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# Reorganization of the Genus *Erythromicrobium*: Description of "*Erythromicrobium sibiricum*" as *Sandaracinobacter sibiricus* gen. nov., sp. nov., and of "*Erythromicrobium ursincola*" as *Erythromonas ursincola* gen. nov., sp. nov.

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**The results of investigations on the morphology, physiology, pigment composition, light-harvesting antenna and reaction center organization, and electron carriers of five *Erythromicrobium* representatives, and on phylogenetic relations among them, are summarized. On the basis of clear phenotypic differences and distinct phylogenetic positions shown by 16S ribosomal DNA analysis, the tentative species "*Erythromicrobium sibiricum*" and "*Erythromicrobium ursincola*" are formally described as the type species of two new genera: *Sandaracinobacter sibiricus* gen. nov., sp. nov., and *Erythromonas ursincola* gen. nov., sp. nov., respectively. The genus *Erythromicrobium* is at present composed of the type species, *E. ramosum*, and two species, "*E. hydrolyticum*" and "*E. ezovicum*," whose nomenclature is yet to be validated. All species studied group within the α-4 subclass of *Proteobacteria*.**

Obligately aerobic bacteriochlorophyll *a* (Bchl *a*)-containing bacteria have been found at several geographical places in different ecological niches (8, 23, 24, 35, 36). They are represented by marine species of the genera *Erythrobacter* and *Roseobacter* (21, 22) and by freshwater species of the genera *Erythromicrobium*, *Roseococcus* (32, 36, 37, 40), and *Porphyrobacter* (8). The main features of this group are inability to grow photosynthetically under anaerobic conditions despite the presence of Bchl *a*, low levels of photosynthetic units in cells, and strong inhibition by light of Bchl *a* synthesis (12, 34).

The first representative of freshwater obligately aerobic Bchl *a*-containing bacteria, isolated from thin microbial mats formed near underwater hydrothermal vents of the Bol'shoi River (Baykal Lake region) in Russia, was ascribed to the marine genus *Erythrobacter* (22) and named "*Erythrobacter sibiricus*" (31). This assignment was made on the basis of Bchl *a* production connected with strict aerobiosis. However, since that time "*E. sibiricus*" was reclassified into the genus *Erythromicrobium*, a new genus for freshwater obligately aerobic anoxygenic photosynthetic bacteria (30, 36).

Based on phenotypic similarities, the five recently isolated species "*E. sibiricum*," "*E. ursincola*," "*E. ezovicum*," "*E. hydrolyticum*," and *E. ramosum* were included in the same genus, *Erythromicrobium* (36). However, DNA-DNA hybridization data showed that DNA from the species "*E. sibiricum*" and "*E. ursincola*" had very low homology (11 to 27%) with the other three species of this genus (36). It was proposed that additional physiological, biochemical, and ribosomal DNA (rDNA) sequence analyses might demonstrate clear differences between "*E. sibiricum*" and "*E. ursincola*," on the one hand, and the other orange freshwater bacteria, on the other (36). In agreement with this proposal, an analysis of 5S rRNA sequences confirmed the genetic heterogeneity of the genus *Erythromicrobium* (28).

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In this paper we exclude "*E. sibiricum*" and "*E. ursincola*" from the genus *Erythromicrobium* and describe them as the members of two new genera, *Sandaracinobacter* and *Erythromonas*, respectively, on the basis of results of morphological, biochemical, physiological, molecular biological, and phylogenetic analyses.

## MATERIALS AND METHODS

**Bacterial strains.** The following species were investigated: the type species of genus *Erythromicrobium*, *E. ramosum* E5, "*E. sibiricum*" RB16-17, "*E. ursincola*" KR-99, "*E. ezovicum*" E-1, and "*E. hydrolyticum*" E4(1) (31, 33, 36, 37, 40) (from the personal collection of V. Yurkov).

**Culture media.** The strains were cultivated in Erlenmeyer flasks aerobically in the dark at 30°C and at pH 7.6 to 7.8 in a medium containing the following (in grams per liter): yeast extract, 1.0; Bacto Peptone, 1.0; sodium acetate, 1.0; KCl, 0.3; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.05; $NH_4Cl$, 0.3; $K_2HPO_4$, 0.3. The medium was supplemented with 20 μg of vitamin $B_{12}$/liter and 1.0 ml of a trace element solution/liter (4).

**Isolation of soluble cyt's, membranes, and pigment-protein complexes.** Cells were harvested by centrifugation at the end of the exponential-growth phase, washed with 20 mM Tris-HCl buffer (pH 7.8), and disrupted by three passages through a French pressure cell operated at 16,000 lb/in$^2$. Unbroken cells and large debris were removed by centrifugation at 12,000 × *g* for 15 min. The supernatant fluid was centrifuged at 150,000 × *g* for 3 h to separate soluble proteins from membranes, which were individually assayed for cytochrome (cyt) *c* content.

The method of fractionation of cell membranes and isolation of light-harvesting complex II (LHII) and reaction center (RC)-LHI complexes has been described elsewhere (38, 39).

