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Microbial diversity and microbial abundance in salt-saturated brines: Why are the waters of hypersaline lakes red?

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Microbial Diversity and Microbial Abundance in Salt-Saturated Brines: Why are the Waters of Hypersaline Lakes Red?

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ABSTRACT

Salt-saturated lakes such as the North Arm of Great Salt Lake and saltern crystallizer ponds contain 10^7-10^8 and more red microorganisms ml^{-1}. Even the Dead Sea occasionally turns red due to microbial blooms. Three types of organisms may contribute to the coloration: the alga *Dunaliella salina* rich in β-carotene, halophilic Archaea (family *Halobacteriaceae*) containing 50-carbon bacterioruberin carotenoids and sometimes also retinal proteins (bacteriorhodopsin, halorhodopsin), and the recently discovered *Salinibacter* (*Bacteroidetes*) which contains pigment salinixanthin (an unusual acylated C_{40}-carotenoid glucoside) as well as different retinal pigments. Bacteriorhodopsin and halorhodopsin enable the cells to directly use light energy for respectively the outward pumping of protons driving ATP generation and for the inward transport of chloride ions. The carotenoid pigments (β-carotene, α-bacterioruberin and derivatives, salinixanthin) primarily appear to protect the cells against photooxidative damage. Salinixanthin also acts as a light harvesting antenna for xanthorhodopsin, the proton-pumping retinal pigment of *Salinibacter.* Quantitative assessment of the relative importance of the different pigments in the coloration of red brines of natural salt lakes and solar saltern crystallizer ponds suggests that α-bacterioruberin and other carotenoids contributed by members of the *Halobacteriaceae* are generally responsible for most of the color of the waters. The quantity of β-carotene present in *Dunaliella* cells often greatly exceeds that of the haloarchaeal bacterioruberin pigments. However, the large amounts of β-carotene contribute only little to the optical properties of the brines because of the dense packing of the pigment in little globules within the chloroplast. Presence of salinixanthin and of bacteriorhodopsin and derivatives in the biomass can often be demonstrated as well, but these pigments have never been shown to contribute greatly to the overall optical properties of the waters. Thus, carotenoids of the bacterioruberin group appear to be the main factor causing the characteristic red color of hypersaline brines worldwide.

INTRODUCTION

> "An embankment is made and ditches to draw clear sea water. It is left for a long time until the color becomes red. If the south wind blows with force during the summer and autumn the salt may grain over night. If the south wind does not come all the profits are lost."

The above lines were derived from the 'Peng-Tzao-Kan-Mu', a compilation of ancient Chinese pharmacology by Li Shih-Chen (1518-1593), as cited in an essay on the history of salt making by Baas Becking (1931) and attributed by him to much earlier times. Red colors are associated with many natural and man-made salt lakes whenever the NaCl concentration approaches saturation (Javor 1989). A more recent essay on salt and salt production was entitled "Red, the magic color for solar salt production" (Litchfield 1991). Such statements clearly show how prominent the color of salt-saturated brines often is. Figure 1 depicts a crystallizer pond of the salterns of Eilat, Israel; similar bright colors are characteristically found worldwide in salt production facilities. Such red brines are by no means restricted to man-made ponds for the production of solar salt. The North Arm of Great Salt Lake, Utah, with salt concentrations above 300 g l^{-1} shows similar colors (Post 1977; Baxter et al. 2005), and even the Dead Sea, a lake that is dominated by divalent cations (currently about 2.45 M [Mg^{2+} + Ca^{2+}] vs. about 1.75 M [Na^+ + K^+]) has occasionally turned red when rainwater floods reduced the salinity of the upper water layers (Oren 2002a).

Figure 1–A crystallizer pond of the Israel Salt Company at Eilat, photographed on April 27, 2008.

