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A gas vesiculate planktonic strain of the purple non-sulfur bacterium *Rhodospirillum rubrum* isolated from Lake Fryxell, Dry Valleys, Antarctica

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Abstract A moderately psychrophilic purple non-sulfur bacterium, *Rhodospirillum rubrum* strain Fryx1, is described. Strain Fryx1 was isolated from the water column under the ice of the permanently frozen Lake Fryxell, Antarctica. Cells of Fryx1 are long thin rods and contain gas vesicles, the first report of such structures in purple non-sulfur bacteria. Gas vesicles are clustered at 2–4 sites per cell. Surprisingly, the 16S rRNA gene sequence of strain Fryx1 is nearly identical to that of *Rfx. antarcticus* strain AB, a short, vibrio-shaped phototroph isolated from an Antarctic microbial mat. Although showing physiological parallels, strains AB and Fryx1 differ distinctly in their morphology and absorption spectra. DNA–DNA hybridization shows that the genomes of strains AB and Fryx1 are highly related, yet distinct. We conclude that although strains AB and Fryx1 may indeed be the same species, their ecologies are quite different. Unlike strain AB, strain Fryx1 has adapted to a planktonic existence in the nearly freezing water column of Lake Fryxell.

Keywords Purple anoxygenic phototrophic bacteria · Lake Fryxell · Antarctica · *Rhodospirillum rubrum* · Gas vesicles · Psychrophily

Dedicated to Prof. Dr. Hans Günter Schlegel on the occasion of his 80th birthday.

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Introduction

Anoxygenic phototrophic bacteria inhabit a variety of extreme environments, including extremes of temperature, pH, and salinity (Madigan 2003). Previous work from this laboratory showed that purple phototrophic bacteria inhabit the water column of permanently frozen lakes and surrounding microbial mats of the McMurdo Dry Valleys, Antarctica (Madigan et al. 2000; Achenbach et al. 2001; Karr et al. 2003). In the Vestfold Hills of East Antarctica, purple and green sulfur bacteria inhabit a series of hypersaline lakes (Burke and Burton 1988a, b; Madigan 1998). However, in Lake Fryxell, located in the Taylor Valley near McMurdo, field studies of pigments (Lizotte and Priscu 1998) or photosynthesis genes (Achenbach et al. 2001) yielded evidence for purple bacteria only (Karr et al. 2003).

The gene *pufM* encodes a highly conserved photosynthetic reaction center polypeptide in all known purple bacteria (Nagashima et al. 1997). Using *pufM* as a functional gene probe, we detected a large diversity of purple non-sulfur bacteria belonging to the alpha- and beta-Proteobacteria in the water column of Lake Fryxell (Karr et al. 2003). In this study, two enrichment cultures of purple bacteria were also obtained. Morphotype A varied from thin long rods to filaments, while morphotype B was a bullet-shaped rod. Both organisms were suspected of being gas-vesiculate from the presence of irregularly shaped, highly refractile areas in the cells (Karr et al. 2003).

In this paper, we characterize pure cultures of morphotype A, which we now designate as *Rhodospirillum rubrum* strain Fryx1. We show that this organism indeed does produce gas vesicles, but surprisingly, is phylogenetically nearly identical to *Rfx. antarcticus* strain AB^T (Madigan et al. 2000), despite several phenotypic differences. We conclude that these two strains of *Rfx. antarcticus* are ecologically distinct and that the special properties of strain Fryx1 make it ideally suited to life as a planktonic phototroph.

Materials and methods

Samples and enrichment cultures

Water samples were obtained from several depths within Lake Fryxell in November (1999, 2001) through a sampling hole drilled through 4.5 m of ice cover. Water samples, obtained with a 5-l Niskin bottle, were transferred to dark plastic bottles in dim light and kept at 4°C for several hours before enrichment cultures were established. Water at a depth below 9 m contained traces of sulfide, while water near the sediments (18 m) contained over 1 mM sulfide (Karr et al. 2003).

Enrichments were established by adding 0.05% (final concentration) Difco yeast extract directly to lake-water samples (from 10 m) in completely filled 10-ml screw-capped tubes. The latter were then incubated at 4°C and incandescent illumination of approx. $1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Although sulfide was not added to these enrichments, lake-water at 10 m contained about 100 μM sulfide (Karr et al. 2003).

Isolation and growth of pure cultures

Strain Fryx1 was purified by repeated streaking of agar plates and phototrophic incubation in GasPak anoxic jars at 15°C and 5–10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Care was taken to keep cultures below 20°C during all manipulations. Strain Fryx1 has been deposited in the American Type Culture Collection as ATCC BAA-1023.

Pure cultures of strain Fryx1 were grown in a modification of medium RCVB (Tayeh and Madigan 1987) called medium Fryx1. The latter was medium RCVB in which 0.2–0.4% (w/v) pyruvate was added in place of malate and the medium supplemented with 0.1% NaCl and 0.1% yeast extract. For tests of carbon source utilization, medium Fryx1 minus pyruvate was used and supplemented with various substrates at the concentrations listed in the text; and in these experiments, yeast extract was reduced to 0.01% and 0.1% NaHCO_3 was added. For sulfide tolerance experiments, medium Fryx1 containing 4 mM sulfide (pH 7) was prepared and other sulfide levels were made by dilution with Fryx1 medium lacking sulfide. Sulfate was assayed by the BaSO_4 method of the APHA (1971).

