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EXPERIMENTAL ARTICLES

Characterization of a New Strain of a Purple Nonsulfur Bacterium from a Thermal Spring

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Abstract—A new budding purple nonsulfur bacterium of the genus *Rhodobacter* (strain Ku-2) was isolated from a mat of a moderately thermal spring (Baikal rift zone, Buryatia, Russia). The bacterium had lamellar photosynthetic membranes, which are typical of only one *Rhodobacter* species, *Rba. blasticus.* The cells contained spheroidene carotenoids and bacteriochlorophyll *a* (Bchl *a*). In vivo absorption spectrum of the cells, with the major maximum at 863 nm and an additional peak at 887 nm, is characteristic of the pigment–protein complexes of Bchl *a*-containing membranes. The previously described *Rba. blasticus* strains do not exhibit the 887-nm maximum. The new isolate was photoheterotrophic, with optimal growth occurring at 35°C, 3 g/L NaCl, and pH 7–8. The DNA G+C content was 64.4 mol %. The similarity between the 16S rRNA gene sequences of strain Ku-2 and the *Rba. blasticus* type strain was 98.7%. The PufM amino acid sequences of strain Ku-2 and the earlier studied *Rba. blasticus* type strain were 89.5 % identical. Thus, strain Ku-2 belongs to the genus *Rhodobacter* and is phylogenetically close to *Rba. blasticus.*

Keywords: anoxygenic phototrophs, proteobacteria, family *Rhodobacteraceae, Rhodobacter blasticus*

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The group of purple nonsulfur bacteria (PNB) includes species that are diverse phenotypically and phylogenetically. Most of PNB species grow photoheterotrophically, utilizing various organic sources of carbon under anaerobic conditions in the light. Only a few PNB species are capable of chemoheterotrophic growth in the dark under microaerobic or aerobic conditions.

PNB belong to alpha- and betaproteobacteria. *Rhodobacter blasticus* belongs to alphaproteobacteria. It differs from other species of the genus *Rhodobacter* by its replication mode (budding) and by the circular-lamellar structure of its photosynthetic membranes [1]. *Rba. blasticus* cultures grown anaerobically have an orange-brown color due to the presence of the spheroidene carotenoid in cells. In the presence of oxygen, spheroidene is transformed to spheroidenone, which imparts purple coloration to the cells. The in vivo absorption spectrum of the cells of *Rba. blasticus* type strain exhibits maxima at 378, 418, 476, 506, 590, 795, and 862 nm [1]. Strain *Rba. blasticus* Rb-5 that we earlier described [2] is similar to the type strain in the absorption maxima of its cells. Strain Rb-5 is phylogenetically close to the type strain *Rba. blasticus* ATCC 33485^T (99.7% 16S rRNA gene sequence identity).

From the Kuchiger thermal spring (Buryatia, Russia), we have isolated a new strain (named Ku-2) of a budding PNB phenotypically and phylogenetically close to the species *Rhodobacter blasticus.* Phototrophically grown cells of strain Ku-2 exhibited the major maximum of bacteriochlorophyll *a* at 863 nm, as well as a minor maximum (shoulder) at 887 nm. Such absorption spectra are typical of some other *Rhodobacter* species but not of known strains of *Rhodobacter blasticus* [1].

The aim of the present work was to study this new isolate with unusual spectral characteristics.

MATERIALS AND METHODS

Strains and cultivation conditions. In addition to the new PNB isolate Ku-2, PNB strain Rb-5, isolated from a microbial mat in the Bol'shaya Reka hydrothermal vent (the Baikal region) [2], was used for the purpose of comparison.

For PNB cultivation, medium of the following composition was used (g/L): NH_4Cl, 0.4; KH_2PO_4, 0.5; $MgCl_2$, 0.2; Na_2SO_4, 0.5; yeast extract, 1; sodium acetate, 1; sodium pyruvate, 1; NaCl, 1; $Na_2S_2O_3 \cdot 5H_2O$, 1; KCl, 0.5; $NaHCO_3$, 1; vitamin B_{12}, 10 μg/L; Pfennig's trace element solution, 1 mL/L [3]. The solutions of $NaHCO_3$ (10%), yeast extract (5%),

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sodium acetate (10%), sodium pyruvate (10%), and sodium thiosulfate (10%) were prepared and sterilized separately and were introduced into the medium immediately before adjusting pH to 7.5–8 and inoculation. Cultivation was performed anaerobically in the light in 20-mL penicillin vials completely filled with liquid medium and closed with rubber stoppers, as well as aerobically in the dark in 500-mL conical flasks. Pure cultures were obtained on plates of agarized (2%) medium in Petri dishes by several consecutive transfers of aerobically grown individual colonies. Purity of the cultures was controlled microscopically.

