

# The contribution of halophilic Bacteria to the red coloration of saltern crystallizer ponds<sup>1</sup>

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## Abstract

Analysis of the pigments extracted from solar saltern crystallizer ponds in Santa Pola near Alicante and on the Balearic island of Mallorca, Spain, showed that 5–7.5% of the total prokaryotic pigment absorbance could be attributed to a novel carotenoid or carotenoid-like compound. This unidentified pigment was identical to the sole pigment present in *Salinibacter ruber*, the only described member of a newly discovered genus of red halophilic Bacteria related to the *Cytophaga-Flavobacterium-Bacteroides* group. On the basis of fluorescence in situ hybridization experiments it has been shown that *Salinibacter* is an important component of the microbial community of Spanish saltern ponds. The red color of saltern crystallizer ponds may thus not only be due to red halophilic Archaea and to  $\beta$ -carotene-rich *Dunaliella* cells as previously assumed, but may contain a bacterial contribution as well. The *Salinibacter* pigment was not detected in samples collected from crystallizer ponds of the salterns of Eilat, Israel, and only traces of it may have been present in the Newark, CA, USA, salterns. The community structure of the prokaryote community inhabiting saltern crystallizers thus shows significant geographic variations. Polar lipid analyses of the biomass collected from the Santa Pola salterns showed that the total contribution of *Salinibacter* and other Bacteria to the total biomass was minor, the most important component of the community being halophilic Archaea. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** *Salinibacter*; Archaeon; Bacterium; Carotenoid; Bacterioruberin; Saltern

## 1. Introduction

Hypersaline lakes approaching NaCl saturation, natural as well as man-made such as saltern crystallizer ponds, are generally colored red due to the presence of dense communities of halophilic microorganisms.

Two types of carotenoid-rich microorganisms have generally been implicated in causing the red coloration: halophilic Archaea of the family Halobacteriaceae, and the unicellular green alga *Dunaliella salina*. The main pigments of the Halobacteriaceae are C-50 carotenoids, mainly  $\alpha$ -bacterioruberin and derivatives [1,2], while *Dunaliella* ac-

cumulates massive amounts of  $\beta$ -carotene under suitable conditions. The relative contributions of red Archaea and  $\beta$ -carotene-rich *Dunaliella* cells to the coloration of saltern crystallizer ponds have been studied in the past.  $\beta$ -Carotene was often found in quantities greatly exceeding the archaeal bacterioruberins. In spite of this, the optical properties of the saltern brines were determined primarily by the archaeal community. This apparent discrepancy was explained by the extremely small in vivo optical cross-section of the  $\beta$ -carotene in *Dunaliella* cells. As the carotenoid is densely packed in granules within the algal chloroplast, the presence of even large amounts of the pigment may contribute much less to the overall red color than the archaeal pigments, which are distributed evenly over the cell membrane [3,4].

It was recently recognized that a significant proportion of the prokaryotes present in saltern crystallizer ponds may belong to the domain Bacteria. Sequencing of bacterial 16S rRNA genes amplified from Spanish crystallizer

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<sup>1</sup> Non-standard abbreviations: The names of genera of the family Halobacteriaceae were abbreviated using three-letter abbreviations as recommended in Int. J. Syst. Evol. Microbiol. 50, 1405–1407.

ponds indicated the presence of a novel type of rod-shaped Bacteria, belonging to the *Cytophaga-Flavobacterium-Bacteroides* group [5]. Its closest known relative is *Rhodothermus marinus*, a red thermophilic bacterium isolated from deep-sea hydrothermal vents. In situ hybridization experiments using fluorescent probes designed to react with the new bacterial phylotype showed that the organism harboring this phylotype is rod-shaped, and may contribute between 5 and 25% of the total prokaryotic community in Spanish crystallizer ponds. The new type of halophilic Bacteria was originally described on the basis of these environmental studies as '*Candidatus Salinibacter*' [5]. The rod-shaped bacteria seen in electron micrographs of the biomass sampled from the saltern crystallizer ponds of the Delta de l'Ebre near Tarragona, Spain [6], may possibly belong to the same group.

We recently succeeded in isolating a number of extremely halophilic Bacteria from saltern ponds in Mallorca and Santa Pola, Alicante, Spain, with 16S rRNA sequences nearly identical to the '*Candidatus Salinibacter*' phylotype. These isolates are among the most halophilic Bacteria extant: they require at least 15% NaCl for growth and tolerate up to saturation. All isolates obtained thus far can be classified within a single species, now described as *Salinibacter ruber* gen. nov. sp. nov. [7].

