Extremophiles (2000) 4:259–265

ORIGINAL PAPER

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Comparisons of the polar lipid and pigment profiles of two solar salterns located in Newark, California, U.S.A., and Eilat, Israel

Received: December 10, 1999 / Accepted: April 6, 2000

Abstract The whole community pigments and lipids have been examined during a 5-year period in two commercial solar salterns located in the United States and in Israel. There were significant differences in the complexity of the lipid and pigment patterns within the California saltern system, and these differences were not consistent over the sampling period despite examination of ponds with the same salinity. The solar saltern system in Eilat, Israel, showed greater consistency during this sampling period and compared directly with previous studies. The complexity of the saltern in Newark, California, could be explained on the basis of the prevailing weather conditions (cooler and more rainfall) and the nutrient-enriched source water. The Eilat saltern, however, has an oligotrophic water source and has a considerably warmer and drier climate. This difference resulted in more diverse and more complex pigment and lipid patterns and presumably microbial populations in the Newark, California, plant than in the saltern in Eilat, Israel.

Key words Solar salterns · Halophilic Archaea · Halophilic Bacteria

Communicated by K. Horikoshi

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Introduction

Solar salterns for the commercial production of NaCl consist of a series of ponds through which seawater flows, becoming progressively more concentrated with respect to total salts. At approximately 8% NaCl, gypsum and other calcium salts begin to precipitate. Then, when the NaCl concentration has reached approximately 32%–35% in the pans known as crystallizers, the precipitation of NaCl occurs. Once all the NaCl has precipitated, the mother liquor is removed from the pans and the salt harvested. In addition, there are salt-making salterns for which soda-based brines provide the feedwater. These alkaliphilic salterns contain quite different and distinctive biological communities from those considered in this article.

The NaCl-dominated saltern ponds are inhabited by a rich variety of microorganisms, and each pond contains a characteristic flora adapted to the prevailing salt concentration, from seawater to NaCl saturation. Microorganisms in the brines include both unicellular green algae, especially *Dunaliella* sp. at the higher salinities, a variety of Bacteria, especially *Halomonas* sp. (Vreeland et al. 1980), and at the highest salinities, dense communities of halophilic Archaea of the family *Halobacteriaceae*. These latter organisms often impart a bright red color to the brine, whereas *Dunaliella* sp. often imparts an orange color. In addition, brine shrimp are found at the intermediate salinities. Because this process for salt manufacturing is well established worldwide, it has been assumed that there are very minor, if any, differences in the microbial populations in solar salterns.

Although reports on the biology of saltern systems in various parts of the world suggest a high degree of similarity in the microbiology (Oren 1993), some differences must occur as the result of changes in incident radiation, temperature, nutrient availability, residence time in the ponds, etc. Saltern pond systems at different geographic locations often differ in the microbial community densities encountered, probably because of variability in the levels of inorganic nutrients (Javor 1983a,b). However, no

comparative data on the diversity of the microbial community structure of geographically separated saltern systems have as yet been reported. Therefore, we have undertaken a synoptic investigation into the variability within each saltern system as well as the variability between two salterns located in different parts of the world: the Cargill Solar Salt Plant, Newark, California, USA, and the Israel Salt Co. in Eilat, Israel. This article reports some of the results of that investigation. Specifically, we are reporting here a comparison between the two salterns with respect to the temporal variability of the whole community lipids and pigments.

Materials and methods

Sampling and recovery of whole community biomass

The sampling locations were selected based on prior work by Litchfield et al. (1999) and were designed to encompass major transition zones in the ecology of solar salterns. Consequently, the inlet areas, concentrators containing 6%–8%, 12%–15%, 20%–25% salt, and the crystallizers were generally sampled. In addition, the high-salinity ponds at locations 10A and 26 were routinely included at the Cargill plant. Sampling dates and locations are listed in Table 1, and the sites within the two solar salt facilities are shown in Fig. 1. The specific gravity of each sample was measured in the laboratory using specific gravity hydrometers, and the values were converted to salinity according to Garrigues (1881).