Morphology and ultrastructure. Cell morphology was studied using an Olimpus BX40 (Japan) microscope equipped with a phase contrast device. Total cell specimens negatively stained with a 0.2% aqueous solution of uranyl acetate and ultrathin sections of cells were examined under a Jeol JEM-100C (Japan) electron microscope at an accelerating voltage of 80 kV. Ultrathin sections were obtained with the use of ultramicrotome from cells concentrated by centrifugation, treated according to the Kellenberg method, dehydrated, and embedded in Epon. The sections were negatively stained by the Reynolds reagent [4] after placing them on copper grids coated with a formvar film.

Fatty acid analysis. A 5-mg dry biomass sample was treated with 0.4 mL of a 1 N hydrogen chloride solution in methanol at 80°C for 1 h (acid methanolysis). The methyl esters formed during methanolysis were extracted with hexane and injected into a Microbial Identification System gas chromatograph (MIDI Inc.) [5].

Pigment composition was inferred from absorption spectra recorded on an SF 56A spectrophotometer (LOMO, Russia) within a wavelength range of 350–1020 nm. The spectra were recorded for suspensions of whole cells in 50% aqueous solution of glycerol or for cellular membrane fractions obtained after disrupting cells ultrasonically and removing cell debris by centrifugation. Additionally, spectral characteristics of acetone–methanol (7 : 2) extracts were studied.

Physiological and biochemical properties and conditions of growth. To determine the growth substrate spectrum, the above-described basal medium was used, supplemented with vitamin B_{12} (10 μg/L) and yeast extract (0.1 g/L) as a source of other vitamins and devoid of other organic compounds except the compound tested, introduced to a concentration of 1 g/L. The pH of the medium was adjusted to 8.0.

Tolerance to sodium sulfide and the capacity for its utilization were determined by growing the bacteria anaerobically in the light in the presence of various concentrations of $Na_2S \cdot 9H_2O$: 0, 300, 500, 700, and 1000 mg/L. Cultivation at various NaCl concentrations and pH values was performed anaerobically in the light. To determine the pH growth range, phosphate (pH 6.8–7.4) and carbonate (8.0–9.5) buffers were used. For determination of the optimal growth temperature, cultivation was performed in a gradient thermostat in a temperature range of 10–50°C. Cell yield was estimated from the optical density measured at 650 nm on a KFK-3 photometer. Sensitivity to antibiotics was estimated after aerobic cultivation on Petri dishes from the sizes of the sterile zones around the discs impregnated with particular antibiotics.

Molecular-genetic studies. Isolation of DNA from cells was performed by the Birnboim–Doly alkaline extraction procedure and the Promega Wizard-technology, as described in [6]. DNA content in the solution obtained and its purity were assessed on a Smart Spec 3000 spectrophotometer (BioRad, United States).

16S rRNA gene amplification and sequencing of the obtained PCR products were performed using the universal bacterial primers 27f and 1492r [7]. Amplification of a fragment of the *puf*LM operon and sequencing of the obtained PCR products were performed using a primer system specific to purple bacteria that was described in [8, 9]. The PCR temperature profile was as follows: 94°C for 2 min, 56°C for 30 s, 72°C for 1 min 30 s; 42 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 1 min 30 s; and final extension, 72°C for 5 min. In all PCR variants the volume of the amplification mixture was 50 μL and its composition was as follows: BioTaq DNA polymerase buffer (17 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8, 2 mM $MgCl_2$); 12.5 nmol of each dNTP, 50 ng of template DNA; 5 pmol of each of the appropriate primers, and 3 units of BioTaq DNA polymerase (Dialat LTD, Russia).

PCR products were analyzed by electrophoresis at 6 V/cm in 1.0% agarose gel stained with ethidium bromide. The results of electrophoresis were recorded using the BioDocAnalyze gel documentation system (Biometra, Germany).

Sequencing of the amplification products was performed by the Sanger method on a DNA Analyzer 3730 automatic sequencer (Applied Biosystems, United States) using the BigDye Terminator v. 3.1 Cycle Sequencing Kit according to the manufacturer's recommendations. The obtained sequences were edited using the BioEdit software package [10]. Primary comparison of the de novo determined sequences with sequences available in the GenBank database was performed using the BLAST software [http://www.ncbi.nlm.nih.gov/blast]. Phylogenetic analysis and construction of dendrograms was performed using the MEGA 4.0 software package [11].

