Rhodobacter johrii sp. nov., an endospore-producing cryptic species isolated from semi-arid tropical soils

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An oval to rod-shaped, phototrophic, purple non-sulfur bacterium, strain JA192^T, was isolated from an enrichment culture of a pasteurized rhizosphere soil sample from a field cultivated with jowar (sorghum) collected from Godumakunta village near Hyderabad, India. Strain JA192^T is Gram-negative, motile and produces endospores. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that the strain JA192^T is closely related to *Rhodobacter sphaeroides* 2.4.1^T (99.9 % sequence similarity), *Rba. megalophilus* JA194^T (99.8 %) and *Rba. azotoformans* KA25^T (98.1 %) and clusters with other species of the genus *Rhodobacter* of the family *Rhodobacteraceae*. However, DNA–DNA hybridization with *Rba. sphaeroides* DSM 158^T, *Rba. megalophilus* JA194^T and *Rba. azotoformans* JCM 9340^T showed relatedness of only 38–57 % with respect to strain JA192^T. On the basis of 16S rRNA gene sequence analysis, DNA–DNA hybridization data and morphological, physiological and chemotaxonomic characters, strain JA192^T represents a novel species of the genus *Rhodobacter*, for which the name *Rhodobacter johrii* sp. nov. is proposed. The type strain is JA192^T (=DSM 18678^T =JCM 14543^T =MTCC 8172^T).

At the time of writing, the genus *Rhodobacter* comprises 11 recognized species, *Rhodobacter capsulatus*, *Rba. sphaeroides*, *Rba. azotoformans*, *Rba. blasticus*, *Rba. veldkampii* and six species described by our group, *Rhodobacter vinaykumarii* (Srinivas *et al.*, 2007), *Rba. changlensis* (Anil Kumar *et al.*, 2007), *Rba. ovatus* (Srinivas *et al.*, 2008), *Rba. maris* (Venkata Ramana *et al.*, 2008), *Rba. megalophilus* (Arunasri *et al.*, 2008) and *Rba. aestuarii* (Anil Kumar *et al.*, 2009). In this study, a strain was isolated from semi-arid soils of Andhra Pradesh, India. According to 16S rRNA gene sequence analysis, the isolate, designated strain JA192^T, clustered within the genus *Rhodobacter*. On the basis of phenotypic and molecular analysis, it is proposed that strain JA192^T represents a novel species.

In May 2002, a wet rhizosphere soil sample from a field cultivated with jowar (sorghum) was collected from Godumakunta village, Hyderabad, India (17.28° N 78.35° E), and brought to the laboratory. The soil was first air-dried for 24 h in the laboratory and then pasteurized (80 °C for 30 min in a hot oven) as described previously (Madigan, 1992). Pasteurized soil (0.5 g) was added to a mineral medium (Biebl & Pfennig, 1981) with pyruvate (0.1 %, w/v) and ammonium chloride (0.1 %, w/v) as carbon and nitrogen sources, respectively, in fully filled screw-capped tubes (10 × 100 mm) and incubated at 30 °C and 2400 lx. Olive-green enrichments of heliobacteria

Abbreviations: ICM, internal cytoplasmic membrane; PHA, poly-β-hydroxyalkanoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA192^T is AM398152.

Further micrographs of strain JA192^T, results of FTIR fingerprinting and a whole-cell absorption spectrum are available as supplementary material with the online version of this paper.

(identified through whole-cell absorption maxima for bacteriochlorophyll *g* at 785–790 nm) were observed after 7 days. The enrichment culture was streaked on agar test-tube slants (25 × 150 mm test tubes sealed with subba seals and flushed with argon) and after 48 h of incubation only one type of reddish-brown colonies appeared on the slants. The culture was purified by repeated streaking on the agar slants to yield a pure culture. The strain was maintained in broth and preserved by lyophilization.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony by a boiling method (Englen & Kelley, 2000). PCR amplification was done as described previously (Imhoff *et al.*, 1998b) and 16S rRNA gene sequencing was performed using the BigDye Terminator version 1.1 sequencing kit (Applied Biosystems) in a 3730 DNA Analyzer (Applied Biosystems) as specified by the manufacturer. For sequencing, primers 342f (Lane, 1991), 790f (5′-GATACCCTGGTAGTCC-3′) and 543r (Muyzer *et al.*, 1993) were used. Sequence similarities were determined using the BLAST 2 SEQUENCES program (Tatusova & Madden, 1999). Sequences were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997) using standard settings and the alignment was improved manually. Phylogenetic trees were reconstructed by the neighbour-joining (NJ; Fig. 1) and maximum-parsimony (MP) methods (topologies of the NJ and MP trees were very similar) using MEGA 4.0 software (Tamura *et al.*, 2007). 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).