Three to four liters of aseptically collected water were centrifuged in an RC 5B (Sorvall DuPont) centrifuge at 11 000 rpm and 4° or 20°C. The resulting pellets were collected and frozen until analyzed.

Lipid extractions and analyses

Aliquots of the concentrated whole community samples were subjected to a modified Bligh and Dyer extraction procedure (Kates 1972). The procedure was further modified to permit extraction in polypropylene centrifuge tubes. The pellets were thawed, slurried in double-distilled water with no phosphate added, and extracted first with chloroform:methanol (1:2 v:v), followed by at least three extractions with chloroform:methanol (2:1 v:v). All extracts were evaporated to dryness using a SpeedVac AES 2000 (Savant) at low temperature with cryogenic pumping to recover the chloroform. Dried samples were transferred in chloroform to vials that were stored at −70°C until analyzed.

Thin-layer chromatography was performed using a chloroform:methanol:acetic acid:water mixture (85:22.5:10:4, v:v:v:v) in lined tanks at George Mason University and unlined tanks at the Hebrew University of Jerusalem. Comparisons of the lipid patterns indicated there was no significant difference in the separation due to this minor difference in techniques. Redicoat, 19-channel silica gel-G plates from Fisher or Whatman were used at George Mason University, and Sigma-Aldrich Silica gel plates were used at the Hebrew University of Jerusalem.

Polar lipids were detected using the spray reagents described by Krebs et al. (1969): Dragendorff's for choline; ninhydrin for tertiary nitrogen-containing compounds; orcinol or α-naphthol for sugars; ammonium molybdate for phosphate; and sulfuric acid:water for organic compounds. The ammonium molybdate and orcinol reagents were purchased from Sigma; all other reagents were prepared in the laboratory using the highest grade of chemicals

Table 1. Dates and sampling data for Newark, California, and Eilat, Israel

Location	Sample designation	Date sampled	Density	Percent salt
Newark, California	SF-1-Inlet	28 Dec 93	NT	NT
	SF-1-6		1.049	7.1
	SF-1-12		1.103	14.0
	SF-1-22		1.192	24.2
	SF-1-C		1.224	27.7
	SF-2-Inlet	25 Apr 95	1.024	3.5
	SF-2-9		1.052	7.5
	SF-2-8A		1.072	10.1
	SF-2-7		1.207	25.8
	SF-2-18		1.223	27.6
	SF-3-13	6 Feb 97	1.012	1.8
	SF-3-Inlet		1.018	2.8
	SF-3-8A		1.066	9.5
	SF-3-11		1.082	11.4
	SF-3-10A		1.164	21.5
	SF-3-26		1.163	21.1
	SF-3-2		NT	NT
	SF-3-8		NT	NT
	SF-4-Inlet	15 Dec 97	1.021	3.1
	SF-4-12		1.081	11.3
	SF-4-8A		1.093	12.9
	SF-4-11		1.109	14.8
	SF-4-26		1.214	26.6
	SF-4-10A		NT	NT
	SF-4-18		NT	NT
	SF-5-12	23 June 98	1.049	7.1
	SF-5-8		1.085	11.8
	SF-5-8A		1.098	13.5
	SF-5-26		1.176	22.5
	SF-5-10A		1.223	27.6
	SF-5-11		1.233	28.7
Eilat, Israel	E-1-305	16 Aug 96	1.149	19.5
	E-1-302		1.238	29.2
	E-1-100	17 Aug 96	1.026	3.9
	E-1-200		1.084	11.7
	E-1-300		1.187	23.7
	E-2-100	23 Jan 97	1.045	6.4
	E-2-104		1.080	11.2
	E-2-202		1.110	14.9
	E-2-203		1.190	24.0
	E-2-302		1.241	29.5
	E-3-100	11 Aug 97	1.026	3.9
	E-3-200		1.100	13.6
	E-3-201		1.130	17.4
	E-3-203		1.170	21.8
	E-3-304		1.241	29.5
	E-4-100	16 Feb 98	1.026	3.9
	E-4-200		1.110	14.9
	E-4-202		1.160	20.8
	E-4-304		1.226	27.9

