plasmids, leading to speculation

Table 1. Characteristics that differentiate strain DFL-11^T from species of the genera *Stappia*, *Pannonibacter* and *Roseibium*

Taxa: 1, strain DFL-11^T; 2, *S. aggregata* DSM 13394^T (data from Uchino *et al.*, 1998; Rüger & Höfle, 1992); 3, *S. marina* DSM 17023^T (Kim *et al.*, 2006); 4, *S. alba* DSM 18320^T (Pujalte *et al.*, 2005); 5, *S. stellulata* DSM 5886^T (Uchino *et al.*, 1998; Rüger & Höfle, 1992); 6, *P. phragmitetus* DSM 14782^T (Borsodi *et al.*, 2003); 7, *Rib. denhamense* JCM 10543^T (Suzuki *et al.*, 2000); 8, *Rib. hamelinense* JCM 10544^T (Suzuki *et al.*, 2000). +, Growth or positive reaction; (+), weak reaction; −, no growth or negative reaction; NT, not tested.

Characteristic	1	2	3	4	5	6	7	8
Cell shape	Rods, uneven ends	Large rods, joined together	Club-shaped	Large rods	Rods, star-shaped	Long rods, star-shaped	Rods	Rods
Flagella	Monotrichous	Oligotrichous	Monotrichous	Monotrichous	Monotrichous	Monotrichous	Peritrichous	Peritrichous
Surface cultures	Faint pink	White to cream	NT	Opaque	White to brown	Beige	Pink	Pink
bchl <i>a</i> <i>in vivo</i> peak (nm)	801, 865	−	NT	−	(+)	−	804, 863	804, 863
<i>pufLM</i> genes	+	−	+	NT	+	−	+	+
<i>coxL</i> genes	+	+	+	NT	+	NT	NT	NT
Sea salt range (% w/v)	1–10	0.3–6	3–6	1–8	0.3–6	0–5	0.5–7.5	0–10
Requirement for NaCl	+	+	+	+	+	−	+	−
Reduction of nitrate to nitrite	−	+	+	+	+	+	+	+
Reduction of nitrate to N ₂	−	+	−	+	+	+	−	−
Indole production	−	−	−	−	−	−	+	+
Polar lipid:								
SQDG	+	+	+	+	−	−	+	+
PMME	+	+	+	+	+	+	+	+
DNA G+C content (mol%)	56	59	60	NT	59	65	58–60	59–63
16S rRNA gene sequence similarity to strain DFL-11 ^T (%)	100.0	97.7	98.0	98.0	94.3	95.0	96.1	97.1

Fig. 5. Polar lipids of strain DFL-11^T and related species. 1, strain DFL-11^T; 2, *Stappia aggregata* DSM 13394^T; 3, *S. marina* DSM 17023^T; 4, *S. alba* DSM 18320^T; 5, *S. stellulata* DSM 5886^T; 6, *P. phragmitetus* DSM 14782^T; 7, *Rib. denhamense* JCM 10543^T; 8, *Rib. hamelinense* JCM 10544^T. AL, amino-lipid; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PMME, phosphatidylmonomethylethanolamine; SQDG, sulphoquinovosyldiacylglyceride; ?, unidentified compound.

that they may be transferred horizontally to related species (Pradella *et al.*, 2004). The taxonomic significance of their presence should therefore be viewed in perspective with other traits. Additional studies of further strains and species may support or refute our current taxonomic hypothesis.

Respiratory lipoquinones, polar lipids and fatty acids

All strains produced ubiquinones, a characteristic feature of the majority of the *Alpha*-, *Beta*- and *Gammaproteobacteria*. The predominance of ubiquinone 10 as the single respiratory lipoquinone is a feature of the majority of the members of the *Alphaproteobacteria*.

Thin layer chromatograms of extracts from cells of strain DFL-11^T showed the presence of phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PMME), sulphoquinovosyldiacylglyceride (SQDG) and an unidentified aminolipid (AL) (Fig. 5). A very similar pattern was found for the two *Roseibium* species, *S. aggregata*, *S. marina* and *S. alba*. In some of the strains, the presence of PE could not be unambiguously distinguished from possible slight tailing effects from the lipid which runs above it, PMME. PMME is known to arise as a result of methylation of PE, indicating that this latter lipid must be synthesized, even if it could not be unambiguously detected. Furthermore, it is not known whether growth conditions/growth phase may also affect the relative composition of PE and PMME. *P. phragmitetus* and *S. stellulata* could clearly be differentiated from the other strains studied since SQDG was not present, providing support for their classification as members of separate genera. The presence of the phospholipids PG, DPG, PC, PE and PMME is a feature typical of certain subgroups within the *Alphaproteobacteria*. The taxonomic significance of the polar lipid pattern, including the presence/absence of lipids (e.g. SQDG or additional amino lipids), must be interpreted together with the fatty acid patterns.

