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Production of D-lactate, acetate, and pyruvate from glycerol in communities of halophilic archaea in the Dead Sea and in saltern crystallizer ponds

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Abstract: When glycerol is added to cultures of halophilic archaea, especially representatives of the genera *Haloferax* and *Haloarcula*, massive amounts of acids are formed. HPLC and enzymatic analyses of supernatants of *Haloferax* cultures grown in the presence of glycerol showed that all produced D-lactate and acetate. Cultures of two *Haloarcula* species tested produced pyruvate and acetate from glycerol. In all cases only a small fraction of the added glycerol was converted to organic acids. Both lactate, pyruvate, and acetate can be used as substrates for the growth of many halophilic archaea, including those that produce them, and acid production is possibly an overflow phenomenon, due to the limited capacity of the enzymatic systems responsible for their dissimilation. To test whether lactate is formed also by natural communities of halophilic archaea at low glycerol concentrations such as may be encountered in situ, we incubated samples from the Dead Sea and from the saltern crystallizer ponds at Eilat with 1.5–3 μM [U-^{14}C]glycerol. After depletion of the glycerol, around 10% of the label was found in lactate and acetate in both brine samples. In addition, pyruvate was formed in Dead Sea water. Upon further incubation of the Dead Sea samples after depletion of the glycerol, pyruvate disappeared rapidly, while acetate and lactate concentrations decreased only very slowly. In saltern brines the lactate formed was degraded after depletion of the glycerol, but the concentration of labelled acetate decreased only very slowly.

Key words: Halobacteriaceae; Lactate; Pyruvate; Acetate; Saltern; Dead Sea

Introduction

Substantial evidence has accumulated on the importance of glycerol as a key compound in the carbon cycle of hypersaline environments. Glycerol is produced by the green alga *Dunaliella*, the main or only primary producer in water bodies with salt concentrations exceeding 20–25%, and is accumulated intracellularly in molar concentrations, serving as an osmotic stabilizer. Glycerol leaking out of healthy and/or decaying *Dunaliella* cells is probably the main carbon and energy source enabling the development of the dense communities of halophilic archaea (the *Halobacterium – Haloferax – Haloarcula* group) that generally are found in these environments [1,2]. Blooms of halophilic archaea in the Dead Sea

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occur only after the development of a bloom of *Dunaliella* [3,4]. Uptake and turnover of glycerol in hypersaline environments inhabited by dense communities of halophilic archaea, such as the Dead Sea and saltern crystallizer ponds, was shown to be very fast [5].

When glycerol is added to cultures of halophilic archaea, especially representatives of the genera *Haloferax* and *Haloarcula*, massive amounts of acids are formed, causing a drop of the medium pH to values below 5, which may result in the death of the culture [6]. Indications were obtained that acid production from glycerol may also occur in halobacterial communities under in situ conditions. Small concentrations of radioactively labelled glycerol added to samples collected from saltern crystallizer ponds, or from the Dead Sea, are rapidly utilized [7]. After completion of ^{14}C-glycerol uptake, only about half of the label was found to be incorporated by the cells. A substantial fraction of the label remaining in the supernatant was not in the form of CO_2: $21 \pm 4\%$ and $35 \pm 2\%$ of the label remained in the soluble fraction upon acidification after completion of glycerol uptake in Dead Sea water samples, and in samples from saltern crystallizer ponds, respectively [5].

Qualitative and quantitative studies on the formation of acids by halophilic archaea were performed by the group of Hochstein in the 1970's, using a single organism (*Halobacterium saccharovorum* R6) [8], and a number of sugars as substrates. When grown in the presence of glucose, *H. saccharovorum* excretes acids into the medium, and these were identified as pyruvate and acetate [9]. Sugars are metabolized by a modified Entner-Doudoroff pathway, in which oxidation precedes phosphorylation (glucose → gluconate → 2-keto-3-deoxygluconate → 2-keto-3-deoxygluconate-6-phosphate → pyruvate + 3-P-glyceraldehyde) [10,11]. In the presence of mannose, pyruvate was produced, and only a little acetate was detected. When grown in the presence of galactose or lactose, only a transitory accumulation of acetate and pyruvate was observed [9]. Lactose, arabinose, ribose, and xylose were oxidized to lactobionic acid, and the corresponding aldonic acids [12].