Morphological and physiological characteristics were determined as follows. Gram-staining and motility of the cells by the hanging drop technique were assessed according to Skerman (1967). Flagella and internal cytoplasmic membrane (ICM) structures were determined using transmission electron microscopy by the protocol of Arunasri *et al.* (2005). Sizes of vegetative cells and spores of strain JA192^T were determined from scanning electron microscope images after fixing the cells in glutaraldehyde (2.5 % glutaraldehyde in 0.05 M phosphate buffer, pH 7.2) for 24 h at 4 °C and post-fixing (2 h) in aqueous (2 %) osmium tetroxide. After a process of dehydration, the air-dried sample was gold coated and viewed in a JEOL JSM 5600 scanning electron microscope.

Poly-β-hydroxyalkanoate (PHA) granules were observed by epifluorescence microscopy after Nile blue A staining (Ostle & Holt, 1982). Spore staining was done according to Schaeffer & Fulton (1933) using a Himedia staining kit. Fluorescent staining was done using acridine orange according to the method described by Schichnes *et al.* (2006). *In vivo* absorption spectra were measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). Carotenoid composition was analysed by using HPLC (Takaichi & Shimada, 1992). Fatty acid methyl esters were prepared, separated and identified according to the instructions for the Microbial Identification System (Microbial ID; MIDI; Agilent 6850) (Sasser, 1990); this analysis was carried out by M/s Royal Life Sciences Pt. Ltd (Secunderabad, India). Dipicolinic acid (pyridine 2,6-dicarboxylic acid) was extracted from sporulating cells using the protocol of Janssen *et al.* (1958) and analysed using HPLC. HPLC analysis was performed at room temperature using a Shimadzu SPD-10AVP isocratic system. Methanol/water/acetonitrile (1 : 1 : 0.25) was used as the solvent at 1.0 ml

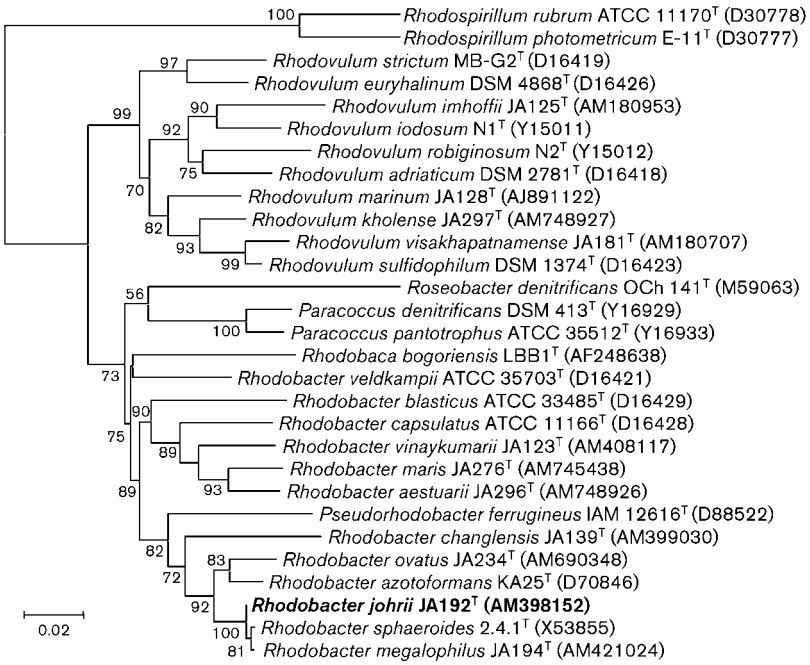

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis of members of the *Rhodobacteraceae* including strain JA192^T and all *Rhodobacter* species with validly published names. The NJ tree was constructed using MEGA 4.0 (Tamura *et al.*, 2007). Numbers at nodes represent bootstrap values (100 replicates). Bar, 2 substitutions per 100 nucleotide positions.

