

Transfer of the Bacteriochlorophyll *b*-Containing Phototrophic Bacteria *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* to the Genus *Blastochloris* gen. nov.

AKIRA HIRAISHI*

Department of Ecological Engineering, Toyohashi University of Technology, Toyohashi 441, and
Laboratory of Environmental Biotechnology, Konishi Co., Tokyo 130, Japan

The phylogenetic positions of the bacteriochlorophyll (BChl) *b*-producing budding phototrophic bacteria *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* were studied on the basis of 16S rRNA gene sequence information. These bacteria formed a tight cluster with the genus *Rhodoplanes* as a sister group within the alpha-2 subgroup of the *Proteobacteria*. Genomic DNA-DNA hybridization assays showed that *R. viridis* and *R. sulfoviridis* were closely related but were different species. Creation of the genus *Blastochloris* gen. nov. is proposed to accommodate these BChl *b*-producing species of phototrophic bacteria.

In 1984 some species of the classically defined genus *Rhodopseudomonas* were reclassified into new genera, including the new genera *Rhodobacter* and *Rhodopila*, on the basis of modern taxonomic criteria (17). In recent years, the taxonomy of the genus *Rhodopseudomonas* has been further reevaluated on the basis of increasing molecular and chemotaxonomic information, and this has led to rejection of the name *Rhodopseudomonas rutila* (10) and the transfer of some *Rhodopseudomonas* species to the genus *Rhodobacter* (19) and to the new genera *Rhodoplanes* (12) and *Rhodobium* (13). Despite these taxonomic changes, the species currently in the genus *Rhodopseudomonas* are still heterogeneous phylogenetically, and both molecular and phenotypic data strongly suggest that only the type species, *Rhodopseudomonas palustris*, should be placed in this genus. The proper taxonomic placement of the bacteriochlorophyll (BChl) *b*-containing *Rhodopseudomonas* species (i.e., *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis*) is a subject for consideration in light of their characteristic features. It has been suggested that one of the BChl *b*-producing species, *Rhodopseudomonas viridis*, is phylogenetically distant from *Rhodopseudomonas palustris* (30) within the alpha-2 subgroup of the *Proteobacteria* and that this organism is more closely related to members of the genus *Rhodoplanes* (12). In the present study the 16S ribosomal DNA (rDNA) sequence similarity of and genomic DNA relatedness between *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* were investigated. The results obtained indicate that these species should be placed in a distinct new genus of phototrophic bacteria, for which the name *Blastochloris* gen. nov. is proposed.

The organisms investigated were *Rhodopseudomonas viridis* ATCC 19567^T (T = type strain), *Rhodopseudomonas sulfoviridis* DSM 729^T, *Rhodoplanes roseus* DSM 5909^T, and *Rhodopseudomonas palustris* ATCC 17001^T, all of which were obtained either from the American Type Culture Collection, Rockville, Md., or from the DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. MYS medium (9), which contained mineral base RM2, 20 mM sodium DL-malate as the carbon source, and 0.05% yeast extract as the growth factor, was used for cultiva-

tion of the organisms. For *Rhodopseudomonas sulfoviridis*, the medium was supplemented with 10 mM glucose, 2 mM sodium sulfide (neutralized), and 2 mM thiosulfate. The medium was supplemented with 10 mM pyruvate (filter sterilized) for *Rhodoplanes roseus*. The organisms were grown at 30°C in screw-cap test tubes under anaerobic conditions in the light. Cells were harvested by centrifugation from cultures at the mid-exponential phase of growth, washed, resuspended in pure water or EDTA-saline, and stored at –20°C until analysis. Genomic DNA was extracted and purified by the method of Marmor (24). DNA base ratios were determined by the high-performance liquid chromatography method (7, 18), and DNA-DNA hybridization assays were performed by photobiotin labeling and colorimetric detection as described previously (7, 10). 16S rDNA fragments were amplified by PCR from the crude cell lysate (11) and were purified by polyethylene glycol precipitation (8, 22). The PCR products were sequenced directly with fluorescent primers and a SequiTherm Long-Read cycle sequencing kit (Epicentre Technologies, Madison, Wis.) and analyzed with a Pharmacia automated DNA sequencer. Sequences were compiled and similarities were calculated with the GENETYX-MAC computer program (Software Development Co., Tokyo, Japan). Multiple alignments of sequences, calculation of nucleotide substitution rates (21), and construction of neighbor-joining phylogenetic trees (27) were performed with the CLUSTAL W program (28). Alignment positions that included gaps and unidentified bases were not taken into consideration for the calculations.

