Strain JA430^T is a Gram-negative, vibrioid to spiral shaped phototrophic purple sulfur bacterium isolated from anoxic sediment of a saltern at Kanyakumari in a mineral salts medium that contained 2% NaCl (w/v). Strain JA430^T grows optimally at 5–6% NaCl and tolerates up to 12% NaCl. Intracellular photosynthetic membranes were of the lamellar type. Bacteriochlorophyll a and carotenoids of the spirilloxanthin series are present as photosynthetic pigments. Major cellular fatty acids are C_{18:1} \omega_7c, C_{16:0}, C_{19:0}cyclo\omega_8c and C_{16:1} \omega_7c/C_{16:1} \omega_6c. Strain JA430^T exhibits photoorganoheterotrophy and chemooorganoheterotrophy and requires para-aminobenzoic acid, pantothenate and pyridoxal phosphate for growth. Phylogenetic analysis on the basis of 16S rRNA gene sequence analysis showed that strain JA430^T forms monophyletic group in the genus Ectothiorhodospira. The highest sequence similarity for strain JA430_T was found with the type strains of Ectothiorhodospira variabilis DSM 21381^T (96.1%) and Ectothiorhodospira haloalkaliphila ATCC 51935^T (96.2%). Morphological and physiological characteristics discriminate strain JA430^T from other species of the genus Ectothiorhodospira, for which we describe this as a novel species, Ectothiorhodospira salini sp. nov. ( = NBRC 105915^T = KCTC 5805^T).

Key Words—Ectothiorhodospira; Gammaproteobacterium; purple sulfur bacterium; 16S rRNA gene based phylogeny
species.

Revision of species delineation in the genus *Ectothiorhodospira* left only 4 species (*Ectothiorhodospira mobilis*, *Ectothiorhodospira shaposhnikovii*, *Ectothiorhodospira marina* and *Ectothiorhodospira haloalkaliphila* [while *Ectothiorhodospira vacuolata* and *Ectothiorhodospira marismortui* are heterotypic synonyms]; Ventura et al., 2000), and a newly described *Ectothiorhodospira variabilis* (Gorlenko et al., 2009) brings the validly published names to five. Members of the genus *Ectothiorhodospira* are widely distributed in habitats like brackish, marine, saline, hypersaline and soda lakes. In this communication, we propose strain JA430<sup>T</sup>, a novel species of this genus that was isolated from a saltern.

**Materials and Methods**

*Isolation of strain JA430<sup>T</sup>*. Strain JA430<sup>T</sup> was isolated from a phototrophic enrichment of a sediment sample [pH ~ 7.0, 30°C, 5.0% (w/v) salinity] collected from a saltern at Kanyakumari, Tamil Nadu, India (GPS positioning of the sample collection site is, 8° 05’ N, 77° 31’ E). Pure culture was obtained by repeated streaking on agar slants (25 x 150 mm test tubes sealed with butyl rubber stoppers and the gas phase was replaced with argon). Purified cultures were grown in Biebl and Pfennig’s medium (Biebl and Pfennig, 1981) with 5.0% (w/v) NaCl in completely filled screw cap test tubes (10 x 100 mm) for phototrophic growth. Culture purity was examined microscopically and by streaking the culture on nutrient agar plates incubated aerobically in the dark at 30°C for 3 days.

*Media and growth conditions*. For routine culturing and for physiological tests, strains were grown phototrophically (anaerobic, light [2,400 lux]) in a mineral salts medium containing [g·L<sup>−1</sup>; pH 7.0]: KH₂PO₄ (0.5), MgSO₄·7H₂O (2.0), NaCl (20.0), NH₄Cl (0.6), CaCl₂·2H₂O (0.15) with yeast extract (0.3), ferric citrate [5 ml·L<sup>−1</sup> from 0.1% (w/v) stock], and trace element solution SL7 (1 ml·L<sup>−1</sup>; Biebl and Pfennig, 1981). Pyruvate (25 mm), NaHCO₃ (12 mm) and Na₂S·9H₂O (2 mm) were used as carbon source/electron donor. Pure cultures were maintained by repeated sub-culturing into fresh medium or as stab cultures and as lyophilized vials. Strain JA430<sup>T</sup> was deposited at NBRC and KCTC.