All *Salinibacter* isolates obtained thus far are brightly red colored. In this paper we demonstrate that the color is caused by a single carotenoid or carotenoid-like compound, which can easily be distinguished from archaeal bacterioruberins and algal  $\beta$ -carotene. We used this property to assess the contribution of the *Salinibacter*-type Bacteria to the prokaryotic community and to the optical properties of red saltern ponds at different geographical locations.

## 2. Materials and methods

### 2.1. Environmental samples and collection of biomass

Brine samples were obtained from crystallizer ponds of the salterns at Santa Pola (Alicante, Spain) and on the Balearic island of Mallorca, of the Israel Salt Company, Eilat (Israel), and of the Cargill Solar Salt Plant, Newark, CA, USA. Data on the samples collected and their proper-

ties are summarized in Table 1. Salt concentrations were estimated by means of hydrometers or by refractometry. To enumerate bacteria, 10-ml portions of brine were centrifuged at room temperature for 15 min at  $12\,000\times g$ . Microscopic examination showed that all bacteria had precipitated to the bottom of the tube, and that no bacteria remained in the supernatant. After removal of about 9.5 ml of supernatant, the cell pellet was resuspended in the remaining liquid, the volume of the final suspension was measured, and its bacterial density was determined with a Petroff-Hauser counting chamber and a microscope equipped with phase contrast optics. For the enumeration of *Dunaliella* cells, samples (2–5 ml) were filtered through Millipore filters (25 mm diameter, 5  $\mu$ m pore size). Filters were placed on microscope slides and cells were counted under a  $10\times$  objective. Cell numbers were calculated from the average number of cells per field and the field diameter.

### 2.2. Bacterial and archaeal cultures

*S. ruber* M31 (DSM 13855<sup>T</sup>), isolated from the Mallorca salterns, and strains Pola-13 and Pola-18 from Santa Pola, Alicante [7], were grown in medium containing ( $\text{g l}^{-1}$ ): NaCl, 200;  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ , 20; KCl, 0.1;  $\text{KH}_2\text{PO}_4$ , 0.3; yeast extract, 0.5; Bacto-peptone, 0.5; casamino acids, 0.5; glucose, 0.5; starch, 0.5; and Na-pyruvate, 0.3. The pH was adjusted to 7.2 with NaOH prior to autoclaving, and readjusted with sterile NaOH after autoclaving.

The following strains of Archaea were included as reference strains for pigment and polar lipid studies: *Halobacterium salinarum* R1, *Haloarcula vallismortis* ATCC 29715<sup>T</sup>, *Haloferax volcanii* ATCC 29605<sup>T</sup>, *Haloferax gibbonsii* ATCC 33959<sup>T</sup>, *Halorubrum saccharovororum* ATCC 29252<sup>T</sup>, and *Natrialba asiatica* JCM 9576<sup>T</sup>. *Hbt. salinarum* was grown in medium containing (all concentrations in  $\text{g l}^{-1}$ ): NaCl, 250; KCl, 5;  $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ , 5;  $\text{NH}_4\text{Cl}$ , 5; yeast extract, 5; pH 7. the medium for *Hfx. volcanii*, *Hfx. gibbonsii* and *Hrb. saccharovororum* contained: NaCl, 175;  $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ , 20;  $\text{K}_2\text{SO}_4$ , 5;  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ , 0.1, yeast extract, 5; pH 7. *Har. vallismortis* was grown in medium composed of NaCl, 206;  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ , 36; KCl, 0.37;  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ , 5;  $\text{MnCl}_2$ , 0.013, and yeast extract, 5, pH 7. The medium for *Nab. asiatica* contained NaCl, 200;

Table 1  
Crystallizer brine samples analyzed in the present study

Sampling site	Sampling time	Salt concentration ( $\text{g l}^{-1}$ )	Prokaryotes (cells $\text{ml}^{-1}$ )	<i>Dunaliella</i> $\text{ml}^{-1}$
Santa Pola, Spain	May 1999	370	ND	$4.5\times 10^3$
Santa Pola, Spain	March 2000	ND	ND	ND
Mallorca, Spain	September 2000	ND	ND	ND
Newark, CA, USA	February 1997	244	$1.5\times 10^8$	$8.9\times 10^3$
Eilat, Israel	April 2000	360	$2\times 10^7$	280
Eilat, Israel	September 200	350	$4.2\times 10^7$	850

ND = not determined.