For chemotrophic growth, cells were grown in 100 ml of inoculated Fryx1 medium contained in a static 2-l cotton-stoppered flask. Incubation was in total darkness at 18°C. Flasks were shaken by hand periodically and then the cells harvested after 2 weeks of growth. Growth of phototrophic cultures was routinely measured as the change in optical density at 660 nm (OD_{660}). Periodic OD readings were taken of all cultures and a time-point chosen for comparative measurements before the most rapidly growing culture had entered the stationary phase. The average OD values for each culture at this time-point were plotted in Fig. 6.

Microscopy and pigment analyses

All methods for phase microscopy and for scanning and transmission electron microscopy (STEM), except for negative staining, were as described by Madigan et al. (2000). Negative staining was done by floating a silicon dioxide filmed grid on a droplet of unfixed cells for 1 min and then transferring to a droplet of 2% phosphotungstic acid for 30 s. The grids were then blotted dry and further dried at 40°C overnight before viewing in a Hitachi model H7100 TEM operating at 75 kV. Cell suspensions were subjected to gentle breakage in a mortar and pestle before applying negative stain to improve the contrast between cells and gas vesicles.

Absorption spectra of intact cells were obtained by suspending centrifuged cells in 30% bovine serum albumin. Solvent extracts of cells were prepared using 100% methanol. All spectra were performed on a Hitachi U-2000 spectrophotometer. Carotenoids were identified and quantitated based on their absorption spectra and retention times in HPLC using a C18 column (Takaichi et al. 2001). Cells grown phototrophically or chemotrophically were lyophilized in Carbondale and shipped to Japan for carotenoid analysis.

Phylogeny and genomic DNA hybridization

Phylogenetic analyses of strain Fryx1 DNA were done by 16S rRNA gene sequencing. The following organisms were included in the phylogenetic analyses: *Rfx. antarcticus* AB^T (AF084947), *Rfx. antarcticus* strain Fryx1 (AY609198), *Rhodospirillum rubrum* FR2^T (D16211), Arctic sea ice bacterium ARK10281 (AF468446), *Rfx. ferrireducens* (AF435948), *Aquaspirillum delicatum* (AF078756), *Pseudomonas lanceolata* (AB021390), *Variovorax paradoxus* (D30793), *Polaromonas vacuolata* (U14585), *Comamonas testosteroni* (M11224), *Aquaspirillum psychrophilum* (AF078755), *Rubrivivax gelatinosus* (D16213), *Rhodocyclus tenuis* (D16209), *Rhodospseudomonas palustris* (D25312), and *Escherichia coli* (J01859).

For DNA hybridization, total genomic DNA was isolated from 1-ml cultures of strain Fryx1, *Rfx. antarcticus* strain AB^T, and *Rfx. fermentans* strain FR2^T, using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minn.). Genomic DNAs were digested with *Sau3AI* and random prime-labeled with digoxigenin-11-dUTP (Roche Molecular Biochemicals, Indianapolis, Ind.). Portions (0.1 μg) of the digested DNA were spotted onto Nylon membranes (MagnaGraph 0.45 μm ; Micron Separations, Westboro, Mass.). The membranes were incubated in a denaturing solution (0.25 N NaOH, 1.5 M NaCl) for 1 min, rinsed with 2 \times SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7) and air-dried. Hybridizations were performed at 46°C (stringent conditions) in EasyHYB solution (Roche Molecular Biochemicals) containing 25 μg digoxigenin-labeled genomic DNA probes/ml and were detected by chemiluminescence using CSPD reagent (Roche Molecular Biochemicals). The resulting images were dig-

itally stored and analyzed using the GeneScan and GeneTools software programs (Spectronics Corp.).

Results

Within 1 month of establishing cold (4°C) enrichment cultures for phototrophic bacteria in anoxic tubes of Lake Fryxell water containing about 0.1 mM autochthonous sulfide and supplemented with low levels of yeast extract, light-brown cell suspensions developed. Replicate tubes set up at 30°C or in darkness showed no growth. A pure culture of a phototrophic purple bacterium was obtained from the cold enrichment by plating and given the strain designation Fryx1.

Morphology and phylogeny

Cells of strain Fryx1 were long thin rods; and some cells could be considered filamentous. Cells measured 0.5 μm in diameter, were straight to slightly curved, and were of variable length, from 5 μm to 30 μm (Fig. 1a). Occasional cells were even longer, forming filaments >50 μm in length; and such cells were more common in stationary phase cultures. Since the cell morphology of strain Fryx1 was highly unusual for a purple bacterium, its phylogenetic position was determined by 16S rRNA gene sequencing. Surprisingly, however, the data showed strain Fryx1 to be >99.8% identical in rRNA gene sequence with *Rfx. antarcticus* strain AB^T (Fig. 1b) over the 1,479 bases aligned in the sequence (Fig. 2). This result led us to immediately repeat the analysis on two separate batches of cells, but the same outcome was achieved.

