Roseateles depolymerans* gen. nov., sp. nov., a new bacteriochlorophyll *a*-containing obligate aerobe belonging to the β -subclass of the *Proteobacteria

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Strains 61A^T (T = type strain) and 61B2, the first bacteriochlorophyll (BChl) *a*-containing obligate aerobes to be classified in the β -subclass of the *Proteobacteria*, were isolated from river water. The strains were originally isolated as degraders of poly(hexamethylene carbonate) (PHC). The organisms can utilize PHC and some other biodegradable plastics. The strains grow only under aerobic conditions. Good production of BChl *a* and carotenoid pigments is achieved on PHC agar plates and an equivalent production is observed under oligotrophic conditions on agar medium. Spectrometric results suggest that BChl *a* is present in light-harvesting complex I and the photochemical reaction centre. The main carotenoids are spirilloxanthin and its precursors. Analysis of the 16S rRNA gene sequence indicated that the phylogenetic positions of the two strains are similar to each other and that their closest relatives are the genera *Rubrivivax*, *Ideonella* and *Leptothrix* with similarities of 96.3, 96.2 and 96.1%, respectively. The cells are motile, straight rods and contain poly- β -hydroxybutyrate granules. Ubiquinone-8 is the predominant quinone. Vitamins are not required for growth. The G+C content of genomic DNA is 66.2–66.3 mol%. Genetic and phenotypic features suggest that the strains represent a new genus in the β -subclass which is evenly distant from known genera. Consequently, the name *Roseateles depolymerans* gen. nov., sp. nov. is proposed for the strains; the type strain of *Roseateles depolymerans* is strain 61A^T (= DSM 11813^T).

Keywords: aerobic photosynthetic bacteria, bacteriochlorophyll *a*, β -subclass, *Proteobacteria*, *Roseateles depolymerans* gen. nov., sp. nov.

INTRODUCTION

There are some species of bacteriochlorophyll (BChl) *a*-containing bacteria that cannot grow photosynthetically under anaerobic conditions even in light of the effective wavelength. These organisms are referred

Abbreviations: BChl, bacteriochlorophyll; FD-MS, field desorption mass spectrometry; LH-I, light-harvesting complex I; PHB, poly- β -hydroxybutyrate; PHC, poly(hexamethylene carbonate); UQ-8, ubiquinone with 8 isoprene units.

The GenBank/EMBL accession numbers for the 16S rRNA gene sequences of strains 61A^T and 61B2 are AB003623 and AB003625, respectively.

to as aerobic photosynthetic bacteria (Harashima *et al.*, 1982; Shiba, 1989; Shimada, 1995). Although the function of their photosynthetic apparatus has not yet been clarified, light-stimulated growth (Harashima *et al.*, 1987; Yurkov & Gemerden, 1993) and photosynthetic activity (Harashima *et al.*, 1982; Kortlüke *et al.*, 1997; Okamura *et al.*, 1986; Shiba, 1984; Takamiya & Okamura, 1984) have been reported in some species of aerobic photosynthetic bacteria. Yurkov & Gemerden (1993) hypothesized an advantage of having both photosynthetic and heterotrophic metabolisms in terms of adaptation to the environment with altering light/dark conditions under aerobic

conditions. Some physiological changes related to adaptation to light/dark have been reported (Takaichi *et al.*, 1991; Tilden *et al.*, 1997).