MgSO<sub>4</sub>·7H<sub>2</sub>O, 20; KCl, 2; tri-Na-citrate, 3; casamino acids, 7.5; yeast extract, 0.5, and FeSO<sub>4</sub>·7H<sub>2</sub>O, 2.3 mg, pH 7. All cultures were grown at 35°C with shaking (200 rpm) in 250-ml Erlenmeyer flasks containing 100 ml of growth medium.

### 2.3. Extraction and characterization of pigments

Biomass from saltern pond samples (2–6 l) was collected by centrifugation (30 min, 5000×g in a Sorvall GS-3 rotor). Samples were preserved when necessary by lyophilization, and/or stored in the dark at –20°C. Reference cultures (20 ml) were centrifuged for 10 min at 12000×g. Cell pellets were extracted in 1 ml methanol–acetone 1:1 (v/v) for 4 h. Absorption spectra were recorded against the solvent in a Hewlett-Packard model 8452A diode array spectrophotometer.

Pigments were separated by means of high performance liquid chromatography, using a Merck-Hitachi HPLC set-up, including pump L-6200A and UV-visible detector L-4200. Pigment extracts were injected through a 100-μl sample loop into a LiChrospher 100 RP-18 column (5 μm, 250×4 mm, Merck) and were eluted at a rate of 1 ml min<sup>–1</sup> by a gradient of acetone and water (from 70–30% to 85–15% in 10 min, from 85% to 100% acetone in the next 5 min, followed by 6 min 100% acetone). The elution of pigments was monitored at 450 nm (a wavelength selected as it enables detection of both carotenoids and chlorophylls), and the eluted peaks were characterized using a Chrom-A-Scope and Chrom-A-Set 500 (Bar-Spec, Rehovot, Israel) spectrum analyzer.

### 2.4. Polar lipid chromatography

Cell pellets of biomass collected from 1–4 l of saltern brines or from 10–30 ml of reference cultures were suspended in 1 ml distilled water and extracted with 3.75 ml methanol–chloroform 2:1 (v/v) for 4 h. The extract was collected by centrifugation, and the pellet reextracted with 4.75 ml methanol–chloroform–water (2:1:0.8, v/v). Chloroform and water (2.5 ml each) were added to the combined supernatants to achieve phase separation, and after centrifugation the chloroform phase was collected and dried in a vacuum desiccator.

Lipids were redissolved in a small volume of chloroform, applied to silica gel plates (Aldrich, 20×20 cm), and separated by single development with chloroform–methanol–acetic acid–water (85:22.5:10:4, v/v) [8]. Lipid spots on the plates were detected by spraying the following reagents: (1) orcinol–ferric chloride spray reagent (Sigma) followed by heating the plates at 150°C (visualizing all lipids while enabling differential detection of glycolipids); (2) molybdenum blue spray reagent (Sigma) for the detection of phospholipids; (3) ninhydrin spray reagent (Sigma), followed by visualization of the spots by heating at 110°C, enabling detection of free amino groups; and (4)

Dragendorff's spray reagent (Sigma) to detect phosphatidylcholine.

## 3. Results

### 3.1. Absorption spectra of extracts of biomass collected from brine samples

Carotenoids dominated the absorption spectra of all crystallizer brines examined (Fig. 1C–F). The shape of the spectra varied, mainly as a result of variations in the amount of β-carotene present in the extracts. The main contributor of β-carotene to the biota in the ponds is the green alga *Dunaliella*. Large, brightly red-orange *Dunaliella* cells were found in all samples examined. The extent to which β-carotene dominates the spectrum depends on the number of *Dunaliella* cells present in the brine and their β-carotene content. β-Carotene dominated the absorption spectra of biomass collected from Santa Pola in May 1999 (Fig. 1C) and from Newark in February 1997