The unexpected phylogenetic results (Fig. 2), coupled with the obvious morphological differences between strains Fryx1 and AB^T (Fig. 1), made the genomic relationship between these organisms an important question. In dot blots using strain Fryx1 DNA as probe, genomic hybridization to strain AB^T DNA was significant, 83% of

the value for the homologous combination (Fig. 2, inset). Hybridization of the Fryx1 probe to DNA from *Rfx. fermentans* FR2^T, a related species of purple bacterium (Hiraishi et al. 1991), was undetectable (Fig. 2, inset), in agreement with previous results using strain AB^T DNA as probe (Madigan et al. 2000).

The phylogenetic results clearly showed that strain Fryx1 was a purple non-sulfur bacterium of the genus *Rhodospirillum* (beta-Proteobacteria) and species *Rfx. antarcticus*. And, although genomic differences between strains AB^T and Fryx1 (Fig. 2, inset) could be detected, their genomes were sufficiently similar for the organisms to be considered as one species (Roselló-Mora and Amann 2001).

Gas vesicles of strain Fryx1

Cells of strain Fryx1 contained one or more phase-bright regions per cell (Fig. 1a). In enrichments, which contained sulfide from the original lake-water samples, the phase-bright regions were thought to be globules of elemental sulfur from the oxidation of sulfide; and thus the organism was a purple sulfur bacterium. However, upon growth in sulfide-free media, cells retained the phase-bright structures, indicating that they were not sulfur globules. The irregular margins of the phase-bright regions in the cell (Fig. 1a) were reminiscent of gas vacuoles (clusters of gas vesicles; Walsby 1972); and TEM confirmed this (Fig. 3). Cells of strain AB^T grown under identical conditions as for strain Fryx1 did not contain gas vesicles.

Gas vesicles of strain Fryx1 showed the typical conical shape characteristic of these structures (Walsby 1972, 1975, 1994). Single vesicles in fixed cells averaged 75–80 nm in width and 130–140 nm in length (Fig. 3a). In unfixed negatively stained cells, the vesicles were swollen and somewhat larger (Fig. 3b,c). This apparent increase in size is an artifact of negative staining and was also seen in gas vesicles from other phototrophic prokaryotes (Walsby and Eichelberger 1968).

In many cells of strain Fryx1, gas vesicles were found localized within the cells, forming 2–4 clusters (vacuoles) per cell (Fig. 3b). Many cells also showed clusters of gas vesicles arranged in a circular fashion surrounding a phase-dark central structure, suggesting that these might

Fig. 1 Morphology of cells of **a** strain Fryx1 and **b** *Rfx. antarcticus* strain AB^T. Phase-contrast photomicrographs. Bars 10 μm