The 16S rDNA sequences determined covered a continuous nucleotide stretch from positions 28 to 1,492 in the *Escherichia coli* numbering system (1). The level of sequence similarity between the 16S rDNAs of *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* was 98.7% (corrected distance, 0.0123). This binary similarity value suggests that the two species are phylogenetically closely related organisms which belong to a single genus but different genspecies. The results of DNA-DNA hybridization assays supported this suggestion, as the genomic DNAs of *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* were related to each other at an average hybridization level of 38% (Table 1). A distance matrix tree was constructed on the basis of the 16S rDNA sequences of *Rhodopseudomonas viridis*, *Rhodopseudomonas sulfoviridis*, and related species of phototrophic and nonphototrophic bacteria that belong to group 2 of the alpha subclass of the *Proteobacteria* (Fig. 1). The two species of BChl *b*-producing pho-

* Mailing address: Department of Ecological Engineering, Toyohashi University of Technology, Tenpaku-cho, Toyohashi 441, Japan. Phone: 81 (532) 44-6913. Fax: 81 (532) 44-6547.

TABLE 1. Genomic DNA relatedness among *Rhodopseudomonas viridis*, *Rhodopseudomonas sulfoviridis*, and some other species of budding phototrophic bacteria

Organism	G+C content of DNA (mol%)	% Hybridization with labeled DNA from:	
		ATCC 19567 ^T	DSM 729 ^T
<i>Rhodopseudomonas viridis</i> ATCC 19567 ^T	66.5 ^a	100	32
<i>Rhodopseudomonas sulfoviridis</i> DSM 729 ^T	67.9	44	100
<i>Rhodoplanes roseus</i> DSM 5909 ^T	66.8 ^b	11	7
<i>Rhodopseudomonas palustris</i> ATCC 17001 ^T	65.0 ^a	6	4

^a Data from reference 10.

^b Data from reference 12.

totrophs formed a tight cluster with the genus *Rhodoplanes* as a sister group within the alpha-2 subgroup of the *Proteobacteria*.

BChl *b* was first discovered in *Rhodopseudomonas viridis* (3, 5) and was later found in some other species of phototrophic purple bacteria, including members of the genera *Thiocapsa* (4) and *Ectothiorhodospira* (15, 16). Among the species of the genus *Rhodopseudomonas* and all other genera of purple non-sulfur bacteria so far recognized, however, *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* are the only species that produce BChl *b* as the photosynthetic pigment. In addition to these species, a BChl *b*-producing budding phototrophic bacterium which may be related to the former species has been isolated from a hot spring (26). The evolutionary and taxonomic significance of the presence of BChl *b* is not clear. However, 16S rDNA sequence comparisons demonstrate that the BChl *b*-producing budding phototrophic bacteria studied here form a monophyletic group that is distinguishable from *Rhodopseudomonas palustris*, other members of the genus *Rhodopseudomonas*, and all other photosynthetic genera established so far in the alpha-2 subgroup. Phylogenetic analysis

