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Full Paper

Rhodovastum atsumiense gen. nov., sp. nov., a phototrophic alphaproteobacterium isolated from paddy soil

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A photoorganotrophic alphaproteobacterium designated strain G2-11^T was isolated from submerged paddy soil. This bacterium had relatively large, oval to rod-shaped cells (2.0–3.0×3.0–10 μm). Cells were motile by means of single polar flagella. The color of phototrophically growing cultures was reddish-brown. The cell extract had absorption maxima at 375, 465, 492, 529, 592, 804, and 844 nm, indicating the presence of bacteriochlorophyll *a* and carotenoides of the spirilloxanthin series. Vesicular intracytoplasmic membranes were present. The main component of cellular fatty acids was $C_{18:1}\omega7c$. Ubiquinone-10 and rhodoquinone-10 were the major quinones. A 16S rRNA gene sequence analysis revealed that the isolate is closest to the acidophilic aerobic photosynthetic bacterium *Acidisphaera rubrifaciens* strain HS-AP3^T (93.3% similarity). The G+C content of genomic DNA is 67.8 mol%. The name *Rhodovastum atsumiense* gen. nov., sp. nov. is proposed for the novel isolate. The type strain is strain G2-11^T (=NBRC 104268^T=KCTC 5708^T).

Key Words——phototrophic bacteria; purple nonsulfurbacteria; *Rhodovastum atsumiense*

Introduction

Most of the genera of phototrophic purple nonsulfur (PPNS) bacteria established so far belong to the class *Alphaproteobacteria*. To date, 18 genera of PPNS bacteria have been described in this major taxonomic group (Imhoff et al., 2005). The phototrophic alphaproteobacteria are quite versatile in cell morphology as well as in physiology. In general, spiral and curved rod-shaped cells are found in PPNS genera of the family *Rhodospirllaceae*, coccoid cells in those of the *Acetobacteraceae*, oval and coccoid cells in those of the *Rhodobacteraceae*, and budding rods in those of the order *Rhizobiales*. The cell size of PPNS bacteria is less than 2 μm in diameter in most cases.

PPNS bacteria are widely distributed in natural aquatic and terrestrial environments including paddy soil. Representatives of PPNS bacteria isolated from paddy fields are those of the genera *Phaeospirillum* (Hiraishi and Kitamura, 1984), *Rhodobacter* (Hiraishi and Kitamura, 1984), *Rhodopseudomonas* (Akiba et al. 1983; Harada et al., 2003; Hiraishi and Kitamura, 1984; Santos et al., 1991), and *Rubrivivax* (Harada et al., 2003; Ramana et al., 2006). During the course of ecological studies on PPNS bacteria in paddy fields in Japan, we isolated a strain that has relatively large, oval to rod-shaped cells measuring ≥ 2 μm in diameter. 16S rRNA sequence comparisons showed that this novel bacterium, designated strain G2-11^T, represents a distinct phylogenetic position within the *Alphaproteobacteria*. In this paper, we report the taxonomic characteristics of strain G2-11^T and propose the name

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Rhodovastum atsumiense gen. nov., sp. nov. for this bacterium.

Materials and Methods

Isolation and cultivation. A sediment mud sample was collected from an edge of a submerged paddy field in Japan (34°43′N, 137°22′E) on April 1998. This sampling site is located in the east of Atsumi Peninsula, Aichi Prefecture. The sediment mud had a pH of 7.0 and a temperature of 20°C in situ. The sample was taken in a polypropylene tube, kept at ambient temperature during transportation, and tested immediately upon return to the laboratory. Small aliquots of the mud sample were inoculated into 20-ml screw capped tubes containing 10 ml of SAYS medium (pH 6.8) (Okubo et al., 2005), which were then completely filled with the same medium and incubated at 30°C under incandescent illumination (8–10 W m^{-2}). After 2 weeks of incubation, all cultures turned red to brown. Microscopic observations confirmed that relatively large oval to rod-shaped cells predominated in these cultures. One of the enrichment cultures was purified anaerobically by using the standard agar shake dilution method followed by repeated streaking of agar plates under aerobic conditions. The organism thus obtained (designated strain G2-11^T) formed convex, reddish-brown colonies on agar media. Strain G2-11^T was subcultured every 3 months in agar stabs of SAYS medium or MYS medium (Hiraishi and Kitamura, 1984).