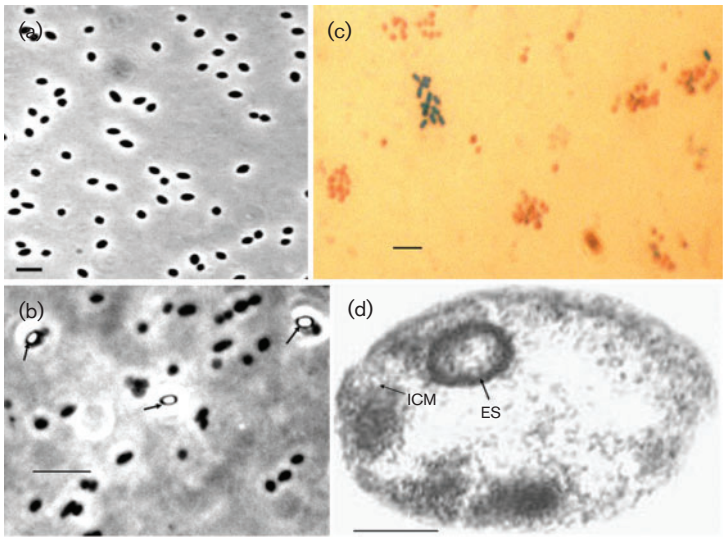

Fig. 2. Micrographs of cells of strain JA192^T. (a) Phase-contrast photomicrograph of vegetative cells. Bar, 2.5 μm. (b) Phase-contrast photomicrograph of sporulating cells (arrows). Bar, 5 μm. (c) Malachite green-stained sporulating cells. Bar, 5.5 μm. (d) Transmission electron micrograph showing sporulated cell along with vesicular ICM structures and thick-walled endospore (ES). Bar, 0.39 μm.

min^{-1} [Luna 5μ C_{18} (2) 100A column; 250 × 4.6 mm] and compounds were detected using a UV-Vis detector at 270 nm. The retention time for calcium dipicolinate was 2.5 min. Endometabolome (fingerprinting) analysis of lyophilized cells of both sporulated and unsporulated cultures of strain JA192^T was carried out using a Fourier-transform infrared (FT-IR) spectrometer (Perkin Elmer) equipped with a KBr beam splitter and a DTGS detector. Spectra were recorded between 4000 and 450 cm^{-1} at a resolution of 4 cm^{-1} and the data were processed using spectrum one software.

For DNA–DNA hybridization, DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out by methods established at the DSMZ as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian).

Individual cells of strain JA192^T were oval to rod-shaped (Fig. 2a), 0.8–0.9 μm wide and 1.5–1.9 μm long (Supplementary Fig. S1a, available in IJSEM Online), motile (polar flagella; Supplementary Fig. S2) and multiplied by binary fission (Fig. 2a). Electron photomicrographs of ultrathin sections of vegetative cells demonstrated the presence of a vesicular type of internal membrane structures (Fig. 2d). Since JA192^T was the only strain of phototroph that grew from the thoroughly pasteurized soil (heliobacteria, though enriched, could not be grown beyond three subcultures), it was interesting to look for endospores in this strain. Vegetative cells of strain JA192^T were grown (60 ml reagent bottles) photoheterotrophically in the medium described above with glutamate [0.1 %, w/v, to induce endospores (Grossman & Losick, 1988)] for 48 h, after which the culture was exposed to 80 °C in dark for 48 h in an oven. About 35 % of the cells contained highly refractile bodies (Fig. 2b) that took up malachite green (spore

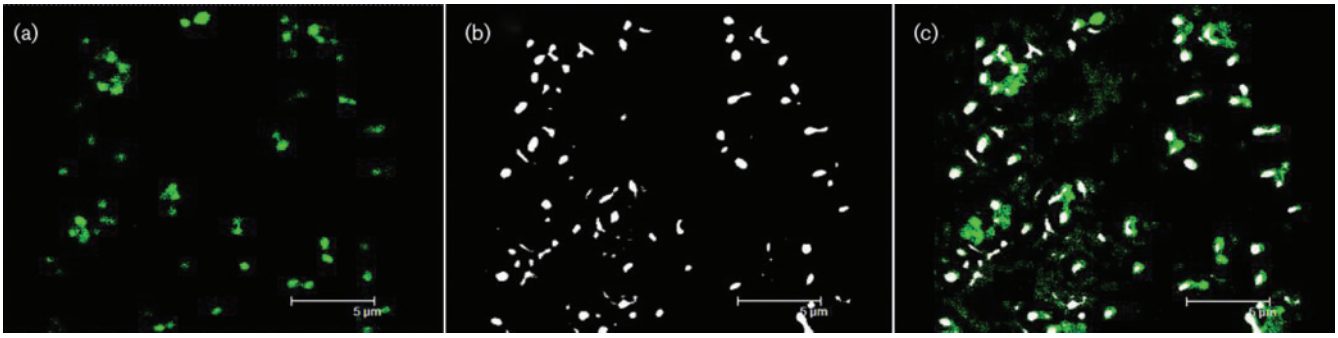

Fig. 3. Fluorescence microscopy of strain JA192^T stained with acridine orange. (a) Fluorescence micrograph showing vegetative cells as highly fluorescent bodies. (b) Transmission electron micrograph showing spores. (c) Overlay of fluorescence and transmission electron micrographs showing vegetative cells as fluorescent bodies and spores as unstained bodies. Bars, 5 μm.

