Phylogenetic Positions of Novel Aerobic, Bacteriochlorophyll *a*-Containing Bacteria and Description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov.

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We analyzed the 16S ribosomal DNAs of three obligately aerobic, bacteriochlorophyll *a*-containing bacteria, "*Roseococcus thiosulfatophilus*," "*Erythromicrobium ramosum*," and new isolate T4^T (T = type strain), which was obtained from a marine cyanobacterial mat. "*Roseococcus thiosulfatophilus*" is a member of the α -1 subclass of the *Proteobacteria* and is moderately related to *Rhodospira globiformis*, *Thiobacillus acidophilus*, and *Acidiphilium cryptum* (level of sequence similarity, 90%). "*Erythromicrobium ramosum*" and isolate T4^T are closely related to *Erythrobacter longus* and *Porphyrobacter neustonensis* (level of sequence similarity, 95%). These organisms are members of the α -4 subclass of the *Proteobacteria*. Strain T4^T is a motile, red or orange bacterium. The major carotenoids are bacteriorubixanthinal and erythroaxanthin sulfate. In vivo measurements revealed bacteriochlorophyll absorption maxima at 377, 590, 800, and 868 nm. Strain T4^T grows in the presence of 5 to 96‰ salinity and uses glucose, fructose, acetate, pyruvate, glutamate, succinate, and lactate as substrates. On the basis of its distinct phylogenetic position and phenotypic characteristics which are different from those of *Erythrobacter longus*, we propose that strain T4^T should be placed in a new species of the genus *Erythrobacter*, *Erythrobacter litoralis*. The descriptions of "*Roseococcus thiosulfatophilus*" and "*Erythromicrobium ramosum*" are emended.

Bacteriochlorophyll (Bchl) *a*-containing, strictly aerobic bacteria were first isolated by Japanese scientists (12, 27, 29). Since then these bacteria have been isolated from a variety of habitats (7, 13, 28, 45–49, 51). The photochemical activity of the photosynthetic apparatus has been studied in *Erythrobacter* sp. strain OCh114 (*Roseobacter denitrificans*) and "*Protaminobacter ruber*" NR-1 (investigated as *Methylobacterium rhodasianum*) (11, 38) (names in quotation marks are not on the Approved Lists of Bacterial Names or validation lists and hence are without standing in nomenclature). The levels of photosynthetic units in cells of aerobic photosynthetic bacteria are consistently low. Light and high oxygen tensions suppress Bchl synthesis. Therefore, the contribution of light to energy metabolism in these predominantly chemoheterotrophic bacteria seems to be small. Under intermittent illumination the growth rate increases at the beginning of the light period at the expense of photosynthetic reactions (10, 50).

Although the obligately aerobic Bchl-containing bacteria are physiologically very different from the anaerobic photosynthetic purple bacteria belonging to the α , β , and γ subclasses of the *Proteobacteria*, the organization of the photosynthetic apparatus in members of the two groups seems to be similar. The primary structures of the Bchl-binding proteins of the reaction centers and the antenna complexes in the strains that have been analyzed are very similar to the respective structures

in anoxygenic purple bacteria (7, 17, 30, 37). Aerobic bacteria containing Bchl *b*, *c*, *d*, or *g* or different types of antenna systems or reaction centers have not been detected. The previously described aerobic Bchl *a*-containing bacteria have been associated with members of the α -subclass of the *Proteobacteria*, including marine *Erythrobacter* and *Roseobacter* species and the freshwater strains of "*Erythromicrobium*," "*Roseococcus*," and *Porphyrobacter* species, or have been classified as rhizobia or methylotrophic bacteria (5, 7, 13, 28, 39, 40, 49). Comparative studies of the sequences of photosynthetic genes and genes for rRNA may help reveal the phylogenetic relationships among the taxa of Bchl-containing bacteria and may help determine the phylogenetic root of the photosynthetic apparatus. In this paper we describe morphological, physiological, and phylogenetic data for three recently isolated strains of aerobic, Bchl *a*-containing bacteria and propose formal descriptions for these organisms.

