***Rhodopseudomonas faecalis* sp. nov., a phototrophic bacterium isolated from an anaerobic reactor that digests chicken faeces**

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Five isolates of a previously undescribed species of purple non-sulfur phototrophic bacteria were characterized. They were Gram-negative, had mobile, budding vibrioid cells and contained lamellar intracytoplasmic membranes. Cultures produced red pigments in the light. Live cells of photosynthetic cultures exhibited absorption maxima at 382, 460–464, 494–496, 534–538, 596, 804–806 and 870–874 nm, indicating the presence of bacteriochlorophyll *a* and carotenoids belonging to the spirilloxanthin series in cells. The new isolates grew anaerobically or microaerobically in the light, but not aerobically in the dark. Optimal growth occurred at 35–40 °C and at pH 6.5–8.5. Various organic compounds were used as photosynthetic electron donors and carbon sources. Sulfate was used as sulfur source for growth. Ubiquinone 10 was synthesized as the major quinone. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain gc^T, a representative of the new isolates, was closest to *Rhodopseudomonas palustris*, with a similarity of 97.5%. DNA–DNA hybridization further distinguished strain gc^T from *Rhodopseudomonas palustris* at the species level. Therefore, the name *Rhodopseudomonas faecalis* sp. nov. was proposed for the new isolates. The type strain is gc^T (= AS1.2176^T = JCM 11668^T).

Keywords: purple non-sulfur phototroph, *Rhodopseudomonas*, 16S rRNA, taxonomy

INTRODUCTION

Purple non-sulfur phototrophs occur widely in environments. Most species of this bacterial group, like the species of the genus *Rhodopseudomonas*, are able to grow anaerobically in the light or aerobically in the dark with different carbon sources and electron donors. In *Bergey's Manual of Systematic Bacteriology*, the genus *Rhodopseudomonas* included seven species: *Rhodopseudomonas palustris*, *Rhodopseudomonas viridis*, *Rhodopseudomonas sulfoviridis*, *Rhodopseudomonas acidophila*, *Rhodopseudomonas blastica*, *Rhodopseudomonas rutila* and *Rhodopseudomonas marina* (Imhoff & Trüper, 1989). Since then, the

taxonomy of *Rhodopseudomonas* has been subject to significant changes on the basis of increasing molecular and chemotaxonomic information. These changes include the rejection of *Rhodopseudomonas rutila* from this genus (Hiraishi *et al.*, 1992), the reclassification of *Rhodopseudomonas rosea* and *Rhodopseudomonas marina* as *Rhodoplanes roseus* and *Rhodobium marinum* (Hiraishi & Ueda, 1994; Hiraishi *et al.*, 1995), and the transfer of *Rhodopseudomonas blastica* to the genus *Rhodobacter* (Kawasaki *et al.*, 1993). *Rhodopseudomonas viridis* and *Rhodopseudomonas sulfoviridis* were transferred to a new genus, *Blastochloris* (Hiraishi, 1997). Most recently, *Rhodopseudomonas acidophila* has been transferred to a newly established genus, *Rhodoblastus* (Imhoff, 2001).

In this paper, we describe the isolation, characterization and identification of a new phototrophic purple non-sulfur bacterium that was isolated from an anaerobic reactor digesting chicken faeces. Based on

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The GenBank accession number for the 16S rRNA gene sequence of strain gc^T is AF123085.

the phenotypic characteristics and molecular studies on the isolates, we classified the bacterium as a new species of the genus *Rhodopseudomonas*, for which the name *Rhodopseudomonas faecalis* is proposed.

METHODS

Bacterial strains, media, and cultivation. Out of five isolates obtained from the effluent of an anaerobic digester treating chicken manure, strain gc^T was studied and characterized in detail. *Rhodopseudomonas palustris* ATCC 17001^T and ATCC 33872 were kindly provided by Professors J. F. Imhoff (Kiel, Germany) and T. Nakase (RIKEN, Japan), respectively.

ATM medium was modified from AT medium (Imhoff & Trüper, 1992) by addition of 0.1 % sodium acetate and 0.1 % sodium succinate, and by deletion of sodium bicarbonate. ATYP medium was created by addition of 0.05 % yeast extract and 0.03 % peptone to ATM medium. ATM was used for physiological and chemical tests, and ATYP medium was used for growth and maintenance of the strains. Agar plates were prepared by addition of 1.5 % agar to the medium. Soft agar was made by reducing the amount of agar to 0.7 %.

Routine cultivation was done anaerobically in screw-capped tubes or bottles filled with medium. Tubes or bottles were flushed with helium or nitrogen gas to remove oxygen. Incubation was conducted at 30–35 °C under incandescent illumination of 1000–3000 lx.