Microscopic examination of red saltern crystallizer brines shows the presence of massive numbers of a variety of microorganisms. Figure 2 shows a typical picture of such a microbial community, consisting of prokaryotic cells, often present in numbers as high as 2 x 10^7–10^8 ml^{-1} and sometimes even higher, as well as red-orange cells of the flagellate unicellular green alga *Dunaliella salina*, typically present in numbers of a few hundreds to a few thousands ml^{-1}. The prokaryotic community is dominated by red Archaea of the family *Halobacteriaceae* (Oren 2006), and in many cases the most abundant type was reported to consist of flat, square or rectangular cells. Many such cells can be recognized in the left panel of Figure 2.

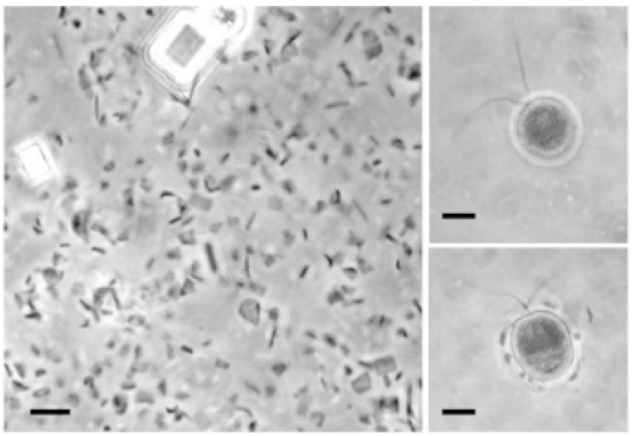

Figure 2–Microorganisms from the crystallizer brine shown in Figure 1: Flat square halophilic Archaea resembling *Haloquadratum walsbyi* and other types of prokaryotes (left panel), and *Dunaliella salina* cells (right panels). Phase contrast. The bars represent 5 μm. The cells shown in the left panel had been concentrated by centrifugation, and therefore no gas vesicles are visible within haloarchaeal cells. Two small halite crystals are seen in the left panel.

The world of halophilic microorganisms is very diverse (Oren 2002a, 2002b, 2002c, 2007), and different types of red, orange or purple pigments are found in many salt-loving organisms. Many, often contradictory statements can be found in the literature about the reasons why salt-saturated brines are red (see Oren et al. 1992). Some authors have attributed the color to the massive presence of β-carotene inside the *Dunaliella* cells. Others have argued that carotenoid pigments of the halophilic Archaea may be the main cause of the red coloration. Yet another idea, brought forward from time to time, is that retinal-containing proteins such as bacteriorhodopsin produced by some halophilic Archaea may be responsible for the color of saltern crystallizer ponds and other salt lakes. In most cases no experimental evidence has been provided to support the statements made, and the nature of the organisms present and their pigments was not further documented. As a result, considerable confusion still exists about the true nature of the optical effects caused by the presence of pigmented microorganisms in the brine.

In this paper I will try to present an overview of our current understanding of the pigments that may contribute to the color of hypersaline brines and the microorganisms that harbor those pigments, and then assess to what extent these pigments indeed contribute to the intense color of saltern ponds such as the one shown in Figure 1. The conclusions are widely applicable. However, it should be realized that it is quite probable that the coloration of brines at different geographical locations and with different chemical and physical properties may be due to different types of pigments and different types of microorganisms.

Dunaliella salina and its β-Carotene Content

Dunaliella salina, first described from salterns near Montpellier in the south of France in 1838 and named in 1905 (Oren 2005), is found worldwide in saltern evaporation and crystallizer ponds as well as in many natural salt lakes. *Dunaliella* is a unicellular eukaryotic alga that taxonomically belongs to the Chlorophyceae. Cells grown in the laboratory under low light and high nutrient concentrations are typically green because of their content of chlorophyll *a* and *b*. However, when exposed to stressful conditions of high light intensities and nutrient limitation, cells become colored orange-red due to the accumulation of large amounts of β-carotene. β-carotene has a characteristic absorption spectrum with a broad peak between 400 and 500 nm and an absorbance maximum at 451 nm (Figure 3). The β-carotene is located in small globules within the interthylakoid space of the cell's single chloroplast (Ben-Amotz et al. 1982, 1988). In some *Dunaliella* strains the pigment may be accumulated in quantities up to 8-10% of the cells' dry weight and possibly even more. The oily globules contain a mixture of the all-*trans* and the 9-*cis* isomers of β-carotene, together with minor amounts of other mono-cis and di-cis stereoisomers (Ben-Amotz et al. 1982). The higher the light intensities to which the cells have been exposed, the higher the ratio between the amount of the 9-*cis* to the all-trans form.