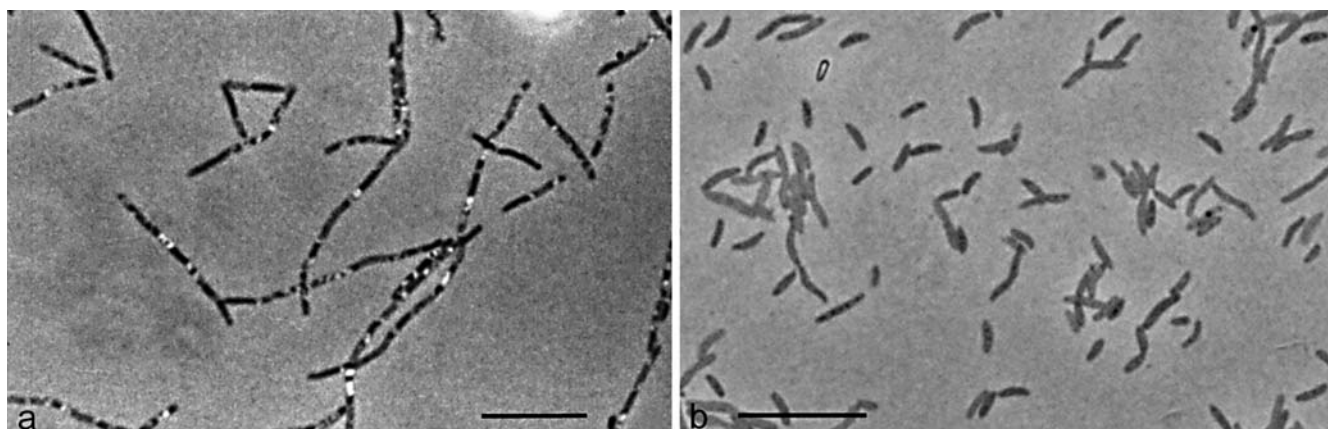
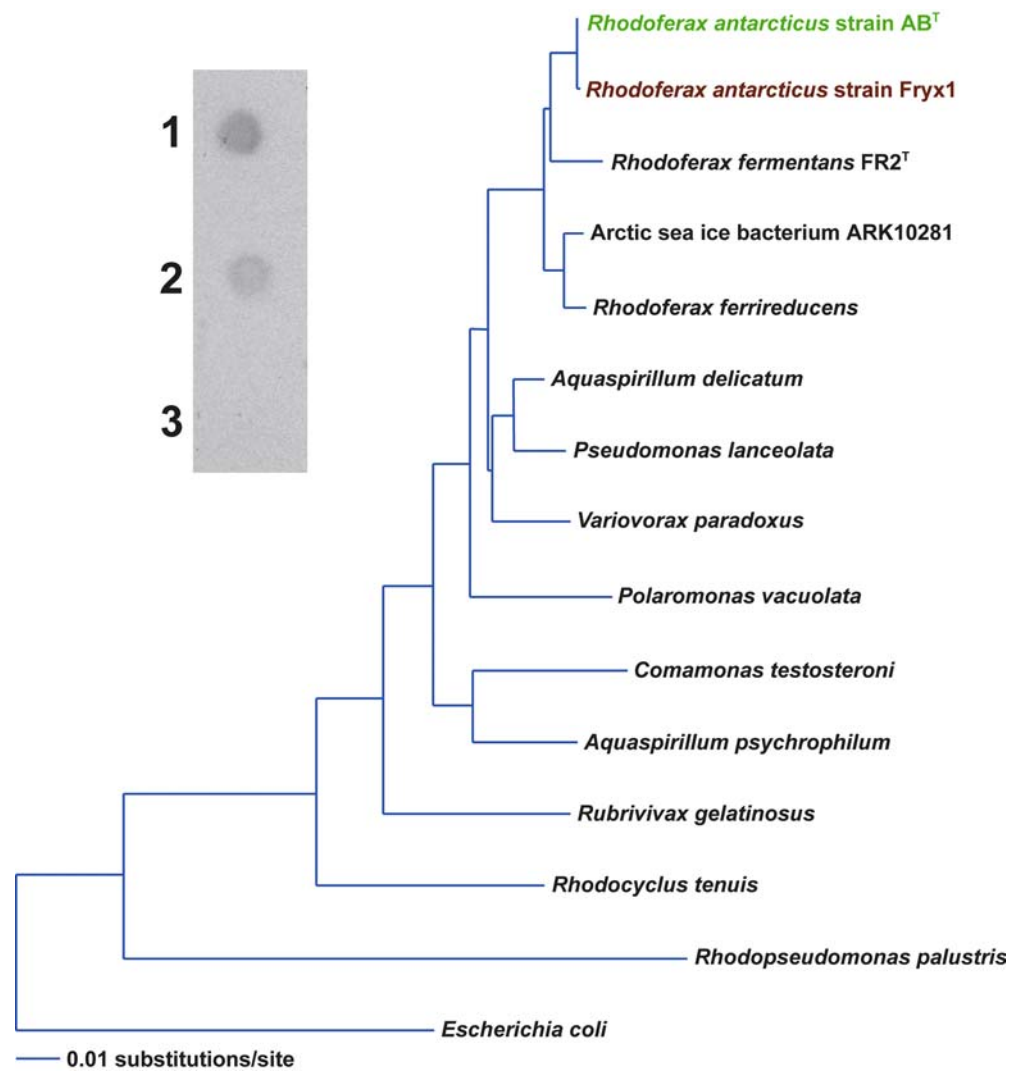


Fig. 2 Small subunit rRNA gene phylogenetic tree of strain Fryx1 and related alpha- and beta-Proteobacteria. *E. coli* (gamma-Proteobacteria) was used as the outgroup. Shown is the dataset resulting from distance analysis on 1,479 characters using the Jukes–Cantor correction. The same topology was obtained using parsimony and maximum likelihood. Bootstrap analysis revealed good support (>75%) for all nodes except the *Aquaspirillum/Pseudomonas/Variovorax* node (58%). All organisms are beta-Proteobacteria except for *Rhodopseudomonas* (alpha-Proteobacteria). *Inset* Dot blots of genomic DNA hybridizations. Genomic DNAs of (1) strain Fryx1, (2) *Rfx. antarcticus* strain AB^T, and (3) *Rfx. fermentans* were hybridized with digoxigenin-labeled genomic DNA from strain Fryx1



be sites of gas vesicle synthesis (Fig. 3c). Similar phenomena were observed with the gas vesicles of certain purple sulfur bacteria (Cohen-Bazire et al. 1969).

Cells of strain Fryx1 were weakly motile from a single polar flagellum (Fig. 3d). Although a few gas-vesiculate bacteria are also able to swim, flagellum synthesis and gas vesicle synthesis in such species typically occur during different growth phases (Pfennig and Trüper 1989; Walsby 1994). In contrast, gas-vesiculate cells of strain Fryx1 could also swim, albeit quite slowly compared with the highly motile strain AB^T (Madigan et al. 2000).

Further evidence that the structures observed were indeed gas vesicles emerged from the finding that cell suspensions of strain Fryx1 were occasionally seen to float to the top of culture tubes and bottles, accumulating in a slimy mass under the cap (Fig. 4a). No clear link between culture conditions and the floating phenomenon was observed. However, floating cell suspensions typically occurred only in older (>2-week-old) cultures.

Pigments

Absorption spectra clearly identified strain Fryx1 as an anoxygenic phototrophic purple bacterium. Major peaks at 798 nm and 836 nm in the spectrum of intact cells signaled the presence of a light-harvesting (LH) II antenna complex (Fig. 5). The shoulder at about 870 nm in the spectrum is likely due to weak absorbance by an LH I antenna photocomplex. Absorbance of methanol extracts of cells of Fryx1 at 770 nm confirmed the chlorophyll pigment as bacteriochlorophyll *a* (Fig. 5). Also shown in Fig. 5 is the absorption spectrum of cells of *Rfx. antarcticus* strain AB^T. Absorption of the LH II components in the two strains were clearly distinct (Fig. 5).