The fatty acid content of strain DFL-11^T was compared with that of *S. stellulata*, *S. aggregata*, *S. alba*, *S. marina*,

P. phragmitetus and the two *Roseibium* species (Table 2 and see Supplementary Table S1 in IJSEM Online). In all species tested, fatty acid 18:1 ω 7c was the main component (49–60 %), which is true for virtually all members of the *Alphaproteobacteria*, a fact usually missed in the majority of species descriptions relating to members of this major evolutionary group. All strains contained 3-OH 14:0, an ester-linked fatty acid, probably located in the lipopolysaccharide (and not derived from the polar lipid fraction). This fatty acid has also been reported in members of the *Agrobacterium/Rhizobium/Ensifer* (formerly the genus *Sinorhizobium*)/*Mesorhizobium* group (Tighe *et al.*, 2000; Quan *et al.*, 2005). Additional 3-OH fatty acids included 3-OH 16:0, 3-OH 18:0, 3-OH 18:1 and 3-OH 20:0, all of which appeared to be amide-linked. The distribution of these 3-OH fatty acids was such that 3-OH 16:0 appeared to be present in strain DFL-11^T, *S. stellulata* and *P. phragmitetus*. Strain DFL-11^T was the only strain in which the 3-OH 16:0 fatty acid appeared to be ester-linked, like the 3-OH 14:0. Fatty acid 3-OH 18:0 was present in all strains examined (albeit in trace amounts in strain DFL-11^T). Fatty acid 3-OH 18:1 was present in *S. stellulata* and *P. phragmitetus* (trace amounts). All strains contained 3-OH derivatives of a C-20 fatty acid. In most cases both the 3-OH 20:0 and 3-OH 20:1 derivatives were present, the exceptions being *S. stellulata* (only 3-OH 20:0) and *P. phragmitetus* (only 3-OH 20:1). In addition, the majority of strains contained a 20:1 ω 7c fatty acid, although the level in *P. phragmitetus* was very low and it was below the level of detection in *S. stellulata*. It is interesting to note that 20:1 ω 7c has also been reported in members of the genus *Nesiotobacter* (Donachie *et al.*, 2006), but it is apparently absent in members of the genus *Pseudovibrio* (Fukunaga *et al.*, 2006). These results, taken together with the polar lipid patterns, provided unambiguous evidence for the chemical heterogeneity within the genus *Stappia* as currently defined and indicated that chemotaxonomy is a valuable parameter in delineating taxa within this group of organisms.

New compound

The unsaturated branched long-chain fatty acid 11-methyl 18:1 ω 6t has been previously identified in bacteria (Kerger

Table 2. Cellular fatty acids of strain DFL-11^T and related species

Taxa: 1, strain DFL-11^T; 2, *S. aggregata* DSM 13394^T; 3, *S. marina* DSM 17023^T; 4, *S. alba* DSM 18320^T; 5, *S. stellulata* DSM 5886^T; 6, *P. phragmitetus* DSM 14782^T; 7, *Rib. denhamense* JCM 10543^T; 8, *Rib. hamelinense* JCM 10544^T. M1 shows ester-linked fatty acids, while M2 shows ester- and amide-linked fatty acids. Values given in bold type indicate taxonomically important fatty acids. Values are percentages of all recorded fatty acids.

Fatty acid	1		2		3		4		5		6		7		8	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
Unidentified 1	1.0	0.7	0.6	0.6	0.8	0.7	0.7	0.8	1.2	0.6	1.0	0.6	0.9	1.1	1.0	0.7
Unidentified 2	1.0	0.5	0.8	0.5	0.5	0.5	0.5	0.4	1.0	0.6	1.5	0.7	0.9		0.7	0.4
Unidentified 3		0.3		0.3			0.3	0.3				0.3			0.5	0.3
3-OH 14:0	3.4	2.1	2.4	2.2	2.4	2.2	2.3	2.4	4.5	2.7	3.8	2.6	3.9	2.0	3.8	2.3
16:1 ω 7c			0.6	0.3	5.7	5.5			0.8	0.5		0.3				
16:0		0.3	0.6	0.7	1.2	1.2	0.4	0.3	8.1	6.6	5.1	4.5	0.7	0.5	0.5	0.5
3-OH 16:0	1.5	1.2								0.6		1.1				
Unidentified 4									0.9	0.7						
18:1 ω 9c	1.0	1.0														
18:1ω7c	71.0	66.0	76.6	63.0	40.7	38.9	64.0	62.3	62.4	55.6	75.5	67.0	68.7	60.4	64.4	59.0
18:0	6.5	7.5	4.8	5.6	7.7	7.5	7.2	7.0	1.8	1.8	1.7	1.7	7.0	8.2	13.6	15.6
11-methyl 18:0ω6t	3.7	4.1	5.4	15.2	12.8	12.3	11.3	11.0	15.8	17.8	10.4	10.7	6.9	7.7	8.5	9.1
Unidentified 5		0.3	0.6	0.3			0.5	0.5		0.8		1.0			0.5	0.6
Unidentified 6									1.2	0.4						
cyclo 19:0					19.2	18.3			2.4	2.6						
3-OH 18:1										0.5		1.6				
3-OH 18:0		0.8		2.3		2.6		1.0		4.7		3.0		1.8		1.5
Unidentified 7											0.9	0.7				
20:1ω7c	9.1	10.4	5.9	4.7	5.8	5.5	8.7	8.7				0.5	7.9	10.5	5.7	6.6
Unidentified 8		0.4														
20:0		0.7		0.3			0.8	1.0		0.6			1.6	2.4		0.5
11-methyl 20:1ω6t							3.1	3.2					1.5	2.2		
Unidentified 3			1.7	1.8												
cyclo 21:0	1.7	2.0			3.1	3.0										
Unidentified 9												0.4			0.7	0.4
3-OH 20:1		0.4		1.2		1.3						1.9		0.5		1.4
3-OH 20:0		0.5		1.1		0.6		1.0		0.7				2.1		1.1
22:1		0.7								0.3				0.6		
22:0										0.8		1.3				
Unidentified 10										1.1						