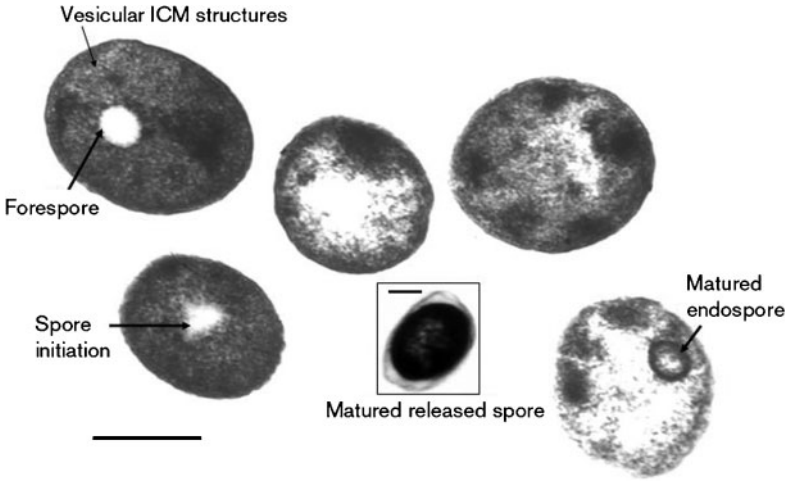

Fig. 4. Transmission electron micrographs showing sporogenesis in strain JA192^T. Bar, 0.4 μm. Inset, Released mature spore; bar, 0.48 μm.

staining; Fig. 2c), and the oval structures (1.1 μm long by 0.8–0.9 μm wide; Supplementary Fig. S1a) did not take up acridine orange, while the vegetative cells did (Fig. 3). The purity of the sporulated culture of strain JA192^T was confirmed by streaking on nutrient agar plates and also on mineral agar medium (Biebl & Pfennig, 1981) and incubating under both aerobic and anaerobic conditions. The purity of the sporulated culture of strain JA192^T was also established from 16S rRNA gene sequencing, which gave a good-quality chromatogram without any background signal.

The refractile bodies are not PHA granules, since they did not take up Nile blue stain, nor did the endometabolome of the sporulated culture show an ester peak (1740 cm^{-1}; Supplementary Fig. S3a). In addition, when the sporulating culture was boiled in trichoromethane for 10 min (to dissolve PHA granules) and stained with acridine orange, the oval structures remained intact and did not take up the stain (Supplementary Fig. S4). Transmission electron micrographs showing sporogenesis (spore initiation, forespore formation, thick-walled endospore; Fig. 4) along with vesicular ICM structures (Figs 2d and 4) provide unequivocal evidence of endospore formation in strain JA192^T, which is further confirmed by the detection of calcium dipicolinate in the sporulated culture (Supplementary Fig S3b). We rule out the possibility of these structures being cysts, which are common in certain phototropic species (Favinger *et al.*, 1989; Zhang *et al.*, 2003; Berleman & Bauer, 2004), where the whole cell is modified and the structures are non-refractile. This is the first report of endospore formation in a phototrophic proteobacterium; however, endospore formation in the non-phototrophic gammaproteobacteria *Serratia marcescens* subsp. *sakuensis* (Ajithkumar *et al.*, 2003) and *Coxiella burnetii* (McCaul & Williams, 1981) and exospore formation in an alphaproteobacterium, *Methylosinus trichosporium* (Reed & Dugan, 1979; Reed *et al.*, 1980), have been reported. Such endospore structures were not observed with a few other type strains of the genus *Rhodobacter* tested under similar conditions in our laboratory (Table 1). Heat-exposed (80 °C for 20 min in a water bath) cultures of strain JA192^T were viable (colonies were observed when plated and broth cultures were grown when these heat-treated cells were inoculated), while the other type strains of the genus *Rhodobacter* were not (heat resistance was 40–45 °C), which distinguishes strain JA192^T from other members of the genus *Rhodobacter*. Sporogenesis could also be induced in strain JA192^T from cells exposed to low (4 °C) temperatures, in old cultures and under nutrient depletion (carbon or nitrogen). Spore germination was induced from heat-exposed (2 ml culture at 80 °C for 20 min in a water bath) cells by transferring into fresh growth medium, followed by phototrophic incubation (10–12 h in 10 × 100 mm fully filled screw-capped test tubes), resulting in the release of spores from vegetative cells (Fig. 5a) and spore germination (Fig. 5b; Supplementary Fig. S1b) along with a germination tube (Fig. 5c).