**Analytical methods.** Membranes were analyzed for the presence of cyt *c* and molecular weights (MW) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (11.5 to 16.5% linear gradient of acrylamide) and were stained with 3,3′,5,5′-tetramethylbenzidine (TMBZ) in the presence of hydrogen peroxide as described elsewhere (27).

The presence of Bchl and carotenoids in *Blastomonas natatoria* was evaluated by using acetone-methanol (7:2) extracts.

**Spectroscopic procedures.** Absorption spectra were recorded at room temperature with a Hitachi U-2000 spectrophotometer.

**Electron microscopy.** Cells from the mid-logarithmic-growth phase were fixed in filtered 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 5.0 mM $CaCl_2$ (pH 7.0) at 4°C for 2 h, embedded in 4.0% agarose, and cut into cubes. Agarose cubes were progressively dehydrated in ethanol solutions from 10 to 96% and then in propylene oxide. Dehydrated cubes were embedded in LR white resin (London Resin Company, Ltd., London, England) and polymerized at 70°C for 9 h. Ultrathin sections were stained with uranyl acetate and lead

TABLE 1. Major characteristics of *Erythromicrobium* representatives[a]

| Characteristic | "*E. sibiricum*" | "*E. ursincola*" | *E. ramosum* | "*E. ezovicum*" | "*E. hydrolyticum*" |
|---|---|---|---|---|---|
| Cell shape and size (μm) | Thin, long bacilli; 0.3–0.5 × 1.5–2.5 | Ovoid; 0.8–1.0 × 1.3–2.6 | Bacilli, branched; 0.7–1.0 × 1.6–2.5 | Long bacilli; 0.6–0.8 × 2.7–2.8 | Bacilli, branched; 0.7–1.1 × 1.8–2.5 |
| Color | Yellow-orange | Orange-brown | Red-orange | Red-orange | Red-orange |
| Major carotenoid in vivo peaks (nm) | 424, 450, 474 | 430, 458, 485 | 466, 478 | 466, 478 | 466, 478 |
| Utilization of: | | | | | |
| Glucose | + | + | + | + | + |
| Maltose | + | + | + | + | + |
| Acetate | + | + | + | + | + |
| Pyruvate | + | + | + | − | + |
| Butyrate | + | + | + | + | − |
| Malate | − | + | + | + | + |
| Citrate | − | + | + | + | + |
| Succinate | − | + | + | + | + |
| Lactate | − | + | + | + | + |
| Ethanol | − | − | + | + | + |
| Methanol | − | − | − | − | − |
| Antibiotic sensitivity to: | | | | | |
| Penicillin | − | − | − | − | ND |
| Tetracycline | − | + | − | − | ND |
| Streptomycin | + | − | + | + | ND |
| Polymyxin B | − | + | − | − | ND |
| Bacitracin | + | − | + | + | ND |
| Kanamycin | + | − | + | + | ND |
| Chloramphenicol | + | + | + | + | ND |
| DNA G+C content (mol%) | 68.5 | 65.4 | 64.2 | 62.5 | 65.2 |

[a] This table is created from the data presented in references 31, 33, 36, 37, and 40. +, substrate is utilized or antibiotic sensitivity. −, substrate is not utilized or antibiotic resistance. ND, not determined.

citrate and were examined with a JEOL transmission electron microscope, model 12000, Ex2, as previously described (42).

**16S rDNA analysis.** Isolation of genomic DNA, PCR-mediated amplification of 16S rDNA, and purification of PCR products were performed as previously described (19). Sequences of purified PCR products were determined by using a *Taq* DyeDeoxy terminator cycle sequencing method (Applied Biosystems Co., Foster City, Calif.) as described in the manufacturer's protocol. The 16S rDNA sequences obtained were aligned manually with sequences of members of the α-4 subclass of the *Proteobacteria*.

Pairwise evolutionary distances were computed by using the correction of Jukes and Cantor (15). The distance matrix method (3) and the neighbor-joining method (20) were used to construct phylogenetic trees from the distance matrices. Bootstrap values calculated for 300 trees were generated by using the algorithms (7).

**Nucleotide sequence accession numbers.** The 16S rDNA sequences determined in this study have been deposited in the EMBL database (Cambridge, United Kingdom) under accession no. Y10678 for *Sandaracinobacter sibiricus* RB16-17[T] and Y10677 for *Erythromonas ursincola* KR99[T]. Because of the many ambiguous nucleotides in the 16S rRNA sequence of *B. natatoria* available in the EMBL data bank under accession no. X73043, a new sequence was obtained from *B. natatoria* DSM 3183 and submitted to the EMBL data bank under accession no. Y13774. The reference sequences were obtained from the European Molecular Biology Laboratory or from the Ribosomal Database Project (16). The nucleotide sequence accession numbers for the reference strains are given as follows (for those described previously, reference numbers are given): *Erythrobacter longus* ATCC 33941[T], M59062; *Erythromicrobium ramosum* DSM 8510[T], X72909; *Rhodobacter capsulatus* B10, reference 16; *Rhodopila globiformis* DSM 161[T], reference 16; *Roseobacter denitrificans* Och114[T], reference 16; *Roseococcus thiosulfatophilus* DSM 8511[T], X72908; *Sphingomonas adhaesiva* JCM 7370[T], X72720; *Sphingomonas capsulata* ATCC 14666[T], 59296; *Sphingomonas rosa* IFO 15208[T], D13945; *Sphingomonas macrogoltabidus* IFO 15033[T], D13723; *Sphingomonas terrae* IFO 15098[T], D13727; *Sphingomonas yanoikuyae* IFO 15102[T], X72725; *Sphingomonas subarctica* HAMBI 2110[T], X94102; *Sphingomonas paucimobilis* DSM 1098[T], X72722; *Sphingomonas paucimobilis* DSM 7562, X94100; *Sphingomonas chlorophenolica* ATCC 33790[T], X87161; *Sphingomonas sanguis* IFO 13937[T], D13937; *Sphingomonas parapaucimobilis* IFO 15100[T], X72721; *Porphyrobacter neustonensis* ACM 2844[T], M96745; *Rhizomonas suberifaciens* IFO 15211[T], D13737; *Sphingomonas aromaticivorans* SMCC F1T99[T], U20756; *Sphingomonas stygia* SMCC B0712[T], U20775; *Sphingomonas subterranea* SMCC B0478[T], U20773.