MATERIALS AND METHODS

Bacterial strains. The following strains were investigated: strain T4^T (T = type strain), which was isolated from a marine cyanobacterial mat (51); "*Erythromicrobium ramosum*" E5^T (46, 47); and "*Roseococcus thiosulfatophilus*" RB3^T (45, 49).

Culture media. Strains RB3^T and E5^T were cultivated in Erlenmeyer flasks semiaerobically in the dark at 30°C and pH 7.6 to 7.8 in medium containing (per liter) 1.0 g of yeast extract (Difco), 1.0 g of Bacto Peptone (Difco), 1.0 g of sodium acetate, 0.3 g of KCl, 0.5 g of MgSO₄ · 7H₂O, 0.05 g of CaCl₂ · 2H₂O, 0.3 g of NH₄Cl, 0.3 g of K₂HPO₄, 20 µg of vitamin B₁₂, and 1 ml of a trace elements solution (4).

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TABLE 1. Physiological properties of *Erythrobacter litoralis* T4^T, *Roseococcus thiosulfatophilus* RB3^T, *Erythromicrobium ramosum* E5^T, and *Erythrobacter longus* OCh101^a

Characteristic	Strain T4 ^T	Strain RB3 ^T	Strain E5 ^T	Strain OCh101
Utilization of carbon sources				
Glucose	+	+	+	+
Fructose	+	—	+	NT
Acetate	+	+	+	+
Pyruvate	+	+	+	+
Glutamate	+	+	+	+
Butyrate	+	—	+	+
Citrate	—	+	+	—
Malate	—	+	+	—
Succinate	+	+	+	—
Lactate	+	+	+	—
Formate	—	—	—	—
Methanol	—	—	—	—
Ethanol	—	—	+	NT
Benzoate	—	—	—	NT
Antibiotic susceptibility				
Chloramphenicol (100 µg/disc)	+	+	+	+
Penicillin (20 U/disc)	—	—	—	+
Streptomycin (50 µg/disc)	—	+	—	—
Fusidic acid (0.5 µg/disc)	+	+	+	+
Polymyxin B (100 U/disc)	—	+	+	—
Hydrolysis of:				
Tween 80	+	—	—	+
Gelatin	—	—	—	+
Starch	—	—	—	—
G+C content of DNA (mol%)	67	70.4	64	57.4

^a —, substrate not utilized, resistant to antibiotic, or compound not hydrolyzed; +, substrate utilized, susceptible to antibiotic, or compound hydrolyzed; NT, not tested.

Strain T4^T was cultivated in the same medium containing 1.5% NaCl.

Morphological and physiological tests. Gram staining was performed by using the method of Gregersen (9). The size and shape of cells were determined by phase-contrast and electron microscopy. The motility of cells was determined by observing a 24-h culture grown in liquid medium. The utilization of organic substrates for growth was investigated under aerobic conditions in liquid medium containing (per liter) 0.3 g of KCl, 0.5 g of MgSO₄ · 7H₂O, 0.05 g of CaCl₂ · 2H₂O, 0.3 g of NH₄Cl, 0.3 g of K₂HPO₄, 15.0 g of NaCl, 0.005 g of yeast extract, 20 µg of vitamin B₁₂, and 1 ml of a trace elements solution (4). The organic substrates were added at a concentration of 1.0 g liter⁻¹ at pH 7.6 to 7.8. The results were recorded 4 days after inoculation.

Susceptibility to antibiotics was detected on agar plates by using antibiotic discs. The concentrations used are shown in Table 1. Tests for oxidase and catalase activities and for the capacity to hydrolyze starch, gelatin, and Tween 80 were performed by using standard procedures (8).

Reduction of nitrate was tested after 7 days of incubation in liquid medium containing 0.2% KNO₃ by using test kits for nitrate and nitrite (Merck, Darmstadt, Germany). Fixation of

CO₂ (autotrophic growth) was investigated on agar plates without organic substrates by using a GasPak system generating a CO₂-H₂ atmosphere (without catalyst) and in a liquid medium containing sodium bicarbonate.

The ability to grow anaerobically was tested in completely filled screw-cap test tubes by using the Pfennig-Lippert media for sulfur and nonsulfur purple bacteria (23).