Morphological and physiological tests. Strain G2-11^T and *Rhodopila globiformis* DSM 161^T were tested for morphological and physiological characteristics. For testing of strain G2-11^T, liquid precultures were made in SAYS or MYS medium under anaerobic or semi-anaerobic conditions in the light. Precultures of *Rpi*. *globiformis* DSM 161^T were grown phototrophically with fructose and gluconate as the carbon sources as described by Pfennig (1974). Unless otherwise indicated, the initial pH of all test media was adjusted to 6.0 for strain G2-11^T and 4.5 for *Rpi*. *globiformis* DSM 161^T, and incubation was at 30°C. Morphology and related properties were studied under an Olympus phase-contrast microscope and a JEOL transmission electron microscope. Ultrathin-section electron microscopy for observing intracytoplasmic membrane systems was performed as described previously (Matsuzawa et al., 2000). Photoassimilation of organic substrates were determined in screw-capped test tubes completely filled with mineral base RM2 (Hiraishi and Kitamura, 1984) supplemented with an organic carbon source and 0.01% yeast extract. Nitrogen source utilization was determined by replacing $(NH_4)_2SO_4$ in MYS medium with different nitrogen sources at a concentration of 0.1% (w/v). Nitrogen-fixing activity was determined in MYS medium in which $(NH_4)_2SO_4$ was replaced with glutamine as the nitrogen source, and H_2 gas production in test tubes with Durham tubes was judged as being positive for nitrogen fixation. Growth was measured turbidometrically at 660 nm, and a final reading was taken after 2 weeks of incubation. All other physiological and biochemical tests were performed as described previously (Hiraishi and Kitamura, 1984; Okamura et al., 2007).

Photopigment and chemotaxonomic analyses. In vivo absorption spectra were measured for the sonicated cell extract with a Shimadzu Biospec 1600 spectrophotometer. Bacteriochlorophyll (BChl) and carotenoids were extracted with an acetone-methanol mixture (7 : 2, v/v) and analyzed spectrophotometrically. Whole-cell fatty acids were analyzed by gas-liquid chromatography of their methyl ester derivatives as described previously (Suzuki and Hiraishi, 2007). Quinones were extracted with a chloroform-methanol mixture, purified by thin-layer chromatography, and analyzed by HPLC as described previously (Hiraishi and Hoshino, 1984).

DNA base composition. Genomic DNA was extracted and purified according to the method of Marmur (1961). The guanine plus cytosine (G+C) ratio of genomic DNA was determined by the HPLC method with external nucleotide standards as described by Mesbah et al. (1989).

16S rRNA gene sequencing and phylogenetic analysis. The 16S rRNA gene of strain G2-11^T was PCR-amplified, purified, and sequenced directly with an automated DNA sequencer as described previously (Hisada et al., 2007). Sequence data were compiled with the GENETYX program (GENETYX Corporation, Tokyo, Japan) and compared with those retrieved from the database. Multiple alignment of sequence data, calculation of the corrected evolutionary distance (Kimura, 1980), and construction of a neighbor-joining phylogenetic tree (Saitou and Nei, 1987) were performed using the CLUSTAL W program ver. 1.83 (Thompson et al., 1994). The topology of the tree was evaluated by bootstrapping with 1,000 resamplings (Felsenstein, 1985).

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain G2-11^T has been deposited under DDBJ accession number AB381935.

