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Phototrophic purple sulfur bacteria as heat engines in the South Andros Black Hole

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Abstract Photosynthetic organisms normally endeavor to optimize the efficiency of their light-harvesting apparatus. However, here we describe two bacterial isolates belonging to the genera *Allochromatium* and *Thiocapsa* that demonstrate a novel adaptation by optimizing their external growth conditions at the expense of photosynthetic efficiency. In the South Andros Black Hole, Bahamas, a dense l-m thick layer of these anoxygenic purple sulfur bacteria is present at a depth of 17.8 m. In this layer the water temperature increases sharply to 36°C as a consequence of the low-energy transfer efficiency of their carotenoids (ca. 30%). These include spirilloxanthin, and related polyene molecules and a novel chiral carotenoid identified as spirilloxanthin-2-ol, not previously reported in purple bacteria. To our knowledge, this study presents the first evidence of such a bacterial mass significantly increasing the ambient water temperature. The transduction of light to heat energy to excess heat may provide these anoxygenic phototropic bacteria with a competitive advantage over non-thermotolerant species, which would account for their predominance within the microbial layer.

Keywords Anoxygenic purple sulfur bacteria · *Allochromatium* · *Thiocapsa* · South Andros Black Hole · Carotenoid · Spirilloxanthin · Energy-transfer

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Introduction

Heat exchange between living cells and their surroundings is a universal phenomenon. It is, however unusual, in natural environments, for microorganisms to significantly raise the temperature of their immediate surroundings since, high cell densities are rarely achieved and metabolic heat generated is usually rapidly dissipated. Notable exceptions to this general rule, however, can be found in artificial high cell density closed systems, such as compost heaps, silage pits, and fermenters (Hunter 1917; Wang et al. 1979; Diaz et al. 1993). In marine environments such as the Baltic, Kahru et al. (1993) using Advanced Very High Resolution Radiometer (AVHRR) satellite images in conjunction with shipboard measurements showed that surface

accumulations of cyanobacteria could cause local increases in seawater temperature of up to 1.5°C. Similarly, Lewis et al. (1983) reported that the deep chlorophyll maximum in the eastern North Atlantic caused the development of a warm layer of water to develop below a colder layer. These studies demonstrate that attenuation of light in the water column by phototropic organisms, if they are present in sufficient numbers, can cause differential heating of the water mass, whereas in their absence this does not occur.

Blue Holes are cave systems that have developed within the Bahamian carbonate platform (Sealey 1994; Palmer 1985). The size of these cave systems can be can be extensive. Laterally cave passages can extend several kilometers and vertically blue holes may range in depth from 10 to several hundred m. These cave systems connect with the sea via the lateral passages. A more intriguing series of cave systems also found in the Bahamas are Black Holes, the most spectacular of which is the South Andros Black Hole which has an almost circular entrance 300 ± 15 m in diameter and is ~47 m deep (Palmer 1985; Schwabe and Herbert 2004). Unlike Blue Holes, the Black Holes have no connection with the sea except via seepage and rock fractures, and fresh water sits on a layer of saline water with little or no mixing. From the air the water of the South Andros Black Hole appears dark blue/black (Fig. 1). In this article we show that the color of the water is due to a 1-m dense layer of purple sulfur purple bacteria located at depth of 17.8 m, which effectively absorbs all the incoming photons. At this depth the ambient water temperature increases sharply. Below the bacterial layer the water temperature cools rapidly. In this study, we describe the processes, which account for the observed increase in water temperature in the bacterial layer.

Fig. 1 Aerial photograph of the South Andros Black Hole cave system, the Bahamas. Reprinted from Quaternary International, 121, Stephanie Schwabe and Rodney A. Herbert, Black Holes of the Bahamas: What are they and why they are black, 3–11, Copyright (2004), with permission from Elsevier

Methods

Cell culture and harvesting

Thiocapsa BH-1 and *Allochromatium* BH-2 isolated from the South Andros Black Hole (Herbert et al. 2005) were grown photosynthetically in the mineral salts medium of Pfennig and Trüper (1992) supplemented with 3% w/v NaCl. Cells were harvested and membranes prepared according to the method described by Evans et al. (1990).