Although glycerol is probably a much more important substrate for halobacteria in nature than glucose, no attempts have been made to identify and quantify the acids produced from glycerol by different representatives of the Halobacteriaceae. In view of the possible importance of simple organic acids in the terminal processes in the carbon cycle of hypersaline ecosystems under anaerobic conditions, we initiated a study of acid formation from glycerol by halophilic archaea, both in culture and under field conditions.

Materials and Methods

Bacterial strains and culture conditions

The following bacterial strains were used in this study: *Haloferax mediterranei* ATCC 35300, *Haloferax gibbonsii* ATCC 33959, *Haloferax volcanii* ATCC 29605, *Haloferax denitrificans* ATCC 35960, *Haloarcula marismortui* ATCC 43049, *Haloarcula vallismortis* ATCC 29715, *Halobacterium cutirubrum* NRC 34001 and *Halobacterium saccharovorum* ATCC 29252.

Glycerol metabolism was studied in growth media containing low concentrations of complex organic carbon sources. The medium for *Halobacterium cutirubrum* contained (g l^{-1}): NaCl, 250; KCl, 5; $MgCl_2.6H_2O$, 5; NH_4Cl, 5; KH_2PO_4, 0.2, and yeast extract, 0.2. The medium for *Haloferax mediterranei*, *Haloferax gibbonsii*, and *Halobacterium saccharovorum* consisted of NaCl, 175; $MgCl_2.6H_2O$, 20; K_2SO_4, 5; $CaCl_2.2H_2O$, 0.1; NH_4Cl, 1.0; KH_2PO_4, 0.2, and yeast extract, 0.2. For the growth of *Haloferax volcanii* and *Haloferax denitrificans* the NaCl concentration of last medium was lowered to 125 g l^{-1}, and the $MgCl_2.6H_2O$ concentration increased to 50 g l^{-1}. The medium for *Haloarcula marismortui* and *Haloarcula vallismortis* contained (g l^{-1}): NaCl, 206; $MgSO_4.7H_2O$, 36; KCl, 0.37; $CaCl_2.2H_2O$, 0.5; $MnCl_2$, 0.013; NH_4Cl, 1.0; KH_2PO_4, 0.2, and yeast extract, 0.2. All media were supplemented with MES (2-[N-morpholino]ethanesulfonic acid) and PIPES (piperazine-N,N′-bis[2-ethanesulfonic acid]) buffer, added from separately sterilized concentrated so-

lutions to final concentrations of 20 mM each. Glycerol and glucose were added as indicated from concentrated sterile stock solutions. Cultures (50 ml portions in 100-ml Erlenmeyer flasks) were grown in a shaking water bath at 35°C.

The growth of cultures was followed by measuring the optical density at 500 nm. The pH of the cultures was measured regularly, and culture supernatants were assayed for glycerol and organic acids.

Dead Sea and saltern crystallizer pond samples

Dead Sea water was sampled at the shore near Ein Gedi on 4 November 1993. Brine from a saltern crystallizer pond was sampled on 26 October 1993. The population densities of *Dunaliella parva* in Dead Sea water, *Dunaliella salina* in the saltern brine, and community densities of bacteria in both samples were determined as described earlier [5]. Table 1 summarizes a number of biological and physical parameters of the samples used. Incubation experiments with ^{14}C-glycerol were started within 2 h of sampling.

Analytical methods

Glycerol was assayed by periodate oxidation, resulting in the formation of 2 mol formaldehyde and 1 mol formate per mol of glycerol, followed by the quantification of formaldehyde by colorimetric reaction with 3-methyl-2-benzathiozolone hydrazone [5]. Lactate, pyruvate, and other components of the growth media did not interfere in this assay.

Organic acids (lactate, acetate, pyruvate, and others) were analyzed by high performance liquid chromatography, using an HPLC set-up consisting of a Merck-Hitachi intelligent pump model L-6200 A, two Lichrospher 100 RP-8 5 μm columns (250 × 4 mm, Merck) connected in series, and a model L-4200 UV-VIS detector, operated at 210 nm. The sample (20 μl) was eluted at a rate of 0.75 ml min^{-1} with buffer (0.52 M KH_2PO_4 and 0.1 M $(NH_4)_2SO_4$, adjusted to pH 2.1 with H_3PO_4) [13].