The newly determined nucleotide sequences have been deposited in GenBank with accession numbers KC967307 and KF182320–KF182322.

The total DNA G+C content was determined by the Owen method [12].

RESULTS AND DISCUSSION

Characterization of strain Ku-2 habitat. The PNB isolation source were samples of cyanobacterial mat formed in the bed of the Kuchiger thermal spring located at the north-western side of the Barguzin ridge 88 km from Kurumkan village (56°52′934″ N, 111°00′050″ E, 570 m above sea level).

The spring water contained trace amounts of sulfide and had a mineralization of 0.45 g/L. The water temperature at the moment of sampling was 40°C, pH 9.6. Microscopic analysis revealed the presence in the bacterial mat of cyanobacteria of the genera *Leptolingbya* and *Sinechococcus*, colorless filamentous sulfur bacteria of the genus *Beggiatoa*, and purple sulfur bacteria of the genus *Thiocapsa*.

Cultural properties and cell morphology and ultrastructure. Under aerobic conditions, strain Ku-2 formed roundish convex colonies that were nearly colorless but turned red with time. In agar columns, small roundish colonies that were light-brown in the anaerobic zone and rose-colored in the aerobic zone formed. In liquid medium, the culture was tinted orange-brown upon anaerobic growth and purple upon aerobic cultivation.

In young cultures, strain Ku-2 cells were nonmotile short oval rods (Figs. 1a and 1b), measuring 1.0 × 1.5 μm. Multiplication occurred by asymmetric division similar to budding (Fig. 1a). Cell wall structure was of the gram-negative type. Upon anaerobic growth in the light, thin-sectioning revealed lamellar intracytoplasmic membranes located along the cell envelope. Cells grown aerobically in the dark were devoid of intracytoplasmic membranes. Storage compounds were represented by roundish electron-transparent inclusions likely to be poly-beta-hydroxybutyrate granules (Fig. 1b). Occasionally, the cells contained small electron-dense granules, apparently composed of polyphosphates. At one of the cell poles, a slimy substance was notable, most probably providing for the attachment of cells to the substrate.

Physiological and biochemical properties. Strain Ku-2 was capable of both photoheterotrophic growth in the light and chemoheterotrophic aerobic growth in the dark. The spectrum of substrates utilized by strain Ku-2 was not essentially different from those of the known strains of *Rba. blasticus* (Table 1). The utilized carbon sources were glucose, fructose, sucrose, ribose, acetate, pyruvate, glutamate, malate, succinate, lactate, propionate, casein hydrolysate, and yeast extract. Neither photoheterotrophic nor chemoheterotrophic growth occurred on pyruvate, benzoate, tartrate, methanol, ethanol, or glycolate as sole carbon sources.

The bacterium did not utilize sulfide or thiosulfate as electron donors either in the light or in the dark. No catalase activity was detected in the cells. Fermentation and denitrification capacities were lacking.

Growth occurred at NaCl concentrations from 0 to 4 g/L, with an optimum at 3 g/L (Fig. 2), and at