et al., 1986; Couderc, 1995; Carballeira *et al.*, 1997; Rontani *et al.*, 2005). The bishomologue (*E*)-13-methyleicosa-14-enoic acid (11-methyl 20:1 ω 6t), which was present in *S. aggregata* (3%) and *Rib. denhamense* (2%), has, to the best of our knowledge, not been reported before from nature.

Taxonomic position of strain DFL-11^T and reorganization of the genus *Stappia*

The taxonomic position of strain DFL-11^T as revealed by neighbour-joining analysis of the 16S rRNA gene sequence alignment is shown in Fig. 6. High similarity was found to *S. aggregata* IAM 12614^T, *S. alba* 5OM6^T and *S. marina* mano 18^T (97.7, 98.0 and 98.0% sequence similarity, respectively) and somewhat lower similarity to *Rib. denhamense* Och 254^T (96.1%) and *Rib. hamelinense* Och 368^T (97.1%). A lower degree of relatedness was exhibited with

the non-bchl-containing species from a soda lake, *P. phragmitetus* (Borsodi *et al.*, 2003) (95.0% similarity) and still less with *S. stellulata* (94.3%). Strain DFL-11^T is distinct from the recently described marine genera *Nesiotobacter* and *Pseudovibrio* (Shieh *et al.*, 2004; Donachie *et al.*, 2006). The adjacent α -2 subgroup of *Proteobacteria* around the genus *Mesorhizobium* was clearly separated (about 90% similarity). The 16S rRNA gene sequence data clearly support the separation of strain DFL-11^T, *S. alba*, *S. marina* and *S. aggregata* from *S. stellulata*, the type species of the genus *Stappia*.

The morphological and physiological traits of strain DFL-11^T are compared with those of the described species of the genera *Stappia* and *Roseibium* and with the genus *Pannonibacter* in Table 1. Differences exist with respect to flagellation, polar lipid distribution, occurrence of

Fig. 6. Neighbour-joining dendrogram of 16S rRNA gene sequence relatedness showing the phylogenetic position of *Labrenzia alexandrii* sp. nov. DFL-11^T within the *Alphaproteobacteria*. Bootstrap values greater than 70% confidence are shown at branching points (percentage of 1000 replicates). GenBank accession numbers are given in parentheses. Bar, 1 substitution per 100 nucleotides.

photosynthetic pigments, reduction of nitrate, indole production, requirement for NaCl and DNA G + C content. Members of the genus *Roseibium* are characterized by peritrichous flagellation, while in all other species tested one or several flagella are inserted at the cell poles. They are also different from all other tested species in their ability to produce indole from tryptophan. The DNA G + C content of the members of the genus *Roseibium* is in the upper range for the group, i.e. 58–63 mol%, surpassed only by members of the genus *Pannonibacter* (65 mol%). In contrast, the DNA G + C content of strain DFL-11^T is the lowest among the investigated species (56 mol%). Strain DFL-11^T also differs from members of the genus *Roseibium* (and all described *Stappia* species) since it is unable to reduce nitrate. The *coxL* genes have been identified in strain DFL-11^T (G. King, personal communication) and in all *Stappia* species tested so far (King, 2003); it is not known if they are present in species of the genus *Roseibium*. Photosynthetic pigments are present in members of the genus *Roseibium* and have

been demonstrated to be present in small amounts in two of the four described *Stappia* species. In *S. marina*, the presence of only the *pufLM* genes was detected.

