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Rhodospirillum sodomense, sp. nov., a Dead Sea *Rhodospirillum* species

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Abstract. A new species of halophilic anoxygenic purple bacteria of the genus *Rhodospirillum* is described. The new organism, isolated from water/sediment of the Dead Sea, was vibrio-shaped and an obligate halophile. Growth was best at 12% NaCl, with only weak growth occurring at 6% or 21% NaCl. Growth occurred at Mg^{2+} concentrations up to 1 M but optimal growth was obtained at 0.05–0.1 M Mg^{2+}. Bromide was well tolerated as an alternative anion to chloride. The new organism is an obligate phototroph, growing photoheterotrophically in media containing yeast extract and acetate or a few other organic compounds. Growth of the Dead Sea *Rhodospirillum* species under optimal culture conditions was slow (minimum t_d ~ 20 h). Cells contained bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series and mass cultures were pink in color. Absorption spectra revealed the presence of a B875 (light-harvesting I) but no B800/B850 (light-harvesting II) photopigment complex. The new organism shares a number of properties with the previously described halophilic phototrophic bacterium *Rhodospirillum salinarum* and was shown to be related to this phototroph by 16S rRNA sequencing. However, because of its salinity requirements, photosynthetic properties, and isolation from the Dead Sea, the new phototroph is proposed as a new species of the genus *Rhodospirillum*, *R. sodomense*.

Key words: Anoxygenic phototrophic bacteria – Purple bacteria – *Rhodospirillum sodomense* – Dead Sea – Hypersaline environments – Photosynthesis

The Dead Sea is a unique hypersaline environment because the dominant cation is Mg^{2+} instead of Na^{+} and because high levels of Ca^{2+} and Br^{-} are present as well (Oren 1988). Although other hypersaline environments have been shown to harbor a diverse and abundant prokaryotic and eukaryotic microflora, with the exception of the green alga *Dunaliella parva*, the Dead Sea is exclusively a prokaryotic realm (Oren 1988).

All of the prokaryotes isolated from Dead Sea water and sediment have been chemoorganotrophs, including Archaea such as *Haloferax* (previously *Halobacterium*) *volcanii* (Mullakhanbhai and Larsen 1975) and *Halobacterium sodomense* (Oren 1983b), and Bacteria such as *Halobacteroides halobius* (Oren et al. 1984) and *Sporohalobacter species* (Oren et al. 1987). Anoxygenic phototrophic bacteria are often abundant in hypersaline environments (Imhoff 1988) but such organisms have not previously been reported from the Dead Sea. In some hypersaline environments, anoxygenic purple bacteria, particularly of the genus *Ectothiorhodospira*, are the major primary producers (Imhoff 1988; Javor 1989). In fact, in saline sulfide springs adjacent to the Dead Sea, microbial mats develop consisting of cyanobacteria and a newly described species of *Ectothiorhodospira*, *E. marismortui* (Oren 1989; Oren et al. 1989). The latter organism requires sulfide but shows a NaCl requirement considerably lower than organisms indigenous to the Dead Sea; this is presumably a reflection of the relatively low salt content of these springs (Oren et al. 1989).

Purple nonsulfur bacteria also inhabit hypersaline environments. These include the moderately halophilic *Rhodospirillum* species, *R. salexigens* (Drews 1981) and *R. mediosalinum* (Kompantseva and Gorlenko 1985), and the more halotolerant species *Rhodospirillum salinarum* (Nissen and Dundas 1984). The latter species is of particular interest because of its extremely broad salinity limits – the organism grows well at both seawater salinities and at salinities above 20% NaCl (Nissen and Dundas 1984).

In anaerobic phototrophic enrichment cultures established using water/sediment samples from the Dead Sea, we obtained a weakly pigmented anoxygenic phototroph that resembled in some respects the previously described species *R. salinarum* (Nissen und Dundas 1984). However, upon characterizing the new organism it became clear that it differed in many properties from that *R. salinarum* and warranted classification as a new species

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of purple bacteria. The new organism, *Rhodospirillum sodomense* strain DSI, is described herein and is named in reference to its natural habitat, the Sea of Sodom, the Talmudic name for the Dead Sea.

Materials and methods

Source of the organism

The new phototroph was isolated from sediment/water samples kindly collected by Dr. David M. Ward (Montana State University) from a site on the western shore of the Dead Sea near Ein Gedi.

Medium and standard growth conditions

Cultures of strain DSI were routinely grown in a medium modified from that used for growth of *Halobacterium volcanii* (Mullakhanbhai and Larsen 1975). The medium, designated as medium DSIC$^-$, contained, per liter: 125 g NaCl, 10 g $MgCl_2 \cdot 6\,H_2O$, 0.2 g $CaCl_2 \cdot 2\,H_2O$, 0.5 g NH_4Cl, 0.6 g KH_2PO_4, 2.5 g K_2SO_4, 1 g $NaHCO_3$, 0.1 g $Na_2S_2O_3 \cdot 5\,H_2O$, 2.1 g MOPS buffer, 20 µg vitamin B_{12} and 1 ml trace elements solution (Wahlund et al. 1991). Organic substrates, usually acetate and yeast extract, were added to medium DSIC$^-$ as specified below. To avoid precipitation, the magnesium and calcium salts and the $NaHCO_3$ were autoclaved as separate solutions. After autoclaving, cooled medium was assembled and aseptically adjusted to pH 7. Plates of DSIC$^-$ medium were solidified with 15 g/l washed Difco (Detroit, Mich., USA) agar. *Rhodospirillum salinarum* was grown in medium SAL, which was identical to medium DSIC$^-$ except that it contained only one half the amount of NaCl.