The β-carotene content of *Dunaliella* is mainly determined by the total irradiance the alga receives during a cell division cycle. High light intensities, nutrient limitation stress or supraoptimal salt concentrations lead to a decrease in the amount of chlorophyll per cell and an increase in its β-carotene content (Ben-Amotz & Avron 1983; Lers et al. 1990). Ultraviolet radiation also has a strong inducing effect on β-carotene biosynthesis.

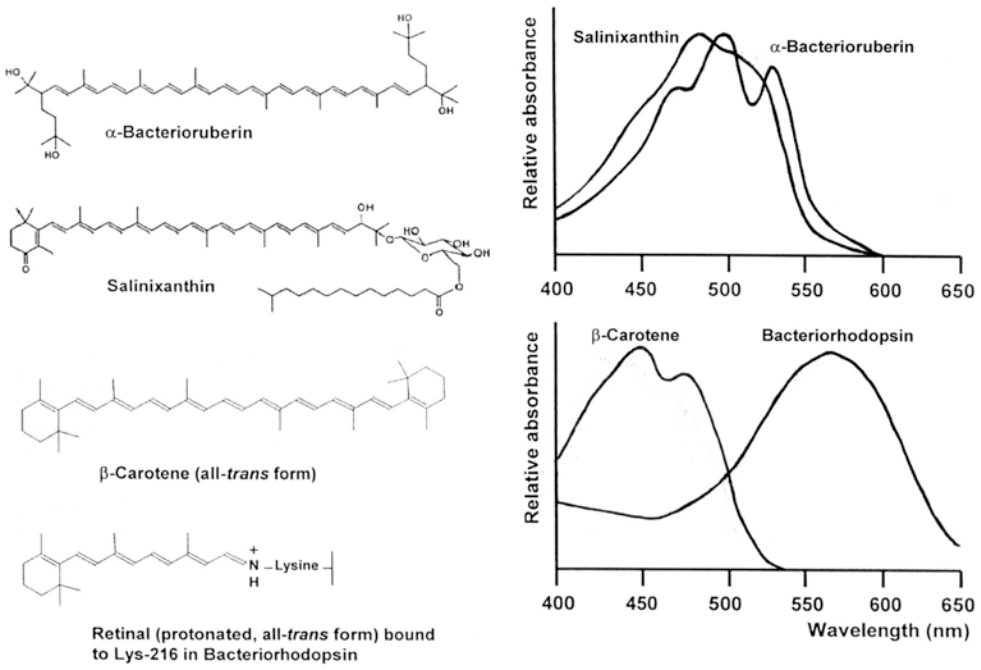

Figure 3–The principal orange, red and purple pigments encountered in the biomass of saltern crystallizer ponds, and their absorption spectra: the C_{50} carotenoid α-bacterioruberin of halophilic Archaea (family *Halobacteriaceae*), salinixanthin, the acylated C_{40}-carotenoid glucoside of *Salinibacter ruber*, β-carotene accumulated by the unicellular green alga *Dunaliella salina*, and bacteriorhodopsin of *Halobacterium* and some other members of the *Halobacteriaceae*.

Dunaliella cells that have accumulated large amounts of β-carotene tolerate much higher light intensities than green cells that had not synthesized major amounts of the pigment. The action spectrum of the photoprotection parallels the absorption spectrum of the carotenoid pigments (Ben-Amotz et al. 1989). The mechanism of the protective effect, however, is still not completely clear. It may be based on the destruction of singlet oxygen radicals produced during photosynthesis under high radiation. However, the β-carotene is located in globules within the interthylakoid space rather than in the thylakoids themselves, and the question should therefore be asked whether the distance between the carotene globules and the thylakoid-bound chlorophyll still allows effective quenching of chlorophyll-generated radicals. It is more probable that the carotene globules may protect the cells against high light damage by acting as a screen that absorbs excess radiation.