Isolation. Samples were obtained from the effluent of an anaerobic digester treating chicken manure, located in the Northwest of China (Shan Xi province). The samples were diluted and inoculated into soft-agar tubes. The tubes were incubated anaerobically in the light. After 1 week cultivation, small red colonies appeared. Colonies were picked up and streaked on agar plates. The plates were placed in anaerobic jars, which were filled with argon and incubated under illumination. This was repeated three to five times to ensure the purity of the culture.

Microscopy. Morphology and ultrastructure of cells grown under illumination were observed by optical microscopy (Olympus BH-2) and by scanning (S-570; Hitachi) and transmission (H-600; Hitachi) electron microscopy.

Absorption spectrum. The absorption spectrum of intact cells grown under illumination was measured with spectrophotometers HP1050 (Hewlett Packard) for 300–820 nm, and Hitachi (model Hitachi) for 800–1400 nm.

Physiological characterization. Tests for photoassimilation of organic substrates were performed in screw-capped tubes containing ATM medium as described in *Bergey's Manual* (Imhoff & Trüper, 1989).

DNA base composition and DNA–DNA hybridization. Genomic DNA was extracted and purified according to the method of Marmur (1961) except for the addition of protease K in the SDS-treating step. DNA base composition was determined by thermal denaturation (Marmur & Doty, 1962). DNA–DNA hybridization was carried out as described by Tindall *et al.* (1984) with a minor modification: DNA fragments were labelled with α -³²P according to the instructions provided with the nick translation kit (Boehringer Mannheim).

Phylogenetic analysis. A 16S rDNA fragment was amplified and sequenced by using the MicroSeq 16S rDNA Gene Kit (ABI Prism, 377 DNA Sequencer, Applied Biosystems). The 16S rRNA gene was amplified by using two primers: 27f (5'-GAG AGT TTG ATC CTG GCT CAG-3') and 1541r (5'-AAG GAG GTG ATC CAG CC-3'). Sequence alignment and analysis of the similarity of the 16S rRNA gene were performed with the CLUSTAL W program (Thompson *et al.*, 1994), and the SEQBOOT, DNADIST, NEIGHBOR-JOIN and CONSENSE programs of the software package PHYLIP version 3.5c (Felsenstein, 1993).

Quinone determination. Quinones were determined following Collins (1985) and Wu *et al.* (1989). The collected bacteria pellet was lyophilized, and treated with chloroform and methanol (2:1, v/v). The quinones were separated on a 254 GF silica plate with n-hexane ethylether as the developing solvent. The number of isoprene units and the relative percentage of components of quinones were determined by HPLC (HP1074; Hewlett Packard) using the column Bondapak C18 (3.9 × 300 mm, Waters). *Rhodopseudomonas palustris* ATCC 17001^T and quinone 10 (standard sample) were used as the control.

Nucleotide sequence accession numbers. The GenBank accession number for the 16S rRNA gene sequence of the test strain gc^T is AF123085. The accession numbers of the reference strains are as follows: *Rhodopseudomonas palustris* ATCC 17001^T, D25312; *Bradyrhizobium japonicum* USDA 110, Z35330; '*Bradyrhizobium lupini*', X87273; *Nitrobacter hamburgensis* X14^T, L35502; *Nitrobacter winogradskyi* ATCC 25381^T, L35506; *Afipia felis* ATCC 53690, M65248; *Rhodoblastus acidophilus* ATCC 25092, M34128; *Blastochloris viridis* ATCC 19567, D25314; *Blastochloris sulfoviridis* DSM 729, D86514; *Rhodoplanes roseus* DSM 5906^T, D25313; *Rhodopseudomonas rhenobacensis*, AJ132402; *Rhodoplanes elegans* AS130, D25311; *Rhodobacter azotoformans* KA25, D70846; *Rhodobacter sphaeroides* ATCC 17023, X53855; *Rhodobacter capsulatus* ATCC 11166^T, D16428; *Rhodobium orientis* JCM 9337, D30792; *Rhodobium marinum* DSM 2698^T, D30790; *Rhodospirillum rubrum* ATCC 11170^T, D30778; *Phaeospirillum fulvum* ATCC 15798, D14433; *Rubrivivax gelatinosus* ATCC 17011^T, D16214.

RESULTS

Isolation

The effluent from an anaerobic reactor digesting chicken faeces became reddish after illumination in a storage tank. We judged that the colour change resulted from the growth of phototrophic purple bacteria. Indeed, five isolates were obtained by direct cultivation of diluted samples of effluent. One isolate, strain gc^T, was studied further.