## RESULTS AND DISCUSSION

**Morphological and physiological differences among *Erythromicrobium* species.** The main phenotypic differences of the five *Erythromicrobium* species are presented in Table 1.

All species synthesize a large amount of carotenoid pigments, which determine the color of the organism. *E. ramosum* was shown to produce at least 10 kinds of carotenoids (38). The two main carotenoids, the orange erythroxanthin sulfate and the red bacteriorubixanthinal, are very polar. According to the in vivo absorption spectra of "*E. ezovicum*," "*E. hydrolyticum*," and *E. ramosum*, the major carotenoid peaks at 466 and 478 nm indicate similar carotenoid compositions in these species, apparent as a similar color of liquid cultures (intensely red-orange). The carotenoid compositions, or at least the major pigments, of "*E. sibiricum*" (absorption at 424, 450, and 474 nm) and "*E. ursincola*" (absorption at 430, 458, and 485 nm) are different from those determined for the three above-mentioned species and different from each other, consistent with the colors of liquid cultures (yellow-orange for "*E. sibiricum*" and orange-brown for "*E. ursincola*") (Table 1).

The cell morphologies and the types of cell division are also significantly different and distinguish "*E. sibiricum*" and "*E. ursincola*" from the other three species and from each other. *E. ramosum*, "*E. ezovicum*," and "*E. hydrolyticum*" are very long rods and produce characteristic thread-like cells, dividing by symmetric or asymmetric constriction. For *E. ramosum* and "*E. hydrolyticum*," ternary fission and branching were demonstrated (33, 37).

"*E. ursincola*" cells are ovoid, and budding or asymmetric division can be used for reproduction (Fig. 1). Thus, budding is a distinctive phenotypic property of "*E. ursincola*."