The Carotenoid Pigments of Halophilic Archaea

Most species of the halophilic Archaea (family *Halobacteriaceae*) are colored pink-red due to a high content of carotenoid pigments in their cell membrane. Only a few species in the group such as *Natrialba asiatica* do not show such a pink color. In some other species the extent of pigmentation depends on the salinity at which the cells had been grown: *Haloferax mediterranei* is only weakly pigmented at high salinity, but produces massive amounts of carotenoid pigments when incubated at suboptimal salinities. However, those species that appear to be most abundant in saltern crystallizer ponds and other salt lakes are all brightly colored, including the square archaeaon *Haloquadratum walsbyi* that is commonly found in salterns worldwide (Bolhuis et al. 2004; Burns et al. 2004).

Extracts of halophilic archaeal cells in organic solvents are pink and show absorbance maxima at 498 and 530 nm with a shoulder at 470 nm (Figure 3). This characteristic absorption spectrum is mainly due to the presence of C_{50} straight-chain derivatives of α-bacterioruberin (Kelly et al. 1970). Different derivatives of α-bacterioruberin are present as well, such as monoanhydrobacterioruberin and bisanhydrobacterioruberin (Kelly et al. 1970; Kushwaha et al. 1975). Several other derivatives have been found in minor amounts (Rønnekleiv et al. 1995). For a more complete account of the carotenoid pigments encountered in the group and their chemical structures see Oren 2002a and Oren 2006.

Studies in the early 1960s using *Halobacterium salinarum* as a model organism have clearly shown the advantages that pigmentation provides to cells. Non-pigmented mutants can easily be isolated, and such mutant strains grow in the dark as well as the red-pink wild type, but when incubated

at light intensities equivalent to full sunlight, the white mutants are rapidly outcompeted by the pigmented parent strain. The carotenoid pigments thus protect the cells against damage by excessive light (Dundas & Larsen 1962). The carotenoid pigments of *Hbt. salinarum* were also claimed to protect the cells against UV radiation and aid in photoreactivation (Wu et al. 1983). A protective role of bacterioruberin by providing resistance to agents that damage DNA such as ionizing radiation or hydrogen peroxide was also shown in *Hbt. salinarum*, a colorless mutant being more sensitive to such agents than the pigmented strain. The frequency of DNA strand-breaks induced by ionizing radiation (^{60}Co γ-rays) was significantly reduced by the presence of bacterioruberin pigments (Shahmohammadi et al. 1998).

The Retinal Pigments of Halophilic Archaea

The retinal pigments present in members of the *Halobacteriaceae* have received much interest since the structure and function of bacteriorhodopsin were elucidated in the early 1970s (Oesterhelt & Stoeckenius 1971). *Halobacterium salinarum* has four such retinal-containing proteins: the outward proton pump bacteriorhodopsin (maximum absorbance at 568 nm) (Figure 3), the inward chloride pump halorhodopsin (578 nm), and two sensory rhodopsins involved in light sensing for phototaxis: a sensor for green light (sensory rhodopsin I) to which the cells are attracted, and a sensor for blue light (sensory rhodopsin II) that acts as a repellant. Not all members of the *Halobacteriaceae* possess genes encoding all these retinal proteins. Many species lack bacteriorhodopsin; *Haloquadratum walsbyi* has two bacteriorhodopsins and one halorhodopsin, but lacks sensory rhodopsins, which would not be useful in these organisms that lack active motility (Bolhuis et al. 2006). Bacteriorhodopsin and halorhodopsin contain seven transmembrane helices with the retinal being covalently attached through the formation of a Schiff base between the aldehyde function of the chromophore and the ε-amino group of a lysine residue in the seventh helix. In *Hbt. salinarum*, bacteriorhodopsin is organized in special patches in the cytoplasmic membrane (the 'purple membrane'), while in certain other species the retinal pigments appear to be more evenly distributed over the cell membrane. More in-depth information on the biology, biochemistry, and biophysics of the haloarchaeal retinal proteins can be found in specialized reviews (Oesterhelt 1988; Lanyi 1990; 1993, Oesterhelt 1995; Lanyi 1998; Lanyi & Luecke 2001; Oren 2002a).