Phototrophically grown cell suspensions of strain Fryx1 were peach-colored, resembling those of strain AB^T (Fig. 4b), but in addition they revealed a slight rose-color when illuminated from behind. Moreover, cell suspensions of strain Fryx1 had a more translucent or milky appearance than did suspensions of strain AB^T, probably due to light scattering by gas vesicles (Fig. 4b).

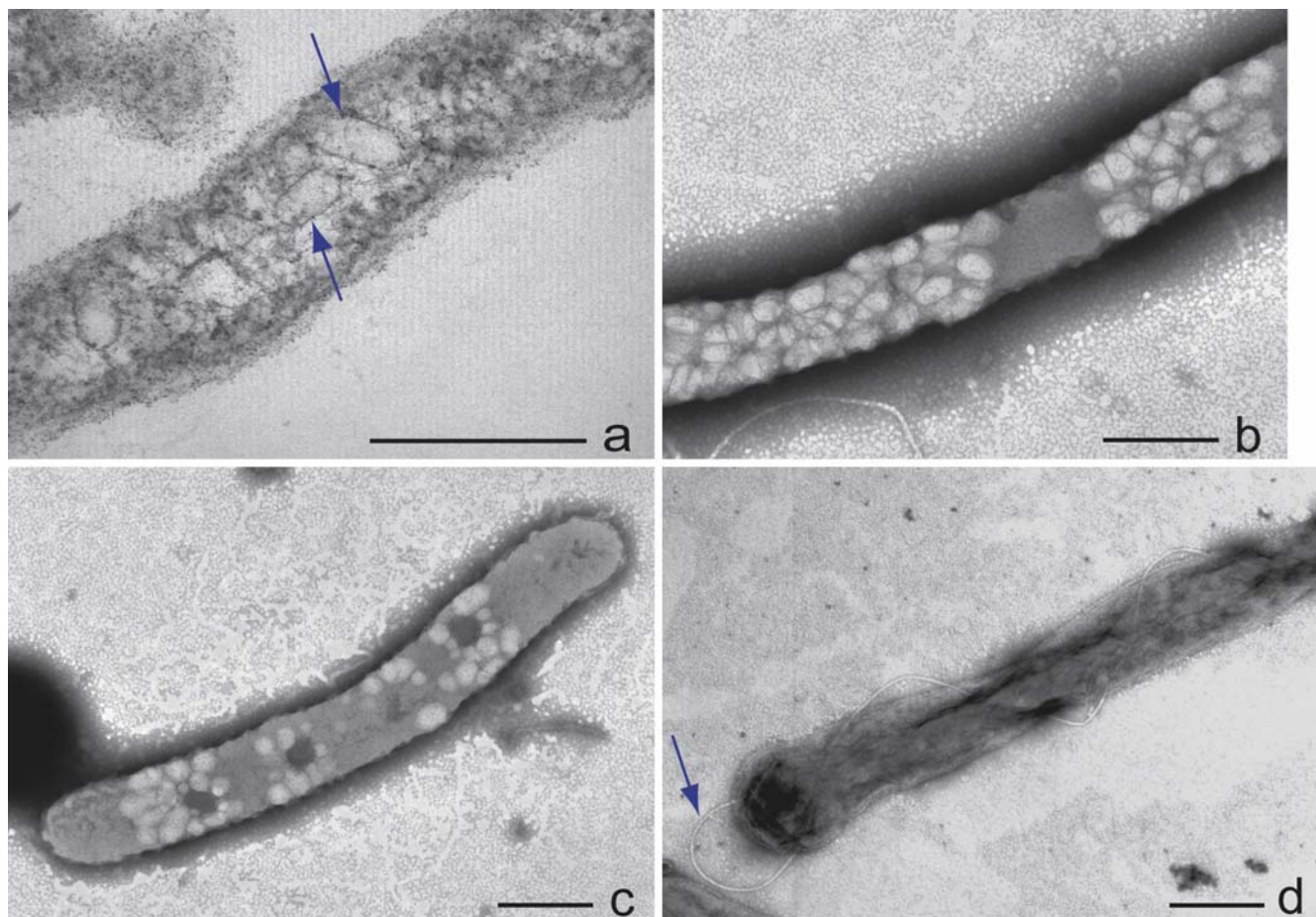


Fig. 3a–d Gas vesicles and flagella of cells of strain Fryx1. **a** TEM of a longitudinal section. Note conically shaped gas vesicles (arrows). **b** Negatively stained TEM showing gas vacuoles (clusters of gas vesicles). **c** Negatively stained TEM showing circular arrangement of gas vesicles at possible sites of synthesis. **d** Negatively stained TEM showing the single polar flagellum (arrow) of a cell of strain Fryx1. Bars, 0.5 μ m

Carotenoid maxima in the spectra of strains Fryx1 and AB^T were similar, although the relative ratios of some pigments were distinct in the two organisms (Fig. 5). The spectra indicated that carotenoids of the spheroidene and normal spirilloxanthin pathways predominate in both organisms. This was confirmed from carotenoid analyses (Table 1). Although the general patterns of carotenoids produced by strains Fryx1 and AB^T were similar, strain Fryx1 contained lower levels of OH-spheroidene and higher levels of OH-spheroidenone than did strain AB^T. Also, the ratio of hydroxylated to non-hydroxylated carotenoids in the two strains differed (Table 1). The carotenoid composition of both Antarctic purple bacteria was distinct from that of *Rfx. fermentans* FR2^T, the type species of the genus (Hiraishi et al. 1991; Table 1).