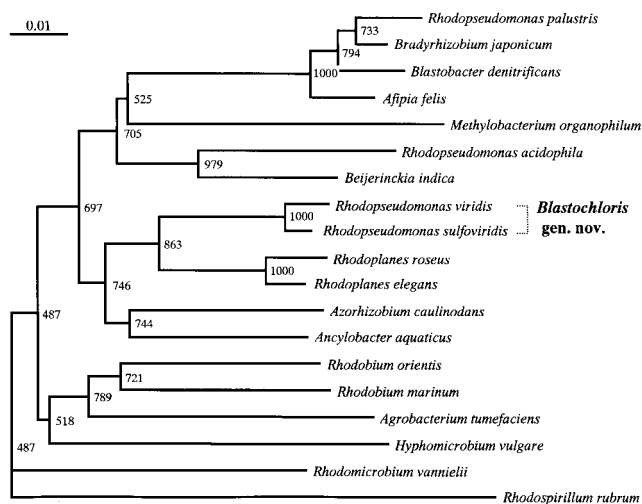


FIG. 1. Distance matrix tree showing phylogenetic affiliations of *Rhodopseudomonas viridis*, *Rhodopseudomonas sulfoviridis*, and related species of phototrophic and nonphototrophic bacteria belonging to the alpha-2 subgroup of the *Proteobacteria*. The 16S rDNA sequence of *Rhodospirillum rubrum* was used as an outgroup to root the tree. Bootstrap confidence values obtained from 1,000 bootstrap trials (6) are given at branch points. Scale bar = 1 nucleotide substitution per 100 nucleotides.

based on amino acid sequences of L and M subunit proteins of photosynthetic reaction centers showed that *Rhodopseudomonas viridis* represents a unique line of descent within the division of purple bacteria (25). *Rhodopseudomonas viridis* is one of the best characterized organisms with respect to structures of the reaction center, as X-ray crystal structures of the reaction center of this organism have been studied (2). In light of the importance of *Rhodopseudomonas viridis* as a key organism in photosynthetic research and the phylogenetic and phenotypic evidence that the BChl *b*-producing budding phototrophs are members of a distinct taxonomic group, these organisms should no longer be included in the genus *Rhodopseudomonas*. Thus, I propose that *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* should be transferred to the genus *Blastochloris* gen. nov., as *Blastochloris viridis* comb. nov. and *Blastochloris sulfoviridis* comb. nov., respectively.

Description of *Blastochloris* gen. nov. *Blastochloris* (Blas.to.chlo'ris. Gr. n. *blastos*, bud shoot; Gr. adj. *chloros*, green; M. L. fem. n. *Blastochloris*, green bud shoot). The characteristics described below are based on information from previous reports (3, 14, 20, 23, 29) and this study. Cells are rod shaped to ovoid and exhibit polar growth, budding, and asymmetric cell division. Rosette-like aggregates sometimes occur. Motile by means of subpolar flagella. Gram negative. Photoheterotrophic, growing anaerobically in the presence of light. Microaerophilic growth in the dark is also possible. Phototrophically growing cells contain intracytoplasmic membranes that are present as lamellae underlying and parallel to the cytoplasmic membrane. Photosynthetic pigments are BChl *b* and carotenoids. The color of photosynthetic cultures is green to olive green. Photoorganotrophy with a number of simple organic compounds as carbon and electron donors is the preferred mode of growth. Both ubiquinones (Q-8 to Q-10) and menaquinones (MK-7 to MK-9) are present. The G+C content of the genomic DNA ranges from 66 to 71 mol%. 16S rDNA sequence information places the genus in the alpha-2 subgroup of the *Proteobacteria*, and the genus *Rhodoplanes* is the closest relative among the photosynthetic genera. The type species is *Blastochloris viridis*.

Description of *Blastochloris viridis* comb. nov. *Blastochloris viridis* (*Rhodopseudomonas viridis* Drews and Giesbrecht 1966, 261^{AL}) (vi'ri.dis. L. adj. *viridis*, green). The description of *B. viridis* is the same as the description given previously for *Rhodopseudomonas viridis* (3, 23, 29). The type strain is strain ATCC 19567 (= DSM 133 = G. Drews F).

Description of *Blastochloris sulfoviridis* comb. nov. *Blastochloris sulfoviridis* (*Rhodopseudomonas sulfoviridis* Keppen and Gorlenko 1975, 258^{AL}) (sul.fo.vi'ri.dis. L. neut. n. *sulfur*, sulfur; L. adj. *viridis*, green; L. adj. *sulfoviridis*, sulfur green). The description of *B. sulfoviridis* is the same as the description given previously for *Rhodopseudomonas sulfoviridis* (20, 29). The type strain is strain DSM 729 (= V. M. Gorlenko P₁).

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S rDNAs of *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* have been deposited in the DDBJ, EMBL, and GenBank databases under accession numbers D25314 and D86514, respectively.

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