The chemical composition of all the strains examined indicated that many of the features present were typical of members of the class *Alphaproteobacteria* [i.e. the presence of Q10 and the dominance of 18:1 ω 7c (+11,12-cyclopropane 19:0)], whereas other features allowed finer differentiation, in particular the presence/absence of SQDG among the polar lipids as well as the distribution and nature of the linkage of the 3-OH fatty acids. In summary, one of the problems that we have encountered is the fact that, in the past, polar lipid composition has not always been taken into consideration when examining the chemotaxonomy of organisms within the *Roseibium*–*Stappia* group. In addition, analysis of the fatty acid content of the strains has not always been as comprehensive as in the studies undertaken here. In particular, the longer chain 3-OH fatty

acids have not been reported (although were probably present in the samples). In the case of the fatty acids 3-OH 20:1 and 3-OH 20:0, the standard identification system offered by MIDI does not presently include them in the peak-naming table. The relevance of the manner in which the 3-OH fatty acids are linked to their parent molecules (i.e. ester- or amide-linked) is an additional feature that allows differentiation, although it is rarely used. Further studies are needed in order to determine the nature of the parent molecule which gives rise to the amide-linked 3-OH fatty acids. The results also indicate that a thorough study of the chemotaxonomy of novel taxa, either within or closely related to this group, is essential in any future taxonomic study and this approach is in line with recommendations made by two *ad hoc* subcommittees (Wayne *et al.*, 1987; Murray *et al.*, 1990).

Based on these traits, the close relationship of strain DFL-11^T with *S. aggregata* as well as to *S. alba* and *S. marina* appears obvious. *S. stellulata* is only distantly related to *S. aggregata* (95.3 % gene sequence similarity), justifying the placement of the former group in a separate genus. *S. stellulata* is the type species (Stapp & Knösel, 1954) which must be retained in the genus and the circumscription emended, while the species *S. aggregata*, *S. alba*, *S. marina* and strain DFL-11^T do not fit within that emended circumscription of the genus *Stappia* and must be placed in a different genus, for which the name *Labrenzia* gen. nov. is proposed. Accordingly strain DFL-11^T represents a novel species of this genus, to be designated *Labrenzia alexandrii* sp. nov., and also serves as the type species.

The description of *S. stellulata* must be extended with respect to the presence of the *pufLM* and *coxL* genes, the ability to produce small amounts of bchl *a*, the lack of SQDG and the fatty acid composition. Members of the new genus *Labrenzia* are differentiated from those of the genus *Roseibium* by their flagellation, slightly lower DNA G+C content, the presence of *coxL* genes, their requirement for NaCl and their lack of indole production, as well as in the details of their chemotaxonomy. Bchl *a* may be present in small amounts. The description of the genus *Pannonibacter* must also be emended in order to cater for the presence of PC (not phosphatidylserine) and to incorporate details of the fatty acid composition.

We do not consider the presence or absence of trace amounts of bacteriochlorophyll or the presence/absence of the *pufLM* genes to be a primary taxonomic marker at the genus level in the organisms under study here, although we do not dispute their potential role in the biology of the organisms concerned.

Emended description of the genus *Pannonibacter* Borsodi *et al.* 2003

In addition to the criteria given by Borsodi *et al.* (2003), the genus circumscription is emended as follows. The polar lipid composition comprises PG, DPG, PMME, PC, an amino

lipid and an unidentified lipid running close to the amino lipid and PMME. PE is not detected, but it is a precursor of PMME. Neither phosphatidylserine nor the glycolipid SQDG are present. The fatty acids comprise 16:1 ω 7c, 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 20:1 ω 7c, 22:0 and the hydroxy fatty acids 3-OH 14:0 (ester-linked), 3-OH 16:0, 3-OH 18:1, 3-OH 18:0 and 3-OH 20:1, all of which are amide-linked. The type species of the genus is *Pannonibacter phragmitetus* with the type strain C6/19^T (= DSM 14782^T = NCAIM B02025^T).

Emended description of the genus *Stappia* Uchino *et al.* 1999

The description of the genus *Stappia* is as given by Uchino *et al.* (1998) with the following additions. The polar lipid composition comprises PG, DPG, PE, PMME, PC and amino lipid. The glycolipid SQDG is absent. The fatty acids comprise 16:1 ω 7c, 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 20:1 ω 7c, 22:0, 22:1 and the hydroxy fatty acids 3-OH 14:0 (ester-linked), 3-OH 16:0, 3-OH 18:1, 3-OH 18:0 and 3-OH 20:0, all of which are amide-linked. The fatty acid 3-OH 18:0 predominates over 3-OH 18:1. *Stappia stellulata* is the type species of the genus.

Emended description of *Stappia stellulata* (Rüger and Höfle 1992) Uchino *et al.* 1999

Has the following characteristics in addition to those described for the genus. The *pufLM* genes of the photosynthesis reaction centre and the *coxL* genes for oxidation of carbon monoxide are present. Acetone extracts of dark-grown cells show a small peak at 772 nm, indicating the presence of bchl *a*. The type strain is ATCC 15215^T (= DSM 5886^T = CIP 105977^T = NBRC 15764^T).