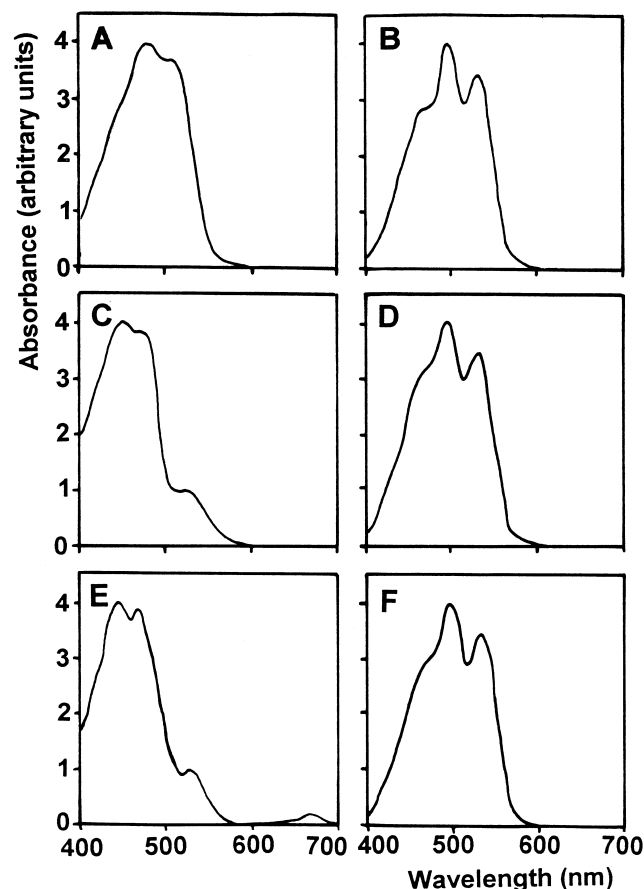


Fig. 1. Absorption spectra of biomass collected from saltern crystallizer ponds in Santa Pola, Alicante (May 1999 and March 2000, C and D, respectively), Newark, CA (February 1997, E), and Eilat (April 2000, F). For comparison the spectra of *S. ruber* Pola-18 (A) and of strain *Har. vallismortis* (B) are shown. All cells were collected by centrifugation and extracted in methanol–acetone (1:1, v/v).

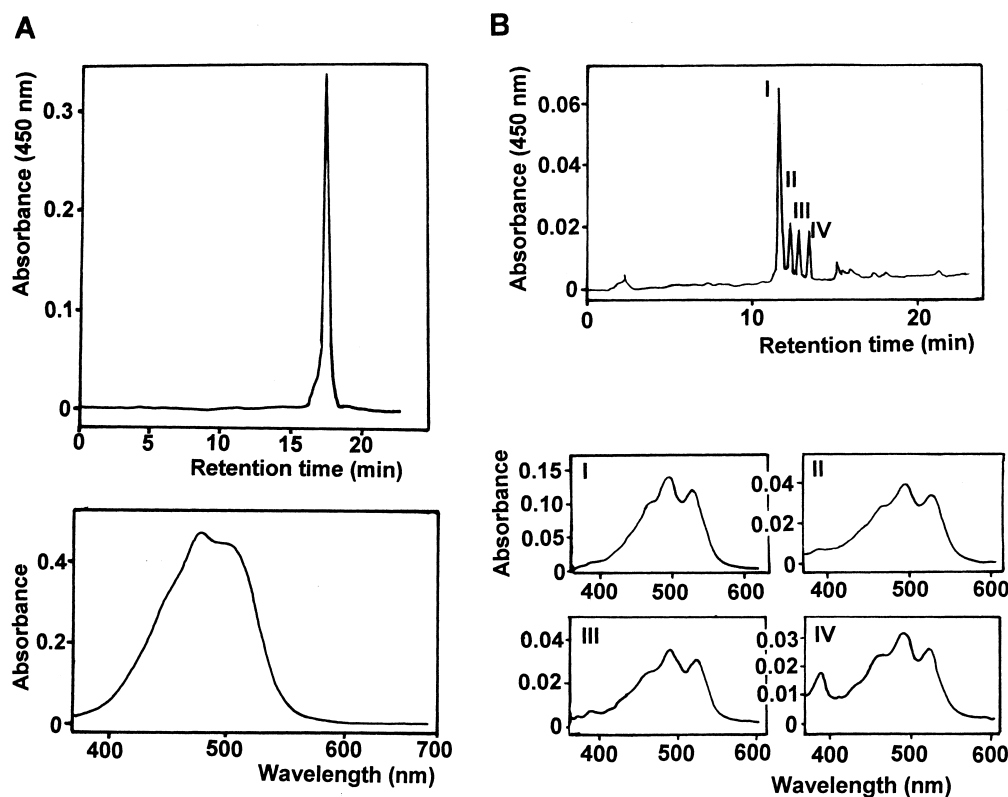


Fig. 2. HPLC separation and characterization of carotenoids extracted from *S. ruber* strain Pola-18 (A) and from *Har. vallismortis* (B). The figure presents the elution patterns as measured at 450 nm and absorption spectra of the separated pigments.