"*E. sibiricum*" has long, thin cells producing long chains

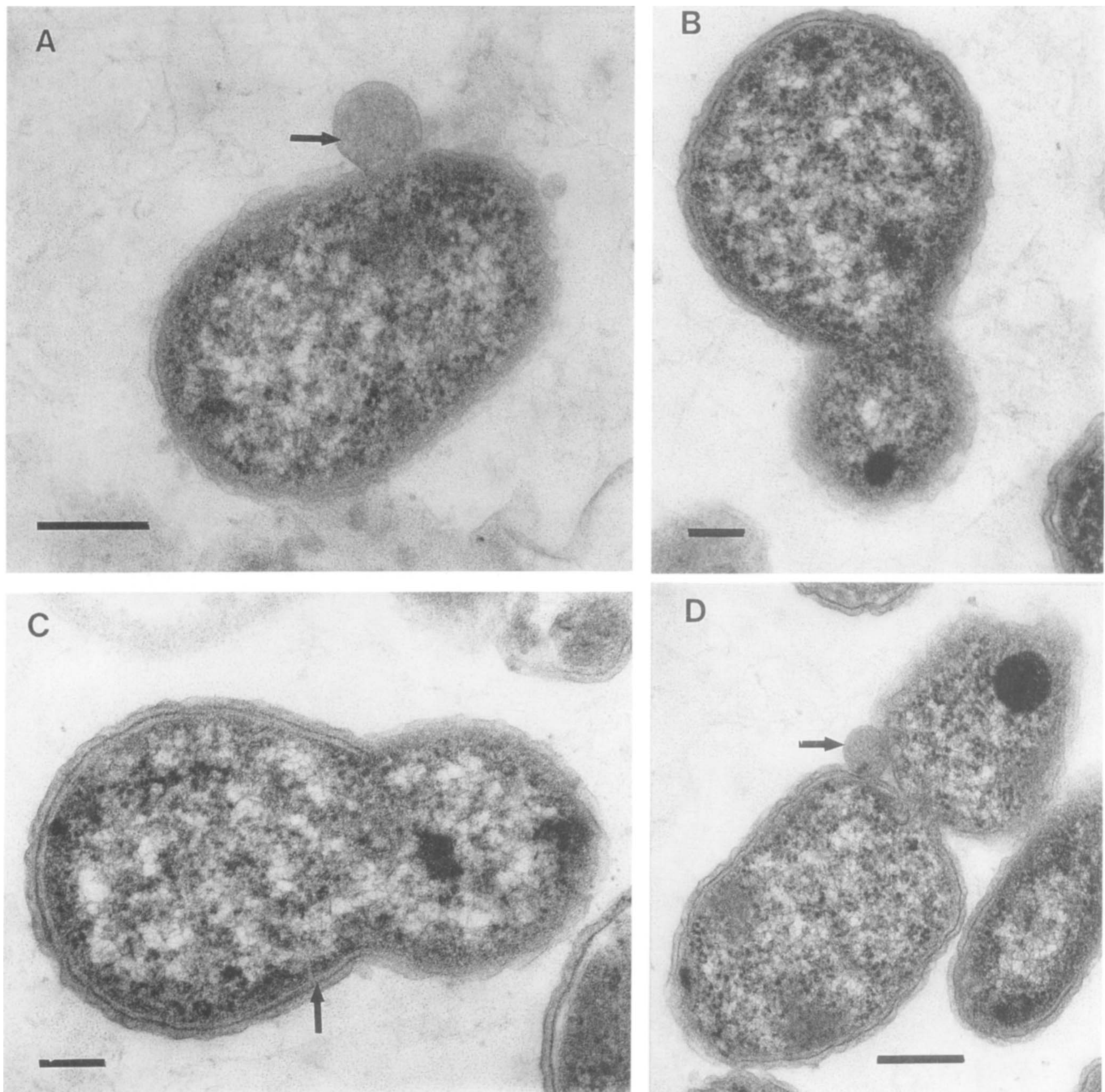


FIG. 1. Electron micrograph of "*E. ursincola*" thin-sectioned cells, showing different stages of bud formation. (A) Initiation of budding. (B) Young bud. (C) Older bud. The cell wall is structured and present in the mother cell only (arrow). (D) The daughter cell is not yet separated, and a new bud initiation point can already be seen (arrow). Bars, 200 nm (A and D) and 100 nm (B and C).

(sometimes up to 10 cells) and reproduces by binary division. Budding or branching has not been observed in "*E. sibiricum*."

The organic compounds utilized as carbon sources and the antibiotic sensitivities of the five species studied in this work are summarized in Table 1.

**Characteristics of the photosynthetic apparatus and electron carriers.** Several photoheterotrophic bacteria have the capability to grow by either photosynthesis or respiration. These two electron transfer systems share some carriers, such as soluble cyt's, quinone molecules, and cyt $bc_1$ complexes. In addition, the photosynthetic apparatus in all purple bacteria contains the photochemical RC center and one or more types of light-harvesting complex (5). The structure and amino acid composition of these components are evolutionarily highly conserved and can be used as a taxonomic marker in bacterial classification (14, 17).

As reported for *E. ramosum* (38, 39), membranes of "*E. hydrolyticum*" and "*E. ezovicum*" also contain two types of antenna complexes, LHI and LHII. We have isolated and purified LHII and RC-LHI particles by detergent treatment of membranes and sucrose gradient fractionation (Fig. 2; Table 2).

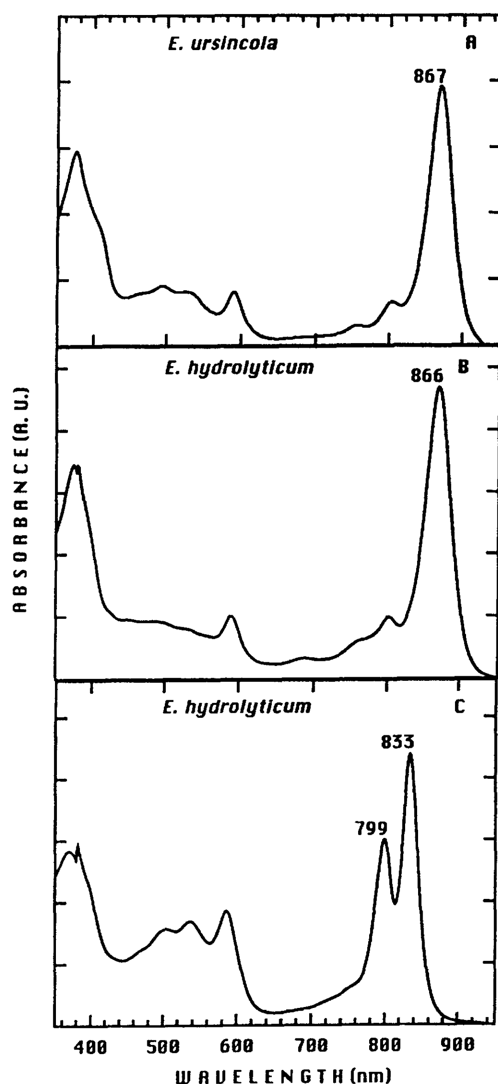


FIG. 2. Room temperature absorption spectra of RC-LHI complexes isolated from "*E. ursincola*" (A) and "*E. hydrolyticum*" (B), and absorption spectrum of LHII complexes purified from "*E. hydrolyticum*" (C).

