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ORIGINAL PAPER

Dihydroxyacetone metabolism in *Salinibacter ruber* and in *Haloquadratum walsbyi*

Rahel Elevi Bardavid · Aharon Oren

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Abstract The extremely halophilic bacterium *Salinibacter ruber* inhabits saltern crystallizer ponds worldwide, together with the square archaeon *Haloquadratum walsbyi*. Cultures of *Salinibacter* have been shown to convert up to 20% of the glycerol added to a not previously characterized overflow product. We here identify this product of incomplete glycerol oxidation by *Salinibacter* as dihydroxyacetone. Genomic information suggests that *H. walsbyi* possesses an efficient uptake system for dihydroxyacetone, and we show here that dihydroxyacetone is indeed metabolized by *Haloquadratum* cultures, as well as by the heterotrophic prokaryotic community of the saltern crystallizer ponds in Eilat, Israel, dominated by *Haloquadratum*-like cells. In the absence of glycerol, *Salinibacter* also takes up dihydroxyacetone. Degradation of glycerol, produced in hypersaline lakes as an osmotic solute by the green alga *Dunaliella salina* may thus involve dihydroxyacetone as an intermediate, which can then be taken up by different types of heterotrophs present in the environment.

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R. Elevi Bardavid · A. Oren (✉)
The Institute of Life Sciences, and The Moshe Shilo Minerva Center for Marine Biogeochemistry,
The Hebrew University of Jerusalem,
Jerusalem 91904, Israel
e-mail: orena@cc.huji.ac.il

A. Oren
Department of Plant and Environmental Sciences,
The Institute of Life Sciences,
The Hebrew University of Jerusalem,
Jerusalem 91904, Israel

Keywords *Salinibacter* · *Haloquadratum* · Dihydroxyacetone · Glycerol · Incomplete oxidation

Abbreviations
DHA dihydroxyacetone
PQQ pyrroloquinoline quinone

Introduction

Crystallizer ponds of solar salterns used worldwide for the production of salt by evaporation of seawater are characterized by dense communities of halophilic microorganisms. The microbial diversity of these NaCl-saturated ponds is limited. The β-carotene rich green alga *Dunaliella salina* is the sole primary producer. The heterotrophic prokaryote community is dominated by the square gas-vacuolate archaeon recently isolated and described as *Haloquadratum walsbyi* (Bolhuis et al. 2004; Burns et al. 2004, 2007). The rod-shaped *Salinibacter ruber* (Bacteroidetes) (Antón et al. 2002) is generally present also in large numbers (Antón et al. 2000; Elevi Bardavid et al. 2007a). Environmental genomics studies in Spanish saltern ponds (Legault et al. 2006) show that most genes recovered from the salt-saturated brines can be attributed to these microorganisms. All three named organisms are pigmented orange or pink-red, and due to their high community densities they impart a red coloration to the brines.

The food web in the saltern environment is relatively simple. *Dunaliella* fixes carbon dioxide to organic compounds. The alga produces massive amounts of glycerol as compatible solute to provide osmotic balance with the outside medium. Glycerol can therefore be expected to be a

key compound for the understanding of the nutrition of the heterotrophic communities (Oren 1993). It was earlier reported on the basis of microautoradiography experiments that the square *Archaea* in Spanish saltern ponds do not take up glycerol (Rosselló-Mora et al. 2003), and cultures of *H. walsbyi* were reported not to use glycerol for growth (Burns et al. 2007); however, a gene annotated as *glpK*—glycerol kinase is present in its genome (Bolhuis et al. 2006). The above-mentioned study of substrate uptake using microautoradiography combined with fluorescence in-situ hybridization also failed to demonstrate incorporation of glycerol by *Salinibacter*, and the species description of *S. ruber* stated that the organism does not grow on glycerol. However, addition of glycerol to *Salinibacter* cultures led to the induction of glycerol kinase activity and to increased cell yields. When radio-labeled glycerol was supplied to the cultures, up to 25% of the label was incorporated into biomass and an additional fraction was respired, but not all the glycerol that had disappeared could be accounted for as cell mass or as CO_2 (Sher et al. 2004). The soluble product excreted by the cells, which accounted for up to about 20% of the radioactivity of the glycerol added, has thus far not been identified.