(Fig. 1E). The last-mentioned sample was unusual in its low salinity, which was the result of severe rains prior to the sampling date. This sample was also the only one in which significant amounts of chlorophylls were found. More in-depth information on the pigment composition of this sample has been published elsewhere [9]. Little  $\beta$ -carotene was found in the cell pellets obtained from Santa Pola in March 2000 (Fig. 1D) and from Eilat in April 2000 (Fig. 1F); the absorption spectra of their extracts were dominated by archaeal pigments of the bacterioruberin series (compare Fig. 1D,F with Fig. 1B). A similar result was obtained with the September 2000 sample from Mallorca (not shown). It has been documented before that cells with a high  $\beta$ -carotene content tend to float during centrifugation, thereby decreasing the relative contribution of algal pigments in the cell pellet collected [4]. This behavior of the *Dunaliella* cells was beneficial in the present study, as it enabled a specific enrichment of bacterial and archaeal carotenoids in the biomass collected.

The absorption spectrum of the *Salinibacter* pigment(s) (a maximum at 482 nm with a shoulder at about 510 nm) overlaps to a large extent with that of the halophilic Archaea (compare Fig. 1A with Fig. 1B). As a result, the presence of minor amounts of the *Salinibacter* pigments cannot easily be detected by analysis of absorption spectra of cell extracts of the biomass without prior separation of the components.

### 3.2. Pigment separation by HPLC

To quantify the possible contribution of *Salinibacter*-derived pigments to the red coloration of saltern crystallizer brines, we separated and quantified the pigments present by HPLC. All *S. ruber* strains showed a single pigment peak, eluting after  $17.3 \pm 0.2$  min, as shown in Fig. 2A for strain Pola-18. The absorption spectrum and elution properties suggest the presence of a novel C-40 carotenoid (J. Hirschberg, Department of Genetics, The Hebrew University of Jerusalem, personal communication). This chemical nature of this pigment is presently under investigation. The elution time and absorption spectrum of this compound are distinct from those of the C-50 carotenoids of halophilic Archaea ( $\alpha$ -bacterioruberin and derivatives such as mono- and bis-anhydrobacterioruberin [1]), as shown in the elution pattern obtained with an extract of *Har. vallismortis* (Fig. 2B).

The differences in elution time and absorption spectra enabled a qualitative and semi-quantitative estimation of the contribution of the *Salinibacter* pigment to the total pigment content of saltern brine biomass samples. The sample collected from Santa Pola, Spain in March 2000 showed a prominent pigment fraction eluting after 17.3 min, having the characteristic absorption spectrum of the *Salinibacter* pigment (fraction VI, Fig. 3A). In addition, bacterioruberin derivatives (fractions I–IV) and  $\beta$ -carotene