NT, not tested

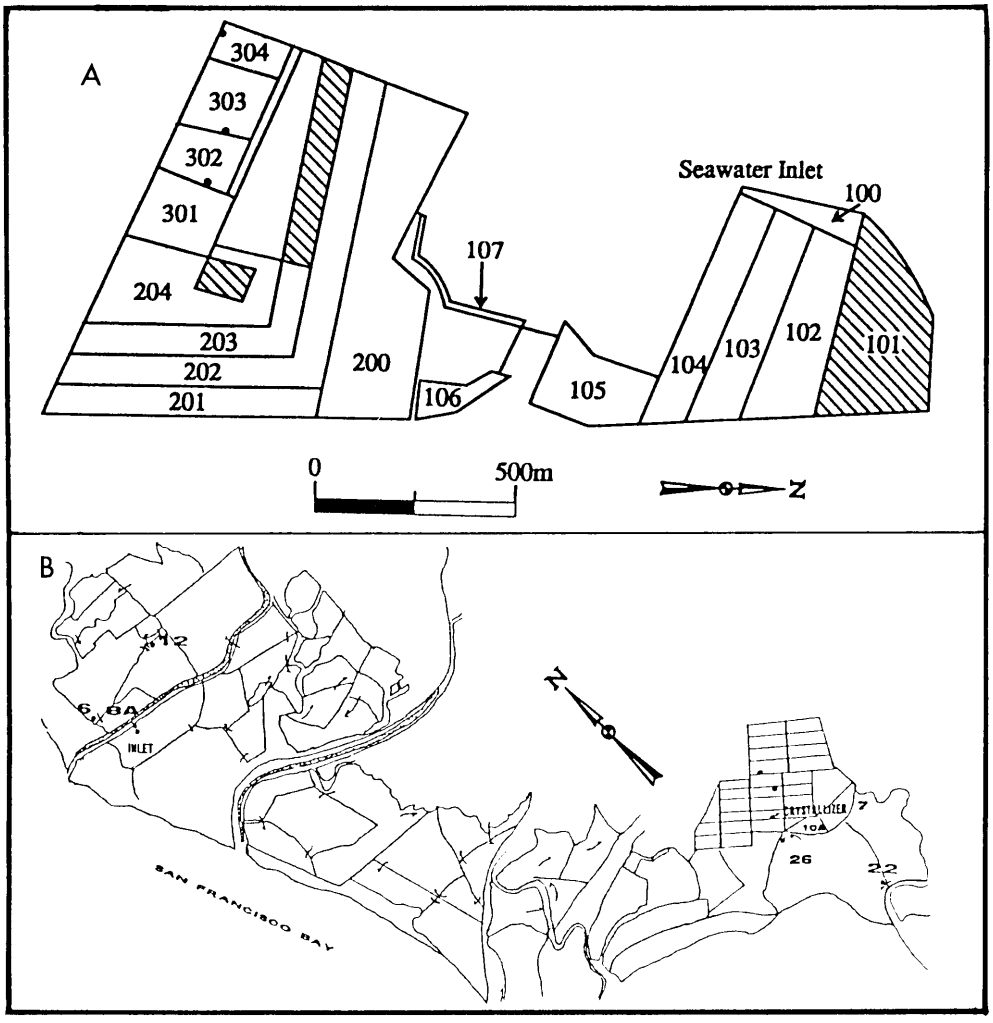

Fig. 1. Location of sampling points in the Eilat, Israel, solar saltern (*upper panel*) and in the Cargill Solar Salt Plant (*lower panel*)

available. Comparisons of the resulting R_f values were made with pure cultures of known halophilic Archaea: *Halobacterium salinarum* NRC 817, *Haloferax mediterranei* ATCC 33500^T, *Haloarcula vallismortis* ATCC 29715^T, and *Natrialba asiatica* JCM 9576 (Kamekura and Dyall-Smith 1995; Kushwaha et al. 1982; Oren 1994; Oren et al. 1996; Torreblanca et al. 1986). Egg-yolk phospholipids and occasionally lipid extracts of *Halomonas elongata* or *Pseudomonas fluorescens* were used to identify bacterial polar lipids. Additionally, commercial standards of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, and cerebroside sulfate (all purchased from Sigma) were also used to identify the lipid spots.