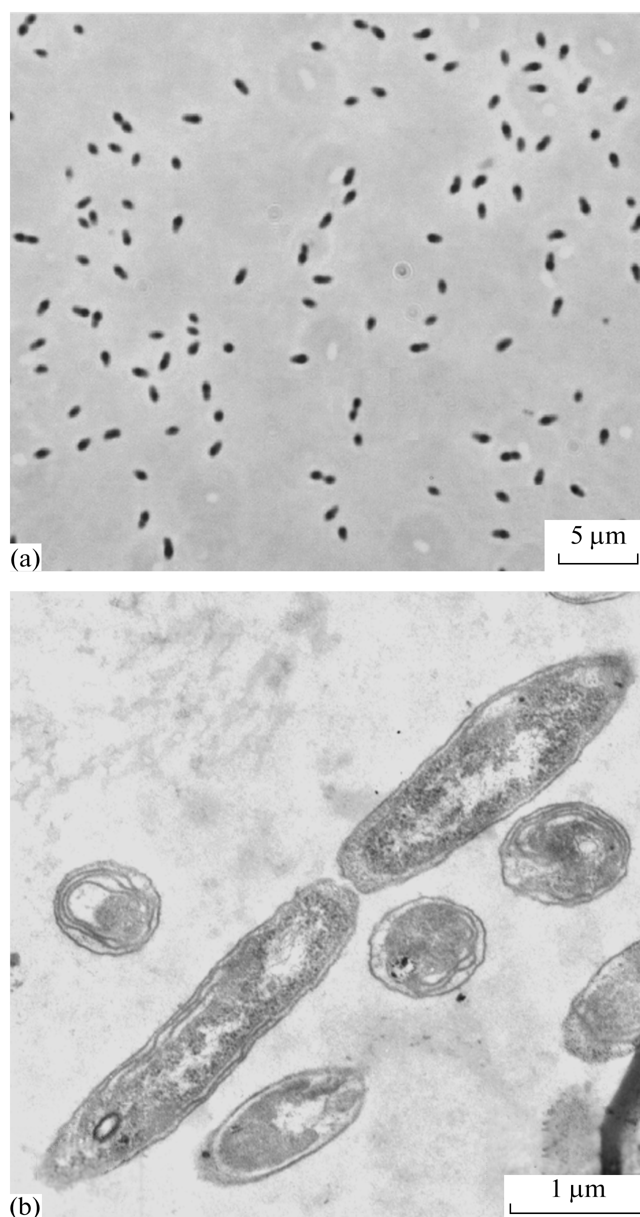

Fig. 1. Morphology and ultrastructure of strain Ku-2 cells: (a) light microscope and (b) electron microscope, ultrathin section showing the lamellar photosynthetic membranes.

pH 6.5–8.5, with an optimum at pH 7.0–8.0. The strain was mesophilic, growing optimally at 25–37°C (Fig. 3). No growth occurred below 10°C or above 42°C.

Like the *Rba. blasticus* type strain ATCC 33485, the new isolate was resistant to the antibiotics ampicillin, tetracycline, aureomycin, vancomycin, and novobiocin and sensitive to amikacin, gentamycin, lincomycin, and mycostatin. However, there were certain differences in the antibiotic sensitivity patterns between *Rba. blasticus* type strain and strain Ku-2, which proved to be resistant to penicillin and nalidixic acid

Table 1. Utilization of organic compounds as carbon sources by strains Rb-5, Ku-2, and *Rba. blasticus* ATCC 33485

Substrates	Rb-5 [3]		*R. blasticus*, ATCC 33485 [1]		Ku-2	
	aerobically	anaerobically	aerobically	anaerobically	aerobically	anaerobically
Glucose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Ribose	+	+	+	+	+	+
Maltose	+	+	+	ND	+	+
Arabinose	+	+	+	ND	+	+
Acetate	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+
Glutamate	+	+	+	+	+	+
Butyrate	+	+	+	+	+	+
Malate	+	+	+	+	+	+
Citrate	+	–	+	+	–	–
Succinate	+	+	+	+	+	+
Lactate	+	+	+	+	+	+
Formate	–	–	–	–	+	+–
Fumarate	–	–	–	+	+	+
Propionate	+	+	+	+	+	+
Benzoate	–	–	–	–	–	–
Tartrate	+	–	+	–	–	–
Ethanol	–	–	–	–	–	–
Methanol	–	–	–	–	–	–
Glycerol	+	+	–	+	–+	+–
Mannitol	+	+	+	+	–	–
Casein hydrolysate	+	+	+	+	+	+
Yeast extract	+	+	+	+	+	+
Glycolate	+	–	–	–	–	–
Catalase activity	+		+			–
Capacity for						
fermentation	–		–			–
denitrification	–		–			–

"+", good growth; "–", no growth; "+–", poor growth; "ND", no data.

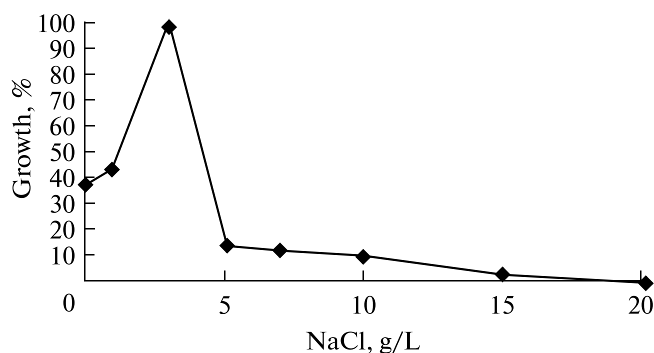

Fig. 2. Strain Ku-2 growth at different NaCl concentrations. The growth at the optimal NaCl concentration was taken as 100%.