Aerobic photosynthetic bacteria have been isolated from various environments: freshwater (Fuerst *et al.*, 1993), marine cyanobacterial mat (Yurkov *et al.*, 1994), cyanobacterial mat from warm/hot spring (Hanada *et al.*, 1997; Yurkov & Gemerden, 1993; Yurkov & Gorlenko, 1992, 1993; Yurkov *et al.*, 1993) and surfaces of seaweeds (Shiba, 1991; Shiba & Simidu, 1982). Currently, aerobic photosynthetic bacteria are classified into the following genera: *Erythro bacter* and *Roseobacter* for marine species; and *Porphyrobacter*, *Erythromicrobium* and *Roseococcus* for freshwater species. Recently, two genera, *Sandaracinobacter* and *Erythromonas*, were separated from *Erythromicrobium* (Yurkov *et al.*, 1997). Furthermore, similar isolates have been reported from soil (Saitoh & Nishimura, 1996), surfaces of plants (Urakami *et al.*, 1993), nodules of legumes (Evans *et al.*, 1990), food, animal feed and sewage sludge (Nishimura *et al.*, 1981), some of which are currently classified into genera which have been considered as typical non-phototrophs: *Acidiphilium* (possessing zinc-substituted BChl *a*) (Wakao *et al.*, 1996), *Bradyrhizobium* (Evans *et al.*, 1990; Young *et al.*, 1991) and *Methylobacterium* (Green & Bousfield, 1983; Urakami *et al.*, 1993). So far, all of the genera described above have been phylogenetically classified into the α -subclass of the *Proteobacteria*. Five genera, *Erythro bacter*, *Erythromicrobium*, *Erythromonas*, *Porphyrobacter* and *Sandaracinobacter*, belong to the α -4 group where aerobic photosynthetic species are predominant and the others belong to the α -1 to α -3 groups, which contain close relatives of typical anoxygenic photosynthetic bacteria (Hanada *et al.*, 1997; Shimada, 1995; Turova *et al.*, 1995; Young *et al.*, 1991; Yurkov *et al.*, 1994, 1997). No aerobic photosynthetic bacterium has been reported outside of the α -*Proteobacteria*.

Two new obligate aerobes containing BChl *a*, strain 61A^T (= DSM 11813^T; T = type strain) and strain 61B2 (= DSM 11814), were isolated from river water by screening aliphatic polycarbonate-degrading microorganisms (Suyama *et al.*, 1998). Phylogenetic analysis based on 16S rRNA comparisons revealed that these strains belong to the β -subclass of the *Proteobacteria*; the strains are thought to be the first aerobic photosynthetic bacteria found in this taxon.

In the present study, morphological, physiological, biochemical and genetic characteristics of the strains are described and a new genus name, *Roseateles depolymerans* gen. nov., sp. nov., is proposed for the isolates.

METHODS

Origin of strains. Strains 61A^T and 61B2 were isolated from the Hanamuro River in Tsukuba, Ibaraki Prefecture of Japan. The strains were isolated with a medium containing

emulsified poly(hexamethylene carbonate) (PHC) as a substrate at 30 °C (Suyama *et al.*, 1998).

The type strain of *Rubrivivax gelatinosus* (DSM 1709^T = ATCC 17011^T = NCIB 8290^T = LMG 4311^T) used in this study as a reference species was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

Culture media. The following media were used in the present study. The agar plates were prepared by adding 1.5% (w/v) Bacto agar (Difco) to the media. PHC medium was used for isolation and maintenance of original culture line. CAV medium was used for the other physiological tests.

The PHC medium contained (l⁻¹): 1 g PHC (emulsified, number-average molecular mass M_n = 2000; Toagosei); 1 g (NH₄)₂SO₄; 0.1 g yeast extract (Difco); 20 mg CaCl₂·2H₂O; 10 mg NaCl; 10 mg FeSO₄·7H₂O; 0.5 mg Na₂MoO₄·2H₂O; 0.5 mg Na₂WO₄·2H₂O; 0.5 mg MnSO₄; 60 mg surfactant (Plysurf A210G; Daiichi Kogyo Seiyaku); 0.2 g KH₂PO₄; and 1.6 g K₂HPO₄ (pH 7.0).

The CAV medium contained (l⁻¹): 2 g Casamino acids (Difco); 0.5 g (NH₄)₂SO₄; 1 ml vitamin mixture; 10 ml basal salt solution; and 25 ml 200 mM potassium phosphate buffer (pH 7.0). The composition of the basal salt solution and the vitamin mixture were previously described (Hanada *et al.*, 1997).

Physiological tests and determination of growth conditions.

The ability of the strains to grow photosynthetically under anaerobic conditions was investigated both on the agar plates and in liquid culture in the light. The anaerobic or semi-aerobic growth on agar plates was assessed in an anaerobic jar with Anaero Pack or Campylo Pack (Mitsubishi Gas Chemical), respectively. The photoheterotrophic growth was ascertained using PE medium (Hanada *et al.*, 1995b), which contains acetate, glutamate and succinate as electron donors. The photoautotrophic growth in the presence of reduced sulfur compounds was determined in a medium containing (l⁻¹): 0.5 g (NH₄)₂SO₄; 0.5 g Na₂S₂O₃·5H₂O; 0.1 g Na₂S·9H₂O; 4.2 g NaHCO₃; 1 ml vitamin mixture; 5 ml basal salt solution (Hanada *et al.*, 1997); and a final concentration of 10 mM potassium phosphate buffer (pH 7.0).