Although we have not studied sporogenesis extensively in strain JA192^T, the following are the major morphological differences from *Bacillus* and other Gram-positive bacteria: (i) absence of asymmetrical division and compartmentalization of the sporulating cell into mother cell and forespore (commonly seen at the onset of sporulation in *Bacillus*; Eldar *et al.*, 2009); (ii) absence of engulfment of forespore; (iii) release of spore is not by complete lysis of mother cell but through a process of opening at the pole (Fig. 5a); and (iv) a multilayered coat (Chada *et al.*, 2003) could not be demonstrated (Fig. 4, inset). Features that are common include: (i) presence of refractile structures (Fig. 2b); (ii) spores are stained by malachite green (Fig. 2c); (iii) formation of forespore (Fig. 4); (iv) detection of calcium dipicolinate (Supplementary Fig. S3b); (v) thickening of forespore (Fig. 4); and (vi) germination through germ tube formation (Fig. 5b and Supplementary Fig. S1b). The pathway of sporogenesis and spore germination in strain JA192^T is shown diagrammatically in Fig. 6.

Strain JA192^T was able to grow photo-organoheterotrophically [anaerobic, light (2400 lx), with different carbon

Table 1. Differentiating characteristics of strain JA192^T and species of the genus *Rhodobacter*

Taxa: 1, strain JA192^T; 2, *Rba. megalophilus* JA194^T; 3, *Rba. sphaeroides* DSM 158^T; 4, *Rba. maris* JA276^T; 5, *Rba. veldkampii*; 6, *Rba. vinaykumarii* JA123^T; 7, *Rba. ovatus* JA234^T; 8, *Rba. blasticus*; 9, *Rba. azotoformans* KA25^T; 10, *Rba. aestuarii* JA296^T; 11, *Rba. capsulatus*; 12, *Rba. changlensis* JA139^T. Data in columns 5, 8 and 11 were taken from Imhoff (2005) and Takaichi (1999); other data represent a comparative analysis done in the authors' laboratory unless indicated. Motile species exhibit polar flagella. +, Present; −, absent; ±, variable in different strains; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell width (μm)	0.8–0.9	1.2–1.5	2.0–2.5	0.6–1.0	0.6–0.8	0.8–1.2	0.9–1.2	0.6–0.8	0.6–1.0	0.7–1.0	0.5–1.2	0.8–1.0
Cell length (μm)	1.5–1.9	1.5–2.0	2.5–3.5	1.0–1.5	1.0–1.3	1.5–3.0	1.0–2.0	1.0–2.5	0.9–1.5	1.5–2.0	2.0–2.5	2.0–4.0
Cell shape*	O–R	O	S–O	O–R, C	O–R	R	O	O–R	O–R	O–R, C	O–R, C	O–R, C
Motility	+	−	+	+	−	−	−	−	+	+	+	−
Endospores	+	−	−	−	NR	−	−	NR	−	−	NR	−
Colour of cell suspension†	YB	YB	GB	YB	YB	YB	YB	OB	YB	YB	YB	YB
Carotenoid composition (mol%)‡												
Neurosporene	2	−	−	2	NR	−	−	1	−	3	−	−
DMSE	−	−	−	−	NR	−	−	−	−	2	−	−
SE	82	73	46	89	NR	20	83	47	85	93	83	67
SO	11	18	47	2	NR	77	12	47	−	tr	11	21
OH-SE	2	2	−	4	NR	3	5	5	3	2	4	12
OH-SO	−	−	2	4	NR	−	−	−	1	−	2	−
Me-SE	−	−	−	−	NR	−	−	−	10	−	−	−
Polar§	3	6	7	−	NR	−	−	−	−	−	−	−
Internal membrane system‖	V	V	V	V	V	V	V	L	V	V	V	V
Slime production	+	+	±	−	−	+	−	−	+	−	±	+
NaCl required (optimum)	−¶	−	−#	−	−	+ (1–4)	−	−	−**	−	−††	−
pH for growth												
Range	6.0–8.0	6.0–8.0	6.0–8.5	5.0–8.0	NR	6.0–8.0	6.0–8.0	NR	6.0–8.0	6.0–8.5	6.5–7.5	6.5–8.0
Optimum	7.0	7.0	7.0	6.5–7.0	7.5	6.0–7.5	6.5–7.0	6.5–7.5	7.0–7.5	7.0	7.0	6.5–7.5
Temperature optimum (°C)	30	5–40	30–34	25–30	30–35	20–30	25–30	30–35	30–35	25–30	30–35	20–30
Sulfate assimilation	+	+	+	+	−	+	+	+	+	+	+	+
Denitrification	−	−	±	−	−	−	−	−	+	−	−	−
Vitamin(s) required‡‡	b	t	b, n, t	t	b, *p*-ABA, t	b	b, t	b, n, t, B_{12}	b, n, t	t	t, (b, n)	b, n, t
DNA G+C content (mol%)§§	64–65.7	66.7	70.8–73.6	62.9	64.4–67.5	68.8	70.1	65.3	69.5–70.2	65.1	68.1–69.6	69.4
Cellular fatty acid content (%)‖‖												
$C_{10:0}$ 3-OH	3.3	3.0	3.8	2.1	−	2.5	−	NR	−	2.3	−	3.5
$C_{16:0}$	3.4	4.2	5.1	8.7	4.3	4.6	4.2	NR	3.6	4.5	4.2	8.0
$C_{16:1}\omega7c$	2.1	1.6	1.9	1.3	17.5	1.4	6.4	NR	4.2	1.0	5.8	7.3
$C_{17:0}$	0.6	0.4	0.6	1.2	0.6	0.8	1.0	NR	1.2	−	1.3	0.1