L-lactate and D-lactate were assayed enzymatically by measuring the reduction of NAD in the presence of hydazine to trap any pyruvate formed [14,15]. The reaction mixtures contained 2.5 ml buffer (0.4 M hydrazine + 0.5 M glycine, pH 9.0), 0.2 ml of 40 mM β-NAD, 0.05–0.2 ml of sample, and 20 μl containing 29 units of L-lactic dehydrogenase (rabbit muscle, Sigma), or 20 μl containing 38 units of D-lactic dehydrogenase (from *Staphylococcus epidermidis*, Sigma). After 3 h incubation at 30°C the absorption at 340 nm was measured against blanks in which the sample was replaced by an equivalent volume of water or lactate-free growth medium.

Transformation of radioactively labelled glycerol to organic acids by Dead Sea and saltern pond samples

Samples of Dead Sea or saltern brines were incubated in 10-ml glass vials in the dark in the presence of [U-^{14}C]glycerol (Amersham, specific activity 5.88 GBq mmol^{-1}, added to a final activity of 9.25 or 18.5 kBq ml^{-1}). In some of the experiments the stock solution of labelled glycerol, which contained 50% ethanol, was partially evaporated in a stream of air to reduce the amount of ethanol introduced in the experimental systems. At the beginning of the experiment and after incubation at 35°C for periods between 3 h and 5 days, 0.25 ml samples were removed. Samples were centrifuged for 5 min at 15000 × *g* to remove bacteria, algae, and other particles. The supernatant fractions were transferred to 1.5-ml plastic centrifuge tubes, 10 μl of concen-

Table 1

Bacterial and algal numbers of hypersaline water samples

Sampling site	Date	Specific gravity	*Dunaliella salina* cells ml^{-1}	*Dunaliella parva* cells ml^{-1}	Bacteria cells ml^{-1}
Dead Sea	4 November 1993	1.226	–	72	1.2×10^7
Eilat saltern	26 October 1993	1.235	890	–	5.3×10^7

trated HCl was added, and the tubes were left open for 1 h in a desiccator containing KOH pellets to trap any $^{14}CO_2$ present. Subsequently, 10 μl of a solution of 50 mM Na-acetate, 50 mM Na-lactate and 5 mM Na-pyruvate was added as a carrier, and samples were analyzed by HPLC as described above. Fractions of 5 drops (about 0.11 ml) were collected at the outlet of the detector, and counted by liquid scintillation counting after addition of 2 ml Instagel (Packard) scintillation cocktail. Concentrations of the acids formed were estimated, based on the radioactivity in the acid fractions, taking the number of carbon atoms in the different acids into account.

Results

Pure culture studies

Eight representative halophilic archaeal species, belonging to 3 different genera, were grown in buffered media, containing a low concentration of yeast extract (0.2 g l^{-1}), and 1 g l^{-1} glycerol or glucose. In all strains tested, with the exception of *Halobacterium cutirubrum*, the addition of glycerol or glucose stimulated growth, as shown by the higher culture turbidities obtained (compare also Fig. 1).

HPLC analysis of supernatants of *Haloferax* cultures grown in the presence of glycerol showed that all strains produced lactate and acetate. After growth on glucose, acetate was found in most cases as the sole acid formed. Cultures of the two *Haloarcula* species tested produced pyruvate and acetate from both glycerol and glucose (Table 2). While some unidentified peaks were detected upon injection of culture media with high concentrations of yeast extract, the media used in this study did not interfere in the HPLC procedure, with the exception of the appearance of a large peak (at about 6.3 min, well before elution of the organic acids), attributable to the elution of salts and other medium components not retained by the column. Lack of interference by medium components was also ascertained by the use of internal standards. Only part of the glycerol or glucose added was converted to organic acids (up to 0.58 mmol acid $mmol^{-1}$ glycerol, and up to