The growth rates as a function of temperature were determined in CAV medium. The vitamin requirement was determined at 30 °C with special CAV medium lacking the vitamin mixture and containing vitamin-free Casamino acids (Difco). The final reading was obtained after nine serial transfers. The growth rates at various pH were also determined at 30 °C in a series of CAV media that were buffered by 10 mM sodium citrate (pH 3–6), 10 mM potassium phosphate (pH 7–8) or 10 mM sodium/glycine buffer (pH 9–11).

For the analysis of photosynthetic pigments, colonies formed on PHC agar plate medium incubated for 3 d were used. Pigments were extracted from cells with acetone/methanol (7:2, v/v). The crude extract was analysed with an HPLC system equipped with a μ Bondapak C18 column (8 × 100 mm; Waters) eluting with methanol (Takaichi & Shimada, 1992). Major carotenoids were purified from silica gel TLC (Merck) developed with n-hexane and acetone (4:1, v/v). Absorption spectra were recorded with a photodiode array detector (200–800 nm, MCPD-3600; Otsuka Electronics) attached to the HPLC apparatus (Takaichi & Shimada, 1992). The molar absorption coefficients in methanol (90.6 mM⁻¹cm⁻¹ at absorption maxima for carotenoids

and $54.9 \text{ mM}^{-1}\text{cm}^{-1}$ at 770 nm for BChl *a*) were used for quantification. The molecular masses were determined by field desorption mass spectrometry (FD-MS), using a double-focusing GC-MS equipped with field desorption apparatus (M-2500; Hitachi) (Takaichi, 1993).

Quinones were extracted with chloroform/methanol (2:1, v/v) from cells grown for 1 d in CAV medium. The extract was purified by the method of Hiraishi *et al.* (1996) and analysed by HPLC using a Gold HPLC System (Beckman) with a ZORBAX-ODS column (Shimadzu).

The organic substrate utilization activities were determined with BiOLOG GN and GP Microplates (Biolog) according to the recommended protocol of the manufacturer. Gelatinase activity was determined by the standard method (Smibert & Krieg, 1981). Utilization of hydrogen gas was investigated with 15% (v/v) hydrogen in air using carbon-free medium at 30 °C.

Nitrogen-fixing ability was tested by acetylene reduction assay (Inoue *et al.*, 1996). Overnight culture was prepared in a medium that contained (l^{-1}): 5 g glucose; 1 g Agar Noble (Difco); 10 ml basal salt solution; and 25 ml 200 mM potassium phosphate buffer (pH 7.0). The culture was placed in a rubber-capped tube with 15% (v/v) acetylene and incubated at 30 °C. Reduction of acetylene was assayed with a GC-8A gas chromatograph (Shimadzu) with a Unibeads C 60/80 column (GL Science).

Electron microscopy. Negatively stained cells and ultrathin sections of the cells were observed with a model H-7000 electron microscope operating at 75 kV (Hitachi). The cells were collected from PHC agar plates (3 d) or from CAV liquid medium (1 d). Cells on a collodion membrane were negatively stained with 1% (w/v) aqueous uranyl acetate. Ultrathin sections were prepared by a previously described method (Hanada *et al.*, 1995a). The cells were suspended in Kellenberger buffer (Kellenberger *et al.*, 1958) and embedded in 1% (w/v) agar. The specimen was fixed with 2.5% (w/v) glutaraldehyde and 1% (w/v) OsO_4 , prestained with ruthenium red and uranyl acetate, and embedded in epoxy resin.

DNA composition and phylogenetic analysis. Genomic DNA was extracted and purified by the method of Marmur (1961). Almost full-length 16S rRNA genes were amplified by PCR with a pair of primers designed from positions 8–27 and 1492–1511 of the *Escherichia coli* 16S rRNA gene (Brosius *et al.*, 1978; Weisburg *et al.*, 1991). The PCR products cloned into plasmid vector (pT7Blue T-Vector; Novagen) were sequenced separately by an automated sequencer (ABI model 373A; Applied Biosystems) from both forward- and reverse-strands using twelve kinds of primers. The detailed procedure for the sequencing was described previously (Suyama *et al.*, 1998).

The G+C content was determined by the protocol of Kamagata & Mikami (1991).