Determination of growth temperature profiles

Growth temperature profiles for *Thiocapsa* strain BH-1 and *Allochromatium* BH-2 were determined by incubating the cultures in the mineral salts growth medium of Pfennig and Trüper (1992) supplemented with 3% w/v NaCl. The mineral salts medium was dispensed in 150 × 15 mm diameter screw-top tubes (Corning Life Sciences) which were pre-equilibrated at the designated incubation temperature in thermostatted glass aquarium tanks set at the following temperatures: 5, 10, 15, 20, 25, 30, 35, and 40°C ± 2°C. After equilibration for 1 h each series of tubes was inoculated, respectively with 1 ml volumes of exponentially growing cultures of *Thiocapsa* BH-1 and *Allochromatium* BH-2. The inoculated tubes were replaced in the thermostatted aquaria and continuously illuminated using tungsten bulbs to give a light intensity at the tube surface of 5,000 lux. At 24 h intervals the tubes were removed and vortexed to ensure the cells remained suspended and when appropriate the cultures were fed with neutralized sulfide. The optical density of the elemental sulfur depleted cultures was measured at 650 nm after 7 days incubation, using a Bausch and Lomb Spectronic spectrophotometer.

Spectroscopy

Absorption spectra were collected at 77 K in an SMC-TBT flow cryostat (Air Liquide, Sassenage, Fr.) cooled with liquid nitrogen, or at room temperature, using a Varian Cary E5 Double-beam scanning spectrophotometer. Samples for low temperature absorption measurements contained 60% (v/v) glycerol.

Room-temperature fluorescence excitation spectra were obtained with a SPEX Fluoromax spectrofluorimeter (ISA, Longjumeau, France) equipped with a red sensitive photomultiplier R406 (Hamamatsu Hamamatsu Electronics, Japan). The efficiency of carotenoid (Car) to bacteriochlorophyll (Bchl*a*) energy transfer (ET) in the photosynthetic membranes was calculated not from the absorption spectrum, but from the fractional absorption

spectrum ($1 - T$, where T is the transmittance of the sample) and the fluorescence (Fl) excitation spectrum, as described previously (Cogdell et al. 1981; Noguchi et al. 1990). The efficiency of Car → Bchl*a* ET, ascribed as the ratio of $Fl/(T - 1)$, was averaged over the 0-0 and 0-1 bands of the carotenoid molecules where the bacteriochlorin molecules do not absorb after the spectra were normalized to the Q_x-transition of the Bchl*a* molecules situated at ca. 590 nm. It was assumed that the ET from the Q_x transition to the Q_y transition of Bchls was 100%. The room-temperature fluorescence excitation and fractional absorption spectra were measured with the same spectral bandwidth of 2 nm. The FL emission was measured at the peak position with the slits wide open (20 nm) to ensure that all the fluorescence was detected.

Carotenoid isolation, purification and structure determination

Pigments were removed from cells by two extractions with acetone/methanol (7:2, v/v) and each extraction was accompanied by sonication of the mixture. Then the solvent was removed by evaporation. The pigment extracts were analyzed by HPLC equipped with a μBondapak C18 column (8×100 mm, RCM type; Waters, USA) and eluted with methanol (1.8 ml/min). Anion exchange chromatography (DEAE-Toyopearl 650M, Tosoh, Japan) was used to separate the bacteriochlorins and polar lipids from the carotenoids (Takaichi et al. 2001). Absorption spectra of the carotenoids were recorded with a photodiode-array detector (MCPD-3600; Otsuka Electronics, Japan) attached to the HPLC system. The molar absorption coefficient at maximum wavelength for each carotenoid in the eluent of methanol was assumed to be the same. Relative molecular masses were determined by field-desorption mass spectrometry using a double-focusing gas chromatograph/mass spectrometer equipped with a field-desorption apparatus (M-2500, Hitachi, Japan). The ^{1}H-NMR (500 MHz) spectra in $CDCl_3$ at 24°C were measured with a UNITY *INOVA*-500 (Varian, USA) system. The peak assignments of NMR spectra were made on the basis of ^{1}H–^{1}H COSY and NOESY analysis. The circular diochroism (CD) spectrum was measured by a J-820 spectropolarimeter (JASCO, Japan) in diethyl ether/*i*-pentane/ethanol (5:5:2, by vol.) at 20°C (Takaichi et al. 2001).