When bacteriorhodopsin is excited by light of a suitable wavelength, a complex photocycle is initiated that lasts about 10 milliseconds. During the process, the retinal group undergoes isomerization from the all-*trans* to the 13-*cis* isomer. In addition, the Schiff base between the retinal and lysine-216 is deprotonated and protonated again. As the proton is released at the outer side of the membrane but replenished from the internal side, the completion of the photocycle results in transport of a proton from the cell to the surrounding medium and the generation of a proton gradient across the cell membrane. This proton gradient can then drive the synthesis of ATP and other energy-requiring processes. Light can thus relieve energy starvation at low nutrient and low oxygen concentrations (Brock & Petersen 1976), and even anaerobic photoheterotrophic growth is possible with light serving as the energy souce (Hartmann et al. 1980).

Under suitable conditions *Halobacterium salinarum* produces large quantities of bacteriorhodopsin. The color of the culture then changes from red-pink or red-orange (due to the presence of bacterioruberin carotenoids) to purple. Genome sequencing (Ng et al. 2000) and molecular biological studies have contributed much to our understanding of the biosynthesis of bacteriorhodopsin and its regulation. *Hbt. salinarum* has a cluster of three genes: *bop*–the gene encoding the bacterio-opsin (the protein backbone of bacteriorhodopsin), *brp*–a bacterio-opsin-related protein, and *bat*–the bacterio-opsin activator. Expression of the *bop* gene cluster is induced by low oxygen tension and by light (Shand & Betlach 1991), and much information has accumulated on the factors that influence the biosynthesis of the protein and of the retinal moiety, all coordinated in a regulon that reacts to changes in light intensity and oxygen concentration (Baliga et al. 2001; DasSarma et al. 2001).

In view of the obvious advantages that the possession of bacteriorhodopsin may bestow on halophilic Archaea, it is surprising that attempts to quantify its presence and importance in natural communities of halophilic Archaea have been so rare. The color of haloarchaeal blooms in salterns and natural salt lake is generally red-pink rather than purple. Occurrence of bacteriorhodopsin was reported in a community of *Halobacteriaceae* that developed in the Dead Sea in 1980–1982, and concentrations of the pigment were then estimated to be in the range of 0.6-0.7 nmol l^{-1} at the time the prokaryote community contained 4-5 x 10^6 cells ml^{-1} (Oren & Shilo 1981). Indications were also obtained that this bacteriorhodopsin may have contributed to light-dependent fixation of CO_2 fixation in a mechanism that still has not been fully elucidated (Oren 1983). Javor (1983) found 2.2 nmol l^{-1} bacteriorhodopsin in the archaeal community in the crystallizer ponds of the salterns of Exportadora de Sal, Guerrero Negro, Baja California, Mexico. Flash spectroscopy provided evidence for the presence of both bacteriorhodopsin and halorhodopsin activity in a community of halophilic Archaea in a shallow coastal hypersaline salt flat on the Sinai peninsula, Egypt (Stoeckenius et al. 1985).