The isolated LHII complexes from "*E. hydrolyticum*" and "*E. ezovicum*," as well as those from *E. ramosum*, showed Bchl absorption maxima at 798 to 800 and 832 to 833 nm, indicating the presence of new types of LHII (Table 2). The long-wavelength band of LHII at 832 to 833 nm is about 20 nm less than that usually observed in purple bacteria. LHII complexes absorbing at similar wavelengths were shown for *Rhodopseudomonas acidophila* and *Rhodopseudomonas palustris* cells grown under special temperature or light intensity conditions (1, 6, 10, 25). These peculiar spectral features found for the antenna complexes of aerobic photosynthetic bacteria indicate an unusual protein environment for Bchl *a* molecules, which is supported by preliminary Raman spectroscopy data (9).

The species "*E. sibiricum*" and "*E. ursincola*" synthesize only the LHI type of antenna, which absorbs maximally at 867 nm (Table 2; Fig. 2).

The analysis of the light-induced difference spectra in the presence of oxygen in whole cells of *Erythromicrobium* representatives, as well as redox titrations and gel electrophoresis of their soluble, membrane, and LHI-RC purified fractions (41), led us to distinguish two clearly different groups on the basis of their behaviors and cyt composition (Table 2).

The very fast cyt photooxidation observed after flash excitation in the cells of "*E. ursincola*" and "*E. sibiricum*" (41) indicates that the immediate electron donor to the RC in these species is an RC-bound cyt *c* of 44.0 and 40.0 kDa in "*E. sibiricum*" and "*E. ursincola*," respectively (Table 2). The species *E. ramosum*, "*E. hydrolyticum*," and "*E. ezovicum*" do not possess an RC-bound cyt *c*, and the photooxidized special pair ($P^+$) of the RC is directly reduced by a soluble cyt *c* (41).

A detailed cyt composition analysis of the soluble and membrane fractions of *Erythromicrobium* representatives shows that despite the existence of RC-bound cyt *c* in "*E. sibiricum*" and "*E. ursincola*," their total cyt *c* compositions are significantly different (Table 2). "*E. ursincola*" contains a more complex soluble and membrane-bound cyt *c* population, whereas the "*E. sibiricum*" soluble (one cyt *c* of 14.0 kDa) and membrane (two cyt *c*'s, including the RC-bound one) fractions have fewer types of cyt. Interestingly, "*E. ursincola*" contains an unusually small soluble cyt *c* of 6.5 kDa. Such a small cyt *c* is rarely found in bacteria and is present in *Hydrogenobacter thermophilus* (cyt $c_{550}$ of 6.0 kDa), *Methylomonas* strain A4 (cyt $c_{554}$ of 4.0 kDa) (29), and the obligately aerobic photosynthetic bacteria *Roseococcus thiosulfatophilus* RB3 (cyt $c_{549}$ and cyt $c_{552}$ of 6.5 and 4.0 kDa, respectively) (41).

The difference in quinone composition between "*E. sibiricum*" and "*E. ursincola*" has been described elsewhere (11). "*E. ursincola*" seems to lack quinone $Q_9$ and contains only quinone $Q_{10}$. In "*E. sibiricum*" both quinones $Q_9$ and $Q_{10}$ are present (Table 2).

From the taxonomic point of view, the compilations of properties described in the above paragraph are useful. On the one hand, in the genus *Erythromicrobium*, the photosynthetic ap-

TABLE 2. Comparative data on photosynthetic apparatus organization and electron transfer carriers[a]

| Species | RC | Absorption peaks (nm) | | cyt *c* | | | Ubiquinone[b] (mmol/g of dry cells) | |
|---|---|---|---|---|---|---|---|---|
| | | LHI | LHII | RC bound (kDa) | Soluble (no. [kDa]) | Membrane bound (no. [kDa]) | $Q_9$ | $Q_{10}$ |
| "*E. sibiricum*" | + | 867 | Absent | 44.0 | 1 (14.0) | 2 (30.0, 44.0) | 0.06 | 0.71 |
| "*E. ursincola*" | + | 867 | Absent | 40.0 | 3 (6.5, 9.0, 14.0) | 4 (14.3, 21.0, 24.0, 40.0) | ND | 0.11 |
| *E. ramosum* | + | 868 | 798, 832 | Absent | 2 (8.0, 14.3) | 3 (8.0, 26.0, 30.0) | 0.09 | $0.19^M$ |
| "*E. ezovicum*" | + | 868 | 800, 832 | Absent | 2 (8.0, 14.3) | 2 (30.0, 34.0) | 0.02 | 0.3 |
| "*E. hydrolyticum*" | + | 866 | 799, 833 | Absent | 1 (14.3) | 2 (21.0, 30.0) | 0.02 | $0.01^M$ |

[a] Species were cultivated in the dark under aerobic conditions. +, present.
[b] Data are cited from reference 11. ND, not detected. A superscript "M" appended to a value means that in addition to the common ubiquinone $Q_{10}$, its methylated form was revealed.

parati of "*E. sibiricum*" and "*E. ursincola*" are composed of RC with a bound tetraheme cyt *c* and only one type of light-harvesting antenna, LHI. On the other hand, *E. ramosum*, "*E. hydrolyticum*," and "*E. ezovicum*" do not possess an RC-bound cyt *c*, and a soluble cyt $c_2$ seems to complete the cyclic electron transfer in the photosynthetic pathways of these three species, which contain an unusual B798-832 LHII complex. These differences in photosynthetic apparatus organization and composition of electron carriers are consistent with the heterogeneity of the genus *Erythromicrobium* deduced from DNA-DNA hybridization data (36) and 5S rRNA sequence comparisons (28), supporting a separation of "*E. ursincola*" and "*E. sibiricum*" from the genus *Erythromicrobium*.