Analysis of the genome of *H. walsbyi* led to the insight that this archaeon contains a unique transport system for dihydroxyacetone (DHA). The system is based on the translocation of DHA over the membrane via facilitated diffusion and its subsequent incorporation into the cellular metabolism via dihydroxyacetonephosphate through a phosphoenolpyruvate-dependent phosphotransferase system (Bolhuis et al. 2006). The authors of the study stated that *H. walsbyi* can grow on DHA and that DHA may be available to the organism as a product of *Salinibacter*. However, no experimental evidence supporting these claims was provided.

In view of the potential importance of DHA in the metabolism of *H. walsbyi*, as suggested by the analysis of its genome, we have re-evaluated the glycerol metabolism of *Salinibacter* and investigated the metabolism of DHA in *Salinibacter* and in *Haloquadratum* cultures. Here we report the identification of the overflow product of *S. ruber* during glycerol transformation as DHA, and we further show that DHA can be consumed by both *H. walsbyi* and *S. ruber*, as well as by the mixed microbial community in saltern crystallizer brines.

Materials and methods

Organisms and growth conditions

Salinibacter ruber strain M31^T (DSM 13855^T) was grown at 35°C in 1 l portions of medium in 2 l Erlenmeyer flasks in an illuminated New Brunswick Innova 44 shaker (100 rpm). The medium composition was (g l^{-1}): NaCl, 195; $MgSO_4 7H_2O$, 25; $MgCl_2 6H_2O$, 16.3; $CaCl_2 2H_2O$, 1.25; KCl, 5.0; $NaHCO_3$, 0.25; NaBr, 0.625; and yeast extract, 1.0; pH 7.0. The medium was sterilized by autoclaving. For the growth of *S. ruber* cells for glycerol uptake studies, glycerol (5 mM) was added to the medium. *Haloquadratum walsbyi* strain C23^T (JCM 12705^T) (Burns et al. 2007) was grown under similar conditions in medium containing (g l^{-1}): NaCl, 240; $MgCl_2 6H_2O$, 30; $MgSO_4 7H_2O$, 35; KCl, 7, to which 5 ml l^{-1} of 1 M NH_4Cl; 2 ml l^{-1} 0.25 M K_2PO_4 buffer, pH 7; 4.4 ml l^{-1} 25% Na-pyruvate; 10 ml l^{-1} 0.5% peptone (Oxoid) + 0.1% yeast extract; 3 ml l^{-1} vitamin solution; and 1 ml l^{-1} trace element solution SL10 (Widdel et al. 1983) were added. The pH was adjusted to 7 and the medium was sterilized by filtration through 0.2 μm pore-size cellulose acetate filters. The composition of the vitamin solution was (mg l^{-1}): *p*-aminobenzoate, 13; biotin, 3; nicotinic acid, 33; hemicalcium D-(+)-pantothenate, 17; pyridoxamine–HCl, 50; thiamine–HCl, 33; cyanocobalamin, 17; D,L-6,8-thioctic acid, 10; riboflavin, 10; and folic acid, 4.

Saltern brine sample

Saltern crystallizer brine was collected from pond 304 of the salterns of the Israel Salt Company in Eilat (Oren 1990) on 12 June 2006. The brine had a density of 1.235 g cm^{-3}, a total dissolved salt content of 368 g l^{-1}, and contained 2,140 *Dunaliella* cells ml^{-1} and 5.8×10^7 prokaryotes ml^{-1}, enumerated microscopically as outlined by Oren and Rodríguez-Valera (2001) and Oren et al. (1996).

Incubation experiments

Late-exponential growth phase cells were collected by centrifugation (2,700*g*, 10 min at room temperature), and the pellets were gently suspended in growth medium to yield cultures with OD_{600} between 1.95 and 3.25 (3.5–9×10^9 cells ml^{-1}, as counted microscopically using a Petroff–Hauser counting chamber and a microscope equipped with phase-contrast optics). For DHA uptake experiments, *S. ruber* and *H. walsbyi* cells were grown in their respective standard growth media, concentrated by centrifugation and resuspended in their growth media to OD_{600} 1.95 and 0.58, respectively, corresponding to 5×10^9 and 5×10^8 cells ml^{-1}. Prokaryotic cells from the Eilat saltern brine were concentrated by centrifugation (20 min, 5,100*g*) to a final density of 4.4×10^9 cells ml^{-1}. *Dunaliella* cells, being lighter than the brine, float during centrifugation, and are not collected in the cell pellet.