Analytical procedures. (i) **Pigments.** Bchl and carotenoids were extracted from whole cells with acetone-methanol (7:2, vol/vol). The total amount of Bchl was determined by using the method of Clayton (1). For quantification of carotenoids a molar extinction coefficient of 128.0 mol · liter⁻¹ cm⁻¹ was used (2). Protein content was measured by the method of Lowry et al. (18).

(ii) **DNA analysis.** DNAs were extracted and purified by the method of Marmur (19). The guanine-plus-cytosine (G+C) contents of the DNAs were calculated from thermal melting point data (20). Amplification of the gene encoding the 16S rRNA and purification of the PCR products were performed as described previously (24, 25). The double-stranded PCR products of "*Roseococcus thiosulfatophilus*" and "*Erythromicrobium ramosum*" were sequenced by using the method of Dorsch and Stackebrandt (3). The ribosomal DNA (rDNA) of strain T4^T was sequenced by the automated method, as described by Rainey and Stackebrandt (25). The sequence analysis was performed by using previously described primers (31).

Data analysis. The 16S rDNA regions of "*Roseococcus thiosulfatophilus*," "*Erythromicrobium ramosum*," and strain T4^T analyzed were 1,426, 1,115, and 1,114 nucleotides long, respectively. The three sequences, which have been deposited in the EMBL data library, were aligned with the sequences in a data base containing the sequences of small-subunit rRNAs of members of the alpha subclass of the *Proteobacteria* (21), as well as sequences that were published recently. Regions whose sequences were not determined in one or more of the reference organisms and regions of alignment uncertainty (i.e., positions 1 to 97, 502 to 558, and 898 to 1072 and from position 1199 to the 3' terminus [*Escherichia coli* nomenclature]) were omitted from the analysis (mainly the short sequences for *Acidiphilium* and *Thiobacillus* strains available). Pairwise evolutionary distances (expressed as the estimated number of changes per 100 nucleotides) were computed from percentages of similarity by using the correction of Jukes and Cantor (15). Phylogenetic trees were generated from the distance matrices by the neighbor-joining method (26). Bootstrap values, based on the results of an analysis of 500 trees at 438 polymorphic sites, were obtained by using the programs of the PHYLIP package (6). Sequence alignment and data comparison were performed by using a SUN SPARC IPC workstation.

Membrane isolation. Cells were harvested at the end of the exponential growth phase with a continuous-flow centrifuge (Padberg, Lahr, Germany), washed with 10 mM Tris-HCl buffer (pH 7.8), and disrupted in a French pressure cell. Unbroken cells and large debris were removed by centrifugation at 12,000 × g for 20 min in a Sorvall refrigerated centrifuge equipped with a type SS34 rotor.

The supernatant was layered on a discontinuous gradient consisting of 0.6, 1.0, 1.2, and 1.5 M sucrose in 10 mM Tris-HCl buffer (pH 7.8). After centrifugation for 16 h at 90,000 × g in a Beckman type Ti60 rotor, the membrane fractions were collected, washed in Tris-HCl buffer, and analyzed.

Carotenoid analysis. Strain T4^T cells and membrane fractions were extracted with acetone, and the pigments were transferred into petroleum ether-diethyl ether (1:1) by partition. The upper phase was analyzed by thin-layer chromatog-

FIG. 1. Absorption spectrum of membrane fraction isolated from cells of *Erythrobacter litoralis* T4^T grown semiaerobically in the dark.

raphy on silica gel, using petroleum ether-diethyl ether-acetone (40:10:10). Single bands of carotenoids were scraped from the plates and compared with previously characterized pigments obtained from "*Erythromicrobium ramosum*" (44).

Electron microscopy. For electron microscopy the bacteria were embedded in Epon (Roth, Karlsruhe, Germany) after fixation with 1% glutaraldehyde and then with 1% osmium tetroxide as described by Kellenberger et al. (16). Preparations were negatively stained with 1% aqueous uranyl acetate. Micrographs were taken with a Philips model EM 400 electron microscope at 80 kV.