**Phylogenetic analysis.** Nearly complete 16S rDNA sequences were determined for the strains "*E. ursincola*" and "*E. sibiricum*." The sequences were aligned with homologous sequences of members of the α subclass of *Proteobacteria*, and the phylogenetic positions were determined. All nonambiguous positions between positions 49 and 1376 (*E. coli* numbering; the actual number of bases was always higher than 1,000) (2) were included in the phylogenetic analysis. The sequences of a subset of 20 strains of *Erythromicrobium*, *Erythrobacter*, *Porphyrobacter*, *Blastomonas*, and related taxa (i.e., members of *Sphingomonas*, *Rhizomonas*, *Zymomonas*, and some misclassified strains) (16, 18) were used to generate a matrix of similarity.

With respect to the phylogenetic positions of the two nonvalid strains of *Erythromicrobium*, all algorithms used in this study (3, 7) invited the same conclusions: these two strains do not cluster with *E. ramosum* and relatives, but they form two separate sublines on the evolutionary tree of *Sphingomonas* species with a binary similarity value of 92.5%. The branching point of "*E. ursincola*" is between *Sphingomonas* subcluster 2 and subclusters 1 and 3 (Fig. 3), while that of "*E. sibiricum*" is between subcluster 4 and subclusters 1, 2, and 3. The lack of high bootstrap values found for the branching of these subclusters indicates low statistical significance of the order of the several subclusters, which has been previously reported (26). The two species investigated here show lower than 96% 16S rDNA identity to *Sphingomonas* reference organisms. While "*E. ursincola*" shows the highest sequence identity to *B. natatoria* (99.8%), "*E. sibiricum*" stands phylogenetically isolated, showing less than 93.5% sequence identity with any of the reference organisms included in this study. The dendrogram (Fig. 3) includes the phylogenetic positions of some other obligately aerobic Bchl *a*-containing bacteria (*R. thiosulfatophilus* and *R. denitrificans*) of the α subclass of the *Proteobacteria*, the positions of which have been shown previously (40).

This phylogenetic analysis of the 16S rDNA of the two nonvalidly described species of *Erythromicrobium* clearly reveals that they cannot be considered members of this genus. However, it does not follow from their affiliation with members of the genus *Sphingomonas* that they should be considered novel species of *Sphingomonas*. The phylogenetic heterogeneity of *Sphingomonas* has been noted before, as several *Sphingomonas*-unlike organisms, such as *Rhizomonas suberifaciens*, *B. natatoria*, and *Zymomonas mobilis* have been found to branch between or close to the four recognized *Sphingomonas* species subgroups (18, 26). The branching of the two Bchl *a*-containing strains of *Erythromicrobium* within the *Sphingomonas* group adds a novel biochemical trait which has not been detected before in members of this group.

"*E. sibiricum*" forms a single subline of descent, whereas "*E. ursincola*" clusters with *B. natatoria* (13). In our opinion, the significant phenotypic and physiological differences between "*E. ursincola*" and *B. natatoria* preclude their assignment to the same genus. *B. natatoria* is a nonphotosynthetic species containing carotenoid pigments and lacking Bchl, whereas "*E. ursincola*" is physiologically related to obligately aerobic anoxygenic photosynthetic bacteria producing carotenoids and Bchl *a*. "*E. ursincola*" contains Bchl *a* incorporated into photochemically active RC and light-harvesting complexes, and it contains specific electron transfer carriers of a cyclic photosynthetic pathway (such as cyt *c* bound to the RC, soluble cyt $c_2$, the RC $Q_A$ primary electron acceptor, and the "special pair" P of the RC). High 16S rDNA sequence similarity between these two species indicates a close phylogenetic relationship and suggests that they have a common ancestor; conceivably one is an evolutionary progenitor of the other. However, due to the existence of significant physiological differences (photosynthesis is a restricted mode of energy generation), they should not be designated members of the same genus for taxonomic purposes.