Cell suspensions (40 ml in 100 ml Erlenmeyer flasks) were supplemented with glycerol (10 or 20 mM) or DHA (1–5 mM) as indicated, and incubated with shaking as above. After different incubation periods, samples were withdrawn, centrifuged for 4 min at 12,000*g*, and the supernatants were stored at –20°C until analysis.

Analytical methods

The colorimetric assay for glycerol is based on oxidation of glycerol with periodate to formaldehyde and formic acid, followed by reaction of formaldehyde with acetylacetone and ammonium ions to give the yellow 3,5-diacetyl-1,4-dihydrolutidine (Ben Amotz and Avron 1978). To 1 ml sample or standard glycerol solution (up to 0.5 µmol glycerol), 1 ml of periodate reagent (65 mg Na-meta-periodate, 90 ml distilled water, 10 ml acetic acid, 7.7 g ammonium acetate) was added. After 5 min incubation at room temperature, 5 ml acetylacetone reagent (1 ml acetylacetone + 99 ml isopropanol) was added, and the absorbance was measured at 410 nm after 20 min incubation at 50°C. We also used a specific, enzyme-based assay. In a final volume of 1 ml we added sample or standard (up to 0.5 µmol glycerol); 25 µmol glycine–NaOH buffer, pH 10; 5 units of *Cellulomonas* glycerol dehydrogenase (Sigma); and 1.5 mg NAD. After 4 h of incubation at 30°C, the absorbance was measured at 340 nm. A system that did not receive enzyme served as control.

Dihydroxyacetone was assessed colorimetrically according to Burton (1957). To 0.5 ml sample or standard (up to 5 µmol DHA), 2 ml of 20 g l^{-1} resorcinol in 10 M HCl was added, and after incubation overnight at room temperature the absorbance was measured at 490 nm. This method will detect all ketoses, and is therefore not specific for DHA. Glycerol does not interfere in the assay, nor does pyruvate, a compound produced by some extreme halophilic *Archaea* during incomplete oxidation of different sugars and glycerol (Oren and Gurevich 1994; Tomlinson and Hochstein 1972; Tomlinson et al. 1974). To quantify DHA we also developed a specific enzymatic assay, based on the oxidation of NADH during reduction of DHA by glycerol dehydrogenase (Burton 1955). To a final volume of 1 ml; we added 10 µmol Tris–HCl buffer, pH 7.5; 0.15 or 0.2 µmol NADH; and culture supernatant sample or DHA standard solutions (up to 0.2 µmol). The absorbance was measured in a BioMate 3 Thermo Spectronic spectrophotometer. Then 5 units of *Cellulomonas* glycerol dehydrogenase (Sigma) were added, and the decrease in absorbance was recorded at room temperature until a stable value was obtained within 2–3 min (Fig. 1). The equilibrium of the reaction glycerol + NAD ⇆ DHA + NADH is in the direction of glycerol oxidation rather than DHA reduction, and therefore quantitative conversion of DHA is not achieved (if it was, addition of 100 nmol DHA should result in a decrease in OD_{340} of 0.63, based on the specific absorbance of NADH of 6.3×10^3 M^{-1} cm^{-1}).

Appropriate control experiments were performed to all tests for the possible interference of salts and other medium components in the colorimetric and enzymatic assays used.

Results

When dense cell suspensions of *S. ruber* were supplemented with 10–20 mM glycerol and incubated with shaking at 35°C, glycerol concentrations dropped at rates between 0.54 and 1.3 µmol 10^9 $cells^{-1}$ day^{-1} (Fig. 2a–c). Use of glycerol by *Salinibacter*, as earlier shown by Sher et al. (2004), was thus confirmed. For quantification of