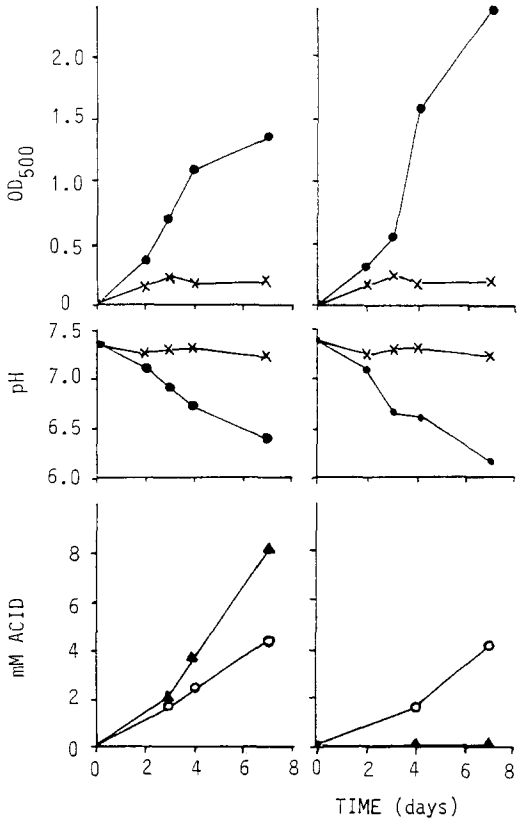

Fig. 1. Growth and formation of lactate (▲) and acetate (○) in cultures of *Haloferax mediterranei* grown in medium buffered with 20 mM MES and 20 mM PIPES, in the presence of 10 g l^{-1} glycerol (left panels) or 10 g l^{-1} glucose (right panels) in addition to 0.2 g l^{-1} yeast extract (●), as compared with a culture grown with 0.2 g l^{-1} yeast extract only (×).

0.79 mmol acids $mmol^{-1}$ glucose). In a culture of *Haloferax mediterranei* grown in the presence of a large excess of glycerol (10 g l^{-1} = 111 mM), up to 8 mM lactate and 4 mM acetate were detected (Fig. 1). At the end of the experiment (after 7 days of incubation) less than 10% of the glycerol added had been consumed, as shown by chemical assay of glycerol in the culture supernatant.

To determine the stereochemical conformation of the lactate produced by *Haloferax mediterranei* and *Haloferax volcanii*, we performed enzymatic assays for L- and D-lactate by testing for the reduction of NAD in the presence of L- and D-lactic dehydrogenase and hydrazine

Table 2

Identification and quantitative determination of acids formed by different halophilic archaea upon growth on glycerol and glucose. Cells were grown in buffered growth media in the presence of 0.2 g l^{-1} yeast extract and 1 or 2 g l^{-1} glycerol or glucose. The formation of lactate, acetate, and pyruvate was tested by means of HPLC in culture supernatants after 3 and 6 days of incubation at 35°C

Strain	Concentration (mM) [a]					
	Glycerol-grown			Glucose-grown		
	Lactate	Acetate	Pyruvate	Lactate	Acetate	Pyruvate
Halobacterium cutirubrum	–	–	–	–	–	– [b]
Halobacterium saccharovorum	–	1.3	0.4	–	–	0.1
Haloferax mediterranei	4.7	1.1	–	–	0.1	–
Haloferax gibbonsii	5.0	2.7	–	2.5	3.1	–
Haloferax volcanii	1.0	1.2	–	–	0.1	±
Haloferax denitrificans	4.9	1.3	–	–	1.6	–
Haloarcula marismortui	–	0.4	1.6	–	3.3	1.1
Haloarcula vallismortis	–	±	0.1	–	±	0.1

[a] Concentrations given are representative values; the concentrations measured varied somewhat in replicate experiments, but the ratios between the concentrations of the different acids formed were similar.

[b] – = not detected; ± = detected in trace concentrations below 0.1–0.2 mM.