Glassware and plasticware were prewashed in Microclean II if previously used and rinsed three times in chloroform, methanol, and finally acetone.

Pigment extractions

Pigments were extracted using the procedure of Oren (1994) and Oren and coworkers (1992). The absorption spectra of the acetone:methanol extracts were read immediately in either a Beckman DU-6000 or a Hewlett-Packard model 8452A diode array spectrophotometer. HPLC, used to separate the various pigment fractions, was operated as described in Oren and Gurevich (1995). Briefly, it included an elution rate of 1 ml min^{-1} using an acetone:H_2O gradient of 70%–30% and 85%–15% in 10 min, followed by 85%–100% acetone for 5 min, and completed by 6 min in 100% acetone. Spectra were analyzed using a Chrom-A-Scope and Chrom-A-Set 500 spectral analyzer (Bar-Spec, Rehovot, Israel).

Results

Whole community pigment analyses

Typical spectral scans for the whole community pigment composition are shown in Fig. 2A,B for the Eilat, Israel, and Newark, California, plants, respectively. From these scans there are apparent differences in the spectral patterns at each location, not only within each series of salterns but also

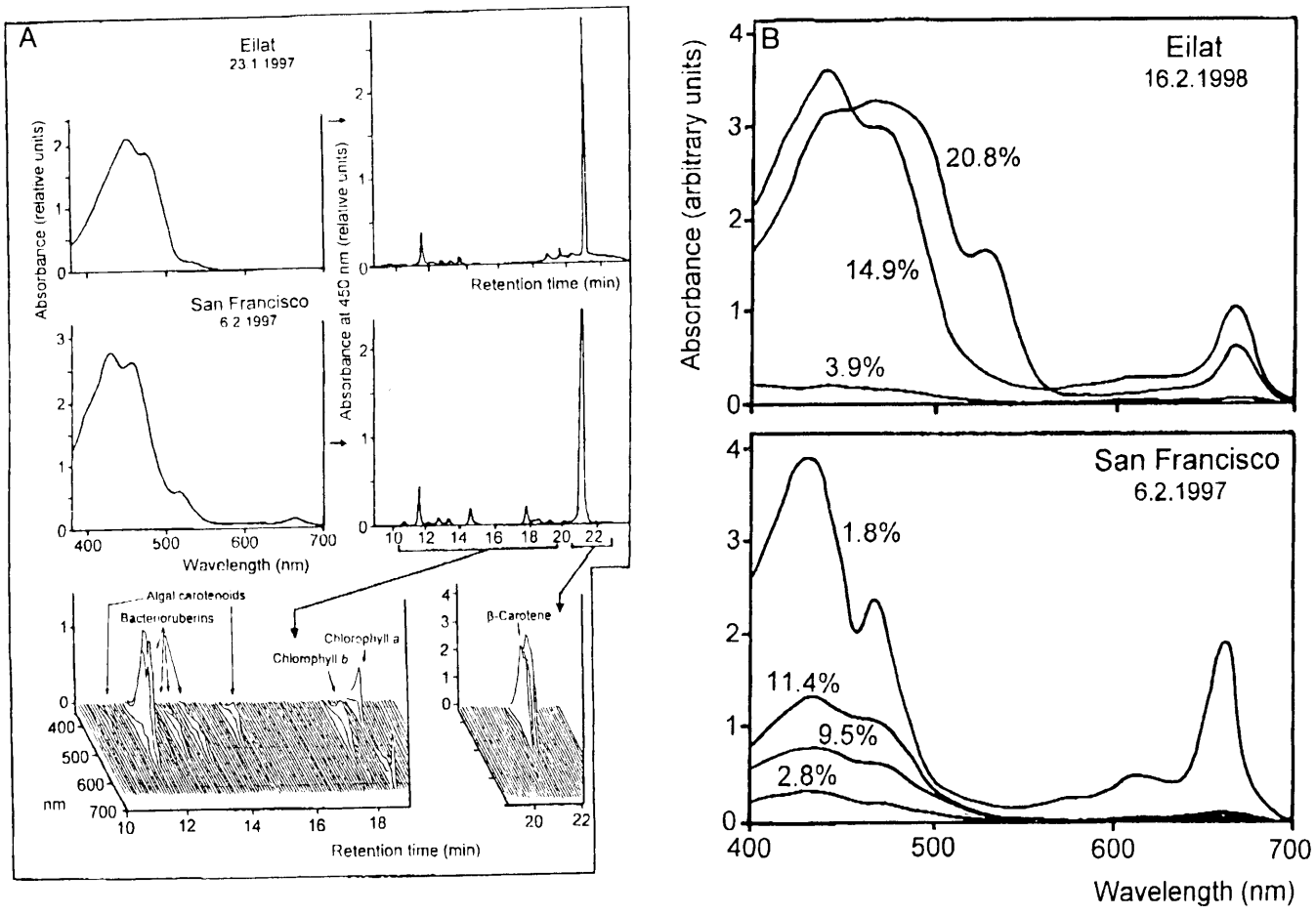

Fig. 2. **A** Analysis of the pigments in the biota of the crystallizer ponds of the Eilat, Israel, saltern (January 23, 1997; *upper panel*) and Cargill Solar Salt Plant (February 6, 1997; *lower panel*). Cells were collected by filtration on glass fiber filters (Whatman GF/C), extracted with methanol:acetone (1:1, v:v), and the absorption spectra were recorded. The pigments were then separated by HPLC and identified according to their retention time and absorption spectra. **B** Semiquantitative absorption spectra of the biota collected from saltern ponds in Eilat (February 16, 1998; *upper panel*) and Cargill Solar Salt Plant (February 6, 1997; *lower panel*). Cells from ponds of different salinities as indicated were collected by centrifugation, extracted with methanol:acetone (1:1, v:v), and the absorption spectra recorded

between the salterns. Although these figures show only one sampling period, they are typical of the scans obtained at the other sampling periods (data not shown).