Emended description of the genus *Roseibium* Suzuki *et al.* 2000

In addition to the criteria given by Suzuki *et al.* (2000), the genus circumscription is emended as follows. The polar lipid composition comprises PG, DPG, PE, PMME, PC, the glycolipid SQDG and amino lipid. The fatty acids comprise 16:0, 18:1 ω 7c, 18:0, 20:1 ω 7c and the hydroxy fatty acids 3-OH 14:0 (ester-linked), 3-OH 18:0, 3-OH 20:0 and 3-OH 20:1, all of which are amide-linked. The level of 20:1 ω 7c is usually in the range 7–11 %. The fatty acid 11-methyl 18:1 ω 6t is produced by all species of the genus and 11-methyl 20:1 ω 6t is produced by some strains. The genus currently comprises *Roseibium denhamense* and *Roseibium hamelinense*, the former being the type species.

Emended description of *Roseibium denhamense* Suzuki *et al.* 2000

The description is the same as that given by Suzuki *et al.* (2000) with the addition that the polar lipid composition is consistent with that of the emended genus circumscription. The fatty acids comprise 16:1 ω 7c, 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 11-methyl 20:1 ω 6t, cyclopropane 19 ω 7,

20:1 ω 7c, 11-methyl 20:1 ω 6t, 22:1 and the hydroxy fatty acids 3-OH 14:0 (ester-linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide-linked. The type strain is OCh 254^T (=ATCC BAA-251^T=CIP 107047^T=JCM 10543^T=NBRC 16782^T).

Emended description of *Roseibium hamelinense* Suzuki *et al.* 2000

The description is the same as that given by Suzuki *et al.* (2000) with the addition that the polar lipid composition is consistent with that of the emended genus circumscription. The fatty acids comprise 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 11-methyl 20:1 ω 6t, 20:1 ω 7c and the hydroxy fatty acids 3-OH 14:0 (ester-linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide-linked. The type strain is OCh 368^T (ATCC BAA-252^T=CIP 107048^T=JCM 10544^T=NBRC 16783^T).

Description of *Labrenzia* gen. nov.

Labrenzia (Lab.ren'zi.a. N.L. fem. n. *Labrenzia* from the name Labrenz, honouring Dr Matthias Labrenz, a German marine microbiologist who described many interesting bacterial isolates from hypersaline Ekho Lake, Antarctica, including three new genera of aerobic anoxygenic phototrophs, using a polyphasic approach).

Cells are Gram-negative rods. Motile by means of one or several polarly inserted flagella. Colonies are white to cream, but may become pink if incubated in the dark under appropriate conditions. Ability to produce bchl *a* in small amounts may be present. NaCl is required for growth. Optimum salinity range is 1–10 %. Optimum pH is 7.0–8.5. May be able to reduce nitrate to nitrite or to N₂. Growth is chemoheterotrophic and non-fermentative under aerobic or anaerobic conditions. Indole is not produced. The major respiratory lipoquinone is Q10. The polar lipid composition comprises PG, DPG, PE, PMME, PC, the glycolipid SQDG and an amino lipid. The fatty acids comprise 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 20:1 ω 7c and the hydroxy fatty acids 3-OH 14:0, 3-OH 18:0 and 3-OH 20:0, all of which are amide-linked. The level of fatty acid 20:1 ω 7c is usually in the range 5–10 %. DNA G+C content is 56–60 mol%. The type species is *Labrenzia alexandrii*.

Description of *Labrenzia alexandrii* sp. nov.

Labrenzia alexandrii (a.le.xan'dri.i. N.L. gen. n. *alexandrii* of *Alexandrium*, the genus name of the dinoflagellate *Alexandrium lusitanicum*, the source of isolation of the type strain).

Displays the following characteristics in addition to those given in the genus description. Gram-negative rods of 0.5–0.7 $\times$ 0.9–3.0 μ m, motile by means of a single, subpolarly inserted flagellum. Cell ends often unequal. Star-shaped aggregates occur. Colonies on Marine Agar 2216 (Difco) are beige to slightly pink, almost transparent, smooth and with

entire margin. Strictly aerobic, non-fermentative heterotroph. Growth occurs within a salinity range of 1 to 10 %, a temperature range of 15 to 34 °C (optimum 26 °C) and a pH between 6.0 and 9.2 (optimum 7.0 to 8.5). The type strain uses acetate, butyrate, succinate, fumarate, malate, citrate, glutamate, pyruvate, glucose and fructose, but not methanol, ethanol or glycerol. Biotin and thiamine are required as growth factors. Gelatin is hydrolysed, but not starch, alginate or Tween 80. Nitrate is not reduced. Indole is not produced from tryptophan. Dark-grown cells contain small amounts of bchl *a* and a carotenoid. Cells are weakly resistant to potassium tellurite. The chemical composition of the cells is consistent with that of the genus circumscription. In addition, cells contain 3-OH 16:0 (ester-linked) as well as the following fatty acids: 16:0, 18:1 ω 9c, 20:0, cyclo 21:0, 3-OH 20:1 and 22:1. The DNA G+C content of the type strain is 56.1 %.