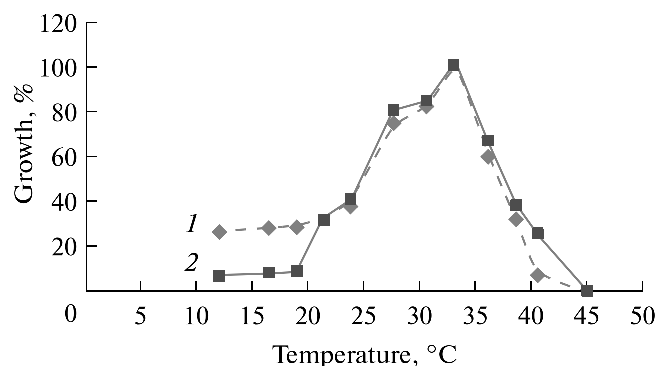

Fig. 3. Strain Ku-2 growth at different temperatures. The growth at the optimal temperature was taken as 100%. *1*, growth under anaerobic conditions; *2*, growth under aerobic conditions.

and sensitive to streptomycin, polymyxin, erythromycin, bacitracin, kanamycin, and neomycin (Table 2).

Pigments. Spectrometric studies of whole cells, membrane fraction, and acetone–methanol extract showed that strain Ku-2 cells contained bacteriochlorophyll *a* and carotenoids of the spheroidene series. In vivo spectrum of whole cells and spectrum of the membrane fraction (Figs. 4–6) exhibited absorption maxima at 451, 479, and 508–510 nm, indicating the presence of carotenoids of the spheroidene series. The maxima at 797–798 and 860–865 nm and the shoulder at 887 nm indicated presence of bacteriochlorophyll *a* in the light-harvesting complexes LH2 and LH1.

Phylogenetic position. Phylogenetic analysis of 16S rRNA gene sequences showed that strain Ku-2 formed a separate cluster together with *Rba. blasticus* strains (Fig. 7). The similarity level between strain Ku-2 and the type strain *Rba. blasticus* ATCC 33485 was 98.7%.

The topology of the phylogenetic tree of amino acid sequences of PufM proteins was similar to the topology of 16S rRNA gene tree. Strain Ku-2 PufM formed a coherent cluster with PufM proteins of *Rba. blasticus* strains (Fig. 8). The difference in PufM amino acid sequences of strain Ku-2 and the type strain *Rba. blasticus* ATCC 33485 was 10.5%.

The PNB strain Ku-2 studied in this work is morphologically and physiologically similar to known strains of the species *Rba. blasticus.* All of these strains, including Ku-2, are similar in the spectra of utilized substrates and in their pH, mineralization, and temperature growth optima. Strain Ku-2 is capable of both anaerobic photoheterotrophic growth in the light and aerobic chemoheterotrophic growth in the dark. It does not utilize sulfide or other reduced sulfur compounds as electron donors for photosynthesis.

It should be mentioned that, in addition to the main maxima typical of known representatives of *Rba. blasticus*, the absorption spectra of strain Ku-2 pigments also exhibited a shoulder at 887 nm, peculiar to *Rhodobacter* representatives whose chromatophores are vesicular. Interestingly, this shoulder turned to a pronounced maximum upon cultivation with periodic illumination (at a light : darkness ratio of 1 : 2). These differences between the absorption spectra are most probably strain-specific.

Comparative analysis of 16S rRNA gene sequences showed that strain Ku-2 is phylogenetically close to *Rba. blasticus*, although its position with respect to other *Rba. blasticus* strains is peripheral. The 16S rRNA gene sequence of strain Ku-2 was 98.7% identical to that of the type strain *Rba. blasticus* ATCC 33485 and 99.1% identical to that of strain *Rba. blasticus* Rb5.

Comparative analysis of PufM amino acid sequences confirmed close relatedness of strain Ku-2 to the species *Rba. blasticus.* However, the difference between PufM amino acid sequences of strain Ku-2 and the type strain ATCC 33485 was appreciable (10.5%).

It can be concluded that strain Ku-2, our isolate from a hydrogen sulfide thermal spring, belongs to the genus *Rhodobacter* and is phylogenetically close to the

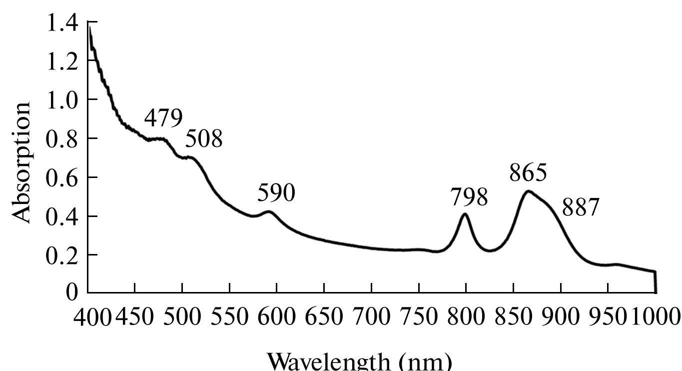

Fig. 4. Absorption spectrum of strain Ku-2 cells upon incubation in the dark.