Nucleotide sequence accession numbers. Accession numbers for the reference 16S rRNA gene sequences were as follows: *Alcaligenes latus*, D88007; *Brachymonas denitrificans*, D14320; *Comamonas testosteroni*, M11224; *Ideonella dechloratans*, X72724; *Leptothrix cholodnii*, X97070; *Leptothrix discophora*, L33974; *Leptothrix mobilis*, X97071; *Polaromonas vacuolata*, U14585; *Rhodocyclus tenuis*, D16208; *Rhodferax fermentans*, D16212; *Rubrivivax gelatinosus*, D16213; *Sphaerotilus natans*, Z18534; *Thiobacillus thermosulfatus*, U27839; and *Variovorax paradoxus*, D30793.

RESULTS

Strains and growth conditions

Two strains, 61A^T (soft and smooth colony) and 61B2 (harder and larger colony than that of 61A^T), were isolated from the Hanamuro River (Ibaraki Prefecture, Japan) as two PHC-degrading strains; the strains co-metabolically utilized PHC (Suyama *et al.*, 1998). Both of the strains formed pink colonies on PHC agar plates within 2–3 d, making clear zones caused by degradation of PHC around their colonies. Other biodegradable plastics, poly(ϵ -caprolactone) and poly(tetramethylene carbonate) (Suyama & Tokiwa, 1997), were degraded in the same way.

The strains were able to grow in CAV medium buffered at pH 5–8 (Fig. 1a). Growth was observed at 5–43 °C, and an incubation temperature of 45 °C was lethal (Fig. 1b). Optimal pH and temperature for the growth of these strains were pH 6.5 and 35 °C, respectively. No vitamins were required for growth; subculturing strains 61A^T and 61B2 in CAV medium lacking vitamins had no effect on growth.

Both of the strains produced a musty odour and mucus. Strain 61B2 tended to make white floc in liquid culture whereas strain 61A^T did not.

Fig. 1. Growth rate of strains 61A^T (●) and 61B2 (○) as a function of pH at 30 °C (a) and as a function of temperature at pH 7.0 (b). Cells were grown in CAV medium under aerobic conditions in the dark.

Fig. 2. Absorption spectra of (a) intact cells of strain 61A^T grown on PHC agar plates (solid line) and CAV agar plates (dashed line) for 5 d; and (b) ultrasonically disrupted membrane in potassium phosphate buffer, pH 6.8 (solid line), and acetone/methanol (7:2, v/v) extract (dotted line) of the cells grown on PHC agar plates. Spectra were recorded with a Beckman DU-640 spectrophotometer.

Photosynthetic pigments

The strains grew on agar plates of CAV, nutrient broth or 0.1% skim milk media, but the colonies were colourless. When the strains were grown on agar plates with a lower concentration of carbon sources (e.g. in the presence of 0.02% Casamino acids), weak pigmentation was observed. *In vivo* absorption spectra of intact cells of strain 61A^T (pigmented and non-pigmented) are shown in Fig. 2(a). The cells grown on PHC agar plates showed a large absorption peak at 870 nm, whereas those grown on CAV agar plates showed no significant peak around this wavelength. No pigmentation was observed in liquid-cultured cells.

The strains did not grow anaerobically and photosynthetically under conditions used for purple sulfur or non-sulfur bacteria. Growth and the production of pigments by the strains were blocked under anaerobic conditions. The strains were able to grow and produced the pigments in semi-aerobic conditions (6–10%, v/v, O₂).

At present, the PHC agar plate is the best medium for pigment production by strains 61A^T and 61B2. Greater pigment production was observed in dark-grown cells compared to light-grown cells. Ultrasonically disrupted cells had absorption maxima at 482, 515, 550, 590, 800 and 870 nm (solid line; Fig. 2b). The

Fig. 3. Electron micrographs of negatively stained cells of strain 61A^T grown in CAV medium for 1 d (a) and ultrathin cross-sections of cells of strain 61A^T grown on a PHC agar plate for 3 d (b). The outer membrane (OM), cytoplasmic membrane (CM) and intracellular granules (G) are shown in (b). Cells were post-stained with uranyl acetate and lead citrate. No invaginations of intracytoplasmic membranes were observed. Bar, 1 μ m.

absorption peaks of the pigments extracted with acetone/methanol (7:2, v/v) were shifted as indicated in the figure (dotted line; Fig. 2b). BChl *a* with phytol ester was confirmed by the retention time on HPLC and absorption spectrum. Analysis of carotenoids using HPLC and FD-MS revealed that the major carotenoid of these strains was spirilloxanthin (*M_r* 596; 89 and 88 mol% of total carotenoids in strains 61A^T and 61B2, respectively). The precursors of spirilloxanthin, i.e. OH-spirilloxanthin (2 and 3 mol%, respectively), anhydorrhodovibrin (8 and 7 mol%) and 3,4-dehydorrhodopin (1 and 2 mol%), were also detected as minor components. The molar ratios of total carotenoids/BChl *a* were 0.65 (strain 61A^T) and 0.58 (strain 61B2). Spheroidene (or sphero-

idenone), which is one of the major carotenoids of *Rubrivivax gelatinosus*, the phylogenetic neighbour described below, was not detected.

The production of photosynthetic pigments in *Rubrivivax gelatinosus* cells was determined under the same conditions as the two strains using CAV or PHC medium. *Rubrivivax gelatinosus* produced BChl *a* and carotenoids in liquid CAV medium or on CAV agar plates. However, pigment production was not observed on PHC agar plates.

Morphology and ultrastructure

Strains 61A^T and 61B2 had similar morphological characteristics. Cells of strain 61A^T grown in CAV medium were motile (motility was observed only in the early exponential phase in a liquid culture), Gram-negative rods that were 0.5 × 2 µm with flagella (Fig. 3a). No intracytoplasmic membranes were observed in ultrathin sections of pigmented cells of strain 61A^T (Fig. 3b). Intracellular granules were observed, some of which were considered to be identical to the poly-β-hydroxybutyrate (PHB) granules determined by Nile Blue A staining (Suyama *et al.*, 1998). No remarkable differences were observed in ultrathin sections between the cells with and without photosynthetic pigments.

Phylogenetic analysis

The obtained sequence of the 16S rRNA genes of each isolate consisted of 1452 bp. The sequences of strains 61A^T and 61B2 were identical except for a single base (i.e. guanine at *E. coli* position 648 in strain 61A^T was replaced by adenine in strain 61B2).

Fig. 4 shows the phylogenetic positions of the strains among representative members of the β-subclass of the *Proteobacteria*, determined by the neighbour-joining method (Saitou & Nei, 1987). The sequence of *Rhodocyclus tenuis* was used as the outgroup reference. *Alcaligenes latus*, *Ideonella dechloratans*, *Rubrivivax gelatinosus*, *Sphaerotilus natans* and the *Leptothrix* species were the phylogenetic neighbours of strains 61A^T and 61B2. *Rubrivivax gelatinosus* was the only phototrophic species among closely related organisms. The closest relatives of the two strains were *Rubrivivax gelatinosus*, *Ideonella dechloratans*, *Leptothrix cholodnii* and *Leptothrix discophora* with similarities of 96.3, 96.2, 96.1 and 96.1 %, respectively.

Physiological and other properties

The results of the nutritional and biochemical tests carried out on strains 61A^T and 61B2 are summarized in Table 1. The reference data for the phylogenetic neighbours are listed together. The strains could utilize the organic acids and carbohydrates listed in Table 1 as sole carbon sources. The strains utilized organic acids (e.g. L-malate, succinate, etc.) and the reducing monohexoses (e.g. glucose, fructose, etc.) well. Some other physiological traits of the two strains were reported previously (Suyama *et al.*, 1998). The major quinone

Fig. 4. Phylogenetic positions of strains 61A^T and 61B2 among neighbouring species selected from the β-subclass of the *Proteobacteria*. Bar, 1 nucleotide substitution per 100 nucleotides in 16S rRNA gene sequences. The numbers at the nodes of the tree indicate bootstrap values (%) for each node of 1000 bootstrap resamplings.

component of strains 61A^T and 61B2 was UQ-8 (ubiquinone with 8 isoprene units). The G + C contents of the strains, determined by HPLC, were 66.3 (strain 61A^T) and 66.2 (strain 61B2) mol %.

DISCUSSION

Strains 61A^T and 61B2 were characterized previously as physiologically new strains that co-metabolically utilized aliphatic polycarbonates (Suyama *et al.*, 1998). The two strains grew only under aerobic conditions with production of BChl *a*. Light did not support growth of the strains under anaerobic conditions. These features are similar to those of aerobic photosynthetic bacteria belonging to the α-subclass of the *Proteobacteria* (Evans *et al.*, 1990; Fuerst *et al.*, 1993; Green & Bousfield, 1983; Shiba, 1991; Shiba & Simidu, 1982; Urakami *et al.*, 1993; Wakao *et al.*, 1996; Young *et al.*, 1991; Yurkov *et al.*, 1993, 1997; Yurkov & Gorlenko, 1992). The absence of intracytoplasmic membranes as shown in Fig. 3(b) has also been reported in some other aerobic photosynthetic bacteria (Hanada *et al.*, 1997; Yurkov *et al.*, 1994). The 16S rRNA gene sequences indicate that the strains are distinguishable from the α-subclass of the *Proteobacteria* and that they belong to the β-subclass. The predominant presence of UQ-8 in the isolates as the major quinone component supports their taxonomic position in the β-subclass of the *Proteobacteria* (Hiraishi *et al.*, 1996).

Absorption maxima at 870 and 800 nm in Fig. 2(b) indicate the presence of BChl *a* in the light-harvesting complex I (LH-I) and the photochemical reaction

Table 1. Characteristics of the species phylogenetically close to strains 61A^T and 61B2

+, ≥ 90% positive; −, ≤ 10% positive; d, 11–89% of the strains positive; NT, not tested. The following references were used: Imhoff & Trüper (1989); Kersters & De Ley (1984); Malmqvist *et al.* (1994); Suyama *et al.* (1998); and Willems *et al.* (1991). 1, Strains 61A^T and 61B2; 2, *Ideonella dechloratans*; 3, *Rubrivivax gelatinosus*; 4, *Alcaligenes latus*; 5, *Leptothrix discophora*; and 6, *Sphaerotilus natans*.

Characteristic	1	2	3	4	5	6
Motility	+	+	+	+	+	+
Flagellation	Several, polar	Several, polar	1, polar	5–10, peritrichous	1, polar	Polar bunch
Cytochrome <i>c</i> oxidase	+	+	NT	+	NT	+
Catalase	−	+	NT	NT	NT	+
Formation of sheaths	−	−	−	−	+	+
Phototrophic growth	−	−	+	−	−	−
Autotrophic growth with hydrogen	−	NT	+	+	−	NT
Nitrogen fixation	−	NT	+	+	NT	NT
Nitrate reduction	−	+(or chlorate)	NT	−	NT	NT
Gelatinase	+	NT	+	+	NT	+
Accumulation of PHB	+	NT	NT	+	+	+
Pigments:						
BChl <i>a</i>	+	−	+	−	−	−
Carotenoids	Spirilloxanthin*	−	Spheroidene, OH-spheroidene, spirilloxanthin†	−	−	−
Growth factors required	−	+	Biotin, thiamin	NT	Vitamin B ₁₂	Vitamin B ₁₂
Carbon sources utilized:						
Acetate	d	+	+	−	−	+
Pyruvate	+	+	+	−	+	+
Lactate	+	+	+	+	−	+
L-Malate	+	NT	+	+	+	+
Succinate	+	+	+	+	+	+
Citrate	+	NT	+	d	−	+
D-Ribose	−	NT	NT	−	−	NT
D-Glucose	+	+	+	+	+(cannot grow)	+
D-Fructose	+	+	+	+	−	+
D-Galactose	+	NT	NT	−	NT	+
Sucrose	−	NT	NT	+	+(not all strains)	+
Glycerol	−	NT	−	+	+	+
Mannitol	+	NT	−	−	NT	+
Sorbitol	−	NT	−	−	NT	+
Isolation source	Water	Water	Mud	Soil	Water	Water
G+C content (mol%)	66.2–66.3	68.1	70.0–72.5	69.1–71.1	67.8–71.1	69.9

* The pigments are produced by cells grown aerobically on PHC agar medium in the dark.

† The pigments are produced by cells grown anaerobically in the light.

‡ See reference by Malmqvist *et al.* (1994).

centre. In the pigmented cells, the molar ratio of carotenoids/BChl *a* in the strains was always about 0.6. This stoichiometry is quite similar to the LH-I in *Rhodospirillum rubrum* (Cogdell *et al.*, 1982), and a major proportion of the carotenoids is considered to be bound to the complex. Although most of the species of aerobic photosynthetic bacteria are known to contain carotenoids which do not bind to the photosynthetic apparatus (e.g. erythroxanthin sulfate), such components were not detected in strains 61A^T and 61B2.

The species listed in Table 1 are the phylogenetic neighbours of the strains suggested by analysis based on the 16S rRNA gene sequences (Fig. 4). The presence of motility, flagellation, PHB granules, oxidase and gelatinase are common characteristics of the species in this taxon. The absence of sheaths clearly distinguishes the strains from the genera *Sphaerotilus* and *Leptothrix*. The difference in the utilization pattern of carbon sources (Table 1) suggests that the strains are distinct from known species. The G+C contents of the two

strains are comparatively low in the phylogenetic cluster and distinguish the strains from relatives in this taxon. The formation of photosynthetic pigments is the most remarkable characteristic of strains 61A^T and 61B2. Although *Rubrivivax gelatinosus* is the only species containing BChl *a* among the phylogenetic neighbours, the growth physiology and the production of pigments in *Rubrivivax gelatinosus* were different from those of the two strains. *Rubrivivax gelatinosus* is a typical anoxygenic phototrophic bacteria and grows under both aerobic and anaerobic conditions. *Rubrivivax gelatinosus* has the spheroidene and spirilloxanthin series as major carotenoid species while the two strains have only the spirilloxanthin series. Nitrogen fixation and autotrophic growth on hydrogen were reported for *Rubrivivax gelatinosus* (Imhoff & Trüper, 1989), but were not found in the two strains. *Rubrivivax gelatinosus* requires biotin and thiamin for growth, whereas the strains could grow in a vitamin-free medium.

The 16S rRNA gene sequence similarities to the

phylogenetic neighbours, *Rubrivivax gelatinosus*, *Ideonella dechloratans* and the *Leptothrix* species, were 96.3, 96.2 and 96.1%, respectively. The distances to each of the genera are sufficient to consider creation of a new genus. The bootstrap probabilities indicated in Fig. 4 show that the genera around strains 61A^T and 61B2 are equally dispersed under the branch with a reliability of 100%. Genetic and phenotypic features suggest that strains 61A^T and 61B2 represent a new genus in the β -subclass of the *Proteobacteria* which is evenly distant from known genera. The name *Roseateles depolymerans* gen. nov., sp. nov. is therefore proposed for the strains.

Description of *Roseateles* gen. nov.

Roseateles (ro.se.a.te'les. L. adj. *roseus* rose-coloured, pink; Gr. adj. *ateles* defective, incomplete; N.L. masc. n. *roseateles* the rose-coloured incomplete photosynthetic bacterium).

Cells are motile, Gram-negative straight rods. Cells possess PHB granules as a storage material and reproduce by binary fission. The bacterium grows heterotrophically under aerobic conditions. BChl *a* with phytol ester and carotenoid pigments are formed. Colonies are pink in colour under conditions suitable for photosynthetic pigment production. The bacterium cannot grow anaerobically even in the light. The major quinone is UQ-8. On the basis of the results of a 16S rRNA gene sequence comparison, the bacteria belong to the β -subclass of the *Proteobacteria*. DNA G+C content is 66.2–66.3 mol%. The type species is *Roseateles depolymerans*.

Description of *Roseateles depolymerans* sp. nov.

Roseateles depolymerans (de.po.ly'me.rans. N.L. v. *depolymerare* depolymerize; N.L. part. adj. *depolymerans* depolymerizing).

Characteristics are the same as those given in the description of the genus. Lives in river water. BChl *a* with phytol ester and spirilloxanthin are present along with the LH-I when cells are grown aerobically on agar plates supplemented with low levels of carbon sources, e.g. 0.02% Casamino acids. Grows well heterotrophically with rich media containing 0.2% Casamino acids, 0.8% nutrient broth or 0.1% skim milk, under aerobic conditions. In this case, BChl *a* and carotenoids are hardly produced. Hydrogen is not utilized for autotrophic growth. Positive for gelatinase and oxidase. Negative for catalase, nitrogen fixation, nitrogen reduction and denitrification. No vitamins are required for growth. Optimum pH for the growth is pH 6.5 and optimum temperature is 35 °C. The culture is not maintained at 45 °C. The bacterium can grow on D-glucose, D-fructose, D-galactose, mannitol, pyruvate, lactate, L-malate, succinate, citrate, Casamino acids or yeast extract as a sole carbon source. Degrades PHC, poly(tetramethylene carbonate) and poly(e-

caprolactone) by means of co-metabolism. Type strain is 61A^T, which has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 11813^T.

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