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
$C_{18:0}$	7.9	5.4	13.9	0.1	6.5	13.4	9.5	NR	8.9	7.2	6.1	9.7
$C_{18:1}\omega7c$	74	73	77.2	68.3	69.4	61.4	70.1	NR	66.8	62.2	79.8	57.0
11-Methyl $C_{18:1}\omega7c$	1.9	3.4	1.0	0.1	–	1.6	2.2	NR	3.2	–	–	6.2
$C_{19:1}\omega7c/\omega6c$/19 cyclo	–	–	–	6.1	–	–	0.6	NR	–	–	–	–

*C, Chains; O, ovoid; R, rod-shaped; S, spherical.

†GB, Greenish brown; OB, orange–brown; YB, yellowish brown.

‡DMSE, Demethylspheroidene; SE, spheroidene; SO, spheroidenone; OH-SE, hydroxyspheroidene; OH-SO, hydroxyspheroidenone; Me-SE, methoxyneurosporene; Polar, polar carotenoid; tr, trace.

§Polar carotenoid with nine conjugated double bonds.

‖L, Lamellar; V, vesicular.

¶NaCl not required, but optimal growth occurs at 3 % NaCl.

#Optimal growth in the absence of NaCl but growth occurs at 3 % NaCl.

**Optimal growth in the absence of NaCl but growth occurs at 5 % NaCl.

††Data from Hiraishi *et al.* (1996).

‡‡b, Biotin; B_{12}, vitamin B_{12}; n, niacin; *p*-ABA, *p*-aminobenzoic acid; t, thiamine; (b, n), a few strains require biotin and/or niacin.

§§Ranges represent values for more than one strain, including the type strain.

‖‖–, Not detected/not reported.

Fig. 5. Phase-contrast photomicrographs of strain JA192^T. (a) Release of matured spore. Bar, 0.5 μm. (b) Germinating spores. GT, Germ tube. Bar, 2 μm. (c) Formation of germ tube (GT). Bar, 900 nm.

Fig. 6. Diagrammatic representation of sporogenesis and spore germination in strain JA192^T. Based on transmission electron microscopy.

sources] and chemo-organoheterotrophically [dark, aerobic, with pyruvate (0.3 % w/v) as carbon source]. Photolithoautotrophy [anaerobic, light (2400 lx) with $Na_2S\,.\,9H_2O$ (1.0 mM) or $Na_2S_2O_3$ (5.0 mM) as electron donors and $NaHCO_3$ (0.1 %) as carbon source], chemolithoautotrophy [dark, aerobic, with $Na_2S\,.\,9H_2O$ (1.0 mM) or $Na_2S_2O_3$ (5.0 mM) as electron donors and $NaHCO_3$ (0.1 %) as carbon source] and fermentative growth [anaerobic, dark, pyruvate (0.3 %, w/v) as fermentable substrate] could not be demonstrated in strain JA192^T. Utilization of various organic/inorganic compounds as carbon/electron donors and utilization of various nitrogen sources by strain JA192^T are detailed in the species description. The photosynthetically grown cell suspension was yellowish brown. The whole-cell absorption spectrum (Supplementary Fig. S5) of strain JA192^T in sucrose gave absorption maxima at 374, 446, 476, 509, 590, 800 and 854 nm, confirming the presence of bacteriochlorophyll *a* and carotenoids, as determined by HPLC, is given in Table 1, along with other type strains of the genus *Rhodobacter*. Whole-cell fatty acid analysis revealed that $C_{18:1}\omega7c$ is predominant in strain JA192^T, which is similar to most type strains of the genus *Rhodobacter* analysed from cells grown under identical conditions (Table 1).