Morphology and ultrastructure

Strain gc^T had Gram-negative vibrioid or peanut-shaped cells. The cells were 0.6–0.8 µm in width and 1.2–2.0 µm in length (Fig. 1). The cells multiplied by budding and formed rosette-like clusters in old cultures. They were mobile by means of a single polar flagellum (Fig. 2). Electronic microscopy revealed that

Fig. 1. Morphology of strain gc^T . Bar, 6 μ m.

Fig. 2. Polar flagellum of strain gc^T . Bar, 6 μ m.

phototrophically grown cells formed intracytoplasmic membranes of the lamellar type (Fig. 3), typical of the genus *Rhodopseudomonas*.

Photopigments and quinones

Round or lens-shaped colonies were formed in agar cultures or reddish suspension produced in liquid cultures when grown anaerobically under illumination. The absorbance maxima of phototrophically grown cells were 382, 460, 494, 534, 596, 806 and 870 nm, indicating the presence of bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series. Examination of lipid extracts of lyophilized cells revealed that the predominant ubiquinone was ubiquinone 10. No menaquinone was detected.

Fig. 3. Lamellar intracytoplasmic membranes of strain gc^T . Bar, 6 μ m.

Growth and physiological and biochemical characteristics

The isolates were able to grow anaerobically in the light. They could not form colonies in darkness at full atmospheric oxygen tension, which is different from *Rhodopseudomonas palustris*. Strain gc^T grew in the temperature range from 28 to 45 °C, with an optimum of 38 °C. When the five isolates were grown phototrophically in ATYP medium they exhibited a doubling time of 4–6 h. They could not grow under anaerobic conditions in the dark, even when nitrate or DMSO was added as terminal electron acceptor. The pH range for growth was 6.0–8.5, with an optimum of 7.0. No growth was found in the medium with 3 % NaCl. Thiamin was required for growth, but it could be replaced by 0.01 % yeast extract.

Strain gc^T was different from other species of the genus *Rhodopseudomonas* in carbon source assimilation. It showed a strong tendency for photoorganotrophy with simple organic compounds as electron donors and carbon sources (Table 1). Acetate, pyruvate, lactate, malate, succinate and butyrate supported good growth. Fumarate and propionate supported moderate growth. No growth was found with benzoate, citrate, formate, gluconate, tartrate, rhamnose, sorbitol, ethanol, methanol, mannitol, aspartate or glutamate. Strain gc^T grew photoautotrophically with hydrogen and sodium bicarbonate. Sulfide and thio-sulfate were not utilized as the electron donor. Ammonium salts and glutamate were used as nitrogen sources. Sulfate was used as sulfur source for growth.

G + C mol % and DNA–DNA hybridization

The G + C content of the genomic DNA for strain gc^T was 63.6 mol %. Strain gc^T showed low levels of

Table 1. Differential characteristics of strain gc^T, *Rhodopseudomonas palustris* and *Rhodopseudomonas rhenobacensis*

+, Good growth; –, no growth; ±, some strains only; b, biotin; t, thiamin dichloride; p-ABA, *p*-aminobenzoic acid; ND, not done. Data for *Rhodopseudomonas palustris* and *Rhodopseudomonas rhenobacensis* were obtained from Imhoff & Trüper (1989) and Hougardy *et al.* (2000).

Characteristic	Strain gc ^T	<i>Rhodopseudomonas palustris</i> ATCC 17001 ^T	<i>Rhodopseudomonas rhenobacensis</i> AJ132402
Aerobic growth in dark	–	+	+
Optimum pH	7.0	6.9	5.5
Cell diameter (µm)	0.5–0.8	0.6–0.9	0.4–0.7
Cell shape	Vibrioid or peanut shape	Rod	Rod
Growth factor	t	p-ABA, b (some)	p-ABA
Nitrate-reduction	–	–	+
Utilization of:			
Aspartate	–	+*	ND
Benzoate	–	+	–
Ethanol	–	+	+
Formate	–	–	+
Fructose	±	±	–
Gluconate	–	+*	+
Glucose	±	±	–
Glutamate	–	–*	–
Propionate	+	+	–
Tartrate	–	–	+
Xylose	–	+	ND

* Data are modified according to our results.

Fig. 4. Distance matrix tree showing phylogenetic relationships between the new isolate and related organisms. Bootstrap confidence values obtained from 1000 bootstrap replications are given at the branch points.

DNA–DNA hybridization (17.7%) to *Rhodopseudomonas palustris* ATCC 17001^T. The low level of genomic DNA relatedness supported the establishment of a new species of the genus *Rhodopseudomonas*.

Phylogenetic relationships

The 1444 bp representing more than 95 % of the entire 16S rRNA gene were amplified from strain gc^T and the

sequence was determined. It had four degenerate bases at positions 258, 268, 379 and 384 (corresponding to *Escherichia coli* 16S rRNA numbering). A similarity search was done in the Ribosomal Database Project (RDP) (Maidak *et al.*, 2001). The results showed that the sequence of strain gc^T is closest to *Rhodopseudomonas palustris* ATCC 17001^T.

The 16S rDNA sequence of strain gc^T was compared with a dataset consisting of 20 16S rRNA genes available from the RDP, including 15 of purple non-sulfur bacteria and 5 of closely related non-phototrophs of the α -subclass of the *Proteobacteria*. Strain gc^T was closest to *Rhodopseudomonas palustris* (97.5%) and *Rhodopseudomonas rhodobacensis* (97%). It also showed high similarities to five chemotrophic bacteria species (> 97% similarity to *Bradyrhizobium japonicum*, '*Bradyrhizobium lupini*', *Nitrobacter hamburgensis*, *Nitrobacter winogradskyi* and *Afipia felis*), as the other *Rhodopseudomonas* species did. A phylogenetic tree was constructed on the basis of evolutionarily corrected distances matrix (Fig. 4). *Rubrivivax gelatinosus*, which is in the β -subclass of the *Proteobacteria*, was used as outgroup. Strain gc^T formed a close cluster with two species of *Rhodopseudomonas* (*Rhodopseudomonas palustris* and *Rhodopseudomonas rhodobacensis*) and five species of chemotrophic bacteria.

DISCUSSION

Strain gc^T met the characteristic requirements of the genus *Rhodopseudomonas*: anaerobic photoheterotrophs, multiplying by budding, and forming the lamellar type of intracytoplasmic membrane. Cells contained bacteriochlorophyll *a*. Among the four species of *Rhodopseudomonas*, strain gc^T most closely resembled *Rhodopseudomonas palustris* and *Rhodopseudomonas rhodobacensis*. However, substantial physiological differences existed between strain gc^T and the above two species (Table 1). Strain gc^T did not grow aerobically in dark, whereas both *Rhodopseudomonas palustris* and *Rhodopseudomonas rhodobacensis* did. Strain gc^T required thiamin as a growth factor, whereas both *Rhodopseudomonas palustris* and *Rhodopseudomonas rhodobacensis* required aminobenzoic acid as a growth factor. Strain gc^T did not utilize ethanol or gluconate as carbon sources whereas *Rhodopseudomonas palustris* and *Rhodopseudomonas rhodobacensis* did. More differences can be found in Table 1. Therefore, we classified strain gc^T as a new species of the genus *Rhodopseudomonas*, and the name *Rhodopseudomonas faecalis* is proposed.

Phylogenetic analysis and DNA–DNA hybridization further supported strain gc^T to be a new species of the genus *Rhodopseudomonas*. DNA–DNA hybridization results showed that strain gc^T was clearly different from *Rhodopseudomonas palustris* at the species level (only 17.7%), supporting the phenotypic differences. Molecular phylogenetic analysis based on 16S rRNA gene sequences also indicated that strain gc^T was

different enough to be separated from other species of the genus *Rhodopseudomonas* (Fig. 4).

We propose a new name for strain gc^T: *Rhodopseudomonas faecalis*.

Description of *Rhodopseudomonas faecalis* sp. nov.

Rhodopseudomonas faecalis (fae.ca'lis. L. adj. *faecalis* pertaining to faeces, as the organism was found in chicken faeces).

Cells are vibrioid or peanut-like, 0.6–0.8 μ m wide and 1.0–2.0 μ m long. Motile by means of polar flagella. Cells multiply by budding and asymmetric cell division. The daughter cell and mother cell resemble an 'S'-form before separating from each other. Rosette-like clusters can be seen in old cultures. Gram-negative, α -proteobacterium. Phototrophically grown cells contain intracytoplasmic membranes of the lamellar type. The absorption maxima of living cells are 382, 460–464, 494–496, 534–538, 596, 804–806 and 870–874 nm. Thiamin is required as a growth factor and 0.05% yeast extract stimulates growth. Anaerobic phototroph. No growth under aerobic conditions. Ammonium salts are used as nitrogen sources. Sulfate is assimilated. Nitrate or DMSO cannot be used as a terminal electron acceptor. The G + C content of the DNA is 63.6 mol %. Isolated from chicken faeces. The type strain is strain gc^T, which has been deposited in the Chinese Collection of Microorganisms, Beijing (AS1.2176^T), and in the Japan Collection of Microorganisms, RIKEN, Wako (JCM 11668^T).

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