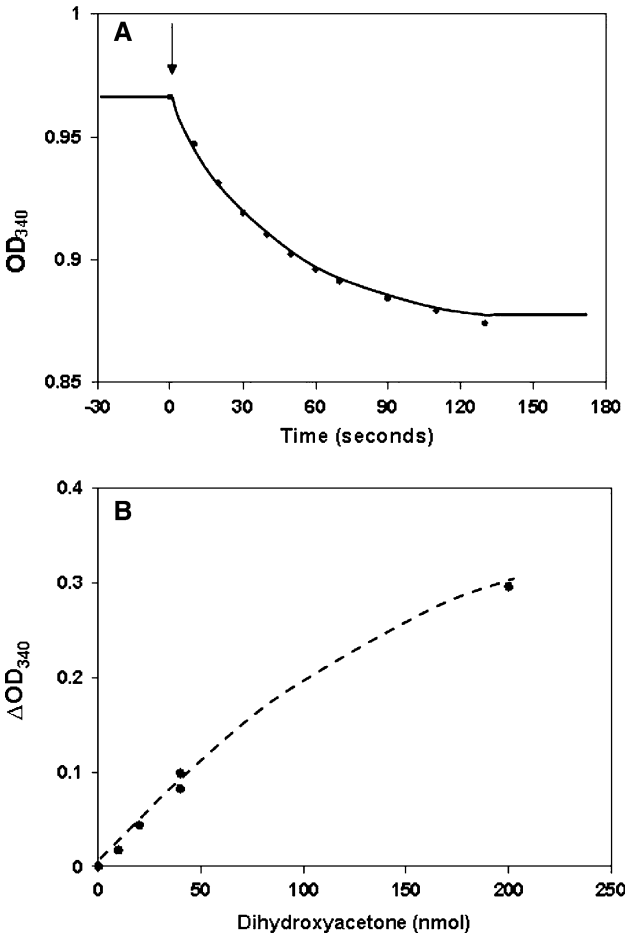

Fig. 1 Enzymatic assay of DHA, based on oxidation of NADH in the presence of glycerol dehydrogenase at room temperature. **a** an example of the kinetics of NADH oxidation in the assay mixture (see "Materials and methods"; total volume 1 ml) containing 40 nmol DHA. At $t = 0$ (*arrow*) glycerol dehydrogenase was added, and the OD was monitored at 340 nm. **b** calibration curve, showing the decrease in OD at 340 nm as a function of the amount of DHA added

glycerol we routinely used an assay based on periodate oxidation, followed by colorimetric determination of formaldehyde produced. This assay has a limited specificity, as positive results are obtained for any substance that yields formaldehyde upon periodate oxidation. DHA is one of these compounds: equimolar quantities of DHA gave about half the color yield obtained with standard glycerol solutions. Therefore we also used a more specific, enzyme-based assay for glycerol, with comparable results.

The decrease in glycerol was in all cases accompanied by the formation of DHA, as assayed using a colorimetric procedure detecting 2-keto sugars (Burton 1957). A decrease in glycerol concentration of 10 mM was accompanied by an apparent increase in DHA between 0.3 mM (Fig. 2c) and 1.1 mM (Fig. 2a, b). In view of the limited specificity of the colorimetric assay used, we developed an enzymatic assay method for DHA, based on oxidation of NADH mediated by glycerol dehydrogenase (Fig. 1). Using this assay, we obtained a value of 1.1 mM for the 104 h sample of the experiment presented in Fig. 2a, for which the colorimetric assay indicated an apparent concentration of 1.3 mM. The identity of at least the greatest part of the keto-sugar formed with DHA was thus confirmed. The zero-time sample from the same experiment did not show any DHA in the enzymatic assay.

The experiment shown in Fig. 2c suggests that the DHA may remain present in the culture at least for some time after glycerol had been depleted. However, experiments in which higher DHA concentrations (1–5 mM) were added to dense suspensions of *Salinibacter* showed that it is also capable of DHA consumption (Fig. 3a). No decrease in DHA was observed in control experiments without cells or in suspensions of cells killed by the addition of 1 mM $HgCl_2$. We confirmed the ability of *Haloquadratum* to take up DHA (Fig. 3b), ability predicted by the analysis of its genome (Bolhuis et al. 2006) but to our knowledge not earlier documented experimentally. *Haloquadratum* did not produce DHA when a dense cell suspension (3×10^9 cells ml^{-1}) was incubated in the presence of 10, 20, or 50 mM glycerol (not shown).

To demonstrate that the natural communities of prokaryotes inhabiting saltern crystallizer ponds may also consume DHA, we followed DHA uptake by cells collected from the Eilat, Israel salterns (Fig. 4). Square *Archaea* morphologically resembling *Haloquadratum* dominated the community. Fluorescent in-situ hybridization experiments confirmed the presence of *Salinibacter* reported earlier on the basis of viable counts on selective culture media (Elevi Bardavid et al. 2007a), using specific 16S rRNA-targeted probes for a sample collected from the same salterns in August 2006, *Salinibacter* was found to make up about 23% of the total number of prokaryote cells (2.1×10^7 cells ml^{-1}) (Elevi Bardavid, unpublished results). DHA disappeared from the cell suspensions at a rate comparable to that obtained in cultures of *H. walsbyi* (Fig. 3b).

Discussion

Annotation of the sequenced genome of *H. walsbyi* (Bolhuis et al. 2006) first suggested the presence of an uptake system for DHA, a system not detected in the genomes of other halophilic *Archaea* sequenced thus far (*Halobacterium*

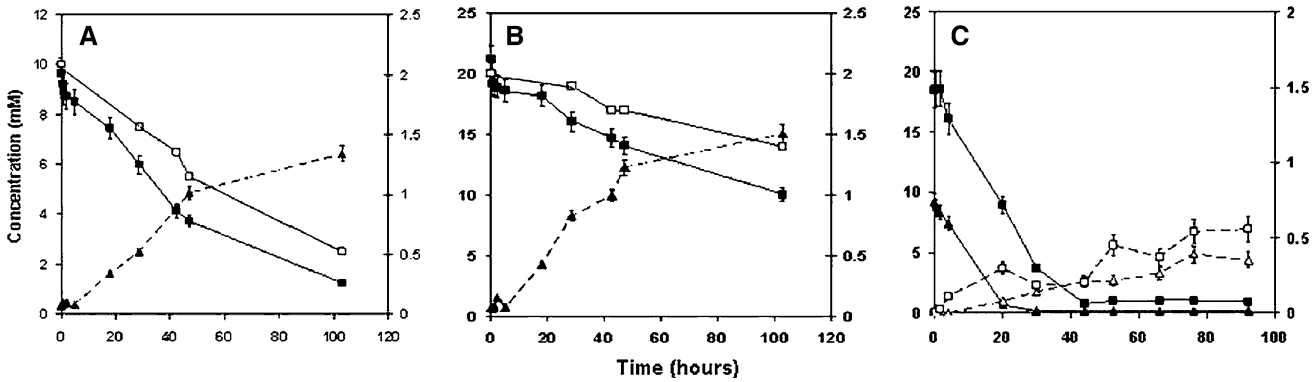

Fig. 2 Formation of DHA by *S. ruber* suspensions incubated in the presence of **a** 10 mM glycerol or **b** 20 mM glycerol. Cells were suspended at a density of 3.5×10^9 cells ml^{-1} (OD_{600} = 1.5). After different periods of incubation at 35°C with shaking in the light, samples were withdrawn and analyzed for glycerol using a colorimetric assay based on periodate oxidation followed by determination of the formaldehyde produced with acetylacetone (*filled square*) and by a specific enzymatic assay (*open square*), and for dihydroxyacetone, using a colorimetric assay for keto sugars (*filled triangle*). **c** a similar experiment in which a higher cell density was used (9×10^9 cells ml^{-1}; OD_{600} = 3.25) with 10 mM (*filled triangle*) or 20 mM glycerol (*filled square*), in which DHA assays (*open triangle* and *open square*, respectively) were continued after the glycerol had been depleted. The left *Y*-axis indicate glycerol concentrations and the right *Y*-axis show DHA concentrations. Most of the assays were performed in duplicates or triplicates, and the error bars show the standard deviation

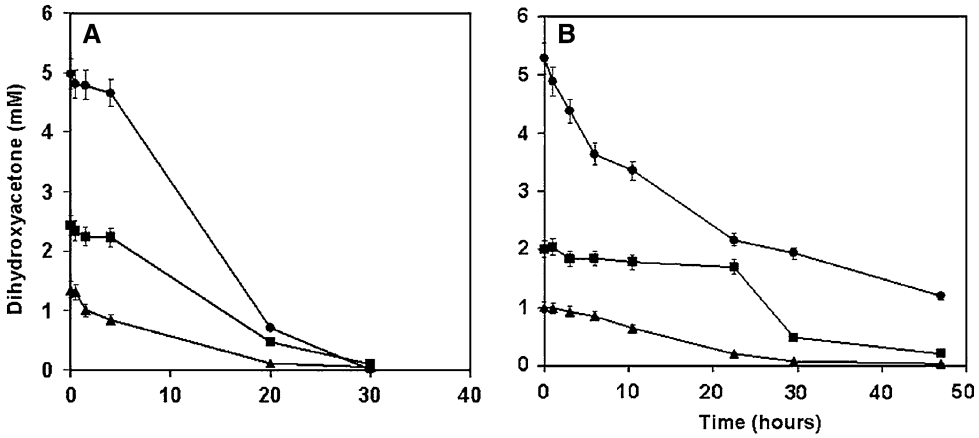

Fig. 3 Consumption of DHA by dense cultures of **a** *S. ruber* and **b** *H. walsbyi*. Cell suspensions (OD_{600} = 1.95 and 0.58; 5×10^9 and 5×10^8 cells ml^{-1}, respectively) in growth medium as described in "Materials and methods" were supplemented with DHA to final concentrations between 1 and 5 mM, and the decrease in DHA was followed during incubation at 35°C. Most of the assays were performed in duplicates or triplicates, and the error bars show the standard deviation

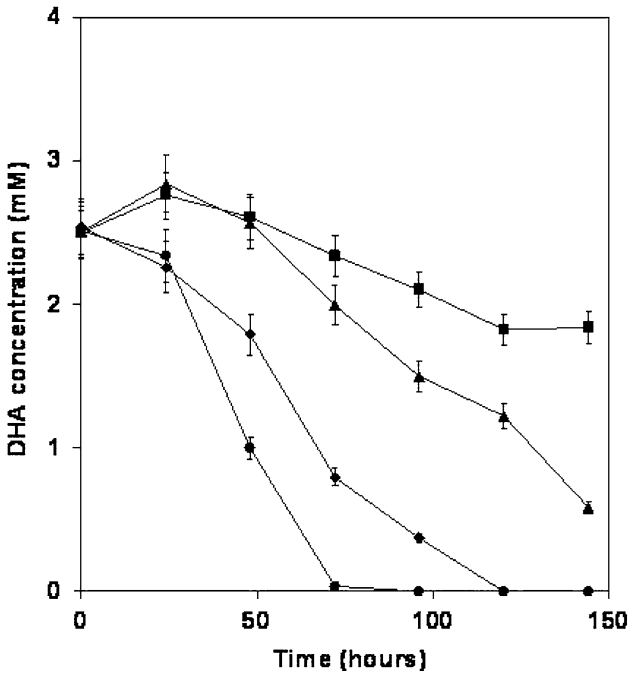

Fig. 4 Consumption of DHA by prokaryotes from a saltern crystallizer pond in Eilat, Israel. Cells were collected in June 2006 from pond 304, concentrated by centrifugation, and suspended to densities of 1.25×10^8 cells ml^{-1} (*filled square*), 3.1×10^8 cells ml^{-1} (*filled triangle*), 6.3×10^8 cells ml^{-1} (*filled triangle*), and 1.25×10^9 cells ml^{-1} (*filled circle*) in saltern brine (density 1.226 g cm^{-3}). Suspensions (10 ml in 50 ml tubes) were supplemented with 2.5 mM DHA, and the decrease in DHA concentration was followed during incubation at 30°C. Most of the assays were performed in duplicates or triplicates, and the error bars show the standard deviation

NRC-1, *Haloarcula marismortui*, *Natronomonas pharaonis*). This led us to initiate the present study to examine the formation and degradation of DHA by the dominant types of heterotrophs in the saltern crystallizer environment.

The finding reported by Bolhuis et al. (2006) is that *H. walsbyi* can grow on DHA as carbon and energy source, as yet without providing the experimental data. Our data confirm uptake and/or chemical modification of DHA by the organism (Fig. 3b). The same article by Bolhuis et al. states that DHA is a putative overflow product of glycerol metabolism in *Salinibacter*, and cites the *S. ruber* genome study by Mongodin et al. (2005). However, DHA metabolism is nowhere mentioned in the Mongodin et al. paper.

The finding by Bolhuis et al. (2004) that the growth of *Haloquadratum* on agar plates is stimulated in the neighborhood of *Salinibacter* colonies suggests some kind of positive interaction between the two organisms. Such a synergistic effect may well be based on the transfer of specific metabolites. The ability of *Haloquadratum* to use DHA as one of its preferred substrates makes DHA a good candidate to explain the nature of the positive interaction between the organisms. In the past we have shown that glycerol metabolism in *Salinibacter* is accompanied by the formation of an overflow product (Sher et al. 2004). We have earlier argued that this overflow product is probably not identical with DHA (Elevi Bardavid et al. 2007b). However, we have now unequivocally identified it as DHA, also using a specific enzymatic assay that we have developed for this purpose.

DHA is not unknown as an overflow product during glycerol metabolism in the bacterial world. Notably, acetic acid bacteria (*Gluconobacter*, *Acetobacter*) produce massive amounts of DHA when grown in the presence of glycerol, and *Gluconobacter oxydans* is used for the commercial production of DHA (Hekmat et al. 2003; Green et al. 2004). In *Gluconobacter*, glycerol oxidation to DHA is mediated by a membrane-bound pyrroloquinoline quinone (PQQ)-linked glycerol oxidase (Prust et al. 2005).

PQQ is found in a limited number of proteobacteria only, and the genome sequence of *S. ruber* does not provide any indication for the presence of PQQ. Therefore, glycerol oxidation to DHA may be attributed to the presence of a soluble NAD-dependent glycerol dehydrogenase, which has been annotated in its genome (Mongodin et al. 2005). However, we earlier reported that we could not detect significant NAD-dependent glycerol dehydrogenase activity in lysates of *Salinibacter* cells grown in the presence or in the absence of glycerol, and that glycerol primarily entered the metabolism through an inductive glycerol kinase (Sher et al. 2004).

The data presented by Sher et al. (2004) suggest that the overflow product formed from glycerol, now identified as DHA, can be slowly taken up by *Salinibacter* after glycerol had been depleted from the medium. Fig. 2c, however, shows no sign of such uptake within the first 2–3 days after the glycerol depletion. On the other hand, the experiment shown in Fig. 3a demonstrates that *Salinibacter*, when supplied with relatively high concentrations of DHA, is capable of rapid uptake. The factors that determine the rate of use of DHA by *Salinibacter* therefore deserve a more in-depth study.

DHA can now be added to the list of overflow products formed by extremely halophilic heterotrophic microorganisms during incomplete oxidation of carbon sources. Certain halophilic *Archaea* of the family *Halobacteriaceae* convert glycerol in part to acetate, pyruvate, and D-lactate (Oren and Gurevich 1994). Production of these acids during sugar metabolism by *Halorubrum saccharovorum* was documented first in the 1970s (Tomlinson and Hochstein 1972; Tomlinson et al. 1974), and the phenomenon appears to be widespread, especially in the genera *Haloferax* and *Haloarcula* (Oren and Gurevich 1994). It is noteworthy that pyruvate is a key component in the growth media used for cultivation of *Haloquadratum* (Bolhuis et al. 2004; Burns et al. 2004). Excretion of these acidic overflow products during glycerol metabolism by halophilic *Archaea* is not restricted to situations in which the cells are flooded with exceedingly high substrate concentrations: formation of acetate and lactate was also found when radiolabeled glycerol was added in micromolar concentrations to saltern crystallizer brines (Oren and Gurevich 1994). It is not clear why so many extreme halophiles, *Archaea* as well as *Bacteria*, incompletely oxidize organic substrates. It is tempting to speculate that the low solubility of oxygen in salt-saturated brines may be one of the causes of the phenomenon.

The possibility that DHA may be derived from the metabolism of the alga *Dunaliella* also deserves to be examined. DHA is an intermediate in the "glycerol cycle" used for the biosynthesis and degradation of its osmotic solute glycerol. Glycerol is synthesized by the reduction of dihydroxyacetone phosphate to glycerol phosphate, which is then dephosphorylated to glycerol; excess glycerol can be returned to the cellular metabolism by its oxidation to DHA and subsequent phosphorylation to dihydroxyacetone phosphate. The enzymes producing and degrading DHA are located in the cytoplasm rather than in the chloroplast (Brown et al. 1982; Gimmler and Lotter 1982). To what extent the cytoplasmic membrane of *Dunaliella* is permeable to DHA is unknown, but the possibility should be taken into account that this compound may leak out of the cells, especially when excess glycerol has to be returned to the cell metabolism after osmotic downshock or when for other reasons, more glycerol has been produced than necessary.

The experiments presented here show that DHA is a substrate of potential interest in the saltern ecosystem and in other hypersaline environments, with *Salinibacter* and potentially also *Dunaliella* as producers of the compound, and different heterotrophs including *Haloquadratum* and *Salinibacter* being able to take it up. The data presented do not provide proof that DHA is indeed produced and turned over in the salterns to a significant extent. Because of the sensitivity limitations of the analytical methods employed, we used much higher concentrations of DHA and other nutrients in our experiments than might be expected to occur in the natural environment. Whether DHA is indeed important in the food chain and the interspecies relationships in hypersaline environments remains therefore to be ascertained.

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References

Antón J, Rosselló-Mora R, Rodríguez-Valera F, Amann R (2000) Extremely halophilic *Bacteria* in crystallizer ponds from solar salterns. Appl Environ Microbiol 66:3052–3057

Antón J, Oren A, Benlloch S, Rodríguez-Valera F, Amann R, Rosselló-Mora R (2002) *Salinibacter ruber* gen. nov., sp. nov., a novel extreme halophilic member of the Bacteria from saltern crystallizer ponds. Int J Syst Evol Microbiol 52:485–491

Ben-Amotz A, Avron M (1978) On the mechanism of osmoregulation in *Dunaliella*. In: Caplan SR, Ginzburg M (eds) Energetics and structure of halophilic microorganisms. Elsevier—North Holland Biomedical, Amsterdam, pp 529–541

Bolhuis H, te Poele EM, Rodríguez-Valera F (2004) Isolation and cultivation of Walsby's square archaeon. Environ Microbiol 6:1287–1291

Bolhuis H, Palm P, Wende A, Farb M, Rampp M, Rodriguez-Valera F, Pfeiffer F, Oesterhelt D (2006) The genome of the square

archaeon "*Haloquadratum walsbyi*": life at the limits of water activity. BMC Genomics 7:169

Brown AD, Lilley RM, Marengo T (1982) Osmoregulation in *Dunaliella*. Intracellular distribution of enzymes of glycerol metabolism. Z Naturforsch 37:1115–1123

Burns DG, Camakaris HM, Janssen PH, Dyall-Smith ML (2004) Cultivation of Walsby's square haloarchaeon. FEMS Microbiol Lett 238:469–473

Burns DG, Janssen PH, Itoh T, Kamekura M, Li Z, Jensen G, Rodríguez-Valera FE, Bolhuis H, Dyall-Smith ML (2007) *Haloquadratum walsbyi* gen. nov., sp. nov., the square haloarchaeon of Walsby, isolated from saltern crystallizers in Australia and Spain. Int J Syst Evol Microbiol 57:387–392

Burton RM (1955) Glycerol dehydrogenase from *Aerobacter aerogenes*. Meth Enzymol 1:397–400

Burton RM (1957) The determination of glycerol and dihydroxyacetone. Meth Enzymol 3:246–249

Elevi Bardavid R, Ionescu D, Oren A, Rainey FA, Hollen BJ, Bagaley DR, Small AM, McKay CM (2007a) Selective enrichment, isolation, and molecular detection of *Salinibacter* and related extremely halophilic *Bacteria* from hypersaline environments. Hydrobiologia 576:3–13

Elevi Bardavid R, Khristo P, Oren A (2007b) Interrelationships between *Dunaliella* and halophilic prokaryotes in saltern crystallizer ponds. Extremophiles, published online 22nd December 2006; DOI 10.1007/s00792-006-0053-y

Gimmler H, Lotter G (1982) The intracellular distribution of enzymes of the glycerol cycle in the unicellular alga *Dunaliella parva*. Z Naturforsch 37:1107–1114

Green SR, Whalen EA, Molokie E (2004) Dihydroxyacetone: production and uses. J Biochem Microbiol Technol Eng 3:351–355

Hekmat D, Bauer R, Fricke J (2003) Optimization of the microbial synthesis of dihydroxyacetone from glycerol with *Gluconobacter oxydans*. Bioprocess Biosyst Eng 26:109–116

Legault BA, Lopez-Lopez A, Alba-Casado JC, Doolittle WF, Bolhuis H, Rodríguez-Valera F, Papke RT (2006) Environmental genomics of "*Haloquadratum walsbyi*" in a saltern crystallizer indicates a large pool of accessory genes in an otherwise coherent species. BMC Genomics 7:171

Mongodin EF, Nelson KE, Daugherty S, