Rubrivivax benzoatilyticus sp. nov., an aromatic, hydrocarbon-degrading purple betaproteobacterium

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A brown-coloured bacterium was isolated from photoheterotrophic (benzoate) enrichments of flooded paddy soil from Andhra Pradesh, India. On the basis of 16S rRNA gene sequence analysis, strain JA2^T was shown to belong to the class *Betaproteobacteria*, related to *Rubrivivax gelatinosus* (99 % sequence similarity). Cells of strain JA2^T are Gram-negative, motile rods with monopolar single flagella. The strain contained bacteriochlorophyll *a* and most probably the carotenoids spirilloxanthin and sphaeroidene, but did not have internal membrane structures. Intact cells had absorption maxima at 378, 488, 520, 590, 802 and 884 nm. No growth factors were required. Strain JA2^T grew on benzoate, 2-aminobenzoate (anthranilate), 4-aminobenzoate, 4-hydroxybenzoate, phthalate, phenylalanine, *trans*-cinnamate, benzamide, salicylate, cyclohexanone, cyclohexanol and cyclohexane-2-carboxylate as carbon sources and/or electron donors. The DNA G+C content was 74·9 mol%. Based on DNA–DNA hybridization studies, 16S rRNA gene sequence analysis and morphological and physiological characteristics, strain JA2^T is different from representatives of other photosynthetic species of the *Betaproteobacteria* and was recognised as representing a novel species, for which the name *Rubrivivax benzoatilyticus* sp. nov. is proposed. The type strain is JA2^T (=ATCC BAA-35^T=JCM 13220^T=MTCC 7087^T).

A small number of low molecular mass aromatic hydrocarbons support the growth of certain purple non-sulfur bacteria (Sasikala & Ramana, 1998) as carbon sources and/or electron donors. Frank & Gaffron (1941) first reported aromatic metabolism in an anoxygenic phototrophic bacterium, *Rhodovibrio parvus* (now placed in *Rhodopseudomonas* as *Rhodopseudomonas palustris*), in which phenylpropionate was transformed to benzoate during phototrophic metabolism. Later, Scher and co-workers (Scher & Allen, 1960; Scher & Proctor, 1960) demonstrated growth of *Rhodopseudomonas palustris* at the expense of benzoate. The discovery of benzoate utilization by *Rhodospirillum fulvum* (Pfennig *et al.*, 1965) [renamed as *Phaeospirillum fulvum* (Imhoff *et al.*, 1998)] led to the investigation of aromatic hydrocarbon degrading capability among other purple non-sulfur bacteria. *Rhodopseudomonas palustris* (Dutton & Evans, 1967; Harwood & Gibson, 1988), *Phaeospirillum fulvum* (Pfennig *et al.*, 1965), *Rhodopseudomonas acidophila* (Yamanaka *et al.*, 1983) [renamed as *Rhodoblastus acidophilus* (Imhoff, 2001)], *Rhodomicrobium vannielii* (Wright & Madigan, 1991), *Rhodobacter capsulatus* (Blasco & Castillo, 1992; Madigan *et al.*, 2001), *Rhodobacter sphaeroides* (Rajasekhar *et al.*, 2000), *Blastochloris sulfoviridis* (Zengler *et al.*, 1999) and the bacteriochlorophyll-containing aerobic phototrophic bacterium *Porphyrobacter sanguineus* (Hiraishi *et al.*, 2002) are phototrophic alphaproteobacteria that are capable of aromatic hydrocarbon metabolism. Phototrophic betaproteobacteria that utilize benzoate include *Rhodocyclus purpureus* (Pfennig, 1978) and *Rhodoferax ferrireducens* (Finneran *et al.*,

Abbreviations: BChl *a*, bacteriochlorophyll *a*; FT-IR, fourier-transform infrared.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA2^T is AJ888903.

Micrographs of cells of strain JA2^T and figures showing whole-cell absorption and FT-IR spectra and a graph showing growth of and utilization of benzoate by strain JA2^T are available as supplementary material in IJSEM Online.

2003). Although *Rhodocyclus gelatinosus* [now *Rubrivivax gelatinosus* (Willems *et al.*, 1991; Imhoff, 1995)] utilizes phloroglucinol for growth and photobiotransforms some other aromatic compounds (Sasikala & Ramana, 1998), to date it is not known whether it can utilize benzoate as a carbon source or electron donor (Whittle *et al.*, 1976). In this paper a new isolate (strain JA2^T) is described, which was obtained through phototrophic enrichments on benzoate. Strain JA2^T clusters with members of the genus *Rubrivivax* of the *Betaproteobacteria* and metabolizes a wide range of aromatic hydrocarbons. Based on phenotypic characteristics and molecular studies, strain JA2^T represents a novel species of the genus *Rubrivivax*, for which the name of *Rubrivivax benzoatilyticus* sp. nov. is proposed.

Strain JA2^T was isolated from the rhizosphere of a flooded paddy field near Eluru, Andhra Pradesh, India. For isolation and cultivation of the isolate, Pfennig's mineral salts medium (Biebl & Pfennig, 1981) supplemented with benzoate (3 mM) as a carbon source and ammonium chloride (0·12 %) as a nitrogen source was used, with incubation under light (2400 lx) at 30 ± 2 °C. Purification of the isolate was achieved by repeated streaking on agar slants that were sealed with suba seals and flushed with argon and incubated phototrophically (2400 lx) at 30 ± 2 °C. For comparative analysis, *Rubrivivax gelatinosus* ATCC 17011^T was used under identical culture conditions. No distinct variation was observed in the colony morphologies of strain JA2^T and *Rubrivivax gelatinosus* ATCC 17011^T. Colonies of strain JA2^T grown photoheterotrophically were round, convex, smooth and dark-orange–brown in colour and surrounded by a thin opaque margin. Microscopical observations, such as cell shape, size, cell division and flagella, were performed using a phase-contrast microscope (Olympus-B201) and the internal membrane structures were viewed with a transmission electron microscope after the cells were processed as described by Hanada *et al.* (2002). Polyhydroxyalkanoates of a culture of strain JA2^T grown for 48 h on Pfennig's medium supplemented with 0·3 % (w/v) pyruvate were stained with Sudan black B (2 % in ethanol) (Smibert & Krieg, 1981) or Nile blue (Ostle & Holt, 1982).

Substrate utilization of carbon sources/electron donors was done in Pfennig's mineral salts medium by replacing benzoate with the test aliphatic substrate at a final concentration of 0·3 % (w/v), in a completely filled screw-cap test-tube (10×100 mm). For utilization of aromatic hydrocarbons as carbon sources/electron donors, the test substrates were used at a concentration of 3 mM, with sodium bicarbonate (0·1 %, w/v) in the medium. A culture grown in the absence of any added carbon source was used as the inoculum. Aromatic hydrocarbons such as cyclohexanol, cyclohexanone, catechol and resorcinol were added after membrane filtration to the autoclaved basal medium containing the nitrogen source. Filter-sterilized bicarbonate was added to the medium after autoclaving and the pH of the medium was adjusted with sterile 1 M NaOH or 1 M HCl to pH 6·8. Nitrogen source utilization was tested by replacing ammonium chloride with various nitrogen sources (0·12 %, w/v). Diazotrophy was tested by growing the cells under a N_2 atmosphere and also by using assays for acetylene reduction activity (Sasikala *et al.*, 1990). Dry weight was determined from an OD_{660} versus dry weight graph, ranging from 0·1 to1·0 OD_{660} prepared for strain JA2^T (OD_{660} of 0·1 was equal to 0·3 mg dry weight per ml). *In vivo* absorption spectra of cells collected from photoheterotrophic (malate; 0·3 %, w/v) cultures grown for 30 h were measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). Absorption spectra were also recorded from pigments extracted with acetone, after elution of the cell suspension with acetone through a 10×200 mm column packed with aluminium oxide. Whole cellular components (fatty acids, intracellular and membrane proteins, polysaccharides, photosynthetic pigments and nucleic acids) of strain JA2^T were distinguished from *Rubrivivax gelatinosus* ATCC 17011^T based on information obtained from fourier-transform infrared (FT-IR) spectroscopy. For FT-IR spectroscopy, 2–3 ml exponentially grown culture was lyophilized and processed with KBr to make a pellet. Spectral characteristics were recorded in the transmission mode between wave numbers 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} and 20 kHz scan speed in a Perkin Elmer FT-IR spectrophotometer (model 128).

Genomic DNA was extracted and purified according to the method of Marmur (1961) and the G+C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989). Cell material for 16S rRNA gene sequencing was taken from 1–2 ml culture. PCR and 16S rRNA gene sequencing were performed as described previously (Shivaji *et al.*, 2000). Sequences were aligned using the CLUSTAL W program (Thompson *et al.*, 1994). The distance matrix was calculated on the basis of the algorithm according to Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1989). Maximum-likelihood phylogenetic trees were established using the DNAML module of the PHYLIP 3.6 program package. Bootstrap analysis was performed. DNA–DNA hybridization analysis was performed at the DSMZ (Germany).

Uprooted paddy at booting stage together with the soil was collected in a polyethylene bag and the rhizosphere soil was used after 3 days for phototrophic enrichment on benzoate. Six colonies obtained during purification were designated strains JA2^T, JA4, JA5, JA6, JA7 and JA8. Colony morphologies of strains JA2^T, JA4 and JA6 were identical: colonies were round, dark-orange–brown in colour with a thin opaque margin and grew on benzoate as a sole source of carbon/electron donor. Strain JA2^T was characterized further using a polyphasic approach.

Cells of strain JA2^T stained Gram-negative. They were rod-shaped (see Supplementary Fig. S1 in IJSEM Online), 0·7–1·0 μm in width and 2·0–6·0 μm in length, motile by a single polar flagellum and formed a rosette-like arrangement. Electron micrographs of ultrathin sections of the cells

revealed the absence of internal membrane structures in strain JA2^T (see Supplementary Fig. S2 in IJSEM Online). Stationary-phase cells of strain JA2^T contained refractive granules. Sudan black and Nile blue staining indicated the presence of polyhydroxyalkanoates. On average, the cells contained 15–30 polyhydroxyalkanoate granules per cell, as observed in ultrathin sections (Fig. 1). In addition, growth of the granules at various stages could be observed (using transmission electron microscopy) and, sometimes, almost the whole cell was filled with a single large granule. Eventually, the granules even burst open the cell and were released as small ovoid granules (Fig. 1). The polyhydroxyalkanoate content of the cells reached up to 80–85 % of the total cell dry mass. The photosynthetically grown cell suspensions were orange–brown in colour. Whole-cell absorption spectra of strain JA2^T had absorption maxima at 378, 488, 520, 590, 802 and 884 nm, confirming the presence of bacteriochlorophyll *a* (BChl *a*) (Supplementary Fig. S3 in IJSEM Online) and most probably the carotenoids spirilloxanthin and sphaeroidene.

Strain JA2^T grew photolithoautotrophically [anaerobically in the light (2400 lx), with H_2 (20 %, v/v)/thiosulfate (0·1 %, w/v) and $NaHCO_3$ (0·1 %, w/v)], photo-organoheterotrophically [anaerobically in the light (2400 lx) with malate (0·3 %, w/v)] and chemo-organoheterotrophically [microaerobically in the dark with malate (0·3 %, w/v)]. Fermentative growth [anaerobically in the dark with glucose/fructose (0·3 %, w/v)] and chemolithoautotrophy [microaerobically in the dark with thiosulfate (0·1 %, w/v) and $NaHCO_3$ (0·1 % w/v)] could not be demonstrated. The substrates that were utilized as carbon sources and electron donors under photo-organoheterotrophic conditions are given in the species description.

Ammonium chloride and molecular nitrogen were the nitrogen sources utilized by strain JA2^T; urea, nitrate or nitrite did not support growth. NaCl was not obligatory for growth of strain JA2^T, but up to 5 % (v/v) was tolerated. In addition, the strain did not require any growth factors (vitamins) and there was no enhancement in the biomass yield in their presence. Production of indole from tryptone was not observed; however, replacement of tryptone with tryptophan resulted in indole production. Gelatin liquefaction was observed. Using FT-IR spectroscopic data (Supplementary Fig. S4 in IJSEM Online), strain JA2^T showed visible spectral differences from *Rubrivivax gelatinosus* ATCC 17011^T, indicating variations in the composition of aromatic compounds (at 980 cm^{-1}), polysaccharides (1300–1100 cm^{-1}) and esters (1726 cm^{-1}).

Aromatic hydrocarbons that supported photo-organoheterotrophic growth of strain JA2^T included benzoate, 2-aminobenzoate (anthranilate), 4-aminobenzoate, 4-hydroxybenzoate, 2-carboxybenzoate (phthalate), phenylalanine, *trans*-cinnamate, benzamide, salicylate (2-hydroxybenzoate), cyclohexanone, cyclohexanol and cyclohexane-2-carboxylate (Table 1). Growth and simultaneous utilization of benzoate

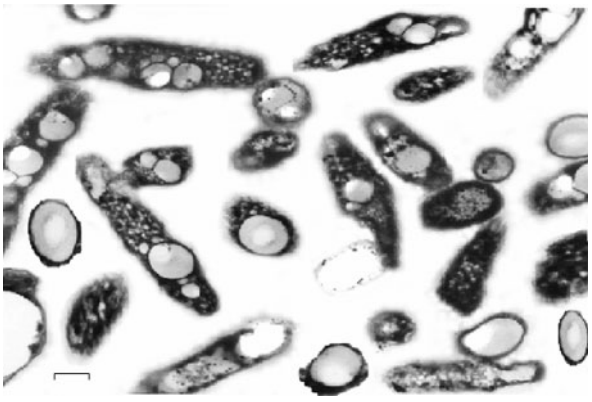

Fig. 1. Electron micrograph of ultrathin section of strain JA2^T showing polyhydroxyalkanoate granules (× 16 240). Bar, 1 μm.

Table 1. Growth and biomass yield of strain JA2^T on various aromatic hydrocarbons as carbon source/electron donors

Values shown are means of experiments performed in duplicate after 7 days of light (2400 lx) anaerobic incubation at 30 ± 2 °C. Phototrophic benzoate-grown cultures were used as the initial inoculum (5 %, v/v). The compounds were utilized at a concentration of 3 mM, in the presence of HCO_3^- (0·1 %, w/v). OD_{660} values: +++, >0·50; ++, 0·3–0·49; +, 0·21–0·29; GI, <0·2 (growth inhibition). NA, Not applicable.

Carbon source/electron donor	Growth
Benzoate	+++
2-Aminobenzoate (anthranilate)	++
4-Aminobenzoate	+
Aniline	GI
Resorcinol	GI
4-Chlorobenzoate*	GI
4-Hydroxybenzoate	+++
2-Hydroxybenzoate (salicylate)	++
Phthalate	++
Diphenylamine	GI
Phenylalanine	+++
Nitrobenzene*	GI
4-Hydroxydiphenyl	GI
trans-Cinnamate	++
Benzamide	++
Sulfanilate	+++
2,4-Dinitrophenol	GI
Phenol	GI
Pyrocatechol	GI
Cyclohexane*	GI
Cyclohexanone	++
Cyclohexanol	++
Cyclohexane-2-carboxylate	+++
Control (without aromatic compound)	NA

*Tested at concentrations of 0·5, 1·0 and 3·0 mM.

is shown in Supplementary Fig. S5 in IJSEM Online. The doubling time of strain JA2^T was about 35 h on benzoate, compared with 8 h on malate.

The G+C content of strain JA2^T was 74·9 mol% (by HPLC). The 16S rRNA gene sequence of strain JA2^T formed a cluster with members of the genus *Rubrivivax* and showed 99 % sequence similarity with *Rubrivivax gelatinosus* DSM 1709^T (=ATCC 17011^T). However, DNA–DNA hybridization of strain JA2^T with *Rubrivivax gelatinosus* ATCC 17011^T revealed a relatedness value of only 54·8 %.

The genus *Rhodocyclus* with three species, *Rhodocyclus purpureus*, *Rhodocyclus tenuis* and *Rhodocyclus gelatinosus*, was described in the first edition of *Bergey's Manual of Systematic Bacteriology* (Imhoff & Trüper, 1989). Based on 16S rRNA gene sequence analysis, even though all three species were grouped in the class *Betaproteobacteria* (Stackebrandt *et al.*, 1988), significant phylogenetic differences separated *Rhodocyclus gelatinosus* from the other two species, thus necessitating the description of a new genus, *Rubrivivax*, with *Rubrivivax gelatinosus* as the type species (Willems *et al.*, 1991). *Rubrivivax gelatinosus* is phylogenetically related to members (genera *Tepidimonas*, *Aquabacterium*, *Ideonella*, *Leptothrix*, *Roseateles*, *Sphaerotilus*, *Thiomonas*, *Xylophilus*) of the family *incertae sedis* (Cole *et al.*, 2003) [*Rubrivivax gelatinosus* was previously included in the family *Comamonadaceae* (Willems *et al.*, 1991; Wen *et al.*, 1999)], separating it from the genera *Rhodoferax* and *Rhodocyclus*. The genus *Rhodoferax*, a later addition to the family *Comamonadaceae*, to date includes two phototrophic species, *Rhodoferax fermentans* (Hiraishi *et al.*, 1991) and *Rhodoferax antarcticus* (Madigan *et al.*, 2000), and the non-pigmented *Rhodoferax ferrireducens* (Finneran *et al.*, 2003). Pairwise sequence comparisons and distance matrix tree analysis showed that the genera *Rhodoferax*, *Rubrivivax* and *Rhodocyclus* formed three separate lineages within the *Betaproteobacteria* (Hiraishi, 1994). Based on the 16S rRNA gene sequence analysis, strain JA2^T clustered closely with members of the family *incertae sedis* within the genus *Rubrivivax* (Fig. 2). However, the morphological, physiological and biochemical traits (Table 2) and DNA–DNA hybridization studies significantly differentiated this strain from the only reported species of *Rubrivivax*, *Rubrivivax gelatinosus*, and therefore strain JA2^T is described here as a novel species, *Rubrivivax benzoatilyticus*. FT-IR spectroscopic data, which are considered to be a connecting link between genomic and phenotypic approaches (Amiel

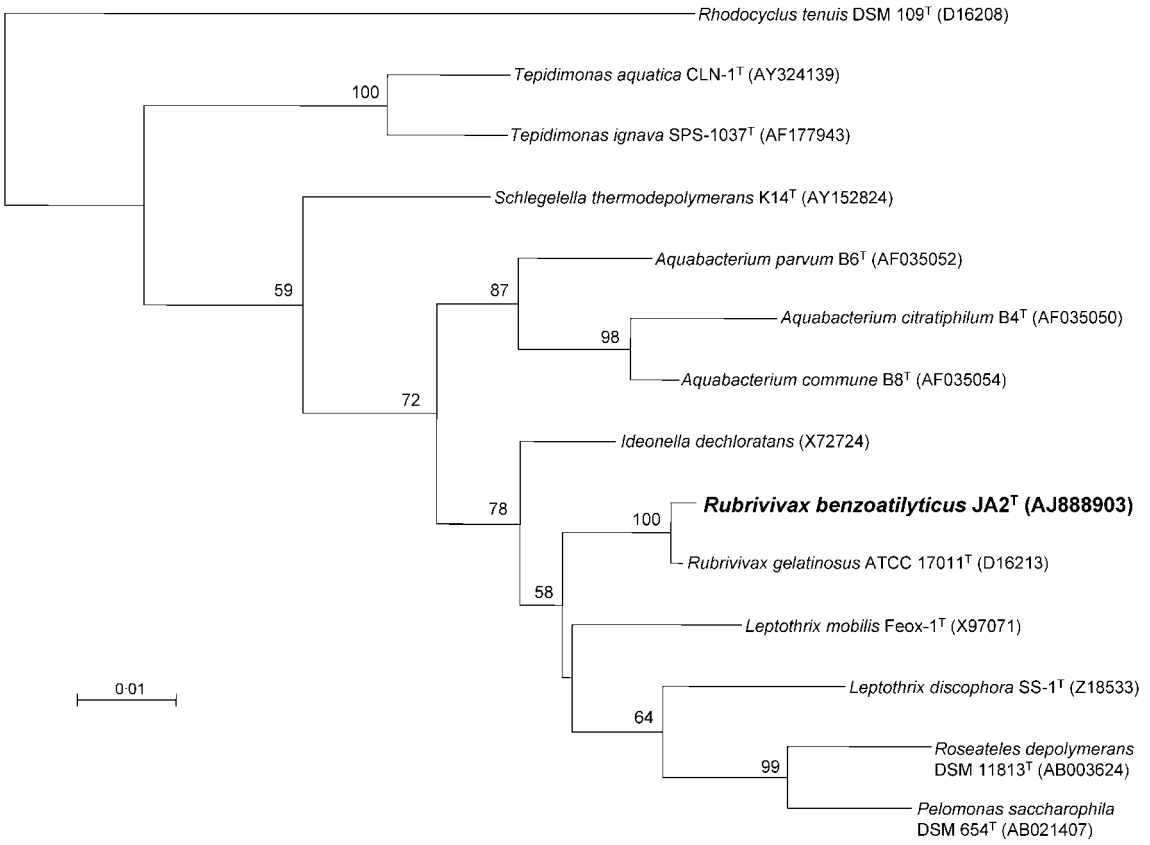

Fig. 2. Dendrogram depicting the phylogenetic relationships of strain JA2^T within the family *incertae sedis* of the *Betaproteobacteria* based on 16S rRNA gene sequence analysis. Numbers at nodes are confidence values of bootstrap analyses with 100 resamplings. Bar, 1 nucleotide substitution per 100 nucleotides.

Table 2. Characteristics that differentiate strain JA2^T from related freshwater purple betaproteobacteria

Strains: 1, Strain JA2^T; 2, *Rubrivivax gelatinosus* ATCC 17011^T; 3, *Rhodoferax fermentans* JCM 7819^T; 4, *Rhodocyclus purpureus* DSM 168^T; 5, *Rhodocyclus tenuis* DSM 109^T. All strains are chemo-organotrophic and utilize acetate, butyrate, succinate and ethanol. +, Positive/substrate utilized; −, negative/not utilized; NT, not tested.

Characteristic	1	2*	3*	4*	5*
Cell shape	Rod	Rod	Curved rod	Half-circle	Spiral
Cell size:					
Width (μm)	0·7–1·0	0·4–0·5	0·6–0·9	0·6–0·7	0·3–0·5
Length (μm)	2·0–6·0	1·0–3·0	1·5–3·0	2·0–3·0	1·5–6·0
Flagella	Single polar	Single polar	Single polar	Non-motile	Single polar
NaCl requirement	−	−	+	−	−
Optimum NaCl concentration	NT	NT	<1 %	NT	NT
Vitamin requirement	−	Biotin and thiamine	Biotin and thiamine	B_{12} and *p*-ABA†	−
Fermentation	−	−	+	−	−
Colour of culture suspension	Orange–brown	Pale-peach/dirty yellowish-brown	Peach–brown	Purple–violet	Brownish-red or purple–violet
Carotenoids	Sphaeroidene series	Sphaeroidene series	Sphaeroidene series	Rhodopinal series	Rhodopinal series
Intracytoplasmic membrane	−	Tubes	−	Tubes	Tubes
Gelatin liquefaction	+	+	−	−	−
DNA G+C content (mol%)	74·9	70·5–72·4	59·8–60·3	64–65	64–65
Utilization of organic substrates:					
Formate	+	+	−	−	−
Propionate	+	+	−	−	+
Valerate	−	+	NT	−	+
Caproate	+	NT	−	+	+
Pyruvate	+	+	+	−	+
Lactate	−	+	+	−	−
Citrate	−	+	−	+	−
Malate	+	+	+	−	+
Fumarate	+	+	+	−	+
Tartarate	+	+	NT	−	−
Glutamate	+	+	+	−	−
Glucose	+	+	+	−	−
Fructose	+	+	+	−	−
Mannitol	−	−	+	−	−
Glycerol	+	−	−	−	−
Methanol	−	+	−	−	−
Benzoate	+	−	−	+	−

*Data from Willems *et al.*, 1991, Hiraishi *et al.*, 1991 and Pfennig & Trüper 1989.
†*p*-ABA, *p*-Aminobenzoic acid.

et al., 2001), have shown differences between strain JA2^T and *Rubrivivax gelatinosus* ATCC 17011^T in a few major cellular components, viz., aromatic compounds, polysaccharides and esters (see Supplementary Fig. S4 in IJSEM Online).

Curved cells commonly observed with *Rubrivivax gelatinosus* (Willems *et al.*, 1991) were not observed with strain JA2^T, the cells of which were always straight rods. One significant difference between strain JA2^T and the other *Rubrivivax* species is the absence of internal membrane structures, which form tubular intrusions in *Rubrivivax gelatinosus* (Willems *et al.*, 1991). In terms of the lack of internal membrane structures, strain JA2^T more probably resembles *Rhodoferax* species (Hiraishi *et al.*, 1991). Preliminary analysis of the carotenoid content of strain JA2^T indicated that it probably has both sphaeroidene and spirilloxanthin series of carotenoids, again similar to *Rubrivivax gelatinosus*. This is in contrast to *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*, which only have carotenoids of the sphaeroidene series (Pinta *et al.*, 2003; Harada *et al.*, 2001; Steiger *et al.*, 2000). The other major difference between strain JA2^T and many other phototrophic members of the *Alphaproteobacteria* and *Betaproteobacteria* is the lack of any vitamin requirement for growth, which is rather rare in this group (Imhoff, 1995). *Rhodocyclus tenuis* and *Roseateles depolymerans* (an aerobic BChl *a*-containing bacterium; Suyama *et al.*, 1999) are the only other species of phototrophic *Betaproteobacteria* that do not have a requirement for vitamins.

Strain JA2^T produced large amounts (up to 85 % of the cell dry weight) of polyhydroxyalkanoates. A similar yield of polyhydroxyalkanoate from *Ralstonia* (*Alcaligenes*) *eutropha* has been reported, with accumulation of up to 96 % of the cellular dry weight (Pedrosalio *et al.*, 1985). This is exploited commercially for biopolyester production (Sasikala & Ramana, 1996).

Strain JA2^T did not produce indole directly from tryptone; however, indole production from tryptophan was observed. This suggests that strain JA2^T is unable to hydrolyse tryptone, in contrast to the extensive proteolytic capabilities of *Rubrivivax gelatinosus* (Tanskul *et al.*, 2003). Therefore we recommend the use of tryptophan for the indole production (tryptophanase) test rather than tryptone. Production of indole from tryptophan, catalysed by the enzyme tryptophanase, is a common diagnostic marker for differentiating members of *Enterobacteriaceae*, including *Escherichia coli*, *Proteus vulgaris*, *Providencia* spp. and *Morganella* spp. (Sonnenwirth, 1980), which belong to the *Gammaproteobacteria*.

Anoxygenic phototrophic bacteria capable of photo-organotrophic utilization of aromatic hydrocarbons mainly belong to the *Alphaproteobacteria* and among the photosynthetic *Betaproteobacteria*, *Rhodocyclus purpureus* and strain JA2^T are capable of benzoate utilization. In addition, anaerobic aromatic hydrocarbon degradation among the *Alphaproteobacteria* is restricted to the photosynthetic members, whereas it is common among members of the *Betaproteobacteria*, including species of *Thauera* and *Azoarcus* (Heider & Fuchs, 1997). Strain JA2^T degraded a variety of aromatic hydrocarbons (Table 1), adding to the aromatic hydrocarbon degrading diversity of phototrophic bacteria (Sasikala & Ramana, 1998).

Description of *Rubrivivax benzoatilyticus* sp. nov.

Rubrivivax benzoatilyticus (ben.zo′at.i.ly′ti.cus. N.L. n. *benzoas -atis* benzoate; Gr. adj. *lutikos* able to loose, able to dissolve; N.L. adj. *lyticus* dissolving; N.L. masc. adj. *benzoatilyticus* dissolving benzoate, named after its utilization of benzoate).

Cells are rod-shaped, 0·7–1·0 μm wide and 2·0–6·0 μm long. Motile by means of a single polar flagellum. Colour of the cell suspension is orange–brown and cells multiply by binary fission. Colony morphology on agar slants is round, convex and smooth, with dark orange–brown colour surrounded by thin opaque margin. Internal membranes are absent. Intact cells have absorption maxima at 378, 488, 520, 590, 802 and 884 nm. Photosynthetic pigments are BChl *a* and probably carotenoids of the alternative spirilloxanthin series with both sphaeroidene and spirilloxanthin. Growth modes are photo- and chemo-organoheterotrophy and photolithoautotrophy. Organic substrates photometabolized include formate, acetate, propionate, butyrate, valerate, succinate, malate, fumarate, oxaloacetate, 2-oxoglutarate, pyruvate, glucose, fructose, tartarate, glutamate, glycerol, ethanol and benzoate. Does not metabolize lactate, citrate, mannitol, methanol or sorbitol. Inorganic electronic donors such as molecular hydrogen and thiosulfate support growth. NaCl and vitamins are not required for growth. The DNA G+C content of the type strain is 74·9 mol% (HPLC).

The type strain is JA2^T (=ATCC BAA-35^T=JCM 13220^T=MTCC 7087^T). Habitat is rhizosphere of flooded paddy.

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