*Morphological characteristics*. Cell morphology (cell shape, cell division, cell size, flagella and visible inter-

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Pigment analysis. In-vivo absorption spectra were measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper and Pfennig, 1981). For pigment analysis, 1 L of the 48 h grown culture was harvested by centrifugation (16,000 rpm × 10 min). The supernatant was discarded and the pellet was washed twice with 0.8% NaCl solution and lyophilized. Pigments were extracted with methanol:acetone (7:2) from the lyophilized cells. Carotenoid composition was determined by C18-HPLC analysis, (eluted with acetonitrile:methanol:ethylacetate, 5:4:1; flow rate 1 ml·min⁻¹; absorption at 450 nm) with a PDA detector.

Quinones. Quinones were analyzed by HPLC using the protocol of Hiraishi and Hoshino (1984) and Hiraishi et al. (1984).

Phylogeny. Cell material for DNA isolation was taken from 1–2 ml of well grown liquid culture. DNA was extracted and purified by using the QIAGEN genomic DNA buffer set. Recombinant Taq polymerase was used for PCR, which was started with the primers 5'-GTTTGATCCTGGCTCAG-3' and 5'-TACCTTGTTACGACTTCA-3' (Escherichia coli positions 11–27 and 1489–1506, respectively). Sequences were obtained by cycle sequencing with the SequiTherm sequencing kit (Biozym) and the chain termination reaction (Sanger et al., 1997) using an automated laser fluorescence sequencer (Pharmacia). The identification of phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarity was achieved using the NCBI-BLAST search (Altschul et al., 1990) and EzTaxon server (Chun et al., 2007). The CLUSTAL_W algorithm of MEGA 4 was used for sequence alignments and MEGA 4 (Tamura et al., 2007) software was used for phylogenetic analyses of both individual and concatenated sequences. Distances were calculated by using the Jukes and Cantor correction in a pairwise deletion procedure. Unweighted pair group with mathematical average (UPGMA), neighbor-joining (NJ), minimum evolution (ME) and maximum parsimony (MP) methods in the MEGA4 software were used to construct phylogenetic trees. Percentage support values were obtained using a bootstrap procedure.

Determination of G+C content of DNA. For DNA G+C, chromosomal DNA was isolated and purified (Marmur, 1961). DNA was hydrolyzed and resultant nucleotides were analyzed by reverse phase HPLC using the methods of Mesbah et al. (1989).

Results and Discussion

Morphological and biochemical characteristics
Cells of strain JA430ᵀ were Gram-negative, 1.0–1.5 μm wide and 2.0–3.5 μm long and vibrioid to spiral in shape. Cell suspension was reddish brown when grown photosynthetically. Cells of strain JA430ᵀ were motile by means of polar flagella (Fig. 1) and multiplied by binary fission. A transmission electron microphotograph of an ultrathin section of strain JA430ᵀ revealed lamellar type internal membrane structures arranged parallel to the cytoplasmic membrane (Fig. 2). Strain JA430ᵀ was able to grow photoorganoheterotrophically [anaerobic, light (30 μE·m⁻²·s⁻¹), with pyruvate (0.3%, w/v)] and chemoorganoheterotrophically [aerobic, dark and pyruvate (0.3%, w/v)]. Photolithoautotrophy [anaerobic, light (30 μE·m⁻²·s⁻¹), Na₂S⁻⁹H₂O (1 mm), Na₂S₂O₃⁻⁵H₂O (2 mm), and NaHCO₃ (0.1%,

![Fig. 1. Electron micrograph of negatively stained cell of strain JA430ᵀ showing polar tuft of flagella. Bar, 667 nm.](image1)