Results and Discussion

Cell morphology and ultrastructure

Strain G2-11^T had Gram-negative, oval to rod-shaped cells measuring 2.0–3.0 μm in width and 3.0–10 μm in length (Fig. 1a). Cells occurred singly or occasionally in pairs and reproduced by binary fission. Phase-contrast microscopy demonstrated that cells in young cultures were motile, and electron micrographs of negatively stained cells revealed the presence of single polar flagella (data not shown). Thin-section electron microscopy showed that phototrophically grown cells formed intracytoplasmic membranes of the vesicular type (Fig. 1b), similar to those of the nearest phylogenetic neighbor of PPNS bacteria, *Rpi*. *globiformis* (Madigan and Imhoff, 2005; Pfennig, 1974) (for phylogenetic information, see below). The formation of vesicular internal membranes by *Rpi*. *globiformis* DSM 161^T was also confirmed by this study. Electron microscopic observations also revealed the presence of poly-β-hydroxybutyric acid-like bodies and electron-dense granules (possibly high-molecular polyphosphate) in phototrophically grown cells of strain G2-11^T.

Photopigments

The color of phototrophic liquid cultures and colonies of strain G2-11^T was red to brown, whereas aerobically grown chemotrophic cultures were colorless. The cell extract from phototrophic cultures had absorption maxima at 375, 465, 492, 529, 592, 804, and 844 nm (Fig. 2), indicating the presence of BChl *a*, the carotenoids of the normal spirilloxanthin series, and the peripheral pigment-protein complex. The presence of BChl *a* was also confirmed by detecting an absorption maximum of the acetone-methanol extract at 770 nm (data not shown).

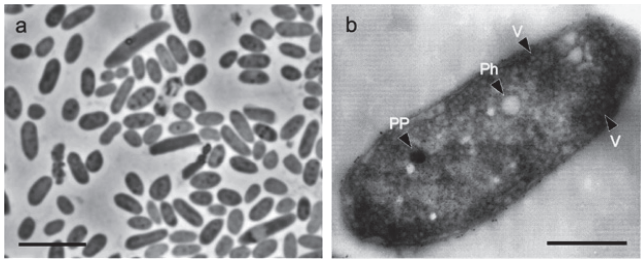

Fig. 1. Cell morphology and ultrastructure of strain G2-11^T. (a) Phase-contrast micrograph of intact cells (scale bar= 10 μm); (b) electron micrograph of ultrathin section of cell (scale bar=1.0 μm). Arrows with letters V, Ph, and PP indicate vesicular intracytoplasmic membranes, a poly-β-hydroxybutyric acid-like body, and a polyphosphate-like granule, respectively.

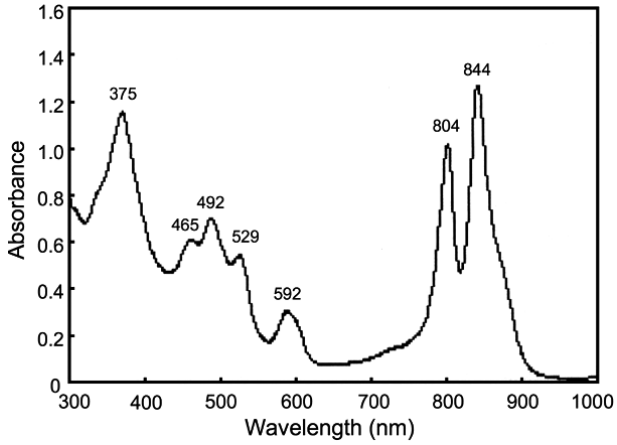

Fig. 2. Absorption spectrum of sonicated cell extract from strain G2-11^T grown phototrophically at a low light intensity (2 W m^{-2}).

Physiology and nutrition

Strain G2-11^T was able to grow anaerobically in the light and aerobically in darkness. Chemotrophic growth was possible at full atmospheric oxygen pressure. Photoautotrophic growth occurred with hydrogen gas (20% head space) but not with 0.5 mM sulfide or 0.5 mM thiosulfate as the electron donor in the presence of 0.01% yeast extract. Neither aerobic chemolithotrophy with thiosulfate nor fermentative growth with fructose or glucose was demonstrated. No anaerobic growth with nitrate as the terminal electron acceptor occurred. The temperature range for growth was 20 to 40°C with the optimum at 30–35°C. The pH range for growth was 5.0 to 8.5 with the optimum 6.0–6.5. Growth occurred in the absence of added NaCl. Little or no growth was found at a NaCl concentration of more than 1.0% (w/v). Biotin and *p*-aminobenzoic acid were required as growth factors.