Although similar volumes were concentrated for each sample, the analyses are semiquantitative because of the differences in biomass recovered from the two salterns. However, there was noticeably more pigment extracted in the same volume of sample from the Cargill works than from the Eilat facility. Pigment levels in the extracts from Cargill were typically higher than those obtained from Eilat saltern ponds of comparable salinity; this appeared to be true throughout the salterns and not just at the inlet or crystallizers. Notable seasonal differences in pigment content were observed in the Cargill salterns. In 1995, there were no definable maxima in either the inlet or concentrator 8A extracts. However, both of these had complex pigment patterns in both the 1997 samplings along with greater numbers of peaks in all the 1997 samples.

HPLC analysis of pigment extracts from the crystallizers and saturated feed brine (Fig. 2A) showed the presence of chlorophylls *a* and *b*, β-carotene, bacterioruberins, and additional carotenoids in both saltern systems. The maximum at 667 nm in the spectral scans (Fig. 2A) is due to chlorophyll *a* and chlorophyll *b* present in *Dunaliella* sp., which was observed microscopically to occur at both locations. The spectra of pigment extracts of the biota collected from the crystallizers were dominated by a combination of *Dunaliella*-derived β-carotene (absorption maximum around 450 nm) and C-50 bacterioruberins from the halophilic Archaea (maximum absorbance at 495 nm with a minor peak around 530 nm) (Oren and Dubinsky 1994; Oren et al. 1992). The β-carotene content of the red-orange *Dunaliella* cells found in Eilat was much higher than that of the *Dunaliella* found in the crystallizers at Cargill. Because of the tendency of β-carotene-loaded *Dunaliella* cells to float upon centrifugation, the absorption spectra of the biomass collected from the Eilat crystallizers by centrifugation was significantly different from that collected by filtration (Oren and Dubinsky 1994). No such differences were observed with the Cargill samples (data not shown).