![Fig. 2. TEM showing lamellar ICM structures in strain JA430ᵀ. Bar, 250 nm.](image2)
Table 1. Differentiating characteristics of species of the genus *Ectothiorhodospira*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain JA430&lt;sup&gt;T, b&lt;/sup&gt;</th>
<th>*E. variabilis&lt;sup&gt;c&lt;/sup&gt;</th>
<th>*E. haloalkaliphila&lt;sup&gt;d&lt;/sup&gt;</th>
<th>*E. marina&lt;sup&gt;d&lt;/sup&gt;</th>
<th>*E. shaposhnikovii&lt;sup&gt;d&lt;/sup&gt;</th>
<th>*E. mobilis&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (μm)</td>
<td>1.0–1.5 × 2.0–3.5</td>
<td>0.8–1.2 × 1.2–1.5</td>
<td>0.7–1.2 × 2.0–3.0</td>
<td>0.8–1.2 × 1.5–4.0</td>
<td>0.8–0.9 × 1.5–2.5</td>
<td>0.7–1.0 × 2.0–2.6</td>
</tr>
<tr>
<td>Color of cell suspensions</td>
<td>reddish brown</td>
<td>purple red/deep red</td>
<td>red</td>
<td>red</td>
<td>red</td>
<td>red</td>
</tr>
<tr>
<td>NaCl optimum (% w/v)</td>
<td>5</td>
<td>5–8</td>
<td>2–6</td>
<td>3</td>
<td>2–3</td>
<td>1–5</td>
</tr>
<tr>
<td>Salinity range (% w/v)</td>
<td>0.5–12</td>
<td>2–20</td>
<td>2.5–15</td>
<td>0.5–10</td>
<td>0–7</td>
<td>1–5</td>
</tr>
<tr>
<td>pH range</td>
<td>7.0–10.0</td>
<td>9.0–9.5</td>
<td>8.5–10.0</td>
<td>7.5–8.5</td>
<td>8.0–8.5</td>
<td>7.6–8.0</td>
</tr>
<tr>
<td>Vitamin requirement</td>
<td>PABA, PA, PY</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Sulfate assimilation</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Photolithoautotrophy</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>MK-7; Q-7</td>
<td>nd</td>
<td>MK-7; Q-8</td>
<td>MK-7; Q-8</td>
<td>MK-7; Q-7</td>
<td>MK-7; Q-8</td>
</tr>
<tr>
<td>(Mo%) G+C of DNA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63 (HPLC)</td>
<td>62.7 (T&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>63.5 (T&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>62.8 (Bd)</td>
<td>62.0 (T&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>67.3 (Bd)</td>
</tr>
<tr>
<td>Substrates used as carbon/e-donor</td>
<td>Hydrogen</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sulfur</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sulfite</td>
<td>–</td>
<td>nd</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Propionate</td>
<td>–</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Butyrate</td>
<td>–</td>
<td>–</td>
<td>nd</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>–</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Fumarate</td>
<td>–</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Succinate</td>
<td>–</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>nd</td>
<td>±</td>
</tr>
</tbody>
</table>

Symbols: +, substrate utilized or present; −, substrate not utilized or absent; ±, variable; (+), weak growth; nd = not determined; Bd, buoyant density; T<sub>m</sub>, thermal denaturation; PABA = para-aminobenzaldehyde; PA = pantothenate; PY = pyridoxal phosphate.

<sup>a</sup>Values for type strains. Substrates in parentheses are utilized by some strains. <sup>b</sup>Organic substrate utilization was tested during photoheterotrophic growth and substrates used as electron donors was tested in the presence of bicarbonate (0.1%, w/v).

All strains are motile and utilize acetate, malate and pyruvate as organic carbon sources. All strains utilize sulfide and thiosulfate as an electron donor. Data taken from: <sup>c</sup>Gorlenko et al. (2009); <sup>d</sup>Imhoff (2005).

Note: *Ectothiorhodospira vacuolata* (Imhoff et al., 1981) is a later heterotypic synonym (Ventura et al., 2000) of *Ectothiorhodospira shaposhnikovii* (Cherni et al., 1969). *Ectothiorhodospira mobilis* (Pelsh, 1936) is an earlier heterotypic synonym (Ventura et al., 2000) of *Ectothiorhodospira marismortui* (Oren et al., 1989).
chemolithoautotrophy [aerobic, dark, Na₂S₂O₃·5H₂O (2 mM) and NaHCO₃ (0.1%, w/v)] could not be demonstrated. Strain JA430ᵀ could utilize only acetate, malate and pyruvate as carbon sources under photosynthetic growth conditions, while the vast majority of organic substrates could not support growth (Table 1). Fermentative growth [anaerobic, dark, with glucose/glutamate/pyruvate (0.3%, w/v)] is absent.

Ammonium chloride and urea were used as nitrogen sources, while nitrate, nitrite, glutamate and glutamine were not utilized by strain JA430ᵀ. Diazotrophic growth could not be demonstrated in strain JA430ᵀ. Catalase reaction was positive. Production of indole from L-tryptophan and phenol from L-phenylalanine was absent. Salt (NaCl) was essential for the growth of strain JA430ᵀ, ranging from 0.5–12% (w/v) with a optimum of 5% (w/v). The pH range for growth of strain JA430ᵀ was 7.0–10.0, with an optimum at 7.5.

PABA, pantothenate and pyridoxal phosphate were required for the growth of strain JA430ᵀ, while all other species of the genus *Ectothiorhodospira* have no vitamin requirement (Table 1).

Photosynthetic pigments and quinones

The whole cell absorption spectra of strain JA430ᵀ exhibited absorption maxima at 311, 377, 512–518, 590–593, 797 and 860 nm and the absorption spectrum for pigments extracted with acetone gave absorption maxima at 493–496 nm, confirming the presence of bacteriochlorophyll a and carotenoids of the spirilloxanthin series. HPLC analysis indicated the presence of spirilloxanthin (53 mol%), rhodopin (22 mol%), anhydrorhodovibrin (13 mol%), tetrahydrolycopene (7 mol%) and rhodovibrin (5 mol%) as major carotenoids. MK-7 and Q-7 are the major quinones of strain JA430ᵀ.

Cellular fatty acid composition

Whole-cell fatty acid analysis of strain JA430ᵀ revealed the predominance of C₁₈:₁ω₇c (56%) with sufficient amounts of C₁₆:₀ (18%), C₁₉:₀cycloω₈c (12%) and C₁₆:₁ω₇c/C₁₆:₁ω₆c (8%).

16s rRNA gene sequence comparison and DNA G+C content

The neighbor joining (NJ) phylogenetic tree constructed by using an almost complete (1,348 nt) 16S rRNA gene sequence of strain JA430ᵀ demonstrated that it belongs to the genus *Ectothiorhodospira* and
showed closest similarity with the type strain *E. variabilis* WN22\(^\text{T}\) (96.1% sequence similarity; Fig. 3). The phylogenetic distances from other type strains of the genus *Ectothiorhodospira* are; *E. haloalkaliphila* BN 9903\(^\text{T}\) (96.2%), *E. marina* DSM 241\(^\text{T}\) (96.6%), *E. shaposhnikovii* DSM 239\(^\text{T}\) (95.1%) and *E. mobilis* DSM 237\(^\text{T}\) (95%). The DNA G+C content of the strain JA430\(^\text{T}\) is 63 mol%. The 16S rRNA gene sequence similarity (96.1%) of strain JA430\(^\text{T}\) with its nearest neighbor *E. variabilis* WN22\(^\text{T}\) is within the recommended standards to delineate a bacterial species (Stackebrandt and Ebers, 2006; Stackebrandt and Goebel, 1994). Strain JA430\(^\text{T}\) also differs phenotypically (Table 1) from other members of the genus *Ectothiorhodospira* in 1. Color of cell suspension, 2. Utilizing limited organic substrates for growth, 3. Lack of sulfate assimilation, 4. Lack of photolithoautotrophic growth and 5. vitamin requirements. These clear differences of strain JA430\(^\text{T}\) from other validated *Ectothiorhodospira* spp. necessitated the description of the novel strain as a new species for which the name *Ectothiorhodospira salini* sp. nov. is proposed.

**Description of *Ectothiorhodospira salini* sp. nov.**

*Ectothiorhodospira salini* (sa.li’ni. L. gen. n. salini, of a salt-cellar). Cells are vibrioid to spiral shaped, 1.0–1.5 \(\mu\)m wide and 2.0–3.5 \(\mu\)m long. Cells are motile by means of polar flagella and divide by binary fission. Growth occurs under anaerobic conditions in the light. Internal photosynthetic membranes are of the lamellar type arranged parallel to the cytoplasmic membrane. Phototrophic cultures are reddish brown. The in vivo absorption spectrum of intact cells exhibits maxima at 593, 797 and 860 nm. Bacteriochlorophyll \(a\) and carotenoids; spirilloxanthin, rhodopin, anhydrorhodovibrin, tetrahydrolycopene and rhodovibrin are present. The type strain is mesophilic (30°C), growing at a pH optimum of 7.5 (range, pH 7.0–10.0) and requiring 5% NaCl for optimal growth (NaCl range, 0.5–12.0%). The preferred mode of growth is photoorganoheterotrophy with a few organic compounds. Good growth occurs on acetate, malate and pyruvate. Photoautotrophic and fermentative growth is absent. PABA, pantothenate and pyridoxal phosphate are required as growth factors. \(C_{18:1}\omega7c\) is the dominant fatty acid; sufficient amounts of \(C_{16:0}\), \(C_{19:0}\omega7c\omega8c\) and \(C_{16:1}\omega6c\omega8c\) are also found. Major quinones are MK-7 and Q-7. The DNA G+C content of the type strain is 63 mol% (by HPLC). Natural habitats are solar salterns. The type strain, JA430\(^\text{T}\) (=NBRC 105915\(^\text{T}\) = KCTC 5805\(^\text{T}\)), was isolated from a sediment sample from a solar saltern at Kanyakumari, Tamil Nadu, India.

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