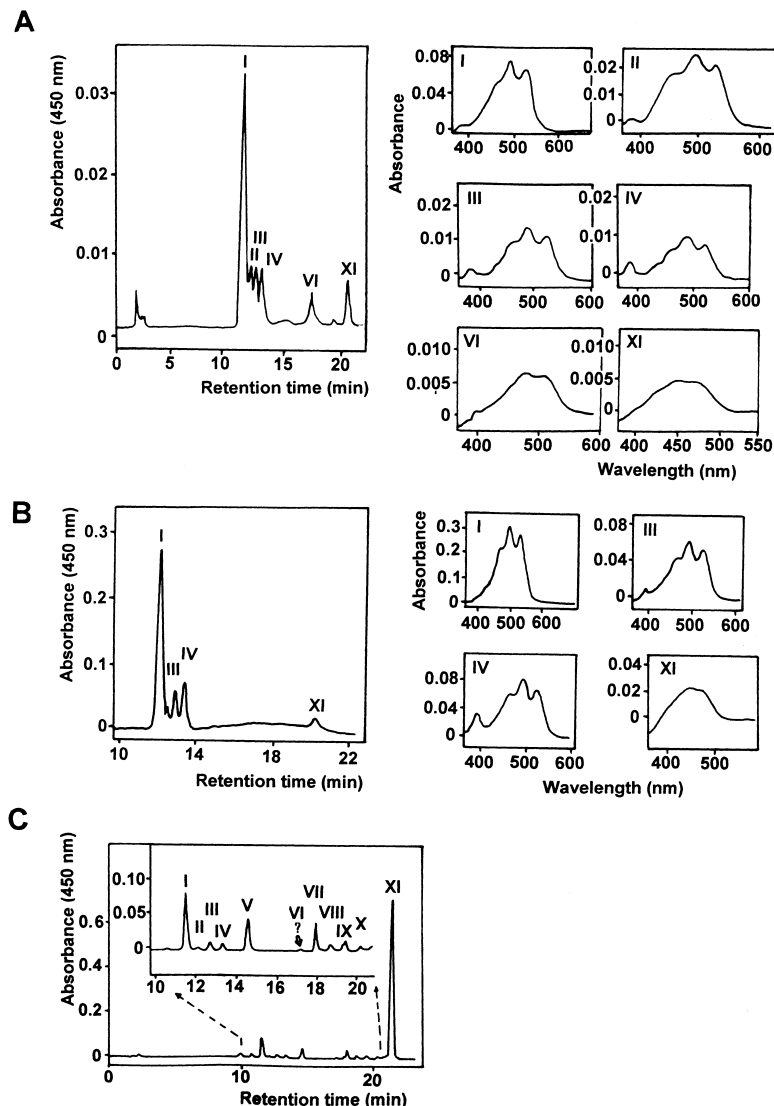


Fig. 3. HPLC separation and characterization of carotenoids and other hydrophobic pigments extracted from saltern crystallizer ponds in Santa Pola, Alicante (March 2000, A), Eilat (April 2000, B), and Newark, CA (February 1997, C). The figure presents the elution patterns as measured at 450 nm and absorption spectra of the separated pigments.

(fraction XI) were detected. From the comparison of the areas of the peaks it was estimated that about 5% of the total prokaryote-derived carotenoids or carotenoid-like pigments could be attributed to *Salinibacter*. In the Mallorca September 2000 sample the contribution of the *Salinibacter* pigment was even 7.5%.

No detectable amounts of *Salinibacter* pigment were found in the brine sample collected from the Eilat saltern in April 2000; the extract of the cell biomass collected by centrifugation only yielded carotenoids of the bacterioruberin series and a small amount of  $\beta$ -carotene (Fig. 3B).

The HPLC elution pattern of the extract of cells obtained from the Newark, CA saltern in February 1997 was more complex (Fig. 3C): in addition to bacterioruberins (fractions I–IV) and massive amounts of  $\beta$ -carotene (fraction XI) we tentatively identified (on the basis of retention time and absorption spectrum) chlorophyll *b* (frac-

tion VII), chlorophyll *a* (fraction VIII), zeaxanthin or lutein (fraction V), and several unidentified – probably algal – carotenoids (fractions IX and X). A small absorption peak was found eluting after 17.3 min (fraction VI). Its size was insufficient to allow spectral analysis, and thus its positive identification as the *Salinibacter* pigment was not feasible.

When attempting to estimate the contribution of red *Salinibacter* cells to the overall number of prokaryotes in the saltern brines on the basis of the HPLC data, it is necessary to obtain information on the amount of pigment per cell. The value of the approach is limited as pigment content per cell within any group may vary greatly with species. Within the family Halobacteriaceae there are strongly pigmented strains, while others – notably members of the genus *Haloferax* – are little pigmented, or may even be colorless, as in the case of *Nab. asiatica* [10]. The