Results and discussion

Microbial layer

In June 1999, during the course of a survey of the previously unexplored South Andros Black Hole system marked temperature anomaly was recorded at 17.8 m depth, where the water temperature increased sharply from 29 to 36°C before decreasing again after a further 1-m increase in depth (Fig. 2). This depth marks the boundary between the oxic brackish upper water mass and the denser anoxic saline lower layer. Since the South Andros Black Hole has no known direct connection to the sea, except through rock fractures and local porosity, water exchange is severely restricted. Hence, the physico-chemical gradients that develop are unusually stable and the boundaries sharp, confirming the absence of mixing (Schwabe and Herbert 2004). In this respect the South Andros Black Hole can be considered analagous to meromictic lake see references (van Gemerden and Mas 1995; Overmann et al. 1991) and references therein. Coincident with the sharp increase in water temperature a 1 m thick gelatinous layer (plate) of anoxygenic phototropic purple sulfur bacteria was observed (Fig. 2, hatched horizontal bar). The dominant purple sulfur bacteria of this warm, saline, and sulfide rich layer have been isolated and identified as members of the genera *Allochromatium* and *Thiocapsa*. The development of dense populations of anoxygenic phototropic bacteria is not uncommon at the pycnocline in stratified lakes (Overmann et al. 1996a, b). However, the thickness of these plates is typically of the order of a few tens-of-centimeters and they are more usually located higher in the water column (van Gemerden and Mas 1995). To our knowledge, this study presents the first evidence of the ability of such bacterial populations to significantly increase the ambient

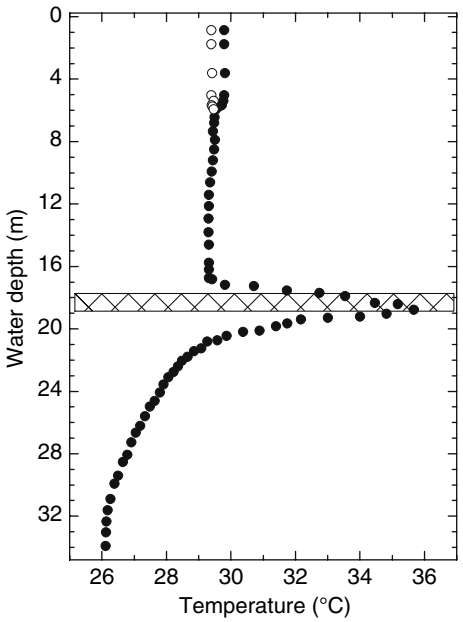

Fig. 2 Day-time (*solid circles*) and night-time (*open circles*) temperature profiles of the water column and spatial location (hatched horizontal bar) of the phototropic purple sulfur bacteria

water temperature. A night temperature profile (Fig. 2, *open circles*) was made and is essentially the same as the daytime profile (Fig. 2, *solid circles*) except that the increased temperature in the surface water mass (0–5 m depth) disappears. This is to be expected as at night there is no solar energy input and hence no surface water heating. The temperature in the microbial layer remains the same as in the day time profile indicating there is little diffusion of heat from this water mass. The dominant phototropic bacteria present in this layer produced considerable amounts of extracellular polymeric material, which further reduces diffusion within the microbial layer. This is very evident in the dive video (see supplementary data), which shows that the body outlines of the SCUBA divers remain after they have passed through this layer. As the divers pass through the viscous layer a purple haze is observed, illuminated by the torches. The accompanying dive video also shows that the water above and below the purple bacterial layer is clear, although the only light source below the bacterial mat is provided by the torches.