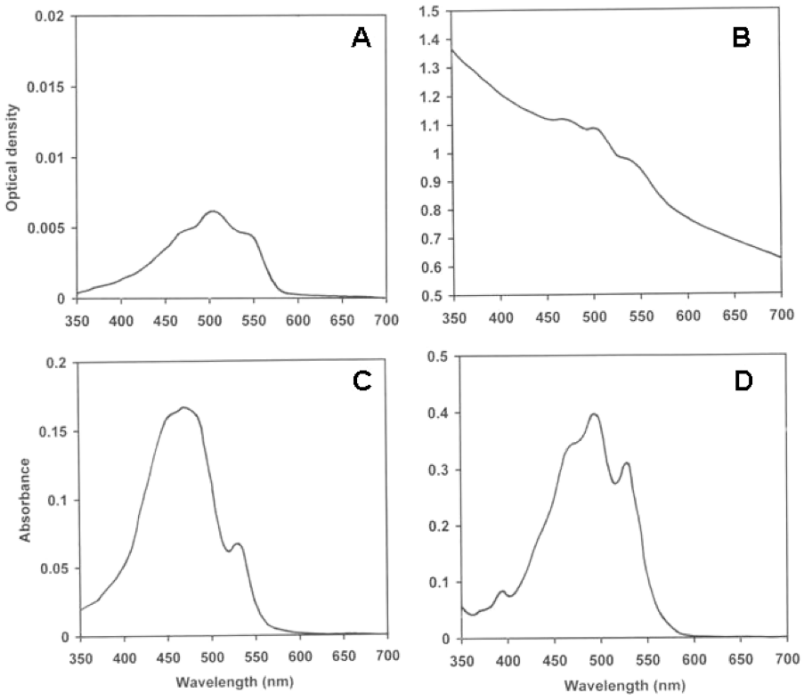

Figure 4–Absorption spectra of brine samples and pigment extracts prepared from them. Brine was sampled from the crystallizer pond of the Israel Salt Company, Eilat, shown in Figure 1. (A) Absorption spectrum of the brine, measured against water to which a small amount of milk was added to adjust for the turbidity of the sample. (B) Absorption spectrum of biomass concentrated from the brine by centrifugation. A portion of 360 ml of brine was centrifuged (30 min, 6000 x g), the pellet resuspended in 4 ml of supernatant brine, and the spectrum was recorded with supernatant brine as the blank. (C) Absorption spectrum of biomass from 50 ml of brine, collected by filtration on GF/C filters and extracted in 3 ml methanol/acetone 1:1 (vol/vol). (D) Absorption spectrum of biomass from 360 ml brine, collected by centrifugation (30 min, 6000 x g), followed by filtration on GF/C filters and extraction in 3 ml methanol/acetone. For explanations see text.

The Carotenoid and Retinal Pigments of *Salinibacter ruber*

Halophilic Archaea of the family *Halobacteriaceae* are not the only pigmented prokaryotes that can contribute to the red coloration of salt lakes and saltern crystallizer ponds. It was recently recognized that a rod-shaped, extremely halophilic representative of the domain Bacteria, phylum *Bacteroidetes*, may also be present in significant numbers in those environments in which red halophilic Archaea thrive. *Salinibacter ruber*, first isolated from Spanish saltern crystallizer ponds (Antón et al. 2002; Oren et al. 2004) now appears to be distributed worldwide in salterns and salt lakes approaching halite saturation. Cultures of *Salinibacter* are orange-red, and contain one major hydrophobic pigment, salinixanthin, identified to be a novel type of acylated C_{40}-carotenoid glucoside (Lutnæs et al. 2002). Its absorption spectrum shows a peak at 482 nm and a shoulder at 506-510 nm (Figure 3). Presence of salinixanthin in the biomass collected from the salterns of Alicante, Spain, could readily be detected by HPLC analysis of the hydrophobic pigments extracted from the cells, and it was estimated that 5-7.5% of the total prokaryote-derived pigment extracted from those salterns originated from bacterial rather than from archaeal extreme halophiles (Oren & Rodríguez-Valera 2001).

Analysis of the genome sequence of *S. ruber* (Mongodin et al. 2005) showed that, although *Salinibacter* is a representative of the Bacteria, it possesses genes homologous to haloarchaeal bacteriorhodopsin, halorhodopsin, and two sensory rhodopsins. While the functioning of the product of the putative halorhodopsin-like gene as a chloride pump still has to be proven, it was shown that the product of the bacteriorhodopsin-like gene, termed xanthorhodopsin, indeed acts as a light-driven proton pump. Further analysis of photochemical processes in *Salinibacter* demonstrated that the carotenoid salinixanthin can transfer absorbed light energy with a high efficiency to the xanthorhodopsin proton pump (Balashov et al. 2005; Balashov & Lanyi 2007). Such direct energy transfer between membranal carotenoids and retinal proteins has never yet been demonstrated in *Halobacterium* or any of the other members of the *Halobacteriaceae.* The finding shows that, at least in some halophilic prokaryotes, the red-orange carotenoid pigments may have additional

functions beyond protection against high light intensities and scavenging of toxic free radicals and to prevent photooxidative damage.

How Do the Different Microbial Pigments Influence the Color of Hypersaline Brines?

As shown above, many different types of red, orange and purple pigments can be produced by halophilic microorganisms: β-carotene of *Dunaliella*, bacterioruberin carotenoids of the *Halobacteriaceae*, salinixanthin of *Salinibacter*, and the different retinal pigments: bacteriorhodopsin and halorhodopsin of haloarchaea and xanthorhodopsin and other retinal proteins of *Salinibacter*. The question should therefore be asked to what extent each of these pigments contributes to the color that we see when we look at red brines of saltern crystallizer ponds (Figure 1), the North Arm of Great Salt Lake, and other similar environments colored by dense communities of halophilic microorganisms.

The answer to the above question may appear simple and straightforward: a simple way to assess the contribution of each pigment could be the extraction of the pigments followed by measurement of the absorption spectrum of the extract. Yet, such a simple approach can easily lead to erroneous conclusions. This is clearly demonstrated by the following case study, based on the same saltern crystallizer brine shown in Figure 1 and its microbial communities illustrated in Figure 2. Microscopic enumeration of the biota gave numbers of 280 *Dunaliella* cells and about 4×10^7 prokaryotic cells ml^{-1} in this brine.

When brine from the crystallizer pond was collected by filtration on glass fiber filters that retain all or at least most of the biomass, and the filters were then extracted with methanol/acetone (1:1, vol/vol), all hydrophobic pigments (carotenoids, chlorophylls) are released, and the components of the resulting extract can be evaluated by measuring the absorption spectrum, if necessary followed by HPLC separation and characterization of each individual pigment fraction. While satisfactory for many purposes, such a protocol does not provide information on the presence of bacteriorhodopsin, halorhodopsin and other retinal proteins, which are not extracted by organic solvents. The resulting spectrum (Figure 4C) shows a very large peak of β-carotene (the broad absorbance maximum around 450 nm). Some bacterioruberin carotenoids are present as well, as witnessed by the small peak at 530 nm. The major bacterioruberin peak at 496 nm is hidden below the large β-carotene peak. The absence of a peak at 665-670 nm shows that the *Dunaliella* cells contained very little chlorophyll. Based on the spectrum shown in Figure 4C the obvious conclusion would be that *Dunaliella*-derived β-carotene contributes most to the light absorption by the brine and is mainly responsible for its color, and that the bacterioruberins of the halophilic Archaea add a minor contribution as well.

An altogether different spectrum was obtained when the biomass was collected by centrifugation instead of by filtration. In this case the brine was centrifugated for 30 minutes at 6000 x g, the pellet was resuspended in a small portion of brine, cells were then collected on glass fiber filters as described above, extracted with methanol/acetone, and the absorption spectrum recorded. The resulting spectrum (Figure 4D) looks like a nearly pure bacterioruberin spectrum (compare Figure 3), and no obvious contribution from β-carotene is seen. Microscopic examination of the cell pellet shows the reason: no *Dunaliella* cells are found in the pellet. Due to the high content of carotene globules the cells are lighter than the brine, and they float upwards rather than sink during centrifugation. When the pellet obtained after centrifugation is resuspended in brine rather than extracted in organic solvent, and the in-vivo absorption spectrum measured against brine cleared of particles (Figure 4B), no straight baseline is obtained. The particles in the suspension not only absorb light but also cause a considerable light-scattering effect. As light scattering is wavelength-dependent, much higher optical densities were measured in the blue part of the spectrum than in the red part. The peaks that rise out of the baseline are those of bacterioruberin pigments. No prominent peak in the area of 570-580 nm is seen, and this proves that bacteriorhodopsin and halorhodopsin do not greatly contribute to the color of the cells (note that in the sample of Figure 3B such pigments would have been preserved, while the treatments applied in Figure 3C and 3D did not extract retinal proteins).

When finally a spectrum was made of brine that was neither filtered not centrifugated, and a correction was made for sample turbidity and light scattering by adjusting the scattering properties of the water blank with a small amount of milk, the true in vivo spectrum of the crystallizer pond biomass was obtained (Figure 4A). Surprisingly, this spectrum resembles that of halophilic Archaea and their bacterioruberin carotenoids, and no contribution of *Dunaliella* β-carotene is noticeable. This is unexpected, as β-carotene was the most abundant pigment in the biomass (compare Figure 4C). The reason why these large amounts of β-carotene contribute so little to the optical properties of the brine is the location of the pigment inside the cells. The haloarchaeal pigments are distributed more or less evenly over the entire cell membrane. This, and the fact that the halophilic Archaea are so abundant in the brine (4×10^7 cells ml^{-1}), enables the bacterioruberin pigments to absorb light, and the resulting spectrum is therefore that of the

haloarchaeal carotenoids. *Dunaliella* cells are rare (only about 280 cells ml^{-1}), and their massive amount of β-carotene is densely concentrated in tiny globules within the chloroplast. In this way the pigment contributes very little to the overall optical properties of the brine. When, however, the algal carotene is extracted with organic solvents, it becomes obvious how much had been accumulated in the *Dunaliella* chloroplasts (Oren et al. 1992; Oren & Dubinsky 1994).

FINAL CONCLUSIONS

1. Different pigments may contribute to the color of red brines in salt lakes: the 50-carbon carotenoid α-bacterioruberin and derivatives synthesized by halophilic Archaea of the family *Halobacteriaceae*, the 40-carbon carotenoid β-carotene that is accumulated within the chloroplast of the alga *Dunaliella salina*, the acylated C_{40}-carotenoid glucoside salinixanthin of the recently discovered *Salinibacter ruber* (*Bacteroidetes*), and the membrane-bound retinal-containing proteins bacteriorhodopsin and halorhodopsin, found in many representatives of the *Halobacteriaceae* as well as in *Salinibacter*.

2. In solar saltern crystallizer ponds and probably in many natural hypersaline salt lakes as well, β-carotene of *Dunaliella* is the most abundant pigment, and its presence dominates the absorption spectrum of biomass extracted with organic solvents that dissolve hydrophobic pigments.

3. However, the color of the brine is, at least in many cases, not primarily due to the algal β-carotene but rather to the presence of archaeal bacterioruberin pigments. This is due to different location of the pigments involved. Halophilic archaea are found in large numbers in the brines, and the bacterioruberin pigments are in most cases evenly distributed over the entire cytoplasmic membrane. On the other hand, the number of *Dunaliella* cells is several orders of magnitude lower, and in these cells the pigment is densely concentrated within globules located between the thylakoids of the chloroplast. Thus the pigment contributes only little to the optical properties of the brines, and its quantity becomes apparent only upon extraction with organic solvents.

4. Presence of retinal pigments (bacteriorhodopsin, halorhodopsin, sensory rhodopsins) within the natural community of halophiles in salt lakes has only rarely been quantified. Such pigments have occasionally been shown to be present, but in vivo absorption spectra of the biomass collected from natural brines never showed a prominent absorption peak that can be attributed to such retinal proteins.

5. Presence of the acylated C_{40}-carotenoid glucoside salinixanthin, a red-orange pigment found in the recently discovered red extremely halophilic bacterial species *Salinibacter ruber*, can be demonstrated at least in some salt-saturated hypersaline brines. Quantitatively its contribution to the pigmentation was always found to be a minor one in comparison with the amount of bacterioruberin pigments from the halophilic Archaea that inhabit the same brines.

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