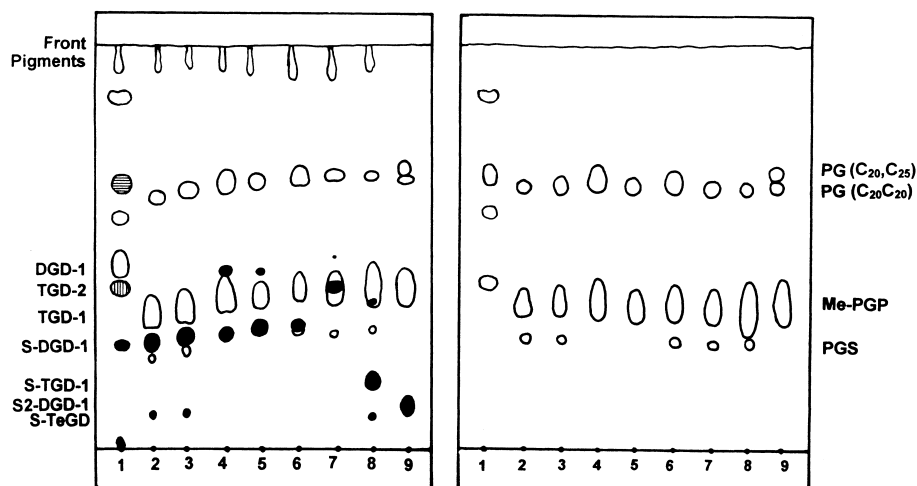


Fig. 4. Thin layer chromatograms of polar lipids extracted from the salterns of Santa Pola, Alicante (March 2000, lane 2) and Eilat (April 2000, lane 3) after staining with orcinol–ferric chloride spray reagent (left panel) or after staining for the presence of phospholipids (right panel). As standards served *S. ruber* strain M31 (lane 1), and the Archaea *Hfx. volcanii* (lane 4), *Hfx. gibbonsii* (lane 5), *Hrb. saccharovorum* (lane 6), *Har. vallismortis* (lane 7), *Hbt. salinarum* R1 (lane 8), and *Nab. asiatica* (lane 9). Glycolipids are indicated as solid black spots in the left panel; spots positive for the ninhydrin stain are hatched horizontally, spots reacting with Dragendorff's reagent vertically. The tentative identification of most archaeal lipid spots detected is indicated. PG = the diphytanyl diether derivative of phosphatidylglycerol; Me-PGP = the methyl ester of the diphytanyl diether derivative of phosphatidylglycerophosphate; PGS = the diphytanyl diether derivative of phosphatidylglycerosulfate; DGD-1, S-DGD-1, S2-DGD-1, TGD-1, S-TGD-1, TGD-2, and S-TeGD refer to different non-sulfated, sulfated, and bis-sulfated diglycosyl, triglycosyl, and tetraglycosyl diphytanyl diether lipids.

amount of pigment per cell may also depend on the nutritional state of the cells. While recognizing the limitations of the approach, we compared the pigment content per cell of *S. ruber* strains Pola-13 and Pola-18 with different representatives of the Halobacteriaceae. Extracts of  $10^9$  *Salinibacter* cells in 1 ml methanol–acetone (1:1) gave peak absorbances of 0.042–0.068 (479 nm). Similar extracts of *Har. vallismortis*, *Hbt. salinarum* R1 and *Hfx. volcanii* yielded values of 0.123, 0.040 and 0.038, respectively (492 nm). Thus, the amount of pigmentation per cell is in the same order of magnitude in the *Salinibacter* isolates and in the aerobic halophilic Archaea.

### 3.3. Polar lipid studies

To examine whether the contribution of Bacteria such as *Salinibacter* to the microbial community in saltern crystallizer ponds can also be assessed according to the presence of specific polar lipids, we separated the lipids extracted from March 2000 Santa Pola sample and the April 2000 Eilat sample by thin layer chromatography (Fig. 4). No detectable amounts of bacterial lipids such as phosphatidylcholine and phosphatidylethanolamine were found. The lipids detected were characteristic of halophilic Archaea of the family Halobacteriaceae, and the patterns found in both salterns were identical. Both salterns showed a single sulfated diglycosyl diether lipid as the major glycolipid; a minor glycolipid spot was found that co-chromatographed with the bis-sulfated diglycosyl diether lipid characteristic of *Nab. asiatica* [10]. In addition, the diether derivatives of phosphatidylglycerol, phosphati-

dylglycerosulfate and the methyl ester of phosphatidylglycerophosphate were found.

### 4. Discussion

There has been a silent consensus that the red color of saltern crystallizer ponds and hypersaline lakes is caused by red halophilic Archaea of the family Halobacteriaceae, whether or not in combination of  $\beta$ -carotene-rich *Dunaliella* cells. Even when *Dunaliella* cells are present in large amounts, and the total amount of  $\beta$ -carotene may exceed that of the archaeal carotenoids, most of the color of the saltern ponds may still be attributed to bacterioruberin pigments. This effect is due to the low in vivo optical cross-section of the  $\beta$ -carotene, which is densely packed in granules in the interthylakoid space within the chloroplast [3,4].

The red color of most members of the Halobacteriaceae has been used as an easily recognizable character to discriminate between archaeal and bacterial members of the prokaryote community. Colony color was used to classify isolates obtained from Spanish salterns into two categories, the red colonies being almost uniquely found at the higher salinity range [11]. It is now becoming clear that colony color itself is not a reliable trait to assess the phylogenetic affiliation of halophilic prokaryotes. Not only have colorless members of the Halobacteriaceae been isolated (e.g. *Nab. asiatica*), but *Salinibacter*, a truly halophilic Bacterium, produces brightly red colonies as well [7].

