Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte *et al.* 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay *et al.* 1995) Uchino *et al.* 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*

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A heterotrophic, antibiotic-producing bacterium, strain T5^T, was isolated from the German Wadden Sea, located in the southern region of the North Sea. Sequence analysis of the 16S rRNA gene of this strain demonstrated close affiliation with *Roseobacter gallaeciensis* BS107^T (99 % similarity), but the results of genotypic (DNA–DNA hybridization and DNA G + C content) and phenotypic characterization revealed that strain T5^T represents a novel species. The novel organism is strictly aerobic, Gram-negative, rod-shaped, motile and forms brown-pigmented colonies. Strain T5^T produces the antibiotic tropodithietic acid throughout the exponential phase which inhibits the growth of bacteria from different taxa, as well as marine algae. Strain T5^T requires sodium ions and utilizes a wide range of substrates, including oligosaccharides, sugar alcohols, organic acids and amino acids. The DNA G + C content is 55.7 mol%. Comparative 16S rRNA gene sequence analysis revealed that strains T5^T and *Roseobacter gallaeciensis* BS107^T group with *Leisingera methylohalidivorans* as their closest described relative within the *Roseobacter* clade (97.9 and 97.6 % sequence similarity, respectively) and with *Ruegeria algicola* (96.6 and 96.5 % similarity, respectively) of the *Alphaproteobacteria*. Comparison of strains T5^T and *Roseobacter gallaeciensis* BS107^T with *Roseobacter denitrificans* and *Roseobacter litoralis* showed striking differences in 16S rRNA gene sequence similarities, chemical composition, pigmentation, presence of bacteriochlorophyll *a* and antibiotic production. On the basis of these results, it is proposed that *Roseobacter gallaeciensis* is reclassified as the type species of a new genus, *Phaeobacter*, as *Phaeobacter gallaeciensis* comb. nov. (type strain BS107^T = CIP 105210^T = ATCC 700781^T = NBRC 16654^T = DSM 17395^T). Strain T5^T (= LMG 22475^T = DSM 16374^T) is proposed as the type strain of a novel species of this genus, *Phaeobacter inhibens* sp. nov. At the same time, emended descriptions are provided of the genera *Roseobacter*, *Ruegeria* and *Leisingera*, as well as reclassifying *Ruegeria algicola* as the type species of a new genus, *Marinovum*, with the name *Marinovum algicola* comb. nov.

Abbreviations: ASW, artificial seawater; Bchl, bacteriochlorophyll; DMS, dimethylsulphate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Phaeobacter inhibens* T5^T is AY177712

INTRODUCTION

The so-called *Roseobacter* clade (Giovannoni & Rappé, 2000) within the *Alphaproteobacteria* includes cultivated and uncultivated organisms found in marine habitats all over the world. Characterization of isolates has demonstrated that the organisms in this group have very diverse metabolisms, including the production of secondary metabolites (Gram *et al.*, 2002; Lafay *et al.*, 1995). Some species of this cluster are even capable of producing antibiotic compounds (Ruiz-Ponte *et al.*, 1999; Brinkhoff *et al.*, 2004; Wagner-Döbler *et al.*, 2004). Recently, it was demonstrated that an isolate from the German Wadden Sea, strain T5^T, and *Roseobacter gallaeciensis* BS107^T were able to produce a new tropolone derivative, tropodithietic acid, which exhibits strong antibiotic properties against marine bacteria of various taxa and marine algae (Brinkhoff *et al.*, 2004). Sequence similarity of the 16S rRNA gene and physiological characteristics of strain T5^T revealed close affiliation with *Rsb. gallaeciensis* BS107^T. On the basis of 16S rRNA gene sequence, these two organisms showed highest similarity values to *Leisingera methylohalidivorans* and *Ruegeria algicola*. However, comparison of strain T5^T and *Rsb. gallaeciensis* BS107^T with *Roseobacter denitrificans* and *Roseobacter litoralis* showed striking differences in genetic similarity, pigmentation, presence of bacteriochlorophyll (Bchl) *a* and production of antibiotics, as well as clear differences in chemical composition. In addition, the type species of the genera *Roseobacter* and *Ruegeria* did not group with these four organisms (T5^T, *Rsb. gallaeciensis* BS107^T, *L. methylohalidivorans* and *Ruegeria algicola*) based on 16S rRNA gene sequence comparisons and there were differences in their chemical composition and physiology. This led us to examine the way in which the genera *Roseobacter* and *Ruegeria* have been defined and to consider the consequences of our studies for the taxonomy of members of the *Roseobacter* clade in general. We have also examined the taxonomic significance of the production of Bchl *a* in members of this group. The inclusion of species which do not produce Bchl *a* in genera with species that do produce Bchl *a* and carry out aerobic photosynthesis simply on the basis of a relatively high degree of 16S rRNA gene sequence similarity has been criticized by Yurkov & Beatty (1998) and Rathgeber *et al.* (2004), who suggest this overemphasizes the significance of 16S rRNA gene sequence analysis.

On the basis of our results, we propose the reclassification of *Rsb. gallaeciensis* as the type species of a novel genus, *Phaeobacter*, and describe strain T5^T as the type strain of a novel species of this genus. In addition, it is necessary to reclassify *Ruegeria algicola* as the type species of a new genus as *Marinovum algicola* gen. nov., comb. nov., and to provide emended descriptions for the genera *Roseobacter*, *Ruegeria* and *Leisingera*.

METHODS

Source of bacteria. Strain T5^T was isolated from a 10⁰ dilution of a most-probable-number series using marine broth 2216 (MB 2216;

Difco) inoculated with surface water from a tidal mud flat (53° 42' 20" N 07° 43' 11" E) of the German Wadden Sea (Brinkhoff *et al.*, 2004). *Rsb. gallaeciensis* BS107^T was obtained from the Collection de l'Institut Pasteur, Paris, France. *Rsb. denitrificans* DSM 7001^T, *Rsb. litoralis* DSM 6996^T, *Ruegeria algicola* DSM 10251^T, *Ruegeria atlantica* DSM 5823^T and *L. methylohalidivorans* DSM 14336^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. All strains were cultured as recommended by the supplier.