With the exception of moderately thermophilic purple sulfur bacteria, such as *Chromatium tepidum* the optimum growth temperature for most members of the family Chromatiaceae lie in the range 20–30°C (Madigan 1986; Pfennig and Trüper 1989). In order to test the hypothesis that these indigenous phototropic bacteria gain an ecological advantage by increasing the temperature of their immediate environment we have determined the optimum growth temperature of both isolates (see "Methods"). Data presented in Fig. 3 show that the optimum growth temperature for both isolates. For clarity, the absorbance data has been normalized to the maximum values for each profile. It is evident from these profiles that under laboratory conditions *Thiocapsa* BH-1 (*closed circles*) and *Allochromatium* BH-2 (*open circles*) have temperature optima of about 35°C, which is similar to the prevailing in situ temperature at 17.8–19 m depth (compare the position of the hatched bars in Figs. 2 and 3). Although this evidence is still preliminary it does nonetheless demonstrate that both isolates have temperature optima attuned to the prevailing thermal conditions which may give them a selective advantage and enable them to out compete other phototropic bacterial populations present at this depth horizon in the water column.

Purple sulfur bacteria belonging to the genera *Allochromatium* and *Thiocapsa* are able to harvest solar energy, using their bacteriochlorophyll and carotenoid chromophores and transduce it into a useable form for the living cell. In vivo absorption spectra (Fig. 4) of both isolates show typical absorption maxima of Bchl*a*, in the blue and near-IR, and carotenoids that absorb between 420 and 550 nm. The carotenoids absorb at wavelengths which are optimal for capturing the photons that penetrate to a depth of

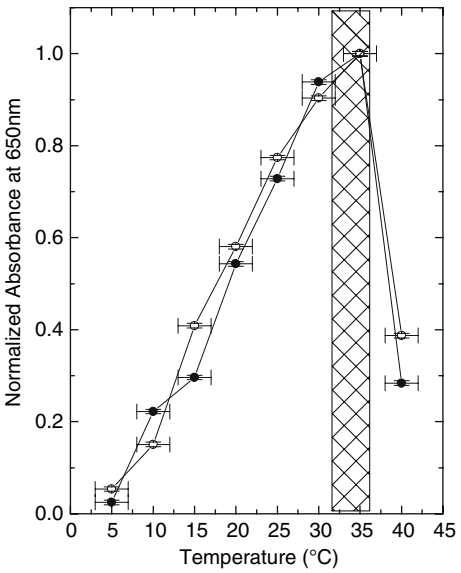

Fig. 3 Growth temperature profiles of isolates *Thiocapsa* BH-1 (*closed circles*) and *Allochromatium* BH-2 (*open circles*). For clarity, the data has been normalized to the maximum absorbance value for each bacterium. The hatched vertical bar represents the in situ temperature domain where the phototropic purple sulfur bacteria are located in the natural water column, see the hatched horizontal bar in Fig. 2

17.8 m. Strong light absorption by the carotenoid-rich cells also prevents light scattering and explains why the water column of the South Andros Black Hole appears black (Fig. 1) not blue as observed in other Bahamian cave systems.

The in vivo absorption spectra of photosynthetic bacteria show several electronic transitions in the near infrared which correspond to their complement of light-harvesting (LH) antenna complexes (Hawthornthwaite and Cogdell 1991; Zuber and Cogdell 1995). In BChl*a*-containing photosynthetic bacteria the transitions between ca. 800 and 860 nm are attributed to peripheral light-harvesting (LH2) complexes. Transitions between ca. 870 and 920 nm are attributed to core light-harvesting (LH1) complexes. All photosynthetic bacteria contain LH1 complexes, however, not all species contain LH2 antennae. It is evident from Fig. 4 that both Black Hole isolates contain absorption peaks attributed to LH2 (*Thiocapsa* BH-1: 798, 821, and 867 nm; *Allochromatium* BH-2: 797, 809, and 874 nm). The absorption maxima for the LH1 complexes are at 914 and 912 nm for *Thiocapsa* BH-1 and *Allochromatium* BH-2, respectively.

The major carotenoid in BH-1 is spirilloxanthin, while in BH-2 it is rhodopin (Table 1). The Car → Bchl*a* efficiencies of ET have been determined by room-temperature steady-state, carotenoid/bacteriochlorophyll excitation-induced, bacteriochlorophyll fluorescence (Cogdell et al. 1981). Laboratory studies using membrane vesicles

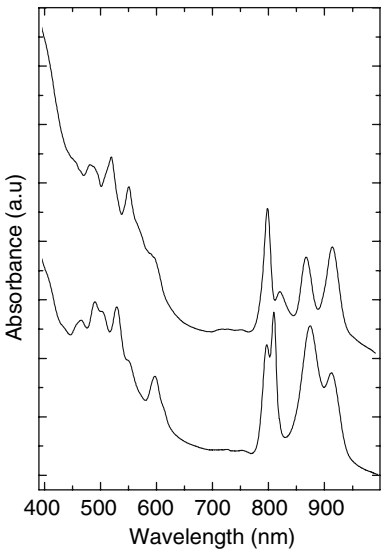

Fig. 4 Low temperature absorption spectra of whole cells of isolates *Thiocapsa* BH-1 (upper plot) and *Allochromatium* BH-2 (lower plot)

Table 1 Carotenoid composition and carotenoid to bacteriochlorophyll energy-transfer (ET) efficiencies from isolates *Thiocapsa* BH-1 and *Allochromatium* BH-2

Carotenoid	Allochromatium BH-2 (%)	Thiocapsa BH-1 (%)
Lycopene	3	n/d
Rhodopin	65	n/d
Anhydrorhodovibrin	7	1
Rhodovibrin	<1	n/d
Spirilloxanthin	25	91
Spirilloxanthin-2-ol	n/d	8
Carotenoid to bacteriochlorophyll ET efficiency	37	28

Carotenoid characterization and quantification (% mol) and ET efficiencies (see also Fig. 5) were determined as previously described (Cogdell et al. 1981; Noguchi et al. 1990)

n/d, Not detected

isolated from intact cells show that both isolates have some of the lowest ET efficiencies for Car → Bchl*a*, yet, reported for purple bacteria: 28% for *Thiocapsa* BH-l and 37% for *Allochromatium* BH-2 (Table 1 and Fig. 5) when compared to comparable bacterial species. For example, the efficiency of Car → Bchl*a* ET is nearly 100% in photosynthetic membranes from wild-type *Rhodobacter sphaeroides*, where the major carotenoid present is spheroidene, (Cogdell et al. 1981; Noguchi et al. 1990; Goedheer 1959). While in *Rhodopseudomans palustris*, where the major carotenoid present is rhodopin (with some anhydrorhodovibrin and spirilloxanthin) (Takaichi 1999) the efficiency of Car → Bchl*a* ET drops to 60%

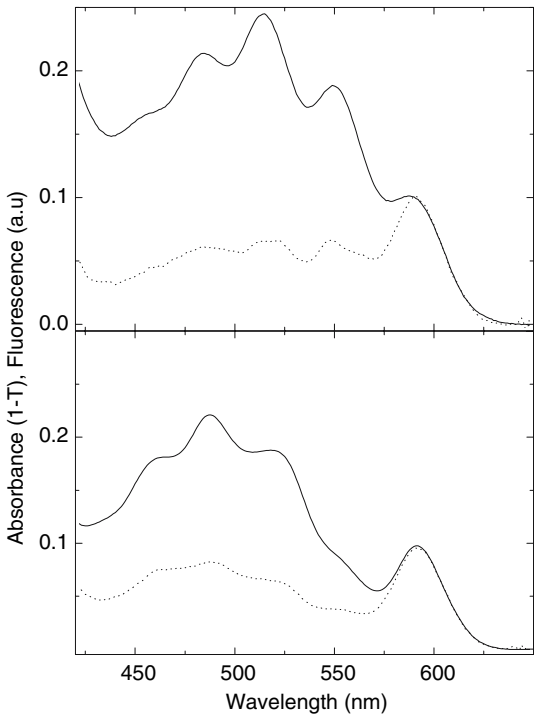

Fig. 5 A comparison of the room-temperature steady-state fractional absorption spectrum (1-T) (*solid lines*) and fluorescence excitation spectrum (*dotted lines*) in isolates *Thiocapsa* BH-1 (upper box) and *Allochromatium* BH-2 (lower box). The spectra were normalized to the Q_x-transition of the bacterioclorophll *a* absorption band as previously described, see Cogdell et al. 1981

(Nishimura and Takamiya 1966). The efficiency of Car → Bchl*a* ET is further reduced in spirilloxanthin-rich species such as *Rhodospirillum rubrum* (nearly 100% spirilloxanthin) to 30–40% (Goedheer 1959). Thus the BH isolates are less efficient at transferring the energy absorbed by the carotenoid molecules to the Bchl molecules, especially when compared to species containing the "same" carotenoids.

Identification of a novel carotenoid

The carotenoid content of the two isolates of purple bacteria was analyzed by HLPC. In most cases the carotenoids could be identified with reference to known carotenoids isolated from *Rhodospirillum rubrum*. Both the *Allochromatium* BH-2 and *Thiocapsa* BH-1 isolates contain carotenoids of the normal spirilloxanthin series (Table 1 and reference (Takaichi and Shimada 1992)), which are optimized for capturing the accessible wavelengths at 17.8 m depth. The identification of anhydrorhodovibrin and spirilloxanthin from BH-1 were confirmed by mass spectroscopy. Their relative molecular masses are 566 and 596, respectively.

There was an unknown carotenoid peak in BH-1 that eluted just after Bchl on the HPLC system. This unknown carotenoid was characterized as follows. The pigment extract was loaded on a column of DEAE-Toyopearl. Carotenoids were eluted with *n*-hexane/acetone (1:1, v/v) while Bchl and polar lipids were retained on the column. Then the unknown carotenoid was collected from the HPLC system. Its absorption spectrum indicates that it is an acyclic carotenoid with 13 conjugated double bonds, which is the same with that of spirilloxanthin (Takaichi 1999; Takaichi and Shimada 1992; Frank and Cogdell 1993). Its relative molecular mass is 612, which is 16 mass units heavier than that of spirilloxanthin, and it changes to a mono-acetyl derivative by acetylation. To obtain further structural information ^{1}H-NMR spectroscopy was used to show that the structure is indeed spirilloxanthin with a hydroxyl group at C-2 (Table 2).

Carotenoid molecules in photosynthetic bacteria (purple, green sulfur, green filamentous, and heliobacteria; except for some aerobic photosynthetic bacteria) do not have chirality (Takaichi 1999). The stereochemistry of the novel carotenoid was probed by circular diochroism spectroscopy and the spectrum found to be the same as that of the chemically synthesized chiral molecule (2′*S*)-plectaniaxanthin (Dumont and Pfander 1984). Therefore, the hydroxyl group is (2*S*) and the novel carotenoid has been determined to be (2*S*)-spirilloxanthin-2-ol (also called (2*S*)-2-hydroxy-spirilloxanthin) (Fig. 6). The IUPAC-IUB semi-systematic name is (2*S*)-1,1′-dimethoxy-3,4,3′,4′-tetradehydro-1,2,1′,2′-tetrahydro-ψ,ψ-caroten-2-ol.