The DNA base composition of strain JA192^T was 65.7 mol% G+C (by HPLC). The phylogenetic relationship of strain JA192^T to other purple non-sulfur bacteria was examined by 16S rRNA gene sequencing. The sequence obtained was classified as belonging to a member of the genus *Rhodobacter* by the Naive Bayesian rRNA Classifier version 2.0 (http://rdp.cme.msu.edu/classifier/classifier.jsp) implemented in the online Ribosomal Database Project, release 10 (http://rdp.cme.msu.edu/index.jsp). The highest sequence similarities of strain JA192^T were found to the type strains of *Rba. sphaeroides*, *Rba. megalophilus* and *Rba. azotoformans* (99.9, 99.8 and 98.1 %, respectively). Phylogenetic analysis confirmed a phylogenetic position of strain JA192^T in close vicinity of *Rba. sphaeroides* 2.4.1^T and *Rba. megalophilus* JA194^T (Fig. 1). In contrast to the high 16S rRNA gene sequence similarities, DNA–DNA hybridization of strain JA192^T with *Rba. sphaeroides* DSM 158^T and *Rba. megalophilus* JA194^T revealed relatedness of only 57 % (with both strains) and 38.4 % relatedness to *Rba. azotoformans* JCM 9340^T, indicating a clear distinction between their genomes, which is strongly supported by differences (7.2, 1.5 and 6.4 mol%, respectively) in their G+C contents. Hybridization was also performed with additional strains [JA193 (73.6 mol% G+C) and JA334 (64 mol% G+C), used as positive controls] that show 99 % 16S rRNA gene sequence similarity to *Rba. sphaeroides* DSM 158^T. The results indicated 81.8 and 33.8 % relatedness (respectively) with *Rba. sphaeroides* DSM 158^T and 46 and 88.8 % relatedness (respectively) with strain JA192^T, indicating their genome relatedness with the respective species.

Low levels of genetic divergence among 16S rRNA gene sequences preclude the identification of novel taxa among cryptic morphospecies (Erwin & Thacker, 2008; Fawley *et al.*, 2004). Genome analysis (DNA–DNA hybridization) together with several other phenotypic traits helped in differentiating *Rba. megalophilus* JA194^T from *Rba. sphaeroides* DSM 158^T (Arunasri *et al.*, 2008) and *Rubrivivax benzoatilyticus* from *Rubrivivax gelatinosus* (Ramana *et al.*, 2006). Other pairs of closely related phototrophic species with nearly 99 % 16S rRNA gene sequence similarity, such as *Phaeospirillum molischianum* and *Phaeospirillum fulvum* (Imhoff *et al.*, 1998a), *Allochromatium minutissimum* and *Allochromatium vinosum* (Imhoff *et al.*, 1998b) and *Blastochloris sulfoviridis* and *Blastochloris viridis* (Hiraishi, 1997), have been maintained due to characteristic phenotypic differences.

The unique character of induction of endospores, NaCl optimum for growth, growth factor requirements, genomic DNA G+C content and DNA–DNA hybridization clearly distinguish strain JA192^T from *Rba. megalophilus* JA194^T, *Rba. sphaeroides* DSM 158^T and other *Rhodobacter* species (Tables 1 and 2). On the basis of these data, strain JA192^T represents a novel species of the genus *Rhodobacter*, for which the name *Rhodobacter johrii* sp. nov. is proposed.

Description of *Rhodobacter johrii* sp. nov.

Rhodobacter johrii (joh′ri.i. N.L. masc. gen. n. *johrii* of B. N. Johri, an eminent and well-known Indian microbiologist).