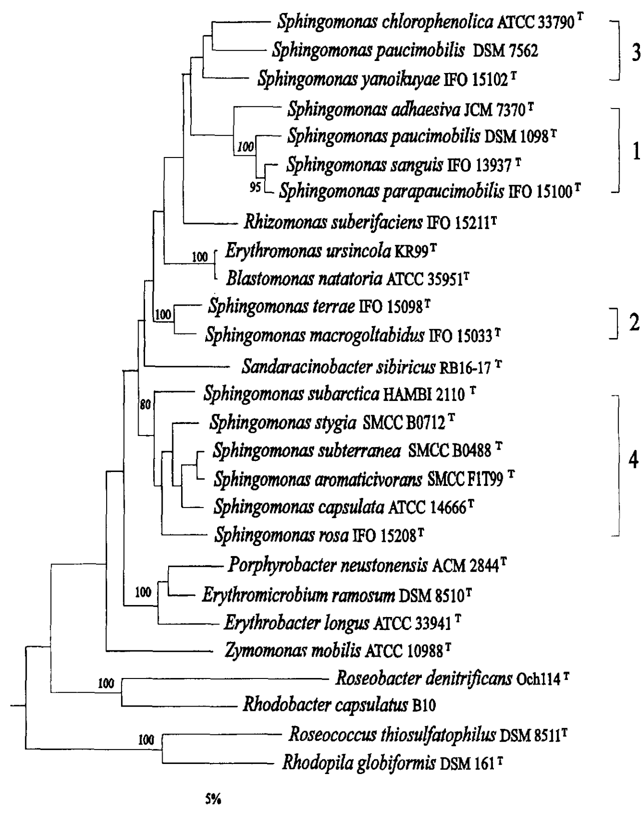


FIG. 3. 16S rDNA dendrogram of relatedness showing the phylogenetic positions of *Erythromonas ursincola* and *Sandaracinobacter sibiricus* on the evolutionary tree of members of the genus *Sphingomonas* and related taxa. The dendrogram was generated by the algorithm of De Soete (3) on the basis of the dissimilarity values. The sequence of *Agrobacterium tumefaciens* was used to root the dendrogram. Numbers refer to bootstrap values, of which only those above 80% are shown. The length corresponding to 5% inferred sequence divergence is indicated at the bottom.

Taken together, the results described in this paper require elevation of "*E. ursincola*" and "*E. sibiricum*" to type species of two new genera of the α subclass of the *Proteobacteria*: *Erythromonas ursincola* gen. nov., sp. nov., and *Sandaracinobacter sibiricus* gen. nov., sp. nov. Thus, the genus *Erythromicrobium* is at present composed of the three species *E. ramosum* (type species), "*E. hydrolyticum*," and "*E. ezovicum*." Descriptions of the three species of the genus *Erythromicrobium* have been given in previous publications (33, 40).

Summary descriptions of the two new genera are given below.

**Description of *Sandaracinobacter* gen. nov.** *Sandaracinobacter* (San.da.ra.ci.no.bac'ter. Gr. adj. *sandaracinos*, orange-colored; Gr. n. *bacter*, rod; M.L. masc. n. *sandaracinobacter*, orange-colored rod).

Cells are thin, long rods, forming chains. Motile by means of subpolar flagella, gram negative. Divide by binary division. Cultures are intensely yellow-orange because of carotenoid pigments.

Contain Bchl *a*. Photosynthetic apparatus consists of RC and LHI. RC contains a tightly bound tetraheme cyt *c*.

Aerobic chemoorganotrophic and facultative photoheterotrophic metabolisms. No growth occurs anaerobically in the light. Ribulose-bisphosphate carboxylase is not detected. No fermentation or denitrification activities detected.

DNA base composition is 68.5 mol% G+C (by thermal denaturation).

The habitat is freshwater. Not halophilic.

Member of the α-4 subclass of the *Proteobacteria.*

The type species is *Sandaracinobacter sibiricus* (formerly "*E. sibiricum*") (37).

**Description of *Sandaracinobacter sibiricus* sp. nov.** *Sandaracinobacter sibiricus* (si.bi'ri.cus. L. adj. *sibiricus*, isolated in Siberia).

Gram-negative, yellow-orange-pigmented, thin, long rods, 0.3 to 0.5 by 1.5 to 2.5 μm or more. Motile by means of subpolar flagella (up to three).

Cells contain Bchl *a* and carotenoid pigments. Carotenoids give main absorption peaks at 424, 450, and 474 nm in vivo. The cytoplasmic membranes contain an RC and LHI with absorption peaks at 750, 799, and 857 nm for RC and 867 nm for LHI. Tetraheme cyt *c* of 44.0 kDa is tightly bound to RC and is its immediate electron donor. Additionally contain soluble cyt *c* of 14.0 kDa and membrane-bound cyt *c* of 30.0 kDa (cyt of $bc_1$ complex). Contains quinones $Q_9$ and $Q_{10}$. Menaquinone is not found (11).

Aerobic chemoorganotroph and facultative photoheterotroph. The best growth substrates are butyrate, sucrose, casein hydrolysate, and yeast extract. Good growth is observed on acetate and maltose; weak growth on media containing glucose, fructose, pyruvate, propionate, or glycerol. No utilization of ribose, sorbitol, benzoate, fumarate, formate, succinate, citrate, malate, methanol, or ethanol.

Optimal temperature for growth is 25 to 30°C. Freshwater bacteria; salinity above 1.0% NaCl/liter of media strongly inhibits growth. The pH optimum is 7.5 to 8.5. The bacteria exhibit oxidase activity and lack catalase activity. Hydrolyze Tween 60 and do not hydrolyze gelatin or starch.