We found significant amounts of the *Salinibacter* pig-

ment in the biomass collected from the Santa Pola saltern, the same saltern in which fluorescence in situ hybridization has shown the presence of large numbers of rod-shaped cells with the *Salinibacter* phylotype [5]. The identification of this pigment was based on its retention time and its absorption spectrum. However, it should be taken into account that minor amounts of C-40 carotenoids have been reported to occur in members of the Halobacteriaceae [12]. Surprisingly high amounts of C-40 ketocarotenoids such as 3-hydroxyechinenone and *trans*-astaxanthin (up to 45–50% of the total carotenoid content) were reported to occur in *Hbt. salinarum*, *Har. hispanica* and *Hfx. mediterranei*, although a thorough chemical characterization of these carotenoid fractions was not performed [13]. Another, yet to be identified archaeon isolated from a saltern near Alexandria, Egypt, produced large amounts of canthaxanthin (up to 34% of the total carotenoid content), this in addition to bacterioruberins (63%) and  $\beta$ -carotene (3%) [14]. In any case, all the above-mentioned pigments have absorption spectra and retention times distinct from those of the *Salinibacter* pigment.

The *Salinibacter* pigment was estimated to contribute about 5% to the prokaryotic pigments found in the March 2000 Santa Pola sample and 7.5% in the biomass collected in September 2000 from the Mallorca crystallizer. Taking into account that the pigment absorbance per cell of this species was found to be of the same order of magnitude as that of red halophilic Archaea, the numerical contribution of *Salinibacter* may be estimated to be around 5–7.5%, agreeing well with the estimate obtained from fluorescence in situ hybridization (between 5 and 25% of the total prokaryote community) [5]. Unfortunately the dominant phylotype of halophilic Archaea present in saltern crystallizer ponds is still awaiting isolation, and therefore the relevant species could not be included as a standard to calibrate the semi-quantitative assessment of the contributions of the Bacteria and Archaea to the biomass in the ponds. HPLC analysis did not reveal the presence of the *Salinibacter* pigment in the Eilat salterns. However, fluorescence in situ hybridization using *Salinibacter*-specific probes showed its presence, albeit in small numbers (J. Antón, Universidad de Alicante, unpublished results).

Activity studies using different antibiotics and other inhibitors to specifically inhibit Archaea and Bacteria have shown that in the Eilat saltern crystallizer ponds essentially all amino acid uptake activity could be attributed to halophilic Archaea; no significant activity of Bacteria was detected at salt concentrations exceeding 250 g l<sup>-1</sup> [15–17]. Similar studies in the Santa Pola and Mallorca salterns are now to be recommended in view of the finding that there, in contrast to in Eilat, halophilic Bacteria of the *Salinibacter* phylotype may contribute significantly to the prokaryote community. Nothing, however, is known as yet about their activities in situ.

The finding of a single sulfated diglycosyl diether lipid as the major glycolipid in the Eilat sample and the pres-

ence of the diether lipid derivative of phosphatidylglycerol sulfate confirmed earlier observations [18,19]. This polar lipid pattern can probably be attributed to the large amount of the – yet uncultured – square flat halophilic Archaea in this pond [18]. The Santa Pola salterns are also dominated by these square Archaea, as recently shown using fluorescence in situ hybridization [20]. As the same phylotype of Archaea dominates both in Santa Pola and in Eilat [21–23], the similarity in polar lipid pattern did not come as a surprise. The finding of an additional minor glycolipid spot, co-chromatographing with the bis-sulfated diglycosyl diether lipid characteristic of *Nab. asiatica* [10], was new, as such a spot has not been detected before in the Eilat salterns. What component of the biomass of the salterns contributed this glycolipid, and why this component had been absent in the past, remains to be determined.

A final conclusion to be drawn from the data presented is that saltern crystallizer ponds at different geographical locations, although superficially similar in biological properties, may show significant differences in the structure of the microbial communities inhabiting them. This point has been stressed previously [9]. There may, of course, also be seasonal differences in the distribution of halophilic Bacteria and Archaea. The apparent differences in the contribution of the *Salinibacter* type of Bacteria to the prokaryotic community in the salterns at Santa Pola, Mallorca, Eilat, and Newark once more demonstrate that comparative studies of salterns in different locations may yield interesting results, thereby deepening our understanding of the diversity of life at high salt concentrations.

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