Strain G2-11^T grew well with simple organic compounds as electron donors and carbon sources. Good carbon sources for phototrophic growth were acetate, pyruvate, succinate, fumarate, malate, malonate, gluconate, D-xylose, D-fructose, L-alanine, L-glutamate, L-glutamine, and yeast extract. Other usable carbon sources were valerate, caproate, lactate, L-arabinose,

D-glucose, D-glactose, D-mannose, trehalose, D-mannitol, D-sorbitol, ethanol, propanol, butanol, L-asparagine, L-aspartate, peptone, and Casamino acids. Not utilized were formate, propionate, butyrate, caprylate, citrate, glycolate, tartrate, palmitate, nicotinate, benzoate, L-rhamnose, lactose, sucrose, D-dulcitol, glycerol, methanol, L-leucine, or L-methionine. The usable nitrogen sources were ammonium salts, nitrate, glutamate, glutamine, and urea. Nitrogen-fixing activity could be demonstrated by observing hydrogen gas production with glutamine as the sole nitrogen source. Nitrite did not support growth. Sulfate was assimilated as the sulfur source.

The results of carbon and nitrogen nutrition tests revealed major phenotypic differences between strain G2-11^T and its phylogenetic relative, *Rpi*. *globiformis* DSM 161^T (see Table 2).

Chemotaxonomic characteristics

A whole-cell fatty acid analysis revealed that the unsaturated component $C_{18:1}\omega7c$ predominated (65.5%) in strain G2-11^T (Table 1). A significant proportion of $C_{16:0}$ (9.5%) was also detected. $C_{18:0}$ 3-OH (2.9%) was the most abundant component of hydroxy fatty acids. These fatty acid patterns resemble those of the phylogenetic relative *Rpi*. *globiformis* (Urakami and Komagata, 1988). Quinone profiling showed that both ubiquinone-10 and rhodoquinone-10 were present as the primary quinone components. The molar ratio of rhodoquinones to ubiquinones detected under phototrophic growth conditions was ca. 0.5. Menaquinones were absent.

Table 1. Cellular fatty acid composition of strain G2-11^T.

Component	Composition (mol%)
Saturated	
$C_{14:0}$	0.4
$C_{15:0}$	0.5
$C_{16:0}$	9.5
$C_{17:0}$	1.2
$C_{18:0}$	4.9
10 Methyl $C_{17:0}$	2.2
10 Methyl $C_{19:0}$	0.3
Unsaturated	
$C_{16:1}\omega5c$	0.8
$C_{16:1}\omega7c$	2.5
$C_{16:1}\omega7c$ alcohol	0.8
$C_{17:1}\omega6c$	3.0
$C_{17:1}\omega8c$	0.3
$C_{18:1}\omega5c$	1.2
$C_{18:1}\omega7c$	65.5
$C_{18:1}\omega9c$	0.5
cyclo-$C_{19:0}\omega8c$	0.5
Hydroxy	
$C_{15:0}$ 3-OH	0.3
$C_{16:0}$ 3-OH	0.6
iso-$C_{16:0}$ 3-OH	0.9
$C_{18:0}$ 3-OH	2.9
Sum in Feature 2[a]	1.3

[a]3OH $C_{14:0}$/$C_{16:1}$ iso I.

Phylogeny and DNA base composition

16S rRNA gene sequence comparisons revealed that strain G2-11^T is most similar to the aerobic photosynthetic bacterium *Acidisphaera rubrifaciens* strain HS-AP3^T (Hiraishi et al., 2000) at a similarity level of 93.3%. Among the type strains of PPNS species, *Rpi*. *globiformis* DSM 161^T is closest to strain G2-11^T (91.3% similarity). The neighbor-joining phylogenetic tree constructed clearly showed that the novel strain is a member of the family *Acetobacteraceae* and represents a distinct phylogenetic position with the genera *Acidisphaera* and *Rhodopila* as its sister group (Fig. 3).