Resistant to chloramphenicol, fusidic acid, streptomycin, amikacin, bacitracin, kanamycin, neomycin, and novobiocin. Sensitive to penicillin, ampicillin, tetracycline, polymyxin B, erythromycin, nalidixic acid, lincomycin, mycostatin, aureomycin, and vancomycin.

Demonstrate a high level of resistance to tellurite. Tellurite resistance depends on medium composition, particularly on organic carbon source. The highest tellurite concentration tolerated is 1,200 μg/ml in media containing acetate or yeast extract as a sole organic source. Tellurite can be reduced and transformed into metallic tellurium accumulated inside cells (42).

Storage compounds: polyphosphates, polysaccharide, and poly-β-hydroxybutyrate.

The DNA G+C content is 68.5 mol%.

Habitat: freshwater algobacterial mat near hydrothermal sulfide-containing vents along the river bottom.

The type strain is RB16-17.

**Description of *Erythromonas* gen. nov.** *Erythromonas* (E.ry.thro.mo'nas. Gr. adj. *erythros*, red; Gr. n. *monas*, a unit, monad; M.L. fem. n. *Erythromonas*, red monad).

Gram negative, ovoid cells, motile by means of a polar flagellum. Do not form chains. Reproduce by budding or asymmetric division.

The cells are orange-brown due to carotenoid pigments. Contain Bchl *a*. Photosynthetic apparatus is organized in RC with tightly bound tetraheme cyt *c* and LHI.

Obligately aerobic, chemoorganotrophic (respiratory metabolism), and facultatively photoheterotrophic. No growth occurs under anaerobic conditions in the light. NaCl is not required for growth.

The DNA G+C content is 65.4 mol% (as determined by thermal denaturation).

Phylogenetically related to members of the α-4 subclass of the *Proteobacteria.*

The type species is *Erythromonas ursincola* (formerly "*Erythromicrobium ursincola*" [37]).

**Description of *Erythromonas ursincola* sp. nov.** *Erythromonas ursincola* (ur.sin'co.la. M.L. adj. *ursincola*, neighbor or compatriot of bears).

The cells are gram negative and ovoid, 0.8 to 1.0 by 1.3 to 2.6 μm. Long chains are not formed. Reproduce by budding or asymmetric division. Cells motile by means of a unique polar flagellum.

The cells contain Bchl *a* and carotenoid pigments. Carotenoids give three main absorption peaks at 430, 458, and 485 nm in vivo, and in combination with Bchl *a* determine the colors of orange in young cultures and dark orange-brown in older liquid or young agar cultures. Bchl *a* is present in membrane-bound protein-pigment complexes, consisting of the RC with absorption peaks at 751, 801, and 853 nm and core LHI absorbing at 867 nm. RC contains tightly bound tetraheme cyt *c* with molecular size of 40.0 kDa. Total cyt *c* of cells growing in the dark is very abundant and represented by soluble cyt *c*'s of 6.5, 9.0, and 14.0 kDa and by membrane-bound cyt *c*'s of 14.3, 21.0, and 24.0 kDa. In cells growing in the dark, only quinone $Q_{10}$ was found (11).

Aerobic, chemoorganotroph, facultative photoorganoheterotroph. The best growth is on media containing glucose, fructose, sucrose, maltose, acetate, glutamate, propionate, casein hydrolysate, or yeast extract. Good growth on pyruvate, butyrate, malate, or succinate. Poor growth on media supplemented with arabinose, citrate, lactate, glycerol, or mannitol. No growth detected in media containing ribose, formate, benzoate, tartrate, methanol, ethanol, or glycolate.

The tricarboxylic acid cycle, and glyoxylate shunt during growth on an acetate-containing medium, is present.

The key enzyme of Calvin cycle, ribulose-bisphosphate carboxylase, is not found. No anaerobic growth in the light, fermentation, or denitrification is found.

Optimal growth temperature is 25 to 30°C. Freshwater organism, does not require NaCl for growth. Optimum pH is 7.0 to 8.0. Yeast extract and vitamin $B_{12}$ satisfy the requirement for growth factors. Oxidase and catalase positive. Tween 80 is hydrolyzed. Lipase activity of 64.9 U/g of biomass. Starch and gelatin are not hydrolyzed.

Sensitive to tetracycline, polymyxin B, amikacin, gentamicin, neomycin, aureomycin, vancomycin, novobiocin, chloramphenicol, and fusidic acid. Resistant to penicillin, ampicillin, streptomycin, erythromycin, nalidixic acid, lincomycin, mycostatin, bacitracin, and kanamycin.

Very resistant to tellurite. Can grow in the presence of tellurite concentrations up to 2,700 μg/ml in acetate-containing

minimal medium. Resistance to tellurite depends on the presence of an organic carbon source in the medium. Tellurite can be reduced and transformed into metallic tellurium accumulated as metal crystals in cell cytoplasm (42).

Storage compounds: polyphosphates.

The DNA GC content is 65.4 mol% (by thermal denaturation).

Habitat: freshwater cyanobacterial mat developing in the thermal springs at pH 6.7 to 7.0 and at temperatures from 34 to 40°C on Kamchatka Island (Russia).

Type strain is KR-99.

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