The type strain, DFL-11^T (=DSM 17067^T=NCIMB 14079^T), was isolated from cultured cells of the marine dinoflagellate *Alexandrium lusitanicum*.

Description of *Labrenzia aggregata* comb. nov.

Basonym: *Stappia aggregata* (ex Ahrens 1968) Uchino *et al.* 1999.

The description is the same as that for *Stappia aggregata* (Uchino *et al.*, 1998) with the addition that the polar lipid composition is consistent with that of the genus circumscription. The fatty acids comprise 16:1 ω 7c, 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 20:1 ω 7c, 20:0 (trace amounts) and the hydroxy fatty acids 3-OH 14:0 (ester-linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide-linked. The level of fatty acid 20:1 ω 7c is usually in the range 5–6 %.

The type strain is B1^T (=ATCC 25650^T=IAM 12614^T=DSM 13394^T=NBRC 16684^T=LMG 122^T=NCIMB 2208^T).

Description of *Labrenzia marina* comb. nov.

Basonym: *Stappia marina* Kim *et al.* 2006.

The description is the same as that given by Kim *et al.* (2006) with the addition that the polar lipid composition is consistent with that of the genus circumscription. The fatty acids comprise 16:1 ω 7c, 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 11-methyl 20:1 ω 6t, cyclopropane 19 ω 7, 20:1 ω 7c, 11-methyl 20:1 ω 6t and the hydroxy fatty acids 3-OH 14:0 (ester-linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide-linked.

The type strain is mano 18^T (=DSM 17023^T=KCTC 12288^T).

Description of *Labrenzia alba* comb. nov.

Basonym: *Stappia alba* Pujalte *et al.* 2006.

The description is the same as that given by Pujalte *et al.* (2005), with the additions that the respiratory lipoquinone and polar lipid compositions are consistent with that of the genus circumscription. The fatty acids comprise 16:1 ω 7c, 16:0, 18:1 ω 7c, 18:0, 11-methyl 18:1 ω 6t, 11-methyl 20:1 ω 6t, 20:1 ω 7c, 20:0, 11-methyl 20:1 ω 6t and the hydroxy fatty acids, 3-OH 14:0 (ester-linked), 3-OH 18:0 and 3-OH 20:0, all of which are amide-linked.

The type strain is 5OM6^T (= DSM 18380^T = CECT 5095^T = CIP 108402^T).