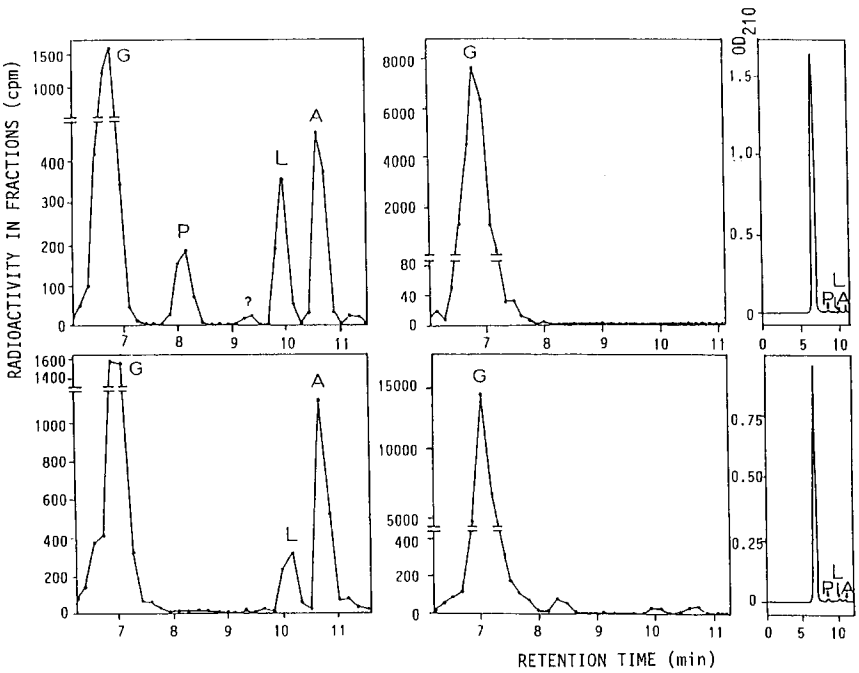

Fig. 2. HPLC elution of Dead Sea water (upper panels) and Eilat saltern pond brine (lower panel) after 24 h and 6 h incubation, respectively, at 35°C in the presence of [U-^{14}C]glycerol (9.25 kBq ml^{-1}) (left panels). The radioactivity in the collected fractions was determined by liquid scintillation counting. For comparison, the results of the elutions of samples collected at the onset of the incubation are shown (middle panels), as are the elution times of unlabelled pyruvate, lactate, and acetate, which were added as carriers to the samples, as followed by measurement of the absorption at 210 nm (right panels). The large peak in OD_{210} after about 6.5 min is due to the elution of salts and other compounds present which are not retarded by the column. Radioactivity peaks can be attributed to glycerol (G), pyruvate (P), lactate (L), and acetate (A).

to trap any pyruvate produced [14,15]. The product present in the supernatants of cultures grown in the presence of glycerol was found to be D-lactate, and no L-lactate was detected.

Formation of acids by Dead Sea and saltern brine samples

To test whether lactate, pyruvate, and acetate are formed also by natural communities of halophilic archaea at low glycerol concentrations, in the order of magnitude encountered in the environment [5], we incubated samples from the Dead Sea and from the saltern crystallizer ponds at Eilat with [U-^{14}C]glycerol (total added glycerol concentration 1.5–3.1 μM), and measured the radioactivity appearing in the different acids after separation by HPLC. While in samples at zero time all radioactivity coeluted with the salt peak, labelled organic acids were detected after incubation of a few hours to 1 day of incubation (Fig. 2). The biota from the Eilat saltern pond transformed part of the glycerol to acetate and lactate at a molar ratio of 4.3–4.8 : 1, and between 8–11% of the decrease in ^{14}C in the glycerol fraction was accounted for by organic acids after 3–6 h of incubation. The Dead Sea water sample converted part (9.5–11.9%) of the glycerol carbons to a mixture of acetate, pyruvate, and lactate (molar ratios obtained after 1 day of incubation between 0.9 and 2.2 acetate and 0.7–1.0 pyruvate per molecule of lactate formed). In several cases a fourth radioactivity peak was detected, eluting after about 9.4 min (Fig. 3). At present the identity of this minor peak is unknown. In both Dead Sea and saltern brines, the fraction of the labelled glycerol that was not recovered as organic acids was in part incorporated into the archaeal cells, and in part released as CO_2. No attempts were made to quantify these fractions in the present work; additional data may be found in a previous study on the subject [5].

When incubation of Dead Sea water was continued after depletion of the labelled glycerol added, the concentration of pyruvate decreased rapidly, however, only a very slow utilization of the acetate and lactate formed was observed (Fig. 3, left panel). In a similar experiment with Eilat saltern brine, the lactate formed disappeared within a day after the depletion of the glycerol, but most of the labelled acetate formed was still present after 2 days of incubation (Fig. 3, right panel).