Table 2. Antibiotic sensitivity of the *Rba. blasticus* type strain and of the two PNB strains under study

Antibiotic	*Rba. blasticus* ATCC 33485 [1]	Strain Rb-5 [3]	Strain Ku-2
Penicillin	–	–	+
Ampicillin	+	+	+
Tetracycline	+	+	+
Streptomycin	+	+	–
Polymyxin	+	+	–
Erythromycin	+	–	–
Nalidixic acid	–	+	+
Amikacin	+	+	–
Gentamycin	+	+	–
Lincomycin	–	+	–
Mycostatin	–	–	–
Bacitracin	+	+	–
Kanamycin	+	+	–
Neomycin	+	+	–
Aureomycin	+	+	+
Vancomycin	+	+	+
Novobiocin	+	+	+

"+", sensitive; "–", insensitive.

species *Rba. blasticus*. However, the conclusion that can be made about the affiliation of strain Ku-2 to this species is tentative and may have to be changed upon analysis of additional close strains.

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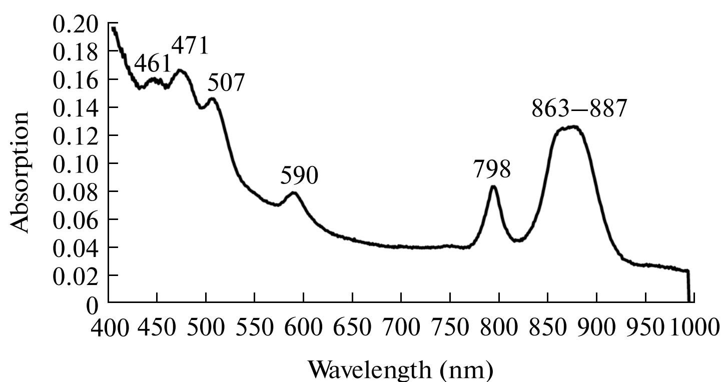

Fig. 5. Absorption spectrum of strain Ku-2 cells upon incubation in the light.

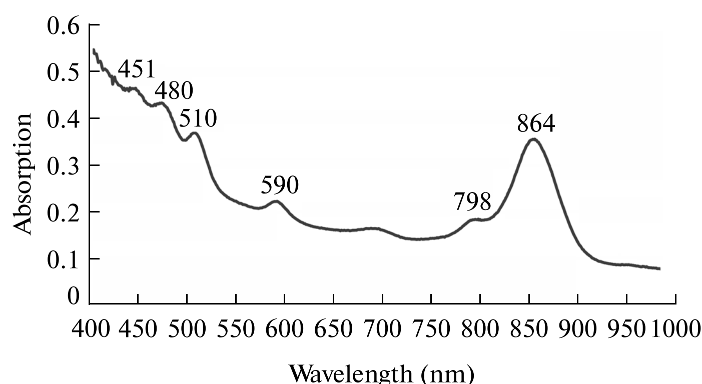

Fig. 6. Absorption spectrum of strain Rb-5 cells.