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REFERENCES

- Allgaier, M., Uphoff, H., Felske, A. & Wagner-Döbler, I. (2003). Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microbiol* **69**, 5051–5059.
- Biebl, H. & Wagner-Döbler, I. (2006). Growth and bacteriochlorophyll *a* formation in taxonomically diverse aerobic anoxygenic phototrophic bacteria in chemostat culture: influence of light regimen and starvation. *Process Biochem* **41**, 2153–2159.
- Biebl, H., Allgaier, M., Tindall, B. J., Koblizek, M., Lünsdorf, H., Pukall, R. & Wagner-Döbler, I. (2005). *Dinoroseobacter shibae* gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. *Int J Syst Evol Microbiol* **55**, 1089–1096.
- Biebl, H., Tindall, B. J., Pukall, R., Lünsdorf, H., Allgaier, M. & Wagner-Döbler, I. (2006). *Hoeflea phototrophica* sp. nov., a new marine aerobic Alphaproteobacterium that forms bacteriochlorophyll *a*. *Int J Syst Evol Microbiol* **56**, 821–826.
- Borsodi, A. K., Micsinai, A., Kovács, G., Tóth, E., Schumann, P., Kovács, A. L., Böddi, B. & Márialigeti, K. (2003). *Pannonibacter phragmitetus* gen. nov., sp. nov., a novel alkali-tolerant bacterium isolated from decomposing reed rhizomes in a Hungarian soda lake. *Int J Syst Evol Microbiol* **53**, 555–561.
- Carballeira, N. M., Sostre, A., Stefanov, K., Popov, S., Kujumgiev, A., Dimitrova-Konaklieva, S., Tosteson, C. G. & Tosteson, T. R. (1997). The fatty acid composition of a *Vibrio alginolyticus* associated with the alga *Cladophora coelothrix*. Identification of the novel 9-methyl-10-hexadecenoic acid. *Lipids* **32**, 1271–1275.
- Carballeira, N. M., Emiliano, A., Sostre, A., Restituyo, J. A., González, I. M., Colón, G. M., Tosteson, C. G. & Tosteson, T. R. (1998). Fatty acid composition of bacteria associated with the toxic dinoflagellate *Ostreopsis lenticularis* and with Caribbean *Palythoa* species. *Lipids* **33**, 627–632.
- Clayton, R. K. (1963). Absorption spectra of photosynthetic bacteria and their chlorophylls. In *Bacterial Photosynthesis*, pp. 495–500. Edited by H. Gest, A. San Pietro & L. P. Vernon. Yellow Springs: Antioch Press.
- Couderc, F. (1995). Gas chromatography/tandem mass spectrometry as an analytical tool for the identification of fatty acids. *Lipids* **30**, 691–699.
- Donachie, S. P., Bowman, J. P. & Alam, M. (2006). *Nesiotobacter exalbescens* gen. nov., sp. nov., a moderately thermophilic Alphaproteobacterium from an Hawaiian hypersaline lake. *Int J Syst Evol Microbiol* **56**, 563–567.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Francke, W., Schulz, S., Sinnwell, V., König, W. A. & Roisin, Y. (1989). Epoxytetrahydroedulan, a new terpenoid from the hairpencils of *Euploea* (Lep. Danainae) butterflies. *Liebigs Ann Chem* **12**, 1195–1201.
- Fukunaga, Y., Kurahashi, M., Tanaka, K., Yanagi, K., Yokota, A. & Harayama, S. (2006). *Pseudovibrio ascidiaceicola* sp. nov., isolated from ascidians (sea squirts). *Int J Syst Evol Microbiol* **56**, 343–347.
- Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. B. (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.
- Golyshina, O. V., Pivovarova, T. A., Karavaiko, G. I., Kondrateva, T. F., Moore, E. R. B., Abraham, W.-R., Lünsdorf, H., Timmis, K. N., Yakimov, M. M. & Golyshin, P. N. (2000). *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the Archaea. *Int J Syst Evol Microbiol* **50**, 997–1006.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Harashima, K., Shiba, T. & Murata, N. (1989). *Aerobic Photosynthetic Bacteria*. Japan Scientific Societies Press. Berlin: Springer.
- Harvey, D. J. (1982). Picolinyl esters as derivatives for the structural determination of long chain branched and unsaturated fatty acids. *Biomed Mass Spectrom* **9**, 33–38.
- Kerger, B. D., Nichols, P. D., Antworth, C. P., Sand, W., Bock, E., Cox, J. C., Langworthy, T. A. & White, D. C. (1986). Signature fatty acids in the polar lipids of acid-producing *Thiobacillus* spp. methoxy, cyclopropyl, alpha-hydroxy-cyclopropyl and branched and normal monoenoic fatty acids. *FEMS Microbiol Ecol* **38**, 67–77.
- Kim, B.-C., Park, J. R., Bae, J.-W., Rhee, S.-K., Kim, K. H., Oh, J.-W. & Park, Y.-H. (2006). *Stappia marina* sp. nov., a marine bacterium isolated from the Yellow Sea. *Int J Syst Evol Microbiol* **56**, 75–79.
- King, G. M. (2003). Molecular and culture-based analyses of aerobic carbon monoxide oxidizer diversity. *Appl Environ Microbiol* **69**, 7257–7265.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Schumann, P. & Hirsch, P. (1999). *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. *Int J Syst Bacteriol* **49**, 137–147.
- Maidak, B. L., Cole, J. R., Lilburn, T. G., Parker, C. T., Jr, Saxman, P. R., Farris, R. J., Garrity, G. M., Olsen, G. J., Schmidt, T. M. & Tiedje, J. M. (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**, 173–174.
- 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.
- Murray, R. G. E., Brenner, D. J., Colwell, R. R., De Vos, P., Goodfellow, M., Grimont, P. A. D., Pfennig, N., Stackebrandt, E. & Zavarzin, G. A. (1990). Report of the ad-hoc-committee on approaches to taxonomy within the Proteobacteria. *Int J Syst Bacteriol* **40**, 213–215.
- Nishimura, Y., Muroga, Y., Saitoh, S., Shiba, T., Takamiya, K. & Shioi, Y. (1994). DNA relatedness and chemotaxonomical feature of aerobic bacteriochlorophyll-containing bacteria isolated from the coast of Australia. *J Gen Appl Microbiol* **40**, 287–296.
- Pfennig, N. & Trüper, H. G. (1992). The family Chromatiaceae. In *The Prokaryotes*, 2nd edn, pp. 3200–3221. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin: Springer.