Nucleotide sequence accession numbers. The 16S rDNA sequences of "*Roseococcus thiosulfatophilus*," "*Erythromicrobium ramosum*," and strain T4^T have been deposited in the EMBL data library under accession numbers 72908, 72909, and 72962, respectively.

RESULTS AND DISCUSSION

Isolation and description of strain T4^T. (i) **Habitat and properties.** Strain T4^T was isolated from a mature cyanobacterial mat located in the supralittoral zone on the West Frisian island of Texel in The Netherlands. The predominant phototrophic organism resembled oxygenic *Microcoleus* strains and the anoxygenic purple sulfur bacterium *Thiocapsa roseopersicina*. Dense populations of colorless sulfur bacteria were also present in the mats. *Thiobacillus* spp. were observed in the upper layer, and representatives of the genus *Thiomicrospira* were found in deeper strata (41). Purple nonsulfur bacteria (presumably representatives of the genus *Rhodobacter*) and marine erythrobacteria (strains T1 through T7) were also isolated; however, in terms of numbers and biomass, members of the latter two taxa were minority groups (51). This microbial mat is flooded twice a month by the North Sea. Because of heavy rainfall and wind-induced evaporation, the salinity of the pore water may vary from 8 to 10‰ to more than 100‰.

The morphology and cultural and physiological properties of strain T4^T are described below in the description of *Erythrobacter litoralis* (see also Table 1). This isolate is not capable of anaerobic growth, either in the light or in the dark, on the media used in our experiments or on the Pfennig-Lippert media for nonsulfur and sulfur purple bacteria (23). Vigorous growth was observed when the bacteria were grown in a liquid

FIG. 2. *Erythrobacter litoralis*. (a) Negatively stained cell, showing flagellum. Bar = 0.7 μ m. (b) Ultrathin cross section, showing the outer membrane (om) and cytoplasmic membrane (cm). No invaginations of intracytoplasmic membranes were detected. Bar = 100 nm.

medium on a shaker and in solid medium on the agar surface in the dark or in the light. We did not observe autotrophic growth on mineral media when CO₂ and O₂ were in the atmosphere.

(ii) **Photosynthetic pigments.** The in vivo absorption spectra of strain T4^T had major maxima at 377, 800, and 872 nm (indicating the presence of Bchl *a*) and at 461 nm with broad shoulders around 488 and 437 nm (indicating the presence of carotenoids). With methanol extracts these peaks shifted to 770 and 368 nm (Bchl *a*) and to 453 and 475 nm (carotenoids) (51). Membrane fractions purified by sucrose gradient centrifugation produced peaks at 800 and 868 nm, indicating that light-harvesting complex I and the reaction center were present (Fig. 1). The cells had a cytoplasmic membrane (Fig. 2) but no intracytoplasmic membrane when they were grown under semiaerobic conditions in the dark.

Strain T4^T produced numerous carotenoids; the carotenoid composition was very similar to that of *Erythromicrobium ramosum* E5^T (44) and that of *Erythrobacter longus* Och101 (34–36). The main carotenoid pigments were bacteriorubixanthin and very polar erythroxanthin sulfate. Strain T4^T cells contained only small amounts of Bchl *a*, but carotenoids were abundant. The Bchl *a* contents of the cells ranged from 0.9

FIG. 3. Phylogenetic positions of representatives of the alpha subclass of the *Proteobacteria*, as determined by the neighbor-joining method. Scale bar = 10% difference in nucleotide sequences. The total distance between two organisms is the sum of the horizontal branch lengths. Reference 16S rRNA and DNA data were obtained from the Ribosomal Database Project (21) or are available from us. The numbers are bootstrap values; values less than 50% were not considered significant and are not shown. The dendrogram was generated by using the algorithm of Saitou and Nei (26), sequence dissimilarity values derived by the algorithm of Jukes and Cantor (15), and the similarity values shown in Table 2.

nmol · mg of protein⁻¹ to 3.9 nmol · mg of protein⁻¹ in the enriched membrane fraction. The ratio of Bchl to carotenoids in the cells was 1:9, and the ratio in the membrane fraction was 1:3.5.