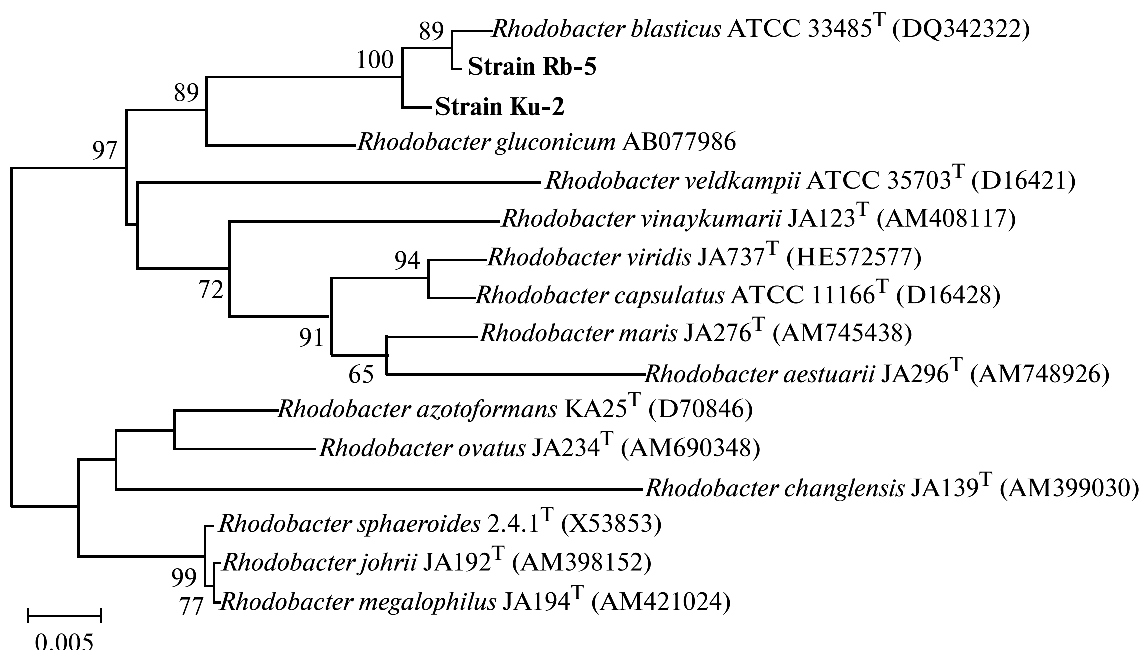

Fig. 7. Phylogenetic dendrogram of strains of *Rhodobacter* species constructed based on 16S rRNA analysis.

Table 3. Comparative characterization of strains Rb-5, Ku-2, and *Rba. blasticus* ATCC 33485

Characteristic	*Rba. blasticus* ATCC 33485 [1]	Strain Rb-5 [3]	Strain Ku-2
Habitat	Eutrophic pond	Cyanobacterial mat in thermal spring	Cyanobacterial mat in thermal spring
Cell division by budding	+	+	+
Cell size and shape, μm	Rods 0.6–0.8 × 1.0–2.5	Rods 0.3 × 1.0–2.0	Rods 1.0 × 1.5
Photosynthetic membranes	Lamellae	Lamellae	Lamellae
Pigments in vivo	378, 418, 476, 506, 590, 795, 862	382, 450, 480, 510, 590, 798, 864	374, 406, 477, 509, 594, 804, 863, 887
Color	Aerobically: orange-red; Anaerobically: rosy-red	Aerobically: orange-red; Anaerobically: rosy-red	Aerobically: orange-red; Anaerobically: rosy-red
Aerobic growth	+	+	+
NaCl	ND	ND	3 g/L
pH	ND	ND	7–8
Temperature, °C	ND	ND	35
G + C, mol %	65.3	66.1	64.4

ND, no data.

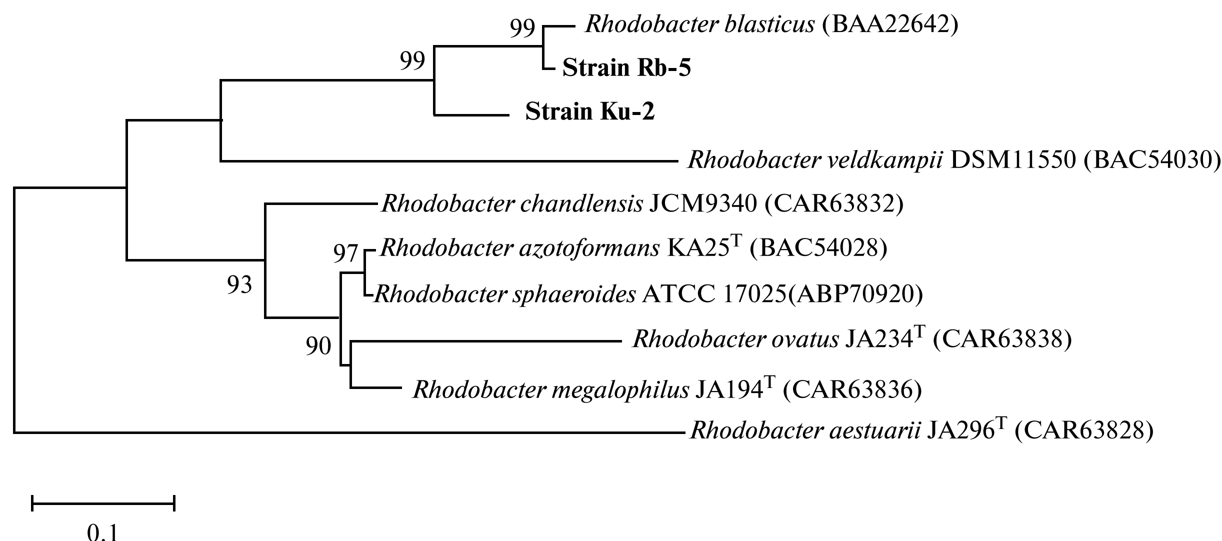

Fig. 8. Phylogenetic dendrogram of strains of *Rhodobacter* species constructed based on analysis of PufM protein amino acid sequences.