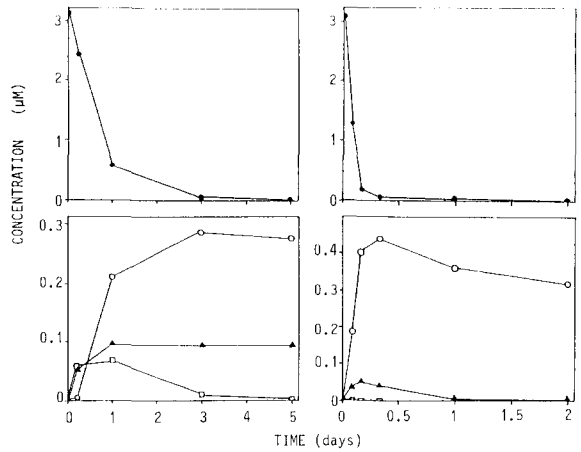

Fig. 3. Kinetics of formation and breakdown of pyruvate, lactate, and acetate produced by the archaeal community in Dead Sea water (left panels) or Eilat saltern brine (right panels) supplemented with [U-^{14}C]glycerol. Radioactively labelled glycerol was added to a final concentration of 3.1 μM, and after different periods of incubation at 35°C the radioactivity in the pyruvate (□), lactate (▲), and acetate fractions (○), as well as in the remaining glycerol (●), was determined after HPLC separation.

Discussion

The formation of acids from glycerol by certain halophilic archaea has been known for a long time, and was first described for a Dead Sea isolate, "*Halobacterium*"(*Haloarcula*) *marismortui* [16,17]. However, in spite of the ecological importance of glycerol in the carbon cycle of hypersaline environments, no reports exist in the literature on the nature of the acids produced by halophilic archaea from glycerol. In the present study we identified acetate formation by most of the organisms tested, and in addition, D-lactate was found to be produced by all *Haloferax* species, and pyruvate by all *Haloarcula* species tested. This is the first time that lactate is reported as a product of halobacterial metabolism. Whether lactate and pyruvate formation is char-

acteristic of all representatives of the genera *Haloferax* and *Haloarcula*, respectively, remains to be ascertained. In all cases only part of the glycerol added was converted to lactate and acetate (Fig. 1, Table 2). The remainder was probably incorporated into cell material, as indicated by the increase in culture turbidity as compared with control cultures not enriched with glycerol. Possibly oxidation to CO_2 also occurred to some extent. No attempts were made to quantitatively estimate the fractions of the substrate that were incorporated or respired. The relatively small amounts of acids formed are sufficient to cause a sharp drop in the pH of unbuffered growth media to toxic values [6].

Not all halophilic archaea produce significant amounts of acids when grown in the presence of glycerol, as shown by *Halobacterium cutirubrum*. This does not mean, however, that glycerol is not used by *H. cutirubrum*. Indeed, it is known that glycerol can be metabolized by related strains [18].

The finding of acetate and pyruvate as products of glycerol metabolism in certain halophilic archaea agrees with the results of the studies of Hochstein and co-workers on glucose metabolism in *Halobacterium saccharovorum* [8–10]. Pyruvate was detected by us also in *Haloarcula marismortui* and *Haloarcula vallismortis* cultures grown in the presence of glycerol or glucose (Table 1). Pyruvate is not a commonly encountered end-product in bacterial fermentations, but overflow of pyruvate has been described in certain fermentative processes, and is probably due to a limited capacity of the enzymatic systems involved in the metabolic pathways downstream of pyruvate [19]. In the case of the halophilic archaea, acid production from glucose and glycerol may be due to similar reasons. It was shown that galactose-grown *Halobacterium saccharovorum* cells or cells grown without carbohydrates also utilized pyruvate and acetate, but the use of these acids by glucose-grown cells was negligible. The extent of acid formation is probably a function of the relative activities of the different enzymes involved in glucose dissimilation. Glucose was found to repress the condensing activities (citrate synthase and malate synthase), associated with the introduction of acetyl-CoA into the tricarboxylic acid cycle. Concomitantly, an increased acetyl-CoA deacetylase activity caused an enhanced hydrolysis of acetyl-CoA to acetate [20].

All three acids detected thus far as products of sugar or polyol transformation in halobacteria (lactate, pyruvate, and acetate) can be used as substrates for the growth of many halophilic archaea, often including those strains that produce them. Most halobacterial isolates were shown to grow well on pyruvate, and excellent growth of *Haloarcula marismortui* on acetate was reported [17,21], while *Haloferax volcanii* also grew moderately well on acetate [21]. Lactate was reported to support growth of *Haloferax mediterranei*, *Haloferax denitrificans*, *Haloarcula hispanica*, *Halobacterium lacusprofundi* and *Halococcus saccharolyticus* [6]. The excretion of acids that can also be utilized by the same bacterium that produces them may be due to the induction and repression of certain enzymatic activities in the presence of excess sugar or glycerol, as was documented in the case of acid production from glucose by *Halobacterium saccharovorum* [20].