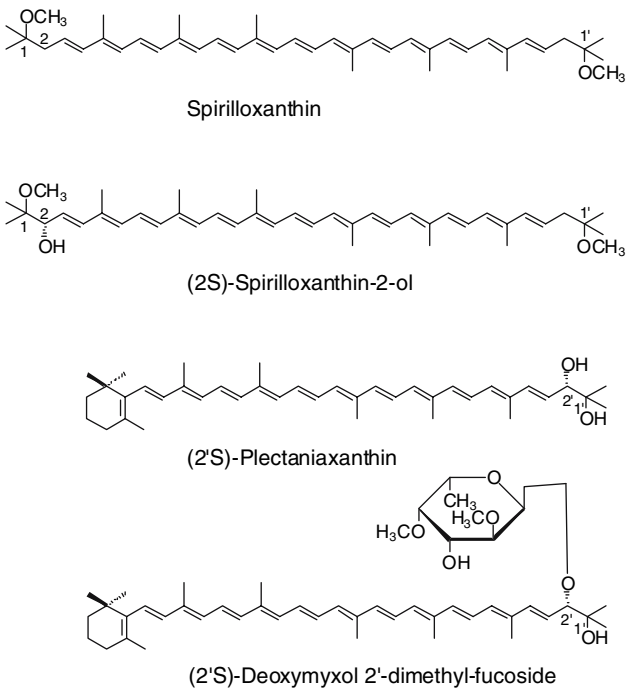

Fig. 6 Structure of (2*S*)-spirilloxanthin-2-ol and other carotenoids

It is interesting to note that the structure of end group of 1-OH and (2*S*)-2-OH is the same as found with the (2′*S*)-myxol groups of carotenoids from cyanobacteria, such as (2′*S*)-deoxymyxol 2′-dimethyl-fucoside (see Fig. 6 for the structure of this Car molecule) from *Synechocystis* sp. PCC 6803 (Takaichi et al. 2001), and not found in purple

Table 2 ^{1}H-NMR data of spirilloxanthin-2-ol in $CDCl_3$ at 24°C

Spirilloxanthin-2-ol				Spirilloxanthin (Lindal and Liaaen-Jensen 1997)	
Proton	δ^a	Proton	δ^a	Proton	δ^a
H_3-16	1.149 s	H_3-16′	1.159 s	H_3-16	1.16
H_3-17	1.115 s	H_3-17′	1.159 s	H_3-17	1.16
H_3-18	1.929 s	H_3-18′	1.929 s	H_3-18	1.93
H_3-19	1.978 s	H_3-19′	1.978 s	H_3-19	1.97
H_3-20	1.987 s	H_3-20′	1.987 s	H_3-20	1.98
H_3C-O-1	3.268 s	H_3C-O-1′	3.235 s	H_3C-O-1	3.23
H-2	4.08 d (7.5)	H_2-2′	2.32 d (7.5)	H_2-2	2.32
H-3	5.66 dd (15.5, 7.5)	H-3′	5.73 dd (15.5, 7.5)	H-3	5.72
H-4	6.39 d (15.5)	H-4′	6.16 d (15.5)	H-4	6.16
H-6	6.20 d (11)	H-6′	6.11 d (11)	H-6	6.11
H-7	6.60 dd (15.5, 11)	H-7′	6.60 dd (15.5, 11)	H-7	6.60
H-8	6.39 d (15.5)	H-8′	6.35 d (15.5)	H-8	6.35
H-10	6.23 d (11)	H-10′	6.23 d (11)	H-10	6.23
H-11	6.65 dd (15.5, 11)	H-11′	6.65 dd (15.5, 11)	H-11	6.65
H-12	6.38 d (15.5)	H-12′	6.38 d (15.5)	H-12	6.38
H-14	6.27 m	H-14′	6.27 m	H-14	6.27
H-15	6.65 m	H-15′	6.65 m	H-15	6.65

[a] δ in ppm, multiplicity, and coupling constant in Hz

bacteria (Takaichi 1999). Thus, spirilloxanthin-2-ol is the first carotenoid with chirality to be identified in anaerobic photosynthetic bacteria. The possible evolutionary and functional significance of this is as yet unknown and requires further study.

Energy budget

In the absence of recent or ongoing igneous/geothermal activity within the Bahamas carbonate platform there is no immediate explanation for the recorded temperature anomaly in the South Andros Black Hole. Therefore, alternative mechanisms must be considered. In formulating a convincing theory to explain the observed temperature anomaly the following points were considered: (i) the physico-chemical gradient boundaries are unusually sharp indicating little mixing, (ii) the temperature anomaly is coincident with the phototropic bacterial plate, (iii) the cells are rich in carotenoids especially spirilloxanthin, and (iv) spirilloxanthin is a known to have a relatively low ET efficiency ($\sim$30%) in antenna complexes (Frank and Cogdell 1993).

We propose, therefore, that the mass populations of anoxygenic phototropic bacteria present at 17.8 m are functioning as heat engines by dissipating excess light energy as heat. It is clear that at this depth in the water column most of the penetrating light is in the 450–550 nm region (Sullivan 1963) and this is the wavelength region where the light is absorbed maximally by the carotenoids (Hawthornthwaite and Cogdell 1991; Zuber and Cogdell 1995; Takaichi 1999; Takaichi and Shimada 1992; Frank and Cogdell 1993). The key question is whether there is sufficient energy dissipation by the carotenoids to account for the recorded increase in temperature?

In order to address this question, we have attempted to estimate the energy budget for the South Andros Black Hole as follows: the volume of the 1 m thick microbial plate at a depth of 17.8 m is approximately 4.9×10^4 m^3, and within this layer the water temperature increases by 7°C. Since 4.186×10^3 J of energy is required to raise 1 Kg of water by 1°C the energy required to raise this water mass by 7°C corresponds to $\sim 14.6 \times 10^{11}$ J. Clearly, this is an over simplification since as the water is brackish/saline, the spatial increase in water temperature is not instantaneous (Fig. 2) and the phototrophs themselves represent a sizable fraction of the total volume. Furthermore, we have no data on how much of this energy input is renewed on a daily basis. However, it clear from the temperature profile of the water column (Fig. 2) that a heat engine which can put out sufficient energy must be present in order to sustain the observed temperature differential of this water mass.

Since, no field measurements are available of the total daily energy input resulting from solar irradiance at the surface of the South Andros Black Hole we have used a similar approach to calculating the daily energy input. On the basis of light attenuation with depth measurements made by Dunstan (1982) on Dancing Lady Reef, Jamaica we have assumed that 66% of the surface light irradiance is attenuated at 17.8 m depth and that all photons are ultimately transduced into heat energy. Pinkney et al. (1995) reported that in Storr's lake, San Salvador, The Bahamas surface light irradiance values measured in mid-summer and at mid-day exceeded 2,200 μEinsteins s^{-1} m^{-2}. If we assume a typical Caribbean summers day consists of 9 h of sunlight (at 2,000 μEinsteins m^{-2} s^{-1}) we estimate that the energy input assuming no light attenuation corresponds to $\sim 7.3 \times 10^{11}$ J at 17.8 m depth. Factoring in a light attenuation value of 66% reduces this value to $\sim 2.4 \times 10^{11}$ J. Since the purple sulfur bacteria have very low carotenoid to bacteriochlorophyll ET efficiencies (ca. 30%, Table 1) then up to 7.2×10^{10} J are potentially available to be released by the bacteria in the form of excess heat on a daily basis. On the basis of these calculations we estimate it would take approximately 21 days to incrementally raise the temperature of the water mass at 17.8 m depth by 7°C, a timescale which we believe is not unrealistic.

A combination of unique factors may account for the origin of the bacterial anomaly that is omnipresent between the brackish and saline water. At the boundary between two water masses there is a steep salinity gradient, oxygen is depleted enabling sulfate-reducing bacteria to grow and generate hydrogen sulfide (H_2S). In turn, this allows the growth of mass blooms of sulfur-oxidizing bacteria. These bacteria occupy a highly specialized niche (saline, sulfide, low light) by exploiting the remaining solar radiation that penetrates deep into the water column, and transforming it into useable chemical potential. After sufficient time (perhaps over a period of years) these bacterial populations have achieved a state of homeostasis with respect to their sulfur cycling, which is manifested by the development of a unique biological phenomenon that is the Heat Engine in the South Andros Black Hole, the Bahamas.

In conclusion, sulfur-based phototropic bacteria isolated from the South Andros Black Hole have evolved a novel ecological adaptation by a self-generated rise in water temperature thereby optimizing their growth conditions in this specialized ecological niche.

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