Strain T4^T was susceptible to chloramphenicol, tetracycline, and fusidic acid and was resistant to penicillin, streptomycin, and polymyxin B (Table 1).

Phylogenetic analysis. The organisms investigated in this study were phylogenetically related to members of the α subclass of the *Proteobacteria*, which also contains the previously described aerobic, Bchl-containing bacteria (7, 33, 42). "*Roseococcus thiosulfatophilus*" RB3^T is a member of subclass α -1 and is moderately related to *Rhodopila globiformis*, *Thiobacillus acidophilus*, and members of the genus *Acidiphilium* (levels of sequence similarity, >90%) (Fig. 3 and Table 2). Also in this group are two organisms whose sequences were retrieved from a clone library obtained from DNAs that were extracted from an acid forest soil sample (32). Both "*Roseococcus thiosulfatophilus*" and *Rhodopila globiformis* contain Bchl *a* and are able to oxidize thiosulfate (14, 45). Both of these species also form pink colonies, and their G+C contents are similar (66.3 and 70.4 mol%). There are, however, consid-

erable differences in the growth conditions which they prefer; *Rhodopila globiformis* prefers to grow anaerobically under photoheterotrophic conditions, while "*Roseococcus thiosulfatophilus*" is obligately aerobic. The antenna systems of the two organisms are also different. *Rhodopila globiformis* seems to have two light-harvesting complexes (890, 862, and 813 nm) (22), while "*Roseococcus thiosulfatophilus*" has only one (855 nm) (44).

"*Erythromicrobium ramosum*" is phylogenetically distantly related to "*Roseococcus thiosulfatophilus*" (level of sequence similarity, 84.9%) (Table 2). "*Erythromicrobium ramosum*" is, however, closely related to *Erythrobacter longus* (level of sequence similarity, 98%) (Table 2 and Fig. 2) and strain T4^T (level of sequence similarity, 97.4%). Together with the recently described taxon *Porphyrobacter neustonensis* (7), these organisms are members of the α -4 subclass of the *Proteobacteria*. *Caulobacter subvibrioides* (33) has also been tentatively assigned to this subgroup. The isolated position of this subgroup and its placement close to the branching point of the alpha subclass from the other subclasses of the *Proteobacteria* have been described previously by Stahl et al. (33) in a study of *C. subvibrioides* and by Fuerst et al. (7) in a study of the other members of the α -4 subgroup.

In a recent publication (43) it was shown that the *Sphingomonas* group belongs to the α -4 subclass of the *Proteobacteria* and is related to *C. subvibrioides* (Fig. 3). The *Erythromicrobium*-*Erythrobacter*-*Porphyrobacter* cluster is most closely related to members of the genus *Sphingomonas*. *Erythrobacter longus* and strain T4^T both contain erythroxanthin sulfate, bacteriorubixanthin, and other minor components, including β -carotene, spirilloxanthin, and zeaxanthin. Both species contain only the core antenna complex that absorbs at 870 nm. A similar carotenoid pattern has also been found in "*Erythromicrobium ramosum*" (34–36, 44). This species, however, contains two light-harvesting complexes, LHI (B870) and LHII (B798–832). The high 16S rDNA similarity values, similar carotenoid patterns, and similar Bchl-protein complexes of *Erythrobacter longus* and strain T4^T argue against separating these two organisms as members of different genera. Strain T4^T grows well at salinity values up to 96‰, while *Erythrobacter longus* grows optimally at salinity values between 17 and 50‰. These two organisms differ in their DNA G+C contents, in their substrate utilization patterns, and in their susceptibility to antibiotics. This justifies separation of strain T4^T from *Erythrobacter longus* as new species, for which the name *Erythrobacter litoralis* is proposed.

"*Roseococcus thiosulfatophilus*" RB3^T and "*Erythromicrobium ramosum*" E5^T have been described recently (45–47). Since these names have not been validated previously and new data have been obtained, more extensive descriptions of these strains are given below.