Growth experiments. Strain T5^T was subcultured in MB 2216 or in artificial seawater (ASW) consisting of: 372 mM NaCl, 49.1 mM MgCl₂, 25.5 mM Na₂SO₄, 9.3 mM CaCl₂, 8.5 mM KCl, 3.2 mM NaHCO₃, 672 µM KBr, 360 µM H₃BO₄, 213 µM SrCl₂, 57 µM NaF, 32 µM Na₂SiO₃, 20 µM (NH₄)NO₃, 0.4 mM ferric citrate, 6 mM NH₄Cl and 0.263 mM K₂HPO₄. ASW medium was supplemented with different carbon sources as described below. Growth was determined by an increase in OD₆₅₀ as measured with a spectrophotometer (DU 520; Beckman). Unless otherwise stated, assays were carried out using cells grown in MB 2216.

Temperature range for growth was determined between 4 and 45 °C at intervals of 5 °C, while the optimum was defined in 2 °C increments between 25 and 35 °C. After plotting the growth curve for each temperature, the maximum growth rate ($\mu_{\max}$) was calculated. The pH range (4–10.5) was tested in increments of 0.5, adjusted with sterile NaOH and HCl solutions (both 1 M). The optimum pH value was determined by growth experiments in a temperature- and pH-controlled, 1.5 l batch fermenter system (FairMenTec) with filter-sterilized medium. Oxygen concentration and pH value were monitored by oxygen and pH electrodes (Mettler Toledo), respectively. In determining optimum pH, the pH was adjusted to 6.0, 7.0, 7.5, 8.0 and 8.5 by automatic titration with NaOH (1 M) or H₂SO₄ (1 M). Each experiment was incubated at 30 °C. After plotting the growth curve, $\mu_{\max}$ was calculated for each pH value.

Experiments to determine optimum salinity were carried out in 250 ml shake flasks at 180 r.p.m. and 25 °C with a culture volume of 50 ml. Composition of the medium was (l⁻¹): 5 g tryptone, 2.5 g yeast extract, 0.05 g K₂HPO₄, 0.034 g SrCl₂ and 1 ml trace element stock solution. The trace element stock solution consisted of (l⁻¹): 80 g KBr, 22 g H₃BO₃, 4 g Na₂SiO₃, 2.4 g NaF and 1.6 g (NH₄)NO₃. For the determination of optimum salinity, a concentrated stock solution (l⁻¹, 72 g NaCl, 2.1 g KCl, 15.5 g MgCl₂, 3.4 g CaCl₂, 12 g Na₂SO₄, 0.9 g NaHCO₃) was used to prepare concentrations of 0, 12.5, 25, 37.5, 50 and 65 g salts solution l⁻¹. Since higher concentrations of some components in the mineral salt stock solution did not dissolve easily, the range of osmotolerance was studied with NaCl only. To determine the salinity range in which strain T5^T was able to grow, a concentrated NaCl solution was added to a minimal medium consisting of 26 mM glucose, 6 mM NH₄Cl, 2.5 mM MgCl₂, 0.263 mM K₂HPO₄ and 1 ml trace element solution SL10 l⁻¹ (Tschech & Pfennig, 1984) to final concentrations of 0.01, 0.05, 0.1, 0.25, 0.37, 0.5, 0.7, 1.0, 1.5 and 2.0 M NaCl. The requirement for Na⁺ was studied in ASW supplemented with 26 mM glucose where Na⁺ was replaced with K⁺.

Cytochrome oxidase and catalase reaction assays were carried out according to the methods described by Smibert & Krieg (1994). Exoenzyme activities (hydrolysis of gelatin, starch and Tween 80) were analysed with MB 2216 solidified with 4% (w/v) gelatin or 1.5% (w/v) agarose and supplemented with 0.2% (w/v) starch and 1% (v/v) Tween 80, respectively, as described previously (Smibert & Krieg, 1994). Reduction of nitrate was tested according to Smibert & Krieg (1994) in ASW supplemented with 26 mM glucose and a mixture of 20 mM lactate and 5 mM glucose. The experiments were analysed after 10 weeks incubation at 20 °C in a 12 h daylight rhythm with *Rsb. denitrificans* DSM 7001^T used as a positive control. Production of Bchl

a was determined by spectrophotometric analysis. Cells grown at 25 °C for 24 h in the dark or with a natural daylight rhythm were collected by centrifugation of 2 ml culture broth (7000 r.p.m. for 5 min) and resuspended in a drop of medium. Pigments were extracted in 1.5 ml ice-cold acetone–methanol solution (7:2 v/v) in the dark for 12 h. Relative absorption was determined with a spectrophotometer (DU 520; Beckman) in the range 400–900 nm. *Rsb. denitrificans* DSM 7001^T was used as a positive control.

To determine the substrate spectrum of strain T5^T, 30 different carbon sources were tested in test tubes. ASW was supplemented with the vitamins nicotinic acid, thiamine, pantothenate, pyridoxal hydrochloride, cyanocobalamin, riboflavin and biotin (all at 0.05 mg l⁻¹). Growth experiments with ASW supplemented with 26 mM glucose showed no growth limitation without the addition of vitamins, but slightly enhanced biomass production took place if all vitamins were supplied. The following carbon sources were added to the medium to a final concentration of 1 g l⁻¹: (+)-D-xylose, (+)-D-glucose, (+)-D-mannose, (+)-D-galactose, (-)-D-fructose, (+)-L-arabinose, (-)-D-ribose, (+)-L-rhamnose, (-)-L-fucose, (-)-D-mannitol, (-)-D-sorbitol, (-)-D-glucosamine, sucrose, maltose, cellobiose, trehalose, lactose, sodium formate, sodium acetate, sodium pyruvate, sodium malate, sodium citrate, disodium succinate, sodium lactate, sodium propionate, starch, inulin, xylan, glycerol and Tween 80. The polysaccharides starch, inulin and xylan were dissolved at 80 °C; only xylan was not completely dissolved and the saturated solution was used. All media were adjusted to pH 7.6 and filter-sterilized (pore size 0.2 µm; Sartorius). Three replicates of 7 ml medium were inoculated with 15 µl cells (washed twice with ASW) from an exponentially growing culture of strain T5^T incubated with MB 2216. Medium without any carbon source was used as a control. Growth was determined by measuring the OD₆₅₀ after incubation at 20 °C and shaking at 100 r.p.m. for 14 days. These experiments were repeated for *L. methylohalidivorans* DSM 14336^T with 11 carbon sources as listed in Table 1.