Cells are Gram-negative, motile (polar flagella), ovals to rods (0.8–0.9 × 1.5–1.9 μm) that multiply by binary fission and have vesicular internal membrane structures. Endospores develop under stress conditions. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 374, 446, 476, 509, 590, 800 and 854 nm, confirming the presence of bacteriochlorophyll *a*. Spheroidene is the major carotenoid. Optimum growth occurs at 30 °C (range 25–35 °C) and pH 6.5–7.0 (range pH 6.0–8.0). NaCl is not required for growth, but cells grow well at 3 % (w/v) NaCl. Photoorganoheterotrophy with a few organic compounds is the preferred mode of growth. Good growth is observed with pyruvate and malate. Growth also occurs with acetate, butyrate, valerate, caproate, lactate, succinate, fumarate, oxaloacetate, 2-oxoglutarate, glucose, fructose, mannitol, sorbitol, glycerol and glutamate. Photolithoautotrophy and chemolithoautotrophy are not observed in the presence of sulfide, thiosulfate or hydrogen as electron donor and $NaHCO_3$ as carbon source. Fermentative growth is not observed in the presence of pyruvate as carbon source. Ammonium chloride, glutamate, glutamine and molecular nitrogen are good nitrogen sources. Biotin is required as growth factor. $C_{18:1}\omega7c$ is the dominant fatty acid along with $C_{18:0}$ and $C_{16:0}$ and lower levels of $C_{16:1}\omega7c$. The DNA base composition of the type strain is 65.7 mol% G+C (HPLC).

The type strain, JA192^T (=DSM 18678^T =JCM 14543^T =MTCC 8172^T), was isolated from a pasteurized rhizosphere soil of a field of jowar (sorghum), collected from a semi-arid soil of Godumakunta village near Hyderabad, Andhra Pradesh, India.

Table 2. Carbon sources and electron donors used by strain JA192^T and species of the genus *Rhodobacter*

Taxa: 1, strain JA192^T; 2, *Rba. megalophilus* JA194^T; 3, *Rba. sphaeroides* DSM 158^T; 4, *Rba. maris* JA276^T; 5, *Rba. veldkampii*; 6, *Rba. vinaykumarii* JA123^T; 7, *Rba. ovatus* JA234^T; 8, *Rba. blasticus*; 9, *Rba. azotoformans* KA25^T; 10, *Rba. aestuarii* JA296^T; 11, *Rba. capsulatus*; 12, *Rba. changlensis* JA139^T. Data in columns 5, 8 and 11 were taken from Imhoff (2005); for other strains, organic substrate utilization was tested during photo-organoheterotrophic growth under similar conditions in our laboratory. All strains utilized pyruvate, but not benzoate or arginine. +, Substrate utilized; −, substrate not utilized; ±, variable in different strains; (+), weak growth; NR, not reported.

Source/donor	1	2	3	4	5	6	7	8	9	10	11	12
Hydrogen	−	−	+	−	−	−	−	+	−	−	+	−
Sulfide	−	−	+	−	+	−	−	−	+	−	+	−
Thiosulfate	−	+	−	−	+	−	−	−	−	−	−	−
Sulfur	−	−	−	−	+	−	−	−	−	−	−	−
Formate	−	+	−	−	−	−	−	−	+	−	+	−
Acetate	+	+	+	(+)	+	+	(+)	+	+	−	+	−
Propionate	−	+	+	(+)	+	−	(+)	+	+	−	+	−
Butyrate	+	+	+	(+)	+	(+)	+	+	+	−	+	−
Valerate	+	+	+	(+)	+	−	+	NR	−	−	+	−
Caproate	+	+	+	(+)	+	−	+	NR	−	−	+	−
Caprylate	−	−	+	−	+	−	−	NR	−	−	+	−
Tartrate	−	+	+	−	−	−	−	−	−	−	−	−
Lactate	+	+	+	(+)	+	−	−	+	+	(+)	+	−
Malate	+	+	+	+	+	−	−	+	+	−	+	−
Fumarate	+	+	+	(+)	+	−	−	+	+	−	+	−
Citrate	−	−	+	−	−	−	−	+	−	−	±	−
Aspartate	+	+	−	−	+	−	−	NR	(+)	−	±	−
Glutamate	+	+	+	−	+	+	−	+	+	−	+	+
Gluconate	+	−	−	−	(+)	−	−	NR	−	−	−	−
D-Glucose	+	+	+	−	+	+	−	+	+	−	+	+
Fructose	+	+	+	−	−	−	−	+	+	−	+	−
Mannitol	+	+	+	−	−	+	+	+	+	−	±	+
Sorbitol	+	+	+	−	−	−	+	+	+	−	±	+
Glycerol	+	+	+	(+)	−	−	+	+	+	−	−	−
Ethanol	−	+	+	−	−	−	−	−	−	−	−	−
Propanol	−	−	(+)	−	−	+	−	NR	−	−	+	−

Acknowledgements

Financial assistance received from the Department of Biotechnology and Department of Science and Technology, Government of India, is acknowledged. Ch. S. thanks the Department of Biotechnology, Government of India, for the award of an Overseas associateship to work at Professor Imhoff's laboratory. Use of infrastructural facilities under the DST, FIST Level-II support and UGC (CAS) to HCU is gratefully acknowledged.

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