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REFERENCES

1. *Bergey's Manual of Systematics Bacteriology,* 1st ed., Staley, J.T., Bryant, M.P., Pfennig, N., and Holt, J.G., Eds., Baltimore: Williams & Wilkins, 1989, vol. 3.

2. Yurkov, V.V. and Gorlenko, V.M., A new strain of the purple nonsulfur bacterium *Rhodopseudomonas blastica*: strain RB-5, isolated from an alkaline sulfide spring, *Mikrobiologiya*, 1992, vol. 61, no. 1, pp. 102–108 (in Russian).

3. Boldareva, E.N., Moskalenko, A.A., Makhneva, Z.K., Tourova, T.P., Kolganova, T.V., and Gorlenko, V.M., *Rubribacterium polymorphum* gen. nov., sp. nov., a novel alkaliphilic nonsulfur purple bacterium from an Eastern Siberian soda lake, *Microbiology* (Moscow), 2009, vol. 78, no. 6, p. 732–740.

4. Netrusov, A.I., Egorova, M.A., Zakharchuk, L.M., Kolotilova, N.N., Kotova, I.B., Semenova, E.V., Tatarinova, N.Yu., Ugol'nikova, N.V., Tsavkelova, E.A., Bobkova, A.F., Bogdanova, A.G., Danilova, I.V., Dinarieva, T.Yu., Zinchenko, V.V., Ismailov, A.D., Kurakov, A.V., Maksimov, V.N., Mil'ko, E.S., Nikitina, E.P., Ryzhkova, E.P., Semenov, A.M., Khomyakova, D.V., Cherdyntseva, T.A., and Yudina, T.G., *Praktikum po mikrobiologii: Ucheb. Posobie dlya stud. vyssh. ucheb. zavedenii* (A Practical Course in Microbiogy: A Manual for Higher Education Students), Moscow: Izdatel'skii tsentr Akademiya, 2005.

5. Tsaplina, I.A., Osipov, G.A., Bogdanova, T.I., Nedorezova, T.P., and Karavaiko, G.I., Fatty acid composition of lipides in thermoacidophilic bacteria of the genus *Sulfobacillus*, *Microbiology* (Moscow), 1994, vol. 63, no. 5, pp. 390–401.

6. Boulygina, E.S., Kuznetsov, B.B., Marusina, A.I., Tourova, T.P., Kravchenko, I.K., Bykova, S.A., Kolganova, T.V., and Galchenko, V.F., A study of nucleotide sequences of *nifH* genes of some methanotrophic bacteria, *Microbiology* (Moscow), 2002, vol. 71, no. 4, pp. 425–432.

7. Lane, D.J., 16S/23S rRNA sequencing, in *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M., Eds., New York: John Wiley and Sons, 1991, pp. 115–175.

8. Beja, O., Suzuki, M.T., Heidelberg, J.F., Nelson, W.C., Preston, C.M., Hamada, T., Eisen, J.A., Fraser, C.M., and DeLong, E.F., Unsuspected diversity among marine aerobic anoxygenic phototrophs, *Nature*, 2002, vol. 415, pp. 630–633.

9. Mao, Y., Wei, J., Zheng, Q., Xiao, N., Li, Q., Fu, Y., Wang, Y., and Jiao, N., *Roseibacterium beibuensis* sp. nov., a novel member of *Roseobacter* clade isolated from Beibu Gulf in the South China Sea, *Curr. Microbiol.*, 2012, vol. 65, pp. 568–574.

10. Hall, T.A., BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symp. Ser.*, 1999, no. 41, pp. 95–98.

11. Tamura, K., Dudley, J., Nei, M., and Kumar, S., MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0, *Mol. Biol. Evol.*, 2007, vol. 24, pp. 1596–1599.

12. Owen, R.J., Hill, L.R., and Lapage, S.P., Determination of DNA base composition from melting profiles in dilute duffers, *Biopolymers*, 1969, vol. 7, pp. 503–516.

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