Aerobic liquid cultures of strain Fryx1 were rose-pink in color, distinct from phototrophically grown cells (Fig. 4b). The color change is catalyzed by a major shift in carotenoid content in aerobic cells from that in phototrophically grown cells. Greater than 95% of the carotenoid pig-

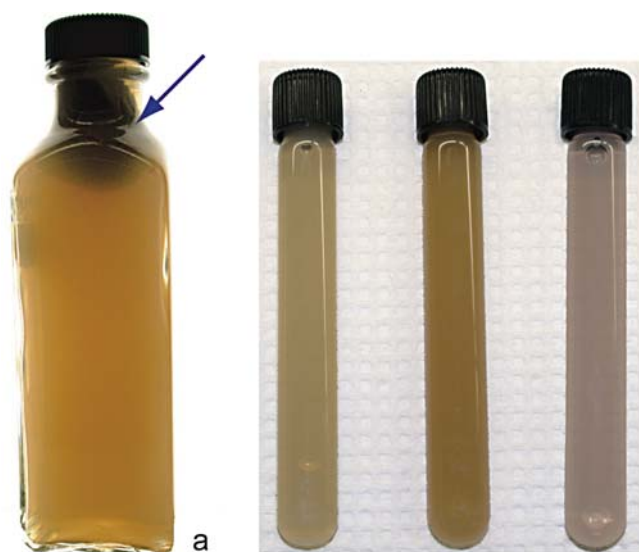


Fig. 4a,b Pigments of strain Fryx1. **a** Bottle culture of phototrophically grown cells. Note the buoyant cell mass near the cap (arrow). **b** Tube cultures of phototrophically grown cells of (left) *Rfx. antarcticus* strain Fryx1, (center) *Rfx. antarcticus* strain AB^T, and (right) aerobic dark grown cells of strain Fryx1

ments produced by aerobic cells of strain Fryx1 were keto-carotenoids, with OH-spheroidenone predominating (Table 1). This is a common observation in cells of spher-

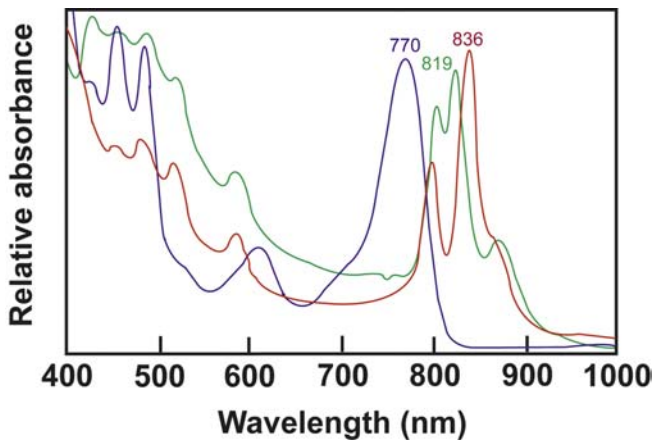


Fig. 5 Absorption spectra of intact cells (*red*) and methanol extracts of cells (*blue*) of *Rfx. antarcticus* strain Fryx1 and of intact cells (*green*) of *Rfx. antarcticus* strain AB^T

oidene pathway purple bacteria exposed to air (Takaichi 1999). Moreover, aerobic cells produced a small amount of a new carotenoid, methoxy-spheroidenone, not seen under phototrophic conditions in strain Fryx1 or any of the other *Rhodospirillum rubrum* species (Table 1). In addition to the color transition in liquid cultures, the surface of phototrophically grown colonies of strain Fryx1 were rose to deep purple in color, while cells just beneath the colony surface displayed more of the brown color of phototrophic liquid suspensions. The significance of this transition was unclear.

Physiology

In a physiological sense, strain Fryx1 appeared to be a typical purple non-sulfur bacterium. Growth occurred either phototrophically or chemotrophically in darkness by respiration. No vitamins were required for growth of strain Fryx1, although low levels (0.01–0.05%) of yeast extract stimulated growth. Photoheterotrophic growth occurred on a limited group of organic substrates, with pyruvate (10–20 mM) supporting the best growth. Moderate photoheterotrophic growth was also achieved on malate,

succinate, fumarate, or lactate (10 mM each, data not shown). Acetate (10–20 mM), a substrate that typically supports robust photoheterotrophic growth of purple non-sulfur bacteria (Pfennig and Trüper 1989), supported only weak growth of strain Fryx1. Other fatty acids tested (C₃, C₄, C₆, each supplied at 1.0–2.5 mM) did not support photoheterotrophic growth of strain Fryx1.

Sugars such as fructose and sucrose were poorly used by strain Fryx1; and glucose did not support growth at all. This is in contrast to *Rfx. antarcticus* strain AB^T where sugars (in particular fructose, glucose) and acetate supported luxurious photoheterotrophic growth (Madigan et al. 2000). Photoautotrophic growth of strain Fryx1 occurred on H₂ + CO₂ (95:5), as was also true for strain AB^T (Madigan et al. 2000). All growth of strain Fryx1 under anoxic conditions was light-dependent.

Growth temperature and salt requirements for strain Fryx1 paralleled those of strain AB^T (Madigan et al. 2000). Best growth of strain Fryx1 was achieved between 15°C and 18°C; and no growth occurred at 25°C or above (Fig. 6). Growth was still good at 10°C and occurred down to 0°C (Fig. 6). Thus, like strain AB^T, strain Fryx1 can be considered mildly psychrophilic. Strain Fryx1 was quite sensitive to salt, growth being significantly inhibited at NaCl concentrations as low as 1% (Fig. 6). This is consistent with the fact that Lake Fryxell contains a slight NaCl gradient (the bottom waters contain about 1% NaCl), but is considered a freshwater lake (Matsumoto 1993).

Strain Fryx1 was surprisingly sulfide-tolerant. Photoheterotrophic growth occurred in pyruvate media containing up to 4 mM sulfide (Fig. 6). Although H₂ supported photoautotrophy in strain Fryx1, no significant sulfide-dependent photoautotrophic growth was observed. Similar results regarding sulfide tolerance were observed in strain AB^T, indicating that sulfide tolerance is a species-specific rather than strain-specific property.

Sulfide measurements indicated that some sulfide oxidation occurred in cultures of strain Fryx1 during photoheterotrophic growth. However, microscopy of sulfide-grown cells showed no evidence for S⁰; and assays for sulfate showed that none of the sulfide consumed was oxidized to sulfate (data not shown). No analyses for other sulfur species were performed.

Table 1 Composition (mol% of total) of carotenoids in phototrophic cells of *Rhodospirillum rubrum* species. *ND* Not detected, *SE* spheroidene, *SO* spheroidenone

| Carotenoid | <i>Rfx. antarcticus</i> strain Fryx1 | <i>Rfx. antarcticus</i> strain Fryx1 ^a | <i>Rfx. antarcticus</i> strain AB ^T | <i>Rfx. fermentans</i> strain FR2 ^T |
|---------------------------|---|--|---|---|
| Spheroidene | 7 | ND | 8 | 44 |
| OH-Spheroidene | 66 | 4 | 72 | 42 |
| Methoxy-spheroidene | 2 | ND | 2 | ND |
| Spheroidenone | 1 | 10 | 1 | ND |
| OH-Spheroidenone | 22 | 83 | 15 | 4 |
| Methoxy-spheroidenone | ND | 3 | ND | ND |
| Spirilloxanthin | 2 | ND | 2 | 10 |
| Keto-spirilloxanthin | <1 | ND | <1 | <1 |
| Diketo-spirilloxanthin | <1 | 1 | <1 | <1 |
| (OH-SE + OH-SO)/(SE + SO) | 12 | 9 | 9 | 1 |
| (SO + OH-SO)/(SE + OH-SE) | 0.32 | 25 | 0.19 | 0.04 |

^aChemotrophic (oxic/dark) cells

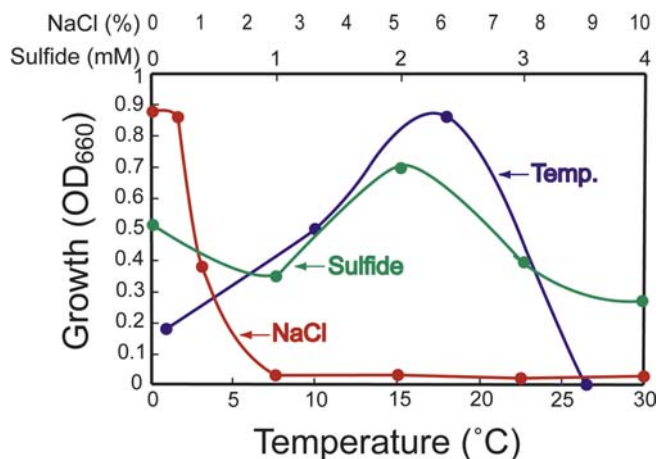


Fig. 6 Effect of temperature, salt, and sulfide on phototrophic growth of *Rfx. antarcticus* strain Fryx1. At time 0 (y-axis), OD₆₆₀ was <0.05 in all cases. Cultures were incubated at 18°C for 6 days (temperature), 13 days (sulfide), or 6 days (salt) before being scored for growth. All results are the means of duplicate measurements. In separate experiments, slow growth of strain Fryx1 at 0°C was achieved

Discussion

Although the molecular results in Fig. 2 showed strains Fryx1 and AB^T to be the same species, the ecologies of these two organisms are likely quite distinct. We conclude that, collectively, the properties of *Rfx. antarcticus* strain Fryx1 are those of a planktonic phototroph. This is in contrast to strain AB^T, which inhabits a microbial mat (Madigan et al. 2000). Because strain Fryx1 is a purple non-sulfur bacterium, this is the first example of a truly planktonic isolate among this group of phototrophic purple bacteria (van Gemerden and Mas 1995).

The presence of gas vesicles in strain Fryx1 and their absence from strain AB^T signals an important ecological distinction between them. Admittedly, it is possible that genes encoding gas vesicle proteins may have been obtained by strain Fryx1 through lateral gene transfer. However, regardless of how strain Fryx1 became gas-vesiculate, strain Fryx1 is not simply strain AB^T with gas vesicles. Morphological, physiological, and genetic differences are apparent between the two strains. As regards morphology, for example, Walsby (1981, 1994) pointed out that the planktonic cyanobacteria that inhabit stratified lakes are gas-vesiculate and are often long and thin; and this tends to maximize their surface area and prevent sinking. This also seems to be the strategy of strain Fryx1, as cells of strain Fryx1 were thinner and many times longer than those of strain AB^T.

Moreover, unlike planktonic purple bacteria such as *Chromatium okenii*, whose cells are highly motile (Schlegel and Pfennig 1961; van Gemerden and Mas 1995), strain Fryx1 is only weakly motile. The presence of gas vesicles and a long thin morphology should greatly improve its buoyancy in the completely unmixed water column of Lake Fryxell. In contrast, cells of strain AB^T are highly motile (Madigan et al. 2000). One could envisage

that, within the microbial mat habitat of strain AB^T, rapid-swimming motility would be advantageous for interactions with other organisms in the mat, while gas vesicles would be superfluous.

Thus, from a nearly identical phylogenetic background, we hypothesize that strains AB^T and Fryx1 diverged, especially as regards motility and morphology, to inhabit quite distinct environments. Indeed, the genomic difference observed between the two strains (as detected by significantly less than 100% hybridization; Fig. 2) is likely a reflection of the different ecological paths taken by these two phototrophs.

Light and temperature conditions in the water column of Dry Valley Lakes should also favor the Fryx1 phenotype. Walsby (1994) calculated the energy costs of cell movement of a phototrophic organism in a water column. His conclusions show that, for slow-growing organisms, the energy costs for maintaining cells in suspension is substantially less for gas-vesiculate than for flagellated organisms (Walsby 1994). In the case of strain Fryx1, growth rates in situ are likely to be strongly limited by temperature and light. For example, at a depth of 10 m in Lake Fryxell, the temperature remains constant at 2°C and light intensity is less than 0.5 μmol photons m² s⁻¹ seasonally (Spigel and Priscu 1998; Karr et al. 2003). From the calculations of Walsby (1994), such conditions should greatly favor gas-vesiculate phototrophs; and they may have been important factors in selecting for the Fryx1 phenotype of *Rfx. antarcticus*. Light and low temperature as controlling factors of gas vesicle synthesis have also been noted in various green sulfur bacteria (Pfennig and Cohen-Bazire 1967; Pfennig and Trüper 1989; Overmann et al. 1991).

Although conditions in Lake Fryxell should be ideal for purple sulfur bacteria, Fryx1 is a purple non-sulfur bacterium. The success of purple non-sulfur bacteria in Lake Fryxell may be due to the highly unusual seasonal nature of Antarctic lakes. Besides the very low light intensities in these lakes (Howard-Williams et al. 1998), light is not available all year round, as the lakes experience nearly 6 months of darkness each year (Spigel and Priscu 1998). The typical versatility of purple non-sulfur bacteria in terms of dark metabolism (Madigan 1988, 1998) may thus favor this physiological group over purple sulfur bacteria in Lake Fryxell.

Other differences between strains Fryx1 and AB^T may also have ecological significance. For example, the absorption spectra of the two organisms are distinct (Fig. 5), indicating that their antenna complexes are not identical. In this regard, sequence analysis of a portion of the *pufM* gene from morphotype A (later designated strain Fryx1; Karr et al. 2003) and strain AB^T showed the gene to differ in only one location over the 229-nucleotide fragment compared (Jung et al. 2003). The differences observed in peripheral antenna (LH II) absorbance in the two organisms (Fig. 5) likely represents the fine tuning of each photocomplex to best use the available wavelengths in their differing habitats.

The sulfide tolerance of strain Fryx1 (Fig. 6) is also noteworthy and stands in contrast to purple non-sulfur

bacteria in general, most of which are quite sulfide-sensitive (Hansen and van Gemerden 1972). Sulfide in Lake Fryxell forms a gradient from 0 mM to more than 1 mM from a depth of 9 m to the sediments (18 m; Karr et al. 2003). Based on the sulfide data of Fig. 6, Fryx1-like phototrophs should be able to inhabit the entire anoxic zone of Lake Fryxell. Interestingly, our molecular probing of *pufM* in the Lake Fryxell water column showed exactly this. Sequences corresponding to strain Fryx1 and three other clones closely related to strain Fryx1 were detected at three depths (9, 11, 17 m) in the Lake Fryxell water column (Karr et al. 2003).

The discovery of strain Fryx1 suggests that other gas vesiculate purple non-sulfur bacteria may exist in nature. Perhaps such organisms coexist with purple and green sulfur bacteria beneath the chemocline in stratified lakes, but have been overlooked in enrichment and isolation studies. The selective isolation of such putative planktonic phototrophs may be facilitated by low light and cold incubation, as these seem to be key factors in the biology of gas vesiculate anoxygenic phototrophic bacteria, species of which now include the purple non-sulfur bacterium *Rfx. antarcticus* strain Fryx1.

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