Experiments with radioactively labelled glycerol as tracer showed that formation of lactate, pyruvate, and acetate also occurs under field conditions at very low added glycerol concentrations (Figs. 2,3), close to those that may be found in Dead Sea and saltern crystallizer brines [5]. Such a result could be predicted in view of the earlier finding that when brines inhabited by halophilic archaea are incubated with [U-^{14}C]glycerol, a substantial part of the radioactivity left in the supernatant after consumption of the labelled compound is not in the form of $^{14}CO_2$ [5]. The formation of organic acids as documented in the present study may account for this fraction. The formation of organic acids in brine samples with relatively low bacterial densities also demonstrates that the occurrence of incomplete oxidation processes is not due to depletion of oxygen by the heterotrophic community.

When incubated with labelled glycerol, acetate and lactate were formed by the saltern crystallizer brine sample; in Dead Sea water, pyruvate was also formed. The difference in the types of acids produced undoubtedly reflects the different

nature of the archaeal communities in both environments. In view of the results obtained with pure cultures, the formation of pyruvate by the Dead Sea community was somewhat unexpected, as the archaeal bloom that developed in the lake in 1992, a remainder of which was still present at the time the experiments were performed, was dominated by representatives of the genus *Haloferax*. This was shown by the presence of the characteristic sulfated diglycosyl diether lipid as the major glycolipid [22], and absence of phosphatidylglycerosulfate [23]. Pyruvate was not detected in the two representatives of the genus *Haloferax* tested (Table 2), but was found to be a major product of glycerol metabolism in *Haloarcula* species. The latter, however, did not contribute significantly to the archaeal community in the Dead Sea, as judged by the absence of their characteristic glycolipid [22]. We do not know whether pyruvate is produced by the (still undescribed) *Haloferax*-like bacterium that dominated the community in the Dead Sea. In addition, the possibility that the nature of the acids excreted depends not only on the species, but also on incubation conditions, deserves an investigation. The archaeal community of the saltern ponds differs from that of the Dead Sea, as shown by the presence of the diether derivative of phosphatidylglycerosulfate as one of the major polar lipids [23].

After the depletion of the glycerol added to Dead Sea water, pyruvate rapidly disappeared, acetate and lactate concentrations, however, decreased only very slowly, if at all (Fig. 3). Previous studies have shown that the rate of metabolism of acetate added to Dead Sea samples was one to two orders of magnitude lower than that of glycerol [7]. Similarly, in water of the north arm of Great Salt Lake, Utah (total salt concentration 22%), glycerol was metabolized about twice as fast as acetate [24]. No information is available in the last case on the contribution of halophilic archaea to the total heterotrophic activity. Similar measurements with lactate and pyruvate have to our knowledge never been performed in any hypersaline environment.

The finding of lactate and pyruvate as products of glycerol metabolism by certain halophilic archaea under field conditions has interesting implications for the terminal processes in the carbon cycle of hypersaline ecosystems under anaerobic conditions. While halophilic sulfate-reducing and methanogenic bacteria that utilize acetate as carbon- and energy source are as yet unknown, the most halotolerant sulfate-reducing bacterium in culture, *Desulfovibrio halophilus*, uses lactate as its preferred electron donor [25]. Pyruvate is also used. This organism grows optimally at 6–7% NaCl, but tolerates NaCl concentrations of up to 18%, well within the salinity range enabling growth of the less salt-requiring types of halophilic archaea (notably the representatives of the genus *Haloferax*) [6]. No data are available on any preference for L- or D-lactate as electron donor by *D. halophilus,* but generally both L- and D-lactate are used by the dissimilatory sulfate reducers [26]. Thus, under suitable environmental conditions, glycerol produced by *Dunaliella* may be in part transformed to lactate and pyruvate by halophilic archaea in hypersaline water bodies, and these compounds may later serve as electron donors for dissimilatory sulfate reduction in the anaerobic sediment.

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