- Pradella, S., Allgaier, M., Hoch, C., Päucker, O., Stackebrandt, E. & Wagner-Döbler, I. (2004). Genome organization and localization of the *pufLM* genes of the photosynthesis reaction center in phylogenetically diverse marine *Alphaproteobacteria*. *Appl Environ Microbiol* **70**, 3360–3369.
- Pujalte, M. J., Macian, M. C., Arahall, D. R. & Garay, E. (2005). *Stappia alba* sp. nov., isolated from Mediterranean oysters. *Syst Appl Microbiol* **28**, 672–678.
- Quan, Z.-X., Bae, H.-S., Baek, J.-H., Chen, W.-F., Im, W.-T. & Lee, S.-T. (2005). *Rhizobium daejeonense* sp. nov., isolated from a cyanide treatment bioreactor. *Int J Syst Evol Microbiol* **55**, 2543–2549.
- Rathgeber, Ch., Beatty, J. T. & Yurkov, V. V. (2004). Aerobic phototrophic bacteria: new evidence for the diversity, ecological importance and applied potential of this previously overlooked group. *Photosynth Res* **81**, 113–128.
- Rontani, J.-F., Christodoulou, S. & Kobizek, M. (2005). GC-MS structural characterization of fatty acids from marine aerobic anoxygenic phototrophic bacteria. *Lipids* **40**, 97–108.
- Rüger, H. J. & Höfle, M. G. (1992). Marine star-shaped-aggregate-forming bacteria: *Agrobacterium atlanticum* sp. nov.; *Agrobacterium meteori* sp. nov.; *Agrobacterium ferrugineum* sp. nov., nom. rev.; *Agrobacterium gelatinovorum* sp. nov., nom. rev.; and *Agrobacterium stellulatum* sp. nov., nom. rev. *Int J Syst Bacteriol* **42**, 133–143.
- Sato, K. (1978). Bacteriochlorophyll formation by facultative methylotrophs, *Protaminobacter ruber* and *Pseudomonas* AM 1. *FEBS Lett* **85**, 207–210.
- Scribe, P., Guezennec, J., Dagaut, J., Pepe, C. & Saliot, A. (1988). Identification of the position and the stereochemistry of the double bond in monounsaturated fatty acid methyl esters by gas chromatography/mass spectrometry of dimethyl disulfide derivatives. *Anal Chem* **60**, 928–931.
- Shiba, T., Shioi, Y., Takamiya, I. K., Sutton, D. C. & Wilkinson, C. R. (1991). Distribution and physiology of aerobic bacteria containing bacteriochlorophyll *a* on the east and west coasts of Australia. *Appl Environ Microbiol* **57**, 295–300.
- Shieh, W. Y., Lin, Y. T. & Jean, W. D. (2004). *Pseudovibrio denitrificans* gen. nov., sp. nov., a marine, facultatively anaerobic, fermentative bacterium capable of denitrification. *Int J Syst Evol Microbiol* **54**, 2307–2312.
- Stapp, C. & Knösel, D. (1954). Zur Genetik sternbildender Bakterien. *Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 2* **108**, 243–259.
- Suzuki, T., Muroga, Y., Takahama, M. & Nishimura, Y. (2000). *Roseibium denhamense* gen. nov., sp. nov. and *Roseibium hamelinense* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from the east and west coasts of Australia. *Int J Syst Evol Microbiol* **50**, 2151–2156.
- Tighe, S. W., de Lajudie, P., Dipietro, K., Lindström, K., Nick, G. & Jarvis, B. D. W. (2000). Analysis of cellular fatty acids and phenotypic relationships of *Agrobacterium*, *Bradyrhizobium*, *Mesorhizobium* and *Sinorhizobium* species using the Sherlock Microbial Identification System. *Int J Syst Evol Microbiol* **50**, 787–801.
- Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Uchino, Y., Hirata, A., Yakota, A. & Sugiyama, J. (1998). Reclassification of marine *Agrobacterium* species: proposals of *Stappia stellulata* gen. nov., comb. nov., *Stappia aggregata* sp. nov., nom. rev., *Ruegeria atlantica* gen. nov., comb. nov., *Ruegeria gelatinovora* comb. nov., *Ruegeria algicola* comb. nov., and *Ahrensia kieliensis* gen. nov., sp. nov., nom. rev. *J Gen Appl Microbiol* **44**, 201–210.
- Wagner-Döbler, I. & Biebl, H. (2006). Environmental biology of the marine *Roseobacter* lineage. *Annu Rev Microbiol* **60**, 255–280.
- 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). International Committee on Systematic Bacteriology. Report of the ad hoc committee on the reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R. B., Abraham, W.-R., Lünsdorf, H. & Timmis, K. N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int J Syst Bacteriol* **48**, 339–348.
- Yurkov, V. V. & Beatty, J. T. (1998). Aerobic anoxygenic phototrophic bacteria. *Microbiol Mol Biol Rev* **62**, 695–724.
- Yurkov, V. V. & van Gernerden, H. (1993). Impact of light/dark regimen on growth rate, biomass formation and bacteriochlorophyll synthesis in *Erythromicrobium hydrolyticum*. *Arch Microbiol* **159**, 84–89.
- Yurkov, V. V., Krassilnikova, E. N. & Gorlenko, V. N. (1993). Effect of light and oxygen on the metabolism of the aerobic bacterium *Erythromicrobium sibiricum*. *Microbiology* (English translation of *Mikrobiologiya*) **62**, 35–38.
- Yurkov, V., Jappé, J. & Verméglio, A. (1996). Tellurite resistance and reduction by obligately aerobic photosynthetic bacteria. *Appl Environ Microbiol* **62**, 4195–4198.