The G+C content of the genomic DNA of strain G2-11^T as determined by HPLC was 67.8 mol%.

Taxonomic consideration

As reported herein, it is clear that the novel strain G2-11^T represents a distinct lineage within the family *Acetobacteraceae* with *Acs*. *rubrifaciens* and *Rpi*. *globiformis* as its phylogenetic neighbors. The 16S rRNA gene sequence similariy between strain G2-11^T and *Acs*. *rubrifaciens* or *Rpi*. *globiformis* seems low enough (<94%) to classify the former strain into a novel genus. This generic allocation for strain G2-11^T is supported unequivocally by the phenotypic data (Table 2). One of the most characteristic features of strain G2-11^T is its large cell size (2–3×3–10 μm). To our knowledge, this bacterium is largest in cell diameter among species of PPNS bacteria so far described. Strain G2-11^T can also be differentiated from *Acs*. *rubrifaciens* and *Rpi*. *globiformis* in a number of phenotypic traits, such as color of cultures, near infrared peak of BChl *a*, optimum pH for growth, carbon nutrition, and quinone composition. A combination of several phenotypic characteristics is useful for differentiation of strain G2-11^T from all other genera of the family *Acetobacteraceae*. Therefore, we propose *Rhodovastum atsumiense* gen. nov., sp. nov. to accommodate strain G2-11^T,

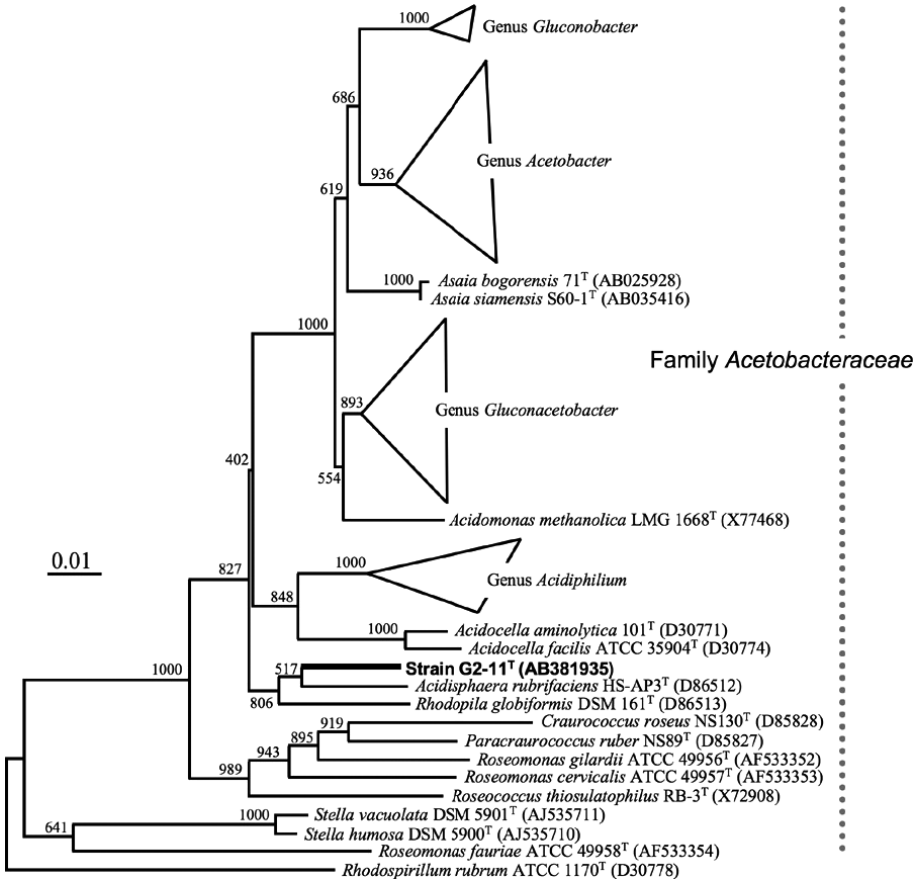

Fig. 3. Neighbor-joining distance-matrix tree showing phylogenetic relationships of strain G2-11^T to other members of the family *Acetobacteraceae* based on 16S rRNA gene sequences.

The 16S rRNA gene sequence of *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup to root the tree. The database accession numbers are given in parentheses following the strain names. Bootstrap confidence values expressed as the percentage of 1,000 bootstrap trials are given at branched points. Scale bar = 1% sequence divergence (K_{nuc}).

which has been deposited with the NITE Biological Resource Center (NBRC), Kisarazu, Japan, as strain NBRC 104268 and the Korean Collection for Type Cultures (KCTC), Daejeon, Korea, as strain KCTC 5708.

Description of *Rhodovastum* gen. nov.

Rhodovastum (Rho.do.vas'tum. Gr. n. *rhodon*, the rose; L. adj. *vastum* empty, vast; M.L. neut. n. *Rhodovastum* the vast rose, referring to the cell color and size).

Cells are Gram-negative ovals or rods. Multiply by binary fission. Motile by means of a single polar flagellum. Facultative phototrophs growing anaerobically in the light and aerobically in darkness. Phototrophic cultures are red to brown. Phototrophically grown cells contain intracytoplasmic membranes of the vesicular type together with bacteriochlorophyll *a* and carotenoides of the normal spirilloxanthin series. Mesophilic, slightly acidophilic, and nonhalophilic. Photoorganotrophy with simple organic compounds is the preferred mode of growth. Sulfide may not be utilized. The main component of cellular fatty acids is $C_{18:1}\omega7c$. 3-OH $C_{18:0}$ is present. Ubiquinone-10 and rhodoquinone-10 are the major quinones. The phylogenetic position is in the family *Acetobacteraceae*. Habitat: paddy fields. The type species is *Rhodovastum atsumiense*.

Description of *Rhodovastum atsumiense* sp. nov.

Rhodovastum atsumiense (a.tsu.mi.en'se. N.L. neut. adj. *atsumiense* pertaining to Atsumi Peninsula, Japan,

Table 2. Differential characteristics of *Rhodovastum atsumiense* gen. nov., sp. nov. and phylogenetically related species of phototrophic bacteria.

Characteristics	*Rhodovastum atsumiense*	*Rhodopila globiformis*[b]	*Acidisphaera rubrifaciens*[c]
Cell shape	Ovals, rods	Cocci	Cocci, short rods
Cell diameter (μm)	2.0−3.0	1.6−1.8	0.7−0.9
Motility by means of flagella	+	+	−
Color of cultures	Red-brown	Purple-red	Salmon-pink
Near IR peak for BChl *a*	844	865	874
Internal membrane system	Vesicular	Vesicular	None
Anaerobic phototrophy	+	+	−
Aerobic growth at full atmospheric tension	+	−	+
Optimum pH for growth	6.0−6.5	4.8−5.0	4.5−5.0
Growth at pH 7	+	−	−
Vitamin required	Biotin, *p*-ABA	Biotin, *p*-ABA	nd
Electron donor/carbon source[a]			
Acetate	+	−	−
Valerate	+	−	−
Caproate	+	−	−
Lactate	+	−	+
Tartrate	−	+	−
Mannose	+	−	d
Sorbitol	+	−	+
Glycerol	−	−	+
Asparagine	+	−	−
Aspartate	+	−	−
Glutamate	+	−	−
Glutamine	+	−	−
Peptone (0.05%)	+	+	−
Casamino acids	+	−	−
Quinone(s)	Q-10, RQ-10	Q-9(10), MK-9(10), RQ-9(10)	Q-10
Mol% G+C of DNA	67.8 (HPLC)	66.3 (Bd)	69.1−69.8 (HPLC)
Habitat	Paddy soil	Acidic freshwater	Acidic spring, acidic mine water

Symbols and abbreviations: +, present or positive; −, absent or negative; d, present or positive in some strains but absent or negative in other strains; nd, not determined; *p*-ABA, *p*-aminobenzoic acid; Q-*n*, ubiquinone with *n* isoprene units; RQ-*n*, rhodoquinone with *n* isoprene units; MK-*n*, menaquinone with *n* isoprene units; Bd, buoyant density.

[a]The substrates utilized by all species are D-fructose, D-glucose, D-mannitol, ethanol, pyruvate, gluconate, fumarate, malate, and yeast extract. The substrates utilized by none of the species are formate, propionate, butyrate, caprylate, citrate, benzoate, L-rhamnose, lactose, sucrose, D-dulcitol, methanol, and L-leucine.

[b]Based on information from Madigan et al. (2005) and this study.

[c]Based on information from Hiraishi et al. (2000).

where the type strain of this organism was isolated).

Cells are Gram-negative and oval to rod-shaped, measuring 2.0−3.0 μm in width and 3.0−10 μm in length. Multiply by binary fission. Motile by means of a single polar flagellum. Internal photosynthetic membranes are of the vesicular type. The color of phototrophic cultures is red to brown, while aerobic cultures are colorless. Phototrophically grown cells have absorption maxima at 375, 465, 492, 529, 592, 804, and 844 nm. Anaerobic photoorganotrophy is the preferred mode of growth. Aerobic growth in darkness occurs at full atmospheric oxygen pressure. The temperature range for growth is 20−40°C (optimum 30−35°C). The pH range for phototrophic growth is 5.0−8.5 (optimum pH 6.0−6.5). Little or no growth occurs in the presence of 1.0% NaCl or more. Biotin and *p*-amino-

benzoic acid are required for growth. Photolithotrophic growth occurs with H_2 but not with thiosulfate or sulfide as the electron donor. The following organic compounds are photoassimilated: acetate, valerate, caproate, lactate, pyruvate, succinate, fumarate, malate, malonate, L-arabinose, D-xylose, D-fructose, D-glucose, D-mannose, D-galactose, glycerol, ethanol, butanol, propanol, asparagine, aspartate, glutamate, glutamine, yeast extract, peptone, and Casamino acids. Not utilized are formate, propionate, butyrate, caprylate, citrate, tartarate, glycolate, nicotinate, palmitate, benzoate, L-rhamnose, sucrose, lactose, trehalose, dulcitol, methanol, leucine, and methionine. Ammonium chloride, molecular nitrogen, nitrate, glutamate, glutamine, and urea are utilized as nitrogen sources, while nitrite does not support growth. Sulfate is assimilated as the sulfur source. The predominant component of cellular fatty acids is $C_{18:1}\omega7c$. $C_{18:0}$ 3-OH is the main hydroxy fatty acid component. The major quinones are ubiquinone-10 and rhodoquinone-10. The G+C content of the DNA is 67.8 mol% (by HPLC). The phylogenetic position is in the family *Acetobacteraceae*. The natural habitat is paddy fields. The type strain is strain G2-11^T (=NBRC 104268^T= KCTC 5708^T).

Acknowledgments

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References

Akiba, T., Usami, R., and Horikoshi, K. (1983) *Rhodopseudomonas rutila*, a new species of nonsulfur purple photosynthetic bacteria. *Int*. *J*. *Syst*. *Bacteriol*., **33**, 551–556.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.

Harada, N., Otsuka, S., Nishiyama, M., and Matsumoto, S. (2003) Characteristics of phototrophic purple bacteria isolated from a Japanese paddy soil. *Soil Sci*. *Plant Nutr*., **49**, 521–526.

Hiraishi, A. and Hoshino, Y. (1984) Distribution of rhodoquinone in *Rhodospirillaceae* and its taxonomic implications. *J*. *Gen*. *Appl*. *Microbiol*., **30**, 435–448.

Hiraishi, A. and Kitamura, H. (1984) Distribution of phototrophic purple nonsulfur bacteria in activated sludge systems and other aquatic environments. *Bull*. *Jpn*. *Soc*. *Sci*. *Fish*., **50**, 1929–1937.

Hiraishi, A., Matsuzawa, Y., Kanbe, T., and Wakao, N. (2000) *Acidisphaera rubrifaciens* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from acidic environments. *Int*. *J*. *Syst*. *Evol*. *Microbiol*., **50**, 1539–1546.