Utilization of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine (final concentration 1 mM) by strain T5^T was tested in MB 2216 without organic carbon sources at 20 °C for 1 week. Growth was inspected daily and determined by an increase in OD₆₅₀.

To determine the differences in sole carbon usage between strain T5^T, *Rsb. gallaeciensis* BS107^T and the methylotrophic *L. methylohalidivorans* DSM 14336^T, growth of these organisms on selected methylated substrates (all except butyrate) was tested in ASW, supplemented with the vitamin solution described above. Butyrate (1 mM), betaine (1 mM), methionine (10 mM), dimethylsulphate (DMS, 0.05 mM), methyl iodide (0.13 mM) and methyl chloride (0.37 mM) were added to the medium in test tubes or in sealed 50 ml serum flasks with an air–liquid ratio of 2:1 to reduce oxygen limitation. Comparison of growth of the organisms with MB 2216 in sealed flasks or cotton-stoppered Erlenmeyer flasks did not reveal growth limitations during 5 days incubation. Methyl iodide was added to sealed culture vessels as an aqueous solution and methyl chloride was added as a gas. Growth was inspected daily by measuring OD₆₅₀ over a 1 week incubation period.

Antibiotic susceptibility of strain T5^T and *Rsb. gallaeciensis* BS107^T was tested as described previously (Brinkhoff *et al.*, 2004).

Microscopic analyses. Colony morphology was studied with a Leitz Aristoplan microscope. Motility of strain T5^T was observed by light microscopy of an exponentially growing culture and the presence of flagella was examined using the staining procedures described by Ryu (1937). The number and type of flagella were examined by transmission electron microscopy (TEM), using cells

from the early exponential phase grown in ASW and supplied with 15 mM glucose. For TEM observations, cells were negatively stained with 1% (w/v) phosphotungstic acid or studied unstained as described previously (Chavez *et al.*, 2004) to preserve the native shape and, after air drying, copper grids (200 mesh; Plano) were examined by TEM (EM 902A; Zeiss).

Antimicrobial activity. Tests for antibiotic production and detection of antimicrobial activity of *L. methylohalidivorans* DSM 14336^T, *Rsb. litoralis* DSM 6996^T and *Rsb. denitrificans* DSM 7001^T followed the method described by Brinkhoff *et al.* (2004).

Chemotaxonomic analyses. Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried cells using the method described by Tindall (1990a, b). Respiratory lipoquinones were separated into their structural classes (menaquinones, ubiquinones etc.) by TLC. Bands were eluted and further separated and identified by HPLC, using an RP₁₈ column (Tindall, 1996). Polar lipids were separated by two-dimensional chromatography and identified on the basis of their R_F values in combination with their reaction with specific staining reagents (Tindall, 1990a, b).

Analysis of fatty acid methyl esters. Fatty acid methyl esters were released from 20 mg freeze-dried cells using methodologies which release only ester-linked fatty acids (M1) or ester- and amide-linked fatty acids (M2) (see Labrenz *et al.*, 1998; Strömpl *et al.*, 1999).

16S rRNA gene sequence analysis. PCR amplification and sequencing of the nearly complete 16S rRNA gene of strain T5^T were performed according to methods described by Brinkhoff & Muyzer (1997). Phylogenetic inferences were performed with the ARB software package [http://www.arb-home.de (Ludwig *et al.*, 2004)]. Only sequences with more than 1300 bp were considered in the calculations. A phylogenetic tree was constructed using neighbour-joining and maximum-likelihood analyses. Alignment positions at which fewer than 50% of sequences of the entire set of data had the same residues were excluded from the calculations to prevent uncertain alignments.

DNA base composition. The DNA G+C content of cells of strain T5^T was determined by extracting genomic DNA according to Cashion *et al.* (1977) and subsequent determination of deoxyribonucleosides by HPLC (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984).

DNA–DNA hybridization. DNA–DNA hybridization analysis was performed using the renaturation method of De Ley *et al.* (1970) with the modifications of Huß *et al.* (1983) and Escara & Hutton (1980). Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992).

RESULTS AND DISCUSSION

Morphology

Colonies of strain T5^T on agar are smooth and convex, with regular edges that become brownish with media containing ferric citrate (e.g. MB 2216) after 24 h incubation at 20 °C. Older colonies become dark brown. Single cells are ovoid rods, 1.4–1.9 × 0.6–0.8 µm (Fig. 1). Strain T5^T was shown to have a Gram-negative cell wall structure by cell lysis after the addition of 3% (w/v) KOH (Gregersen, 1978). Spore formation was not observed. Cells are motile by means of a polar flagellum.

Table 1. Major properties that differentiate *Phaeobacter* (*Roseobacter*) *gallaeciensis* BS107^T, *Phaeobacter inhibens* sp. nov. T5^T and *Marinovum* (*Ruegeria*) *algicola* DSM 10251^T from each other and from related species

Species: 1, *Marinovum* (*Ruegeria*) *algicola* DSM 10251^T (data from Lafay *et al.*, 1995); 2, *L. methylohalidivorans* DSM 14336^T (Schaefer *et al.*, 2002); 3, *Phaeobacter inhibens* sp. nov. T5^T; 4, *Phaeobacter* (*Roseobacter*) *gallaeciensis* BS107^T (Ruiz-Ponte *et al.*, 1998); 5, *Rsb. denitrificans* DSM 7001^T (Shiba, 1991); 6, *Rsb. litoralis* DSM 6996^T (Shiba, 1991). +, Positive result or growth; −, negative result or no growth; V, variable; ND, no data available.

Characteristic	1	2	3	4	5	6
Colony colour	Beige/pinkish	No pigment	Brown	Brown	Pink	Pink
Bchl <i>a</i>	−	−†	−	−	+	+
DNA G + C content (mol%)	60*	60·5	55·7	57·6–58	59·6	56·3–58·1
DNA–DNA hybridization with <i>P. gallaeciensis</i> BS107 ^T (%)	2–4*	<1	16·3–19·6	100	2*	3*
Growth at 4 °C	−	+†	+	−	+	+
Growth at 37 °C	+	−†	−	+	−	−
Nitrate reduction	−	−	−	−	+ (light)	−
Enzyme activities:						
Amylase	(+)	+	−	−	−	−
Tweenase 80	−	−†	−	−	+	+
Gelatinase	+	−†	−	−	+	+
Substrate used:						
Arabinose	+‡	−†	−	−	+‡	V‡
Rhamnose	−/+‡	−†	−	−	+‡	+‡
Fucose	−/+‡	−†	−	ND	+‡	+‡
Cellobiose	−	−†	+	+	−§	−‡
Trehalose	+	−†	+	+	−§	ND
Glucosamine	−	−†	+	−	+‡	+‡
Acetate	−	−	+	+	+	+
Citrate	+	−	+	−	+	+
Succinate	−	−†	+	+	+ / −‡	+ / −‡
Butyrate	−	−†	+	+†	−	−
Tween 80	−‡	−†	−	−†	+‡	+‡
Glycerol	−	−	+	+	−‡	−‡
Sorbitol	−/+‡	−†	+	+	+‡	+‡
Serine	ND	−	+	−	ND	ND
Leucine	−‡	−†	+	+	−‡	−‡
Methyl iodide	ND	+	−	−†	ND	ND
Methyl chloride	ND	+	−	−†	ND	ND
DMS	ND	+	−	−†	ND	ND
Betaine	ND	+	+	−	ND	ND
Vitamin requirement:						
Thiamine	+	−	−	+	+	+
Nicotinic acid	−	−	−	−	+	+
Biotin	+	−	−	−	+	+

*Data from Ruiz-Ponte *et al.* (1998).

†Data from this study.

‡Data from Labrenz *et al.* (1999).

§Data from Lafay *et al.* (1995)

Physiological characteristics

The results of physiological tests demonstrated that strain T5^T is a marine, strictly aerobic and Na⁺-requiring bacterium, with growth occurring between 0·01 and <1·5 M NaCl and optimally between 0·51 and 0·68 M NaCl. Strain T5^T is capable of growth in a pH range of 6·0–9·5, with an

optimum of pH 7·5. Calculated growth rates were maximal between 27 and 29 °C, with $\mu_{\max}$ = 0·43 under optimum conditions during growth in a fermenter. Above 36 °C, no growth was observed.

The following carbon sources supported cell growth of strain T5^T: (+)-D-xylose, (+)-D-glucose, (+)-D-mannose,

Fig. 1. Transmission electron micrographs of cells of *Phaeobacter inhibens* sp. nov. T5^T. Unstained (a) and negatively stained cells (b) showing the typical rod-shaped cell morphology with a single polar flagellum. Bars, 2 µm (a) and 0.5 µm (b).

(+)-D-galactose, (-)-D-fructose, (-)-D-ribose, (-)-D-mannitol, (-)-D-sorbitol, (-)-D-glucosamine, sucrose, maltose, cellobiose, trehalose, sodium acetate, sodium pyruvate, sodium malate, sodium citrate, disodium succinate, sodium lactate, sodium propionate, sodium butyrate, xylan and glycerol. In addition, strain T5^T showed growth with all amino acids tested (see Methods). Further carbon sources that were tested, but not utilized, are listed in Methods.

Growth experiments with (+)-L-arabinose, (+)-L-rhamnose, (-)-L-fucose, (-)-D-sorbitol, D-glucosamine, cellobiose, trehalose, sodium butyrate, disodium succinate, Tween 80, leucine, DMS, methyl iodide, methyl chloride and betaine confirmed earlier results for *L. methylohalidivorans*, indicating that this organism appears to be an obligate methylotroph, capable of growth on only a limited number of substrates (Schaefer *et al.*, 2002). *L. methylohalidivorans* DSM 14336^T utilized all methylated substrates tested (betaine, methionine, DMS, methyl iodide and methyl chloride); however, strain T5^T and *Rsb. gallaeciensis*

BS107^T were not able to grow with DMS, methyl iodide or methyl chloride. In contrast to strain T5^T, *Rsb. gallaeciensis* BS107^T did not grow with betaine.

Strain T5^T and *Rsb. gallaeciensis* BS107^T are capable of producing the antibiotic tropodithietic acid, which inhibits growth of bacteria belonging to a number of different taxa and marine algae (Brinkhoff *et al.*, 2004). In the case of strain T5^T, it was shown that the antibiotic is produced during the exponential growth phase (Brinkhoff *et al.*, 2004). No inhibitory effects could be observed with *Ruegeria algicola*, *Ruegeria gelatinovorans*, *L. methylohalidivorans*, *Rsb. litoralis* or *Rsb. denitrificans* (Brinkhoff *et al.*, 2004; this study). Cells of strain T5^T and *Rsb. gallaeciensis* BS107^T are susceptible to penicillin G, streptomycin sulphate and chloramphenicol. The results of the phenotypic characterization are summarized in Table 1.

Genetic analyses

Analysis of the 16S rRNA gene sequences of *Rsb. gallaeciensis*, *Rsb. denitrificans* and *Rsb. litoralis* indicated that *Rsb. gallaeciensis* does not group with the other *Roseobacter* species, but instead groups with *L. methylohalidivorans* and *R. algicola* as its closest described relatives, regardless of which method of analysis is used (Fig. 2). Other features also differentiate *Rsb. gallaeciensis* from *Rsb. denitrificans* and *Rsb. litoralis*. Members of the genus *Roseobacter* were originally defined as pink-pigmented, aerobic phototrophic bacteria containing Bchl *a* (Shiba, 1991). However, *Rsb. gallaeciensis* is brown-pigmented, not phototrophic and does not contain Bchl *a*. A former member of the genus *Roseobacter*, *Roseobacter algicola* ATCC 51440^T (Lafay *et al.*, 1995), isolated from a culture of the toxin-producing dinoflagellate *Prorocentrum lima*, does not produce Bchl *a* and has since been reclassified as *Ruegeria algicola* (Uchino *et al.*, 1998). *Rsb. gallaeciensis* was placed in the genus *Roseobacter* on the basis that the 16S rRNA gene sequence of the type strain, BS107^T, clustered closest to *R. algicola* at a time when the numbers of described species and available sequences of the *Roseobacter* clade were significantly lower than at present. These findings, as well as the additional physiological differences listed in Table 1, are indicative of the need for the reclassification of *Rsb. gallaeciensis* in a novel genus.

The DNA G + C content of strain T5^T was determined to be 55.7 mol%. This value is similar to that of *Rsb. gallaeciensis* BS107^T (57.6–58 mol%) and *Rsb. litoralis* (56.3–58.1 mol%), but differs from the DNA G + C content of *L. methylohalidivorans* (60.5 mol%) and *Rsb. denitrificans* (59.6 mol%). Since a 10 mol% difference in DNA G + C content has been proposed as a clear differentiating criterion for species at the genus level (Stackebrandt & Liesack, 1993), these results demonstrate no significant distinctive features. However, it is evident that organisms which have diverged fairly recently on an evolutionary time scale will share many genes and, within these genes, codon usage will also be similar, both of these effects being reflected in the overall DNA G + C content. The effect of DNA G + C content on codon usage

Fig. 2. Neighbour-joining tree showing the phylogenetic relationships of *Phaeobacter* (*Roseobacter*) *gallaeciensis* BS107^T, *Phaeobacter inhibens* sp. nov. T5^T, *Marinovum* (*Ruegeria*) *algicola* ATCC 51440^T and representatives of the *Rhodobacteraceae* within the *Alphaproteobacteria* based on 16S rRNA gene sequence similarity. Only bootstrap values >50% (derived from 2000 replicates) are shown. Filled circles indicate nodes also recovered reproducibly with maximum-likelihood. Selected members of the *Gammaproteobacteria* were used as an out-group (not shown) to define the root of the tree. GenBank sequence accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position.

and its significance in an evolutionary framework has been outlined by De Ley (1967).

DNA–DNA hybridization studies revealed low levels of DNA–DNA relatedness, all below 5%, between *Rsb. gallaeciensis* BS107^T and *Rsb. denitrificans* DSM 7001^T, *Rsb. litoralis* DSM 6996^T, *L. methylohalidivorans* DSM 14336^T and *Ruegeria algicola* DSM 10251^T (Table 1). The hybridization results, differences in pigmentation, antibiotic production, exoenzyme activities and utilization of a great variety of substrates, though not of methyl compounds, indicate that *Rsb. gallaeciensis* BS107^T does not belong to the genus *Leisingera*, despite a 16S rRNA gene sequence similarity of 97.6% to *L. methylohalidivorans* DSM 14336^T, or to the genus *Roseobacter*. The 16S rRNA gene sequences of *Rsb. gallaeciensis* BS107^T and strain T5^T differ only at 13 positions (99% similarity). DNA–DNA hybridization analysis, however, revealed a DNA–DNA relatedness of 16.3–19.6% between *Rsb. gallaeciensis* BS107^T and strain T5^T. According to Wayne *et al.* (1987), the definition of members of the same species generally includes DNA–DNA hybridization values of more than 70%. Thus, we conclude that *Rsb. gallaeciensis* BS107^T represents a member of a novel genus, for which further support was sought via analysis of the chemical composition of the cell.

The chemical composition of all strains examined confirmed that ubiquinones were the sole respiratory lipoquinones present and that Q10 predominated. The predominance of Q10 is a feature characteristic of many, but not all, members of the *Alphaproteobacteria*. All members of the *Roseobacter* clade examined to date also have Q10 as the dominant respiratory lipoquinone. The polar lipids of all strains examined in this study consisted mainly of phospholipids, although amino lipids were present in some of the strains (Fig. 3). The polar lipid compositions of *Rsb. denitrificans* DSM 7001^T and *Rsb. litoralis* DSM 6996^T were

very similar, as were the polar lipid compositions of strain T5^T and *Rsb. gallaeciensis* BS107^T. However, there were clear differences between the two groups. The polar lipids of *Ruegeria algicola* DSM 10251^T, *Ruegeria atlantica* DSM 5823^T and *L. methylohalidivorans* DSM 14336^T were not identical to each other or to any of the other strains examined (Fig. 3). The fatty acid composition of all strains was dominated by 18:1 ω 7c, but this is also a feature of the vast majority of species within the *Alphaproteobacteria*. Despite being listed in many recent publications as being a diagnostic characteristic, the presence of this fatty acid in major amounts is neither species- nor genus-specific. The major differences/similarities between the strains were found in the fatty acids that were present in lower concentrations. In particular, distinct differences between strains were found in the distribution of the hydroxy fatty acids and the nature of their linkage (ester- and/or amide-linked). It should be noted that, although 3-oxo-14:0 has been reported to be present in members of the genus *Roseobacter* (Neumann *et al.*, 1995), the compound identified as 3-OH 14:1/3-oxo 14:0 did not co-elute with authentic 3-oxo-14:0, although mass spectrometry indicated that the compound had a molecular mass suggesting that it was a derivative of a 3-OH 14:0 containing one double bond. In the absence of more detailed studies, this compound is listed as 3-OH 14:1/3-oxo 14:0 in Table 2. *Rsb. denitrificans* and *Rsb. litoralis* were indistinguishable on a qualitative basis, as were strains T5^T and *Rsb. gallaeciensis*. Taken together, the chemical data are indicative of the fact that *Rsb. denitrificans* and *Rsb. litoralis* may be grouped together to the exclusion of other species currently assigned to the genus *Roseobacter*. Strain T5^T and *Rsb. gallaeciensis* BS107^T also formed a distinct group. The chemical evidence also provides support for the exclusion of *Ruegeria algicola* from the genus *Ruegeria*. The value of chemical data in both a taxonomic and evolutionary context has been a topic in the literature for almost the last half

Fig. 3. TLC chromatograms of the polar lipids of *Phaeobacter* (*Roseobacter*) *gallaeciensis* comb. nov. and related strains. (a) *Rsb. litoralis* DSM 6996^T; (b) *Rsb. denitrificans* DSM 7001^T; (c) *Phaeobacter* (*Roseobacter*) *gallaeciensis* BS107^T; (d) *Ruegeria atlantica* DSM 5823^T; (e) *Ruegeria algicola* DSM 10251^T; (f) *Phaeobacter inhibens* DSM 16374^T; (g) *L. methylohalidivorans* DSM 14336^T. Chromatograms were run as described in Methods, the first dimension (horizontal, from left to right) in chloroform/methanol/water (65:25:4, by vol.) and the second dimension (vertically, from top to bottom) in chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Total lipids were visualized using 5% ethanolic molybdophosphoric acid (heated for 30 min at 150 °C). Specific stain reagents were used, as described in Methods, for the detection of functional groups. PG, Phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PL1, PL2, PL3, unidentified phospholipids; AL, aminolipid; ?, lipids which did not react in a specific fashion with the spray reagents and remain to be characterized.

Table 2. Fatty acid content (%) of the strains used in this study

Strains: 1, *Marinovum* (*Ruegeria*) *algicola* DSM 10251^T; 2, *L. methylohalidivorans* DSM 14336^T; 3, *Phaeobacter* (*Roseobacter*) *gallaeciensis* CIP 105210^T; 4, *Phaeobacter inhibens* sp. nov. DSM 16374^T; 5, *Ruegeria atlantica* DSM 5823^T; 6, *Rsb. litoralis* DSM 6996^T; 7, *Rsb. denitrificans* DSM 7001^T. M1 and M2 refers to the method of differential hydrolysis (see Methods) which distinguishes between ester- and amide-linked fatty acids. Fatty acids labelled ? were not unambiguously identified. tr, Trace.

Fatty acid	1		2		3		4		5		6		7	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
3-OH 10:0		1.15	1.83	1.83	2.19	1.86	1.70	1.71		0.41	3.25	2.78	2.12	2.14
14:1			3.18	2.27	3.14	2.14	2.80	2.19						
14:0									3.95	3.84				
3-OH 12:0	1.13	1.64		2.27		1.27		1.59	5.52	8.67				
3-OH 14:1/3-oxo-14:0*		0.56				0.89		0.87		1.75		3.58		3.78
16:1 ω 7c	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr		0.36		0.53
16:0			5.16	5.08	3.96	3.79	4.02	3.83	5.94	6.25	1.51	1.11	1.58	1.56
?												0.35	0.86	0.76
?											1.81	1.50		
2-OH 16:0			8.92	6.97	5.05	3.90	4.47	3.10	10.08	10.44				
18:1 ω 9c	0.99	0.84	3.08	2.54	0.92	0.89	1.11	0.76				0.56		
18:1 ω 7c	92.07	86.74	68.43	70.27	76.08	75.51	74.92	73.77	48.21	45.53	91.84	86.44	92.74	87.22
?			2.38				1.81		2.48	2.39				
18:0	2.48	2.73	0.95	0.97	2.60	2.57	2.66	3.14	2.35	2.28	1.59	1.39	2.71	2.75
11-methyl 18:1 ω 7c	3.34	3.17	6.07	6.86	6.06	6.60	6.52	7.45	30.93	30.40		0.54		
?								0.98						
?		2.58		0.95		0.57		0.61		2.30		1.41		1.28

*This fatty acid was not unambiguously identified and is not identified in the MIDI system.

century and this work supports the point of view clearly expressed by Tindall (1994).

In examining the current data, it is appropriate to look also at the way that genera within the *Roseobacter* clade have been defined in the past so that one may be able to avoid continual nomenclatural and taxonomic rearrangements in the future. When originally described, the genus *Roseobacter* consisted of only two species. A third species was added, *Rsb. algicola*, on the basis that it was 'phylogenetically close' and that it also formed a monophyletic group with the other two species. Phenotypic differences were largely ignored in favour of a 'phylogenetic' (i.e. 16S rRNA gene sequence) approach. In conducting further arrangements within the *Roseobacter* clade, Uchino *et al.* (1998) also favoured this approach, reclassifying *Rsb. algicola* as *Ruegeria algicola* and transferring some marine 'agrobacteria' to the genus *Ruegeria* (*Ruegeria atlantica* and *Ruegeria gelatinovora*) based on the fact that they also formed a monophyletic group, despite the clear heterogeneity in the chemical data they presented. Work by Tindall and co-workers (Labrenz *et al.*, 1998, 1999, 2000, 2005; Wagner-Döbler *et al.*, 2004; Biebl *et al.*, 2005a, b) on members of the *Roseobacter* clade has followed the approach outlined here, based on a general principle that evolutionary groups are reflected in the chemical composition of the cell (Tindall, 1994) and also at other levels. This approach has indicated that using 16S rRNA gene sequence data alone for defining taxa has a number of disadvantages. An example is in taxa where the branching order is uncertain and cannot be resolved, such as in the case of members of the genera *Sulfitobacter*, *Staleyia* and *Oceanibulbus* (Labrenz *et al.*, 2000; Wagner-Döbler *et al.*, 2004). Arahall *et al.* (2005) have also noted a similar problem with 'unstable' dendrograms. In such cases, it is also impossible to define monophyletic groups based on these data alone. Where it is possible to define monophyletic groups, it is often forgotten that these are not necessarily genera and that the picture may change as more taxa are added. Given the fact that few more than 5000 prokaryotic species have yet been described and that estimates put the total number at anywhere between 50 000 and 5 million, it is important to be aware that the majority of bacterial species remain to be described (Tindall, 2004). The *Roseobacter* clade is an excellent example of exactly this effect as more taxa have been added and have altered the 'monophyletic nature' of existing genera.

Although it is generally agreed that prokaryote taxonomy is probably best served by a polyphasic approach, the concept, as outlined by Wayne *et al.* (1987), gives priority to the 16S rRNA gene sequence data in determining 'the phylogenetic position of a taxon'. That there may be problems with a taxonomy based primarily on 16S rRNA gene sequences is illustrated in members of the genus *Hyphomonas* (Badger *et al.*, 2005; Lee *et al.*, 2005). Cain & Harrison (1960) clearly indicated that taxonomic systems based on single (or few parameters) are artificial (unless one can prove that one single gene represents the evolution of the whole organism)

and that more broadly based systems give more 'natural' taxonomic systems [see Tindall (2002) for a discussion of the different meanings of 'natural']. It is also interesting to note that the principles followed here, as outlined by Tindall (1994) and put into practice by Labrenz *et al.* (1998, 1999, 2000, 2005), Biebl *et al.* (2005a, b) and Wagner-Döbler *et al.* (2004), are broadly in line with proposals made by Murray *et al.* (1990), a publication which is rarely followed or cited. A further consequence for this approach is the way that different datasets are interpreted and used in circumscribing taxa. Taxa that are difficult to distinguish by 16S rRNA gene sequence or chemotaxonomic data such as *Rsb. litoralis*/*Rsb. denitrificans* or strain T5^T/*Rsb. gallaeciensis* BS107^T can be distinguished by phenotypic (biochemical/physiological) data as well as by indirect comparisons (DNA-DNA hybridization) of their genomes. However, as chemical differences become evident and the 16S rRNA gene sequences diverge to a greater degree, phenotypic (biochemical/physiological) differences may be more difficult to interpret and genome differences also increase. Interestingly, monophyletic groups (*Rsb. litoralis*/*Rsb. denitrificans* or strain T5^T/*Rsb. gallaeciensis* BS107^T) are clearly evident when the chemical data are examined, indicating the significance of such data in helping to define and delineate evolutionary groups (Tindall, 1994). Combining 16S rRNA gene sequence data, chemical data and phenotypic data in this way also has consequences for improving the taxon specificity of gene probes and detecting 'signature sequences' (B. J. Tindall, unpublished). Furthermore, taking such an approach to the circumscription of taxonomic groups also influences the way one evaluates the significance of taxonomy/nomenclature when genomes are compared.

Taken together, the data presented here indicate that *Rsb. gallaeciensis* BS107^T and strain T5^T represent two species in a novel genus. Further consequences of this work are that the description of the genus *Roseobacter* needs to be emended, as do the descriptions of the genera *Leisingera* and *Ruegeria*. The genus *Roseobacter* as originally defined by Shiba (1991) should be emended to take into account the chemotaxonomic data and also to exclude from this genus all species with the exception of *Rsb. litoralis* and *Rsb. denitrificans*. It is also proposed that the genus *Leisingera* as originally defined by Schaefer *et al.* (2002) should be emended to take into account the chemotaxonomic data and also to delineate this genus from all other genera that show a 16S rRNA gene sequence similarity of >95%. The genus *Ruegeria* as originally defined by Uchino *et al.* (1998) should be emended to take into account the chemotaxonomic data and to exclude from this genus all species with the exception of *Ruegeria atlantica*. A direct consequence of this work, emending the description of the genus *Ruegeria* as well as restricting that genus to one species, is that *Ruegeria algicola* (Lafay *et al.*, 1995) Uchino *et al.*, 1999 must be transferred to another genus. The new genus *Marinovum* is proposed to accommodate this species and the name *Marinovum algicola* comb. nov. is proposed for the type species.

Given the fact that all of the emended or new descriptions involve the use of respiratory quinone, polar lipid and fatty acid data, we recommend, as did Murray *et al.* (1990), that future work on this group of *Proteobacteria* (and indeed others) includes all three datasets.

Description of *Phaeobacter* gen. nov.

Phaeobacter (Phae.o.bac'ter. Gr. adj. *phaeos* dark, brown; N.L. masc. n. *bacter* rod; N.L. masc. n. *Phaeobacter* a brown rod).

Cells are Gram-negative ovoid rods that multiply by binary fission. Tendency to aggregate in liquid MB 2216 medium. On marine agar 2216, colonies become brownish to dark brown and a diffusible brownish pigment is produced. The optimum salinity range is from 0.2 to 0.68 M NaCl. The optimum pH is between 7.0 and 7.5. Cells are motile by means of polar flagella. Photosynthetic growth does not occur. Bchl *a* is absent. Metabolism is chemoheterotrophic and obligately aerobic. Not able to reduce nitrate. No growth is observed with methyl iodide, methyl chloride or DMS. Oxidase- and catalase-positive, but negative for amylase, gelatinase and Tweenase. The major respiratory lipoquinone is Q10. The polar lipids comprise phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an amino-lipid and two unidentified lipids. The fatty acids comprise 3-OH 10:0, 14:1, 3-OH 12:0, 3-OH 14:1/3-oxo 14:0, 16:0, 2-OH 16:0, 18:1 ω 7c, 18:0 and 11-methyl 18:1 ω 7c. The 3-OH 10:0 and 2-OH 16:0 fatty acids were ester-linked, whereas the 3-OH 12:0 and 3-OH 14:1/3-oxo 14:0 fatty acids were amide-linked. The type species is *Phaeobacter gallaeciensis*. The genus currently comprises two species, *Phaeobacter gallaeciensis* and *Phaeobacter inhibens*.

Description of *Phaeobacter gallaeciensis* comb. nov.

Phaeobacter gallaeciensis (gal.lae.ci.en'sis. L. masc. adj. *gallaeciensis* pertaining to *Gallaecia* the roman name for Galicia, the north-west region of Spain).

Basonym: *Roseobacter gallaeciensis* Ruiz-Ponte *et al.* 1998.

The description is the same as that for *Roseobacter gallaeciensis* (Ruiz-Ponte *et al.*, 1998) with the following additions. Sensitive to penicillin G. Cells produce the antibiotic tropodithietic acid. Growth also occurs with butyrate and methionine as sole carbon sources. No growth of type strain with betaine.

The type strain is BS107^T (=ATCC 700781^T=CIP 105210^T=NBRC 16654^T=DSM 17395^T).

Description of *Phaeobacter inhibens* sp. nov.

Phaeobacter inhibens (in.hi'bens. L. part. adj. *inhibens* inhibiting).

Exhibits the following properties in addition to those given in the genus description. Cells are 1.4–1.9 μm $\times$ 0.6–0.8 μm .

Colonies on agar are smooth and convex with regular edges that become brownish/ochre on ferric-citrate-containing media after 24 h incubation at 20 °C and dark brown after incubation for 48 h, with diameter up to 0.8 mm. Tendency to aggregate in liquid MB 2216. Cells grow at temperatures ranging from 4 to 36 °C, with an optimum between 27 and 29 °C, and pH ranging from 6.0 to 9.5 (optimum pH 7.5). Cells grow in the presence of Na⁺ concentrations of 0.01 and <1.5 M, optimal salinity is between 0.51 and 0.68 M; no growth is observed without Na⁺. Oxidase- and catalase-positive, but amylase-, gelatinase- and Tweenase-negative. The following substrates are utilized: (+)-D-xylose, (+)-D-glucose, (+)-D-mannose, (+)-D-galactose, (–)-D-fructose, (–)-D-ribose, (–)-D-mannitol, (–)-D-sorbitol, (–)-D-glucosamine, sucrose, maltose, cellobiose, trehalose, sodium acetate, sodium pyruvate, sodium malate, sodium citrate, disodium succinate, sodium lactate, sodium propionate, sodium butyrate, xylan, glycerol, betaine, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. No growth is supported by the following substrates: (+)-L-arabinose, (+)-L-rhamnose, (–)-L-fucose, lactose, sodium formate, starch, inulin, Tween 80, DMS, methyl iodide or methyl chloride. No vitamin requirements observed. Cells produce the antibiotic tropodithietic acid during the exponential growth phase and are susceptible to penicillin G, streptomycin sulphate and chloramphenicol. The DNA G+C content is 55.7 mol%.

The type strain, T5^T (=DSM 16374^T=LMG 22475^T), was isolated from surface water of a tidal mud flat in the German Wadden Sea, a southern region of the North Sea.

Emended description of the genus *Roseobacter* Shiba 1991

The description of the genus *Roseobacter* is as given by Shiba (1991), with the following additions. The major respiratory lipoquinone is Q10. The polar lipids are phosphatidylglycerol, two unidentified phospholipids and an aminolipid. The fatty acids comprise 3-OH 10:0, 3-OH 14:1/3-oxo 14:0, 16:0, 16:1 ω 7c, 18:1 ω 7c and 18:0. The 3-OH 10:0 is ester-linked, while the 3-OH 14:1/3-oxo 14:0 fatty acid is amide-linked. The type species of the genus is *Roseobacter litoralis*. The genus currently comprises two species, *Roseobacter litoralis* and *Roseobacter denitrificans*.

Emended description of the genus *Leisingera* Schaefer *et al.* 2002

Leisingera (Lei.sin'ge.ra. N.L. fem. n. *Leisingera* in honour of Thomas Leisinger, on the occasion of his retirement and for his contributions to our understanding of the biochemistry of bacterial methyl halide metabolism).

The description of the genus *Leisingera* is as given by Schaefer *et al.* (2002) with the following additions. The

major respiratory lipoquinone present is Q10. The polar lipids comprise phosphatidylglycerol, phosphatidylethanolamine, an unidentified phospholipid, two unidentified lipids and an aminolipid. Phosphatidylcholine is not present. The fatty acids comprise 3-OH 10:0, 14:1, 3-OH 12:0, 16:0, 2-OH 16:0, 18:1 ω 9c, 18:1 ω 7c, 18:0 and 11-methyl 18:1 ω 7c. The 3-OH 10:0 and 2-OH 16:0 fatty acids are ester-linked, while the 3-OH 12:0 fatty acid is amide-linked. The type, and currently only, species of the genus is *Leisingera methylohalidivorans*.

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

The description of the genus *Ruegeria* is as given by Uchino *et al.* (1998), with the following additions. The major respiratory lipoquinone present is Q10. The polar lipids comprise phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, two unidentified phospholipids and two unidentified lipids. The fatty acids comprise 3-OH 10:0, 14:0, 3-OH 12:0, 3-OH 14:1/3-oxo-14:0, 16:0, 2-OH 16:0, 18:1 ω 7c, 18:0 and 11-methyl 18:1 ω 7c. The 2-OH 16:0 fatty acid is ester-linked, the 3-OH 12:0 fatty acid appears to be both ester- and amide-linked, while the 3-OH 10:0 and 3-OH 14:1/3-oxo-14:0 are amide-linked. The type, and currently only, species of the genus is *Ruegeria atlantica*.

Description of *Marinovum* gen. nov.

Marinovum (Ma.ri.no'vum. L. adj. *marinus* of or belonging to the sea, marine; L. neut. n. *ovum* an egg, an egg-shape, oval; N.L. neut. n. *Marinovum* a marine egg-shaped bacterium).

Considering that this genus currently comprises only a single species, the description of the genus and type species is identical to that given by Lafay *et al.* (1995) for *Roseobacter algicola*, with additional chemotaxonomic data. The major respiratory lipoquinone present is Q10. The polar lipids comprise phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified phospholipid, a single, unidentified lipid and an aminolipid. The fatty acids comprise 3-OH 10:0, 3-OH 12:0, 3-OH 14:1/3-oxo-14:0, 18:1 ω 7c, 18:0 and 11-methyl 18:1 ω 7c. The 3-OH 12:0 fatty acid appears to be both ester- and amide-linked, while the 3-OH 10:0 and 3-OH 14:1/3-oxo-14:0 fatty acid are amide-linked. The type, and currently, only species of the genus is *Marinovum algicola*.

Description of *Marinovum algicola* comb. nov.

Marinovum algicola (al.gi'co.la. L. n. *alga* alga; L. masc. suff. -cola from L. masc. n. *incola* an inhabitant; N.L. n. *algicola* alga dweller).

Basonym: *Roseobacter algicola* Lafay *et al.* 1995.

Other synonym: *Ruegeria algicola* Uchino *et al.* 1999.

The description of the species is as given for *Roseobacter algicola* by Lafay *et al.* (1995). The type strain is FF3^T (=ATCC 51440^T=CIP 104267^T=DSM 10251^T=IAM 14591^T=NBRC 16653^T).

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