Whole community polar lipid analyses

Representative examples of the polar lipid compositions of the whole communities at the two sites are shown in Fig. 3A–C. Insufficient amounts of lipid material were recovered from the low-salinity ponds at Eilat to permit analysis. At salinities exceeding 14%–15% in the Eilat saltern, a simple pattern was obtained dominated by archaeal lipids: the diphytanyl diether derivatives of diether analogues of phosphatidyl glycerol (PG), the methyl ester of phosphatidyl glycerophosphate (Me-PGP), phosphatidyl glycerosulfate (PGS), and a single glycolipid, chromatographically identical with S-DGD-1, the sulfated diglycosyl diether lipid characteristic of the genera *Haloferax*, *Halobaculum*, and *Halococcus* (Fig. 3A). The pattern obtained was relatively constant during the years of the study, in both the summer and winter samples.

The Newark samples showed a much greater variability in types of polar lipids encountered, both as a function of salinity and with time. Figure 3B is a composite of the polar lipids found in the lower-salinity pans. The most common lipids throughout the system were the unidentified glycolipids and other lipids that were positive for the phosphate test or appeared with sulfuric acid charring. The most noticeable characteristic of these lower-salinity pans, however, is the marked increase in the numbers of lipids found after 1995. The drought ended in the early spring of 1994, and normal to slightly above normal rainfall has been occurring since then, resulting in increased nutrient levels entering the pans. Thus, the culturable microbial populations have been denser; more polar lipids were recovered in 1997 and 1998, and the patterns were more similar than those encountered before May 1995.

In the pans containing greater than 15% salts, both PGP and PG tended to be present regardless of the time of year or when sampled (Fig. 3C). In addition, PGS and S-DGD-1 were commonly present although in a few instances the two compounds could not be resolved (pans 10A and 26 in February 1997). Also, the crystallizers and pans 10A and 26 typically contained PG along with an array of unidentified glycolipids. As in the lower-density pans, there was a large number of spots that only reacted with the phosphate or sulfuric acid sprays. Cochromatography with known standards also did not resolve the identity of these spots. Pans 10A and 26 were more similar to each other than any of the other pans, undoubtedly because they were interchangeably used as brine for washing the harvested salt or contained the bitterns from the crystallizers. Overall, however, the patterns are considerably more complex in the Cargill saltern than in the Eilat salt works.

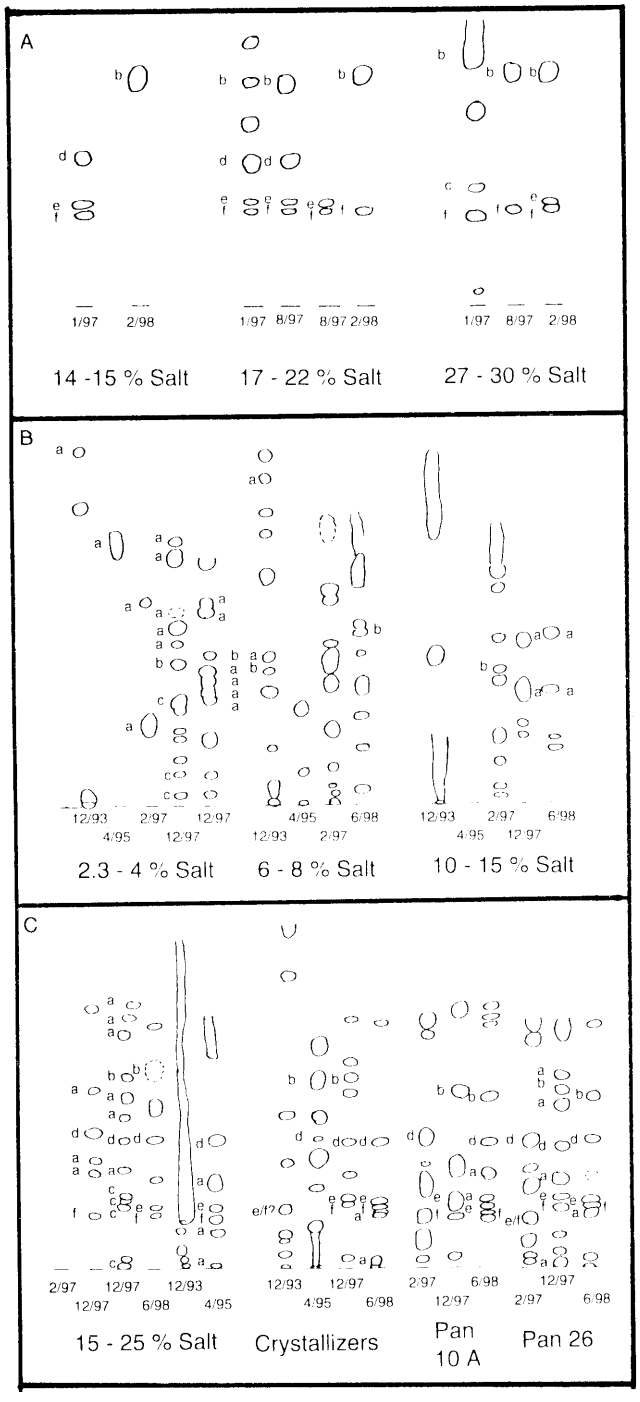

Fig. 3. **A** Polar lipid patterns of the biota from the different salinity ponds in the Eilat, Israel, solar saltern. **B** Polar lipid patterns of the biota from the low-salinity ponds at the Cargill Solar Salt Plant. **C** Polar lipid patterns of the biota from the higher salinity ponds at the Cargill Solar Salt Plant. In all cases, the cells were collected by centrifugation; the lipids were extracted and separated by TLC, and identified by their reaction to specific reagents, relative migration distances, and known standards. *a*, Orcinol positive; *b*, phosphatidyl glycerol; *c*, ninhydrin positive; *d*, methyl phosphatidyl glycerophosphate; *e*, phosphatidyl glycerosulfate; *f*, sulfated diglycosyl diether lipid

Discussion

Saltern pond systems may differ greatly in their biological properties. A comparison of the nutrient-rich Western Salt Co. (Chula Vista, California, USA) and the nutrient-poor Exportadora de Sal (Guerrero Negro, Baja California,

Table 2. A comparison of the lipid characteristics of the two salterns

Characteristic	Newark, California	Eilat, Israel
Seasonal differences	Yes	Little
Chlorophyll content	High	Low
Presence of *Artemia* in higher salt concentrations	Yes	Low
β-Carotene	High	High
Bacterioruberin in crystallizers	High	Low
Polar lipids in crystallizers:		
Phosphatidylglycerol	Yes	Yes
Methyl-phosphatidylglycerolphosphate	Yes	Yes
Phosphatidylglycerosulfate	Yes	Yes
Sulfated glycosyl-diphytanyl ether-1	Yes	Yes
Additional sulfated diglycosyl diphytanyl ether	Yes	No

Mexico) showed great differences in total biomass in the ponds (Javor 1983b). Beyond such obvious quantitative differences, which are to be expected when nutrient levels determine the primary productivity rates and the extent of the development of a heterotrophic bacterial community, solar saltern systems worldwide were generally assumed to support similar microbial communities. The findings presented in this study show that not only do the microbial communities encountered differ as a function of the salt concentration, as monitored by their lipid patterns, but geographic differences may also occur. A comparison between the crystallizer samples from California and from Israel (Table 2) shows that the whole communities of the two solar salterns are indeed different. Although there are similarities in the salt content of the various pans, there are significant differences in the types of lipids found, the chlorophyll content, and the bacterioruberin content, and hence in the microbial community.

Part of this difference no doubt is due to the seasonal fluctuations that occur at the California plant, where not only are the temperatures milder than in Israel, but the rainfall is significantly greater and the source of the water for the Newark plant is San Francisco Bay. The main freshwater flow into the Bay is from the Sacramento River and the San Joaquin River (Conomos et al. 1985), along with runoff from surrounding communities and industries. This pattern undoubtedly results in a higher organic loading than found in Eilat, where the main water source for the saltern is oligotrophic Gulf of Aqaba water. Recently, after the 1997 sampling program, the influent water has been mixed with brine effluent from the reverse osmosis plant.

Whole community lipid analysis has been widely used in the characterization of the microbial communities in sediments (White et al. 1979a, 1993; White 1983), soils (Kieft et al. 1997; White et al. 1983), and detritus (White et al. 1979b), but there have been very few studies examining the pigments and lipids of hypersaline waters with the goal of characterizing the whole microbial community. Volkman et al. (1988) reported the pigment and lipid composition of a saline lake in Antarctica and found a mixture of bacterial and phytoplankton pigments, carotenoids, and an array of polar lipids, sterols, alcohols, free fatty acids, triglycerides, wax esters, ketones, and esters. The ratios of the various lipid fractions to total lipids varied with depth, and the sediments contained the least amounts of total lipids as well as components of the various fractions.

Most of the studies referenced here have used identification and quantitation of fatty acid methyl esters derived from the ester-based lipids to describe the microbial community or its metabolic state. However, the polar lipids of the halophilic Archaea do not have fatty acids, but instead contain diphytanyl ether linkages to the glycerol backbone with various types of phosphate, sulfate, or glycosyl substituents on the third carbon of the glycerol (Kamekura 1993; Kates 1996). The polar lipid composition differs in the various genera of the *Halobacteriales* and has been used to identify the genera (Kamekura 1993; Tindall 1992; Torreblanca et al. 1986). Thus, the approach of using fatty acids methyl esters to characterize the microbial community is not appropriate in hypersaline environments, and polar lipid profiles provide a better indicator of the composition of the whole microbial community. It should be noted that PG and PGP are both found in Bacteria and halophilic Archaea; the former with fatty acids and the latter with phytanyl ether side chains. The dominance of the halophilic Archaea in the higher salinity pans indicates that these two polar lipids are most likely of an archaeal origin rather than a bacterial origin.

Although there have been many studies on the lipids of individual pure cultures of organisms from salterns reported (Kamekura 1993; Tindall 1992; Torreblanca et al. 1986), to date there have been only a few reports that characterized the whole microbial community in a hypersaline environment using polar lipid analysis. Oren and Gurevich (1993) examined the polar lipids occurring in the 1992–1993 halophilic archaeal bloom in the Dead Sea (Oren and Gurevich 1995). They demonstrated that only one major polar lipid was present, a sulfated diglycosyl diphytanyl ether, which is typical of members of the genus *Haloferax*. However, they were not able to culture the organism at that time. Later studies suggested that the dominant archaeon may have been a *Halobaculum* sp. rather than a *Haloferax*; both genera share the same glycolipid (Oren et al. 1995). Earlier studies of Eilat crystallizer ponds (Oren 1994; Oren et al. 1996) yielded a simple pattern with a similar single sulfated diglycosyl diphytanyl ether, accompanied by the diphytanyl diether analogues of PG, Me-PGP, and PGS. This lipid pattern may be attributable to the presence of large numbers of flat-square bacteria (Oren et al. 1996), a type of organism that is yet awaiting isolation. The same lipid pattern was again found in the Eilat crystallizers during the present study (see Fig. 3), showing once more that in Eilat conditions varied little with time.

Although many similarities were encountered in the nature of the lipids and pigments for the biota of the California and Israel salterns, significant differences were also found. The biodiversity in the California salterns is greater than in the Eilat salterns, as shown here and as reflected in the numbers and types of culturable bacteria

(data not presented). When this is coupled with the seasonal differences noted for California, there is a marked temporal effect on the total population and the types of organisms encountered in the California saltern. Because the California saltern is located in a cooler climate, there is a longer retention time of the water in the various pans. With the nutrient-enriched source water, this combination permits the development of a larger microbial population and, at least in the lower-density pans, there is a diverse natural inoculum. On the other hand, Eilat, Israel, is much warmer, and the saltern water has a much shorter retention time. Hence, there is less time for community development, especially as salt harvesting is a year-round activity whereas it occurs only in the summer in California. Therefore, even at the same salt concentrations, different populations of microorganisms have developed in these two saltern systems.

Acknowledgments The authors thank the personnel at the Cargill Solar Salt Plant in Newark, California, USA, and the personnel at the Israel Salt Company in Eilat, Israel, for their assistance in this research. We are also appreciative of the efforts of G. Dimitrov, S. Vinayak, and N. Koval for their assistance in various aspects of this study. Portions of this work were supported by grant No. 95-00027 from the United States–Israel Binational Science Foundation (BSF, Jersualem) and the Halophile Fund of George Mason University Research Foundation.

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