Cultures were grown photosynthetically (anaerobic-light) in completely filled 17-ml screw-cap tubes of medium DSIC$^-$ or SAL supplemented with yeast extract to a final concentration of 0.1–0.2%; Na acetate was often added to 0.1–0.2% as well. Cultures were routinely incubated in a water bath at 37–40 °C and illuminated with a bank of incandescent lights at an intensity of approximately 11.2 klux. Light intensities were measured using a Weston model 756 light meter. Growth was monitored with a Klett-Summerson colorimeter (660 filter). Before incubation in the light, cultures were placed at 37 °C overnight in darkness.

Growth experiments

Photoheterotrophic growth of strain DSI and of *R. salinarum* on individual carbon substrates was determined in mineral media containing 0.05% yeast extract and supplemented with 0.4% (w/v) of an organic carbon source (see 'Results'). If growth occurred, cells were transferred to fresh medium and tested again for growth. Use of a substrate was scored following a second transfer in the same medium. Photoautotrophic growth potential was tested on plates of mineral media containing 0.005% yeast extract incubated under a H_2/CO_2 atmosphere; identical plates incubated under a N_2/CO_2 atmosphere served as control.

Aerobic dark growth potential was tested on mineral media plates supplemented with 0.2% yeast extract and incubated in darkness at 30 °C in air. Microaerobic growth was tested by auxanography (Siefert and Pfennig 1980) in seeded 1% agar tubes of mineral media containing 0.2% yeast extract and exposed to air in darkness.

Nitrogenase

Nitrogenase activity was tested by acetylene reduction. Cells grown in mineral media lacking ammonia but containing 0.05% yeast extract were used to inoculate 100 ml of the same medium supplemented with 0.2% acetate in a 250-ml stoppered side-arm flask with a N_2:CO_2 (98:2) headspace. After 3 days of growth, 20 ml of culture was removed anaerobically and tested for nitrogenase activity as previously described (Madigan et al. 1984; Wahlund et al. 1991).

Pigment, protein, and PHB analyses

Absorption spectra of membrane vesicles (chromatophores) obtained by standard techniques (Madigan and Gest 1979) and suspended in 50 mM Tris · HCl (pH 7) were measured in a Hitachi (Tokyo, Japan) U-2000 recording spectrophotometer. Bacteriochlorophyll *a* was extracted from cell pellets with ice-cold methanol for 1 h (−20 °C in darkness), the resulting suspension centrifuged and the absorbance measured at 770 nm using the extinction coefficient of 46.1 $g \cdot l^{-1} \cdot cm^{-1}$ of Smith and Benitez (1955). Protein assays were performed on cell pellets remaining after methanol extraction using a dye binding assay (Bulletin 1069, BioRAD Laboratories, Richmond, Calif., USA). Poly-β-hydroxybutyric acid (PHB) was assayed by extracting cell pellets of acetate grown cells by the method of Law and Slepecky (1965).

Electron microscopy

Cells were prepared for electron microscopy as previously described (Wahlund et al. 1991). Stained sections were viewed using a Philips (Eindhoven, The Netherlands) model 300 transmission electron microscope. Intact cells were viewed by scanning electron microscopy following critical point drying.

Genetic properties

The percent G + C content of DNA was determined via thermal denaturation following isolation of genomic DNA as described by Wahlund et al. (1991). 16S ribosomal RNA was isolated and sequenced and a phylogenetic tree constructed as previously described (Wahlund et al. 1991).

Results

Isolation of strain DSI

Illuminated anaerobic (37 °C) liquid enrichments using the medium described for growth of *Haloferax volcanii* (Mullakhanbhai and Larsen 1975) containing 0.05% yeast extract and 0.1% sodium acetate and inoculated with samples of Dead Sea water and sediment gave rise within four weeks to a light pink sediment. The pink cell mass was used as inoculum for agar shake tubes of the same medium which yielded light pink colonies within two weeks. Repeated application of the agar shake method eventually yielded a pure culture of a small, thin, vibrio-shaped organism we designated as strain DSI.