Hisada, T., Okamura, K., and Hiraishi, A. (2007) Isolation and characterization of phototrophic purple nonsulfur bacteria from *Chloroflexus* and cyanobacterial mats in hot springs. *Microbes Environ*., **22**, 405–411.

Imhoff, J. F., Hiraishi, A., and Süling, J. (2005) Anoxygenic phototrophic purple bacteria. *In* Bergey's Manual of Systematic Bacteriology, 2nd ed., Vol. 2, The *Proteobacteria*, Part A, Introductory Essays, ed. by Brenner, D. J., Krieg, N. R., Staley, J. T., and Garrity, G. M. (Editor-in-Chief), Springer, New York, pp. 119–132.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J*. *Mol*. *Evol*., **16**, 111–120.

Madigan, M. T. and Imhoff, J. F. (2005) Genus XI *Rhodopila*. *In* Bergey's Manual of Systematic Bacteriology, 2nd ed., Vol. 2, ed. by Brenner, D. J., Krieg, N. R., Staley, J. T., and Garrity, G. M. (Editor-in-Chief), Springer, New York, pp. 83–85.

Marmur, J. (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J*. *Mol*. *Biol*., **3**, 208–218.

Matsuzawa, Y., Kanbe, T., Suzuki, J., and Hiraishi, A. (2000) Ultrastructure of the acidophilic aerobic photosynthetic bacterium *Acidiphilium rubrum*. *Curr*. *Microbiol*., **40**, 398–401.

Mesbah, M., Premachandran, U., and Whitman, W. B. (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int*. *J*. *Syst*. *Bacteriol*., **39**, 159–167.

Okamura, K., Hisada, T., and Hiraishi, A. (2007) Characterization of thermotolerant purple nonsulfur bacteria isolated from hot-spring *Chloroflexus* mats and the reclassification of "*Rhodopseudomonas cryptolactis*" Stadtwald-Demchick et al. 1990 as *Rhodoplanes cryptolactis* nom. rev., comb. nov. *J*. *Gen*. *Appl*. *Microbiol*., **53**, 357–361.

Okubo, Y., Futamata, H., and Hiraishi. A. (2005) Distribution and capacity for utilization of lower fatty acids of phototrophic purple nonsulfur bacteria in wastewater environments. *Microbes Environ*., **20**, 135–143.

Pfennig, N. (1974) *Rhodopseudomonas globiformis* sp. n., a new species of the Rhodospirillaceae. *Arch*. *Microbiol*., **100**, 197–206.

Ramana, Ch. V., Sasikala, Ch., Arunasri, K., Anil Kumar, P., Srinivas, T. N. R., Shivaji, S., Gupta, P., Süling, J., and Imhoff, J. F. (2006) *Rubrivivax benzoatilyticus* sp. nov., an aromatic, hydrocarbon-degrading purple betaproteobacterium. *Int*. *J*. *Syst*. *Evol*. *Microbiol*., **56**, 2157–2164.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol*. *Biol*. *Evol*., **4**, 406–425.

Santos, T. S., Hiraishi, A., Sugiyama, J., and Komagata, K.

(1991) Identification of the diazotrophic bacteria previously referred to as *Protomonas*-like bacteria, and their capacity for nitrogen fixation. *J*. *Gen*. *Appl*. *Microbiol*., **37**, 331–340.

Suzuki, S. and Hiraishi, A. (2007) *Novosphingobium naphthalenivorans* sp. nov., a naphthalene-degrading bacterium isolated from polychlorinated-dioxin-contaminated environments. *J*. *Gen*. *Appl*. *Microbiol*., **53**, 221–228.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequencing weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*., **22**, 4673–4680.

Urakami, T. and Komagata, K. (1988) Cellular fatty acid composition with special reference to the existence of hydroxy fatty acids, and the occurrence of squalene and sterols in species of *Rhodospirillaceae* genera and *Erythrobacter longus*. *J*. *Gen*. *Appl*. *Microbiol*., **34**, 67–84.