Description of *Roseococcus* gen. nov. *Roseococcus* (Ro.se.o.coc'cus. M. L. adj. *roseus*, rose, pink; Gr. n. *coccus*, sphere or spheroidal shape; M.L. masc. n. *Roseococcus*, pink spherical bacterium). The description of the genus below is based on the original description of Yurkov and Gorlenko (45) and new data. Biochemical, morphological, and 16S rDNA sequence data for strain RB3^T (= DSM 8511^T) support the proposal that a new genus and species should be described.

Cells are gram negative, coccoidal, pink, and motile by means of polarly inserted flagella. Bchl *a* and carotenoid pigments are present. Cells divide by binary fission. Obligately aerobic, chemoorganotrophic (respiratory metabolism), and facultatively photoheterotrophic. Some members can utilize thiosulfate as an additional energy source.

TABLE 2. Similarity matrix based on 16S rRNA sequence comparisons

Organism	% Similarity to:																			
<i>Erythrobacter litoralis</i> DSM 8509 ^T	97.2																			
<i>Erythrobacter litoralis</i> DSM 8509 ^T	96.1	97.7																		
<i>Erythromicrobium ramosum</i> DSM 8510 ^T	96.7	97.4	98.1																	
<i>Porphyrobacter neustonensis</i> ACM 2844 ^T	93.8	94.1	95.5	94.6																
<i>Sphingomonas adhaesiva</i> IFO 19099 ^T	94.4	94.4	95.0	95.5	95.0															
<i>Sphingomonas capsulata</i> IFO 14666 ^T	92.9	93.5	93.2	92.2	93.8	93.5														
<i>Sphingomonas parapaucimobilis</i> IFO 13935 ^T	88.4	87.7	87.3	87.7	86.5	87.7	86.8													
<i>Roseobacter denitrificans</i> Och114 ^T	87.0	87.3	86.3	86.2	85.7	87.0	86.8	92.1												
<i>Rhodobacter capsulatus</i> B10	88.2	88.7	87.4	87.4	87.6	88.8	88.7	93.5	95.2											
<i>Paracoccus denitrificans</i> LMG 4218 ^T	89.1	89.0	89.3	88.8	89.3	89.1	90.1	86.3	87.0	88.5										
<i>Ancylobacter aquaticus</i> ATCC 25396 ^T	88.8	89.0	89.0	88.5	89.3	87.6	88.5	86.5	87.1	88.2	92.4									
<i>Rhodopseudomonas palustris</i> GH	88.7	89.1	89.4	89.4	89.3	88.8	88.5	89.0	89.4	90.1	93.0	93.3								
<i>Beijerinckia indica</i> DSM 1715 ^T	90.7	90.2	90.5	90.4	89.4	90.5	90.7	89.3	89.4	91.1	93.8	91.1	92.5							
<i>Agrobacterium tumefaciens</i> DSM 30205 ^T	88.4	88.7	88.8	89.0	88.0	87.0	87.1	87.3	87.9	88.5	89.9	89.6	91.0	91.8						
<i>Pseudomonas diminuta</i> ATCC 11568 ^T	86.6	87.1	87.3	87.4	86.8	86.3	86.2	85.7	87.6	87.4	89.3	89.4	90.1	96.1	85.9					
<i>Caulobacter bacteroides</i> ATCC 15254 ^T	84.0	85.2	84.5	84.6	83.5	85.2	84.6	83.7	84.2	85.2	85.7	86.2	86.5	86.6	86.6	99.1				
<i>Acidiphilium cryptum</i> DSM 2389 ^T	84.2	85.4	84.6	84.8	83.7	85.4	84.6	83.7	84.2	85.2	85.7	86.2	86.5	86.6	86.6	93.2	93.2			
<i>Thiobacillus acidophilus</i> DSM 700 ^T	86.0	86.3	86.5	86.8	85.6	86.8	86.5	85.2	85.1	86.2	88.0	86.0	87.4	89.0	88.2	91.8	91.6	94.1		
<i>Rhodopila globiformis</i> DSM 161 ^T	84.6	84.9	85.1	85.6	84.5	85.6	85.1	84.0	84.2	84.0	86.0	85.1	86.3	86.5	86.6	93.5	93.2	94.1	94.1	
<i>Roseococcus thiosulfatophilus</i> DSM 8511 ^T	86.2	87.6	87.3	87.3	86.0	86.8	87.0	85.9	86.2	87.4	89.3	88.4	88.7	88.4	86.5	87.6	87.6	90.4	90.4	
<i>Rhodospirillum salinarum</i> ATCC 35394 ^T	87.1	87.7	87.9	87.0	88.2	87.3	88.0	86.5	87.9	86.3	89.3	88.4	89.4	89.1	89.0	87.4	87.7	88.7	87.9	88.4
<i>Azospirillum lipoferum</i> DSM 1691 ^T	88.0	88.8	89.4	88.7	89.6	87.6	88.5	86.0	86.3	86.6	89.6	88.8	89.3	89.1	89.8	87.0	87.0	89.1	87.3	92.2

No growth occurs under anaerobic conditions in the light. Methanol is not utilized. NaCl is not required for growth.

The DNA G+C content is 70.4 mol% (as determined by thermal denaturation).

Phylogenetically related to members of the α -1 subclass of the *Proteobacteria*.

The type species is *Roseococcus thiosulfatophilus*.

Description of *Roseococcus thiosulfatophilus* sp. nov. *Roseococcus thiosulfatophilus* (thi.o.sul.fa.to'phi.lus. M. L. adj. *thiosulfatophilus*, thiosulfate liking). The description of the species below is based on the original description of Yurkov and Gorlenko (45) and new data.

Gram-negative, pink cocci that are 0.9 to 1.3 by 1.3 to 1.6 μ m and are motile by means of single polar flagella. Cells contain Bchl *a* and carotenoid pigments. Membranes isolated from cells grown semiaerobically in the dark have absorption maxima at 478, 501, and 505 nm (carotenoids) and at 800 and 855 nm (Bchl). The major carotenoid is C₃₀ carotenedioate. Aerobic, chemoorganotrophic, and facultatively photoheterotrophic.

Growth occurs on yeast extract and when succinate, acetate, pyruvate, citrate, lactate, malate, or glutamate is used as a sole carbon source; weak growth occurs with glucose, maltose, and glycerol. Fructose, sucrose, ribose, arabinose, butyrate, formate, fumarate, propionate, benzoate, tartrate, methanol, ethanol, mannitol, glycolate, and methanol are not used. Casein is hydrolyzed.

The cells can oxidize thiosulfate to sulfate in the presence of organic compounds. Ribulose diphosphate carboxylase activity is not detected. The cells are susceptible to tetracycline, streptomycin, polymyxin B, erythromycin, amikacin, kanamycin, neomycin, aureomycin, vancomycin, chloramphenicol, and fusidic acid, but resistant to gentamicin, lincomycin, nystatin (Mycostatin), bacitracin, and penicillin. Oxidase and catalase positive.

The storage material consists of polysaccharides, poly- β -hydroxybutyrate, and polyphosphate.

The G+C content of the DNA is 70.4 mol%.

Habitat: freshwater cyanobacterial mat of a thermal alkaline sulfide spring (54°C; pH 9.3; 7.4 mg of sulfide per liter; 1.6 mg of oxygen per liter).

The type strain is RB3 (= DSM 8511).

Description of *Erythromicrobium* gen. nov. *Erythromicrobium* (E.ry.thro.mi.cro'bi.um. Gr. adj. *erythrus*, red; Gr. adj. *micros*, small; Gr. n. *bios*, life; N.L. n. *Erythromicrobium*, red microbe). The description of the genus below is based on the original description of Yurkov et al. (47) and new data. Biochemical, morphological, and 16S rDNA sequence data for strain E5^T (= DSM 8510^T) support the proposal that a new genus and species should be described. Gram negative, rod shaped, and usually motile by means of flagella. Branching may occur. The cells are orange, contain Bchl *a* and carotenoids, and multiply by binary division.

Aerobic chemoorganotrophs and facultative photoheterotrophs. No growth occurs anaerobically in the light. Ribulose diphosphate carboxylase is not detected. No fermentation and no denitrification activities occur. Phylogenetically related to members of the α -4 subclass of the *Proteobacteria*.

The DNA G+C content ranges from 62.5 to 68.5 mol%.

The habitat is freshwater. Not halophilic.

On the basis of DNA-DNA hybridization data and phenotypic properties, the following five species have been tentatively identified: "*Erythromicrobium sibiricum*," "*Erythromicrobium ursincola*," "*Erythromicrobium ezovicum*," "*Erythromicrobium hydrolyticum*," and "*Erythromicrobium ramosum*" (49). The type species is "*Erythromicrobium ramosum*."

Description of *Erythromicrobium ramosum* sp. nov. *Erythromicrobium ramosum* (ra.mo'sum. L. adj. *ramosum*, ramifying, referring to the morphology of the cells). Gram-negative, orange rods that are 0.6 to 1.0 by 1.3 to 2.5 μ m. Cells may branch. Multiplication occurs by binary or ternary fission. Motile by means of polar flagella. Bchl *a* and carotenoids are present. The cytoplasmic membrane contains a reaction center and two light-harvesting complexes, one with an absorption maximum at 870 nm and one with absorption maxima at 798 and 832 nm. The major carotenoids are the very polar compound erythroanthin sulfate and bacteriorubixanthinal. Optimal growth occurs at temperatures between 25 and 30°C and at pH values between 7.0 and 8.5.

The optimum substrates for growth are glucose, sucrose, maltose, acetate, pyruvate, butyrate, malate, succinate, fumarate, propionate, glutamate, casein hydrolysate, and yeast extract. Growth also occurs on fructose, citrate, lactate, tartrate, and ethanol. No growth occurs on ribose, arabinose, formate, benzoate, methanol, mannitol, glycerol, and glycolate. Methanol is not utilized. Starch, gelatin, and Tween 80 are not hydrolyzed. Oxidase and catalase positive. The tricarboxylic acid cycle operates. The glyoxalate shunt has been observed in strain E4 (2), but not in strain E5^T.

Susceptible to the following antibiotics: tetracycline, polymyxin B, erythromycin, nalidixic acid, amikacin, gentamicin, nystatin, bacitracin, kanamycin, neomycin, aureomycin, vancomycin, novobiocin, chloramphenicol, and fusidic acid. Resistant to penicillin, ampicillin, streptomycin, and nystatin. The storage compounds are polysaccharides, poly- β -hydroxybutyric acid, and polyphosphates.

The G+C content of the DNA is 63.6 to 64.2 mol%.

Habitat: cyanobacterial mat from an alkaline spring (pH 9.5; 25°C).

The type strain is E5 (= DSM 8510).

Description of *Erythrobacter litoralis* sp. nov. *Erythrobacter litoralis* (li.to.ra'lis. L. adj. *litoralis*, at the beach or coast, referring to the supralittoral habitat). Gram-negative, rod-shaped cells. Short chains up to five cells long may occur. The cells are red or orange, become red or brown in older cultures, and are 1.0 to 1.3 by 0.2 to 0.3 μ m. Motile by means of flagella (Fig. 2). Cells contain Bchl *a* and carotenoids. The main carotenoids are bacteriorubixanthinal and erythroanthin sulfate. The in vivo absorption spectrum of Bchl *a* has maxima at 370, 590, 800, and 868 nm. The cells do not form intracytoplasmic membranes. Aerobic, chemoorganotrophic, and catalase and oxidase positive. Growth occurs on glucose, fructose, butyrate, glutamate, acetate, and lactate, and weak growth occurs on succinate. Does not utilize methanol. Tween 80 is hydrolyzed, but gelatin and starch are not hydrolyzed. Nitrate is not reduced. Susceptible to chloramphenicol, tetracycline, and fusidic acid. Resistant to penicillin, streptomycin, and polymyxin B. The optimal temperature for growth is 25 to 30°C.

Habitat: marine cyanobacterial mat in a supralittoral zone. This organism is able to grow in a very wide salinity range, from 5‰ (freshwater) to 96‰.

The DNA G+C content is 67 mol%.

The type strain is T4 (= DSM 8509).

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