Because the color of cultures of the new organism was similar to that of some halobacteria, i. e., a dull pink, it was initially considered that the new isolate could be a *Halobacterium* species. However, pigment analyses quickly showed that the new organism contained bacteriochlorophyll (Bchl) *a* and was thus an anoxygenic phototroph. Cells of strain DSI measured 0.6–0.7 × 1.6–

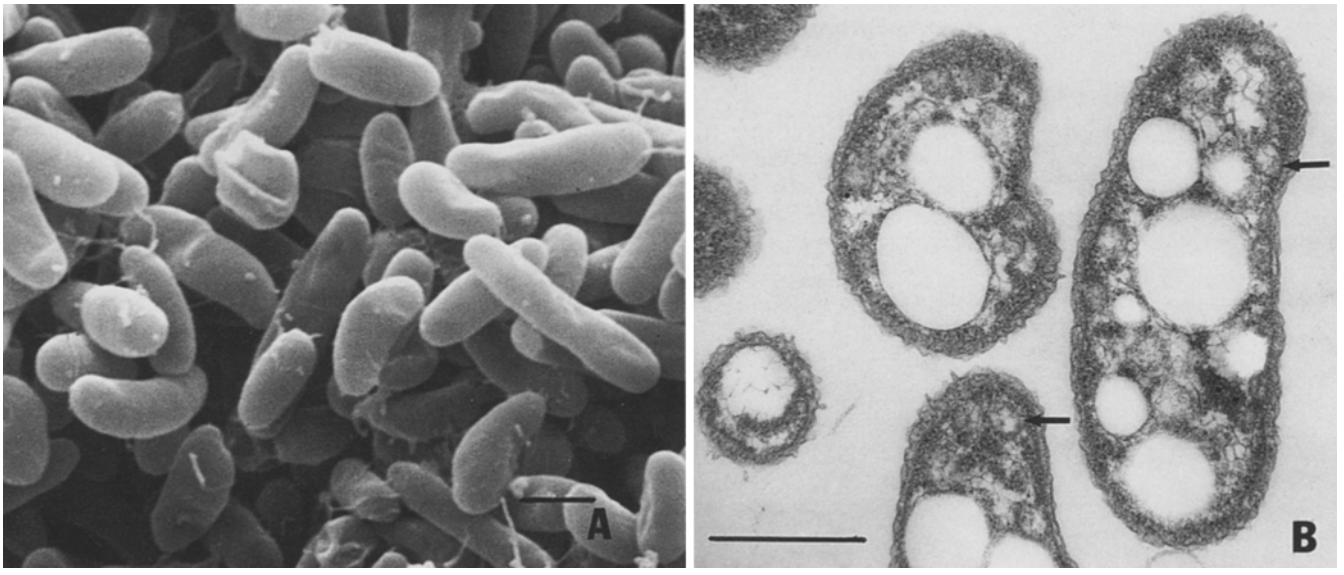

Fig. 1. Scanning **A** and transmission **B** electron micrographs of *Rhodospirillum sodomense* strain DSI. In **B** note large droplets of poly-β-hydroxybutyrate and occasional chromatophores (arrows). *Marker bar* = 1 μm in **A** and 0.5 μm in **B**

2.5 μm, stained gram-negatively, and were weakly motile. Scanning electron micrographs of strain DSI showed vibrio-shaped cells (Fig. 1A) while transmission electron micrographs revealed a wavy outer membrane on cells and the presence of a few internal membrane vesicles (chromatophores), the presumed location of photosynthetic pigments (Fig. 1B). Large granules of poly-β-hydroxybutyric acid were also present in acetate-grown strain DSI cells (Fig. 1B). Similar properties in an anoxygenic phototroph had previously been noted in the halophilic *R. salinarum* (Nissen and Dundas 1984), although cells of this organism were on average somewhat larger than those of strain DSI.

Growth properties

Strain DSI grew under photoheterotrophic conditions in complex media containing yeast extract as carbon source. In mineral media supplemented with 0.05% yeast extract, acetate, malate, succinate, or pyruvate stimulated growth of strain DSI whereas citrate, butyrate, and propionate did not (data not shown). Despite numerous experiments employing vitamin supplements to defined media containing acetate/CO_2 as carbon sources, sustained growth of strain DSI was never achieved in the complete absence of yeast extract; at least 0.005–0.01% yeast extract was required for reasonable growth. Minimum generation times for strain DSI under optimal growth conditions (0.1% each of yeast extract and acetate) were never less than about 20 h (data not shown). On plates, photoheterotrophically-grown strain DSI produced small, stiff, raised pink colonies which were difficult to remove with an inoculating loop. Tests for photoautrophic growth of strain DSI with H_2 or H_2S as electron donor (in the presence of 0.005% yeast extract) were negative.

Strain DSI grew poorly if at all in darkness under aerobic chemoorganotrophic conditions (on plates). Under the same conditions, *R. salinarum*, used for comparative purposes, grew as large, shiny, deep red colonies that were mucoid and easy to remove from a plate. Tests for microaerobic growth of strain DSI in seeded agar tubes were also negative (data not shown), indicating that this organism is probably an obligate phototroph.

Useable nitrogen sources were not easy to identify for strain DSI because of the requirement for yeast extract. However, in low yeast extract media ammonia clearly served as a nitrogen source but no evidence for N_2 fixation was obtained. In the latter connection, suspensions of cells grown in media containing 0.05% yeast extract and 0.1% acetate, conditions which allow for derepression of nitrogenase in other purple bacteria, were completely negative when assayed for nitrogenase by acetylene reduction. In addition, using cloned *Rhodospirillum rubrum nifHDK* probes, no hybridization to strain DSI DNA was obtained (P. W. Ludden, G. P. Roberts, and M. T. Madigan, unpublished results), strongly suggesting that strain DSI is not a diazotrophic phototrophic bacterium.

Photosynthetic pigments

Anaerobic photoheterotrophically grown mass cultures of *R. salinarum* and strain DSI differed dramatically in color. As previously reported (Nissen and Dundas 1984), phototrophic cultures of *R. salinarum* were blood red in color. By contrast, cultures of strain DSI were pink and eventually turned beige in very old cultures or in cultures grown with high levels of yeast extract (>0.2%).

Absorption spectra of membrane vesicles prepared from both organisms showed both to contain Bchl *a* as sole bacteriochlorophyll but revealed differences between them in their light-harvesting pigment-protein complexes

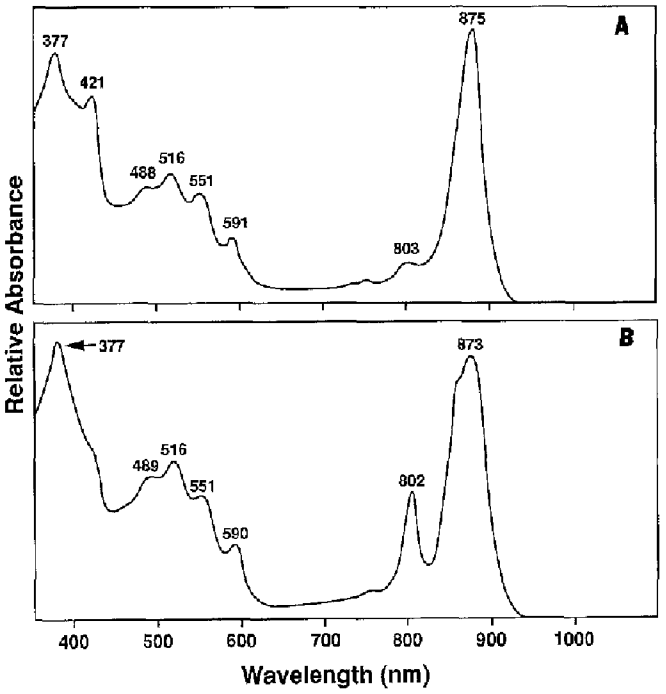

Fig. 2. Absorption spectra of membrane vesicles (chromatophores) of **A** *Rhodospirillum sodomense* strain DSI and **B** *Rhodospirillum salinarum*. Both organisms were grown phototrophically at 13 klux incandescent illumination

(Fig. 2). In *R. salinarum*, strong absorption maxima in the near infrared at 802 and 873 nm with a distinct shoulder at 850 nm (Fig. 2B) indicated the presence of both light-harvesting II (B800/850) and light-harvesting I (B875) photopigment complexes. By contrast, membrane vesicles from strain DSI yielded a spectrum typical of *Rhodospirillum rubrum* (Pfennig and Trüper 1992); only minor absorbance at 803 nm (due to reaction center Bchl *a*) was evident along with a single symmetrical peak at 875 nm, indicating the presence of only the B875 complex (Fig. 2A). Absorption maxima at 489, 516, and 551 nm in spectra of both organisms indicated the presence of carotenoids of the spirilloxanthin series (Pfennig and Trüper 1992).

Absorption spectra of cells grown at different light intensities supported the original contention that strain DSI contained only a single (B875) light-harvesting complex. Although the shoulder in the *R. salinarum* spectrum at 850 nm was more apparent in high light grown cells than in low light grown cells, a major peak at 802 nm in spectra of all such cells clearly signaled the presence of the B800/850 complex. By contrast, in strain DSI, absorbance at 803 nm always remained minor and the 875 peak in cells grown at any light intensity was always symmetrical with no evidence of a shoulder at 850 nm (data not shown).

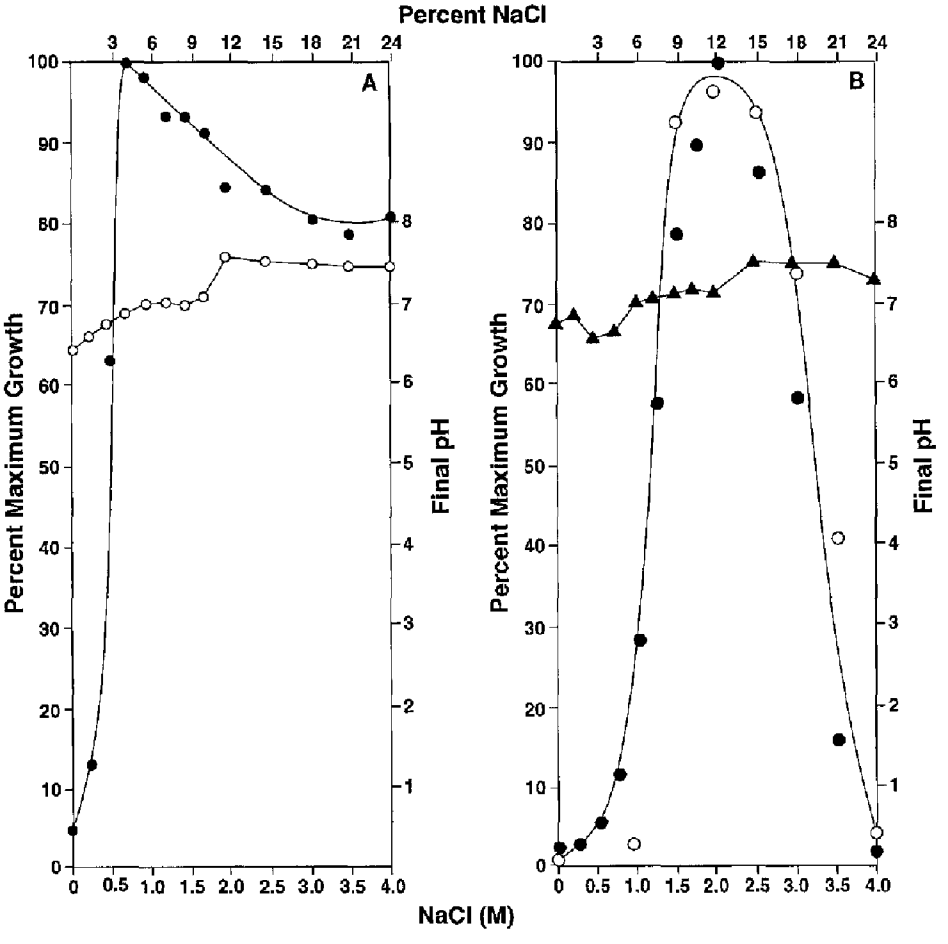

Fig. 3. Effect of NaCl concentration on the growth of **A** *Rhodospirillum salinarum* and **B** *Rhodospirillum sodomense* strain DSI. Each point (○, ●) is the mean of two separate experiments. Final pH (○ in **A**; ▲ in **B**) was taken to insure that significant fluctuations were not occurring during growth

Cells of strain DSI and of *R. salinarum* also differed dramatically in their specific pigment content, perhaps a reflection of the differences between the two organisms in light-harvesting Bchl content. Cells of strain DSI grown at the optimal light intensity (~ 13 klux) contained 19.4 µg Bchl *a*/mg cell protein; cells of *R. salinarum* grown at the same intensity contained over three times this much, 58.7 µg Bchl *a*/mg cell protein. Although not quantitated, it is assumed that the weak pink pigmentation of mass cultures of strain DSI (as compared with the intense red color of *R. salinarum*) is due to a low specific carotenoid content in this organism, since carotenoids are generally synthesized in coordinate fashion with bacteriochlorophyll and other components of the photosynthetic complexes of phototrophic purple bacteria (Wellington et al. 1992).

Salinity optima

R. salinarum was previously described as a halophilic anoxygenic phototroph which had a very broad tolerance for NaCl (Nissen and Dundas 1984). Our results with this organism were in agreement with that, but showed *R. salinarum* to grow optimally (achieve highest cell yields in a defined time period) at relatively low salt concentrations (4–6% NaCl) (Fig. 3A). By contrast, results with strain DSI were quite different. The Dead Sea phototroph showed a clear preference for 12% (2 M) NaCl and grew well only within the range of 10–15% NaCl (Fig. 3B). At 4% NaCl, a concentration that supported optimal phototrophic growth of *R. salinarum* (Fig. 3A), strain DSI was unable to grow (Fig. 3B). No growth of strain DSI was obtained at 4 M (24%) NaCl while good growth of *R. salinarum* at that salinity was still obtained (Fig. 3A).

In similar experiments in which NaCl was present at optimal levels for each organism (12% NaCl for strain DSI, 4–6% NaCl for *R. salinarum*) and the concentration of $MgCl_2$ varied, it was found that both organisms grew best at about 0.1 M Mg^{2+}, but that both organisms could grow, albeit slowly, in media containing up to 1 M $MgCl_2$ (data not shown). In either organism, concentrations of Mg^{2+} above about 0.3 M significantly reduced final cell yield, but surprisingly, this effect seemed more apparent in strain DSI than in *R. salinarum*; at 1 M Mg^{2+} the former organism grew to about 20% of maximal cell yield while the latter grew to about 70% of maximal (data not shown). On the other hand, the Mg^{2+} affinity of strain DSI was low and differed from that of *R. salinarum*, as strain DSI was unable to grow in media containing less than 100 µM Mg^{2+} whereas *R. salinarum* grew normally (data not shown).

NaBr tolerance

The Dead Sea is highly unusual among hypersaline environments because of its high bromide content, over 5 g/l (Oren 1988). Because of this, and because Br^-, like Cl^-, is a halogen, experiments were performed to test the ability of Br^- to replace Cl^- as major anion for growth. The results of these experiments showed clear differences between strain DSI and *R. salinarum* and are shown in Fig. 4. Strain DSI was able to grow to near maximal cell yields in media to which up to 80% of the NaCl was replaced by NaBr while growth of *R. salinarum* showed significant inhibition of growth under these same conditions (Fig. 4). However, some Cl^- is presumably required for strain DSI because although growth initially occurred in media containing 2 M NaBr and no NaCl (Fig. 4), cultures could not be transferred in this medium and continue growing.

Genetic properties

DNA purified from strain DSI had a G + C base ratio of 66.2–66.4 mol % as determined by thermal denaturation. Identical procedures carried out on DNA isolated from cells of *R. salinarum* yielded a value of 65.2 mol %, considerably lower than the published values of 67.4 (buoyant density) −68.1 (thermal denaturation) for DNA from this organism (Nissen and Dundas 1984).

The phylogenetic position of strain DSI relative to that of other purple bacteria was examined by 16S rRNA

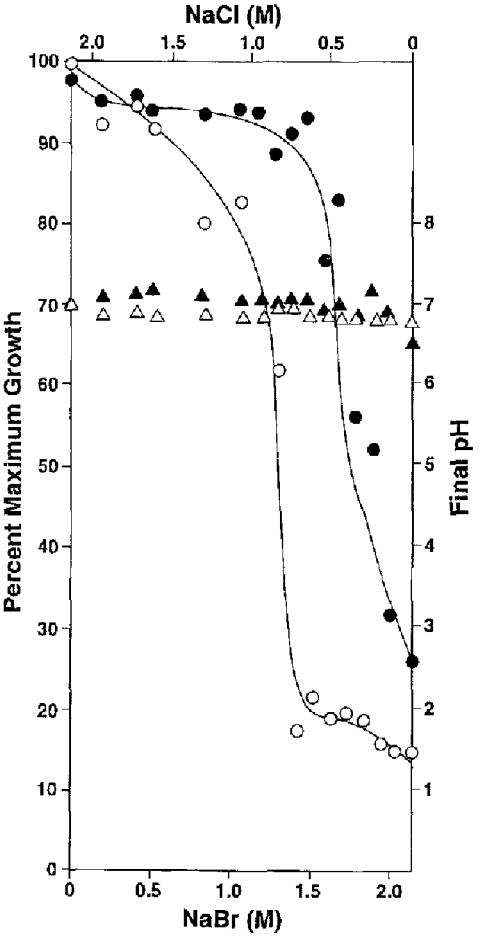

Fig. 4. Effect of the replacement of NaCl by NaBr on the growth of *Rhodospirillum sodomense* strain DSI (●) and *Rhodospirillum salinarum* (○). *Closed* and *open triangles* represent final pH values in each culture, respectively. Each point is the mean of two separate experiments

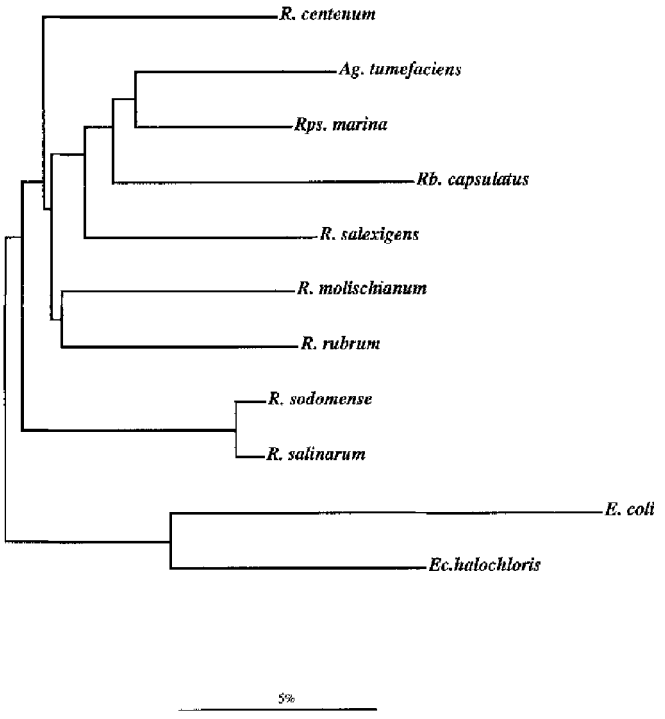

Fig. 5. Phylogenetic position of *Rhodospirillum sodomense* in relation to other *Rhodospirillum* species and selected other organisms. *Scale* indicates distance on the tree equivalent to 5 nucleotide substitutions per 100 nucleotides in 16S rRNA. Phylogenetic tree is based upon evolutionary distances given in Table 1. *Abbreviations: R., Rhodospirillum; Rb., Rhodobacter; Rps., Rhodopseudomonas; Ag., Agrobacterium; E., Escherichia; Ec., Ectothiorhodospira*

sequencing. A phylogenetic tree generated from comparative sequencing data showed strain DSI to be closely related but clearly distinguishable from *R. salinarum*. These two organisms form a cluster distinct from other halophilic purple bacteria including *Rhodospirillum salexigens*, *Rhodopseudomonas marina*, and *Ectothiorhodospira species* (Fig. 5 and Table 1). The strain DSI/*R. salinarum* cluster emerges from the "α-1" purple bacteria (Woese et al. 1984; Woese 1987) as a branch distinct from that of other α-1 purple bacteria such as *Rhodospirillum rubrum* (Fig. 5).

Discussion

Although the Dead Sea *Rhodospirillum* species and the established halophilic species, *Rhodospirillum salinarum*, are phylogenetically related, they are clearly phylogenetically distinguishable and differ in several characteristics sufficient to warrant creation of a new species for strain DSI. We thus propose to create the species *Rhodospirillum sodomense* to describe phototrophs with the properties of strain DSI.

Both *R. sodomense* and *R. salinarum* are halophilic. However, *R. sodomense* showed a rather tight optimum for NaCl of 12%, almost precisely the NaCl content of the Dead Sea (Oren 1988). Such salinity requirements are typical of other Dead Sea prokaryotes as well (Javor 1989; Mullakhanbhai and Larsen 1975; Oren 1983a, b; 1988; Oren et al. 1984; 1989). Thus, according to the definitions of Imhoff (1986), *R. sodomense* should be considered a moderately to extremely halophilic phototroph. This is in sharp contrast to that of *R. salinarum*, where optimal phototrophic growth was obtained in media containing 4% NaCl, while significant growth still occurred in media containing as high as 24% NaCl. Thus, *R. salinarum* can best be described as a slight halophile which is extremely halotolerant (Imhoff 1986).

These findings on the salinity optima of *R. sodomense* and *R. salinarum* have potential ecological ramifications. An ability to grow under a wide range of salinities would be a clear advantage to an organism such as *R. salinarum* living in its saltern habitat (Nissen and Dundas 1984); as salinity increased during the salting out process, the organism could continue to grow at all salinities. By contrast, a halophile residing in the Dead Sea would not experience such fluctuations in salinity and would be expected, as was found herein with strain DSI, to be

Table 1. Evolutionary distance matrix for a collection of bacterial 16S rRNA sequences including *Rhodospirillum sodomense* (see 'Materials and methods')[a, b]

1.	*Rhodospirillum rubrum*										
2.	*Rhodospirillum centenum*	11.9									
3.	*Rhodospirillum molischianum*	11.7	11.3								
4.	*Rhodospirillum salinarum*	14.1	11.8	12.9							
5.	*Rhodospirillum sodomense*	13.9	11.9	13.1	1.5						
6.	*Rhodospirillum salexigens*	13.1	12.3	14.9	12.6	12.3					
7.	*Rhodopseudomonas marina*	11.7	13.5	12.3	12.4	12.1	10.8				
8.	*Agrobacterium tumefaciens*	14.2	14.0	11.3	13.9	14.3	12.6	8.9			
9.	*Rhodobacter capsulatus*	14.2	14.2	15.0	16.2	16.2	13.6	11.9	13.2		
10.	*Ectothiorhodospira halochloris*	18.1	18.0	18.1	15.7	16.0	18.5	18.4	18.1	22.0	
11.	*Escherichia coli*	20.6	22.1	21.8	22.6	22.6	22.2	21.4	23.0	25.7	17.2

[a] Only positions represented by a known nucleotide in all sequences in the alignment are considered in the analysis. The 16S rRNA sequences are from the following sources: *Agrobacterium tumefaciens* (Gen Bank/EMBL accession no. M11223); *Ectothiorhodospira halochloris* (Gen Bank/EMBL accession no. 59152); *Escherichia coli* (J01695); *Rhodopseudomonas marina* (M27534); *Rhodospirillum centenum* (not yet accessioned); *Rhodospirillum molischianum* (M59067); *Rhodospirillum rubrum* (M32020); *Rhodospirillum salinarum* (M59069); *Rhodospirillum salexigens* (M59070); *Rhodospirillum sodomense* (M59072); *Rhodobacter capsulatus* (M60671)

[b] The sequences used herein were obtained from the Ribosome Database Project (RDP) at the University of Illinois

Table 2. Characteristics of halophilic members of the genus *Rhodospirillum*

Species	Cell dimensions	Mol% G + C[f]	Color of Phototrophically – grown mass cultures	Internal membranes	Dark aerobic growth	Optimum growth temp. (°C)	Required growth factors	Optimum NaCl (range)	Nitrogen fixation	Light-harvesting complexes[h]	Habitat
R. salexigens[a]	0.6–0.7 × 0.8 –0.9 µm	64 (B_d)	Red	Lamellar	Very good[a]	35 –40	None	7 (5–20)	Yes[b]	I, II	Pools of evaporating seawater, Oregon, USA
R. salinarum[c]	0.8–0.9 × 2.0 –3.5 µm	67.4 (B_d) 68.1 (T_m) 65.2 (T_m)[e]	Red	Vesicular	Very good[c]	42	Complex	4[c] (3–24)	Yes[g]	I, II	Portugese Saltern; marine (?)
R. mediosalinum[d]	2.2–6 × 0.8–1.0 µm	66.6 (T_m)	Pink to Brown-Red	Vesicular	Microaerophilic	30–35	Pantothenate, B_1, PABA[i]	4 7 (0.5–15)	NR[d]	I, II	Warm saline springs, Russia; marine (?)
R. sodomense[e]	0.6–0.7 × 1.6–2.5 µm	66.2–66.6 (T_m)	Pink	Vesicular	No	37 –40	Complex	12 (6 –20)	No	I	Dead Sea

[a] Drews 1981; Imhoff and Trüper 1989; Trüper and Imhoff 1980; chemotrophic growth under fully aerobic (dark) conditions as fast as phototrophic growth
[b] Rubin und Madigan 1986
[c] Nissen and Dundas 1984; aerobic dark growth rate reported to be faster than phototrophic growth rate
[d] Kompantseva and Gorlenko 1985; nitrogen fixation potential not reported (NR)
[e] This study
[f] B_d = Buoyant density; T_m = Thermal denaturation
[g] Contains *nifHIDK* genes (P. W. Ludden and G. P. Roberts, personal communication) but has not been tested for acetylene reduction
[h] I, B875 complex; II, B800/850 complex (see Brunisholz and Zuber 1992)
[i] PABA, para-aminobenzoic acid

more closely adapted to the relatively constant salinity of its habitat. Indeed, the ability of *R. salinarum* to grow well at seawater salinities and under fully aerobic conditions suggests that it may also inhabit marine environments. This would be impossible for *R. sodomense* because of its obligately phototrophic physiology and inability to grow at seawater salinities.

Because of the high concentrations of Mg^{2+} in the Dead Sea it was predicted that strain DSI would at least tolerate, if not actually grow optimally, at high levels of Mg^{2+}. Although capable of growth at 1 M $MgCl_2$, strain DSI grew better at about 10% of this concentration. However, some other Dead Sea halophiles also show this pattern, and in fact, two groups of Dead Sea prokaryotes can be defined on the basis of their Mg^{2+} requirements. *Haloferax volcanii*, *Halobacterium sodomense*, and *Halobacteroides halobius* all show high (0.5 M) Mg^{2+} requirements for optimal growth and high tolerance (up to 1.8 M in the case of *H. sodomense*) for Mg^{2+} (Mullakhanbhai and Larsen 1975; Oren 1983b; Oren et al. 1984). By contrast, members of the genus *Sporohalobacter* isolated from the Dead Sea (*S. lortetii* and *S. marismortui*), like strain DSI, grow optimally at 0.1–0.3 M Mg^{2+} and grow poorly above 0.6 M Mg^{2+} (Oren 1983a; Oren et al. 1987). On the other hand, the high tolerance of strain DSI for bromide is similar to that of *Haloferax volcanii*, an organism capable of growing in media in which 100% of the original NaCl level is replaced with NaBr (Oren and Bekhor 1989). The dramatic difference in bromide tolerance between strain DSI and *R. salinarum* shows that *R. sodomense* is well adapted to this anion in its Dead Sea habitat.

Clear differences exist in the content of photopigment complexes in strain DSI and *R. salinarum*, with the absence in the former organism of the common B800/B850 light-harvesting complex. The *Rhodospirillum* species *R. rubrum* and *R. centenum* and the *Rhodopseudomonas* species *R. marina* are the only other Bchl *a*-containing phototrophs that contain only a light-harvesting I (B875) complex (Brunisholz and Zuber 1992). Of what adaptive significance possession of only a single light-harvesting complex would confer on an organism living in the Dead Sea is not clear. However, it should be noted that the single photocomplex of *R. sodomense* coupled with its low specific Bchl *a* and membrane vesicle contents suggests that this organism may have unusual photosynthetic properties not readily apparent from the studies performed herein.

Table 2 lists the major properties of strain DSI and compares them with those of other halophilic *Rhodospirillum* species. On the basis of the results of this study (summarized in Table 2) and its isolation from an unique hypersaline habitat, the Dead Sea, strain DSI is hereby proposed as a new species of the genus *Rhodospirillum*.

Description of *Rhodospirillum sodomense*

Rhodospirillum sodomense, sp. nov. (so. do. mense N.L. adj. Pertaining to the Sea of Sodom, the Talmudic name for the Dead Sea). Cells vibrio-shaped, occasionally true spirilla, measuring 0.6–0.7 × 1.6–2.5 μm. Internal membranes few and of the vesicular type.

Photosynthetic cultures are pink and major absorption maxima of living cells are observed at 875–880, 591, 551, 516, 421, and 377 nm, indicating the presence of bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. B875 light-harvesting complex (but not B800/850 complex) present. Obligately halophilic, growth occurs photoheterotrophically in medium containing 6–20% NaCl (sharp optimum at 12% NaCl). Temperature optimum 35–40 °C (range 25–47 °C), pH optimum 7. Photoheterotrophic growth does not occur in the absence of yeast extract; however, in low yeast extract-containing media, growth is stimulated by acetate, malate, pyruvate, or succinate. Very poor to no growth aerobically in darkness. Nitrogen sources used: ammonia and yeast extract. Poly-β-hydroxybutyrate produced as storage polymer. No evidence for N_2 fixation. Extremely bromide tolerant, grows normally with 50–75% of NaCl replaced by NaBr. Habitat: Dead Sea water/sediment.

The mol % G + C of the DNA is 66.2 (T_m).

Deposited in the American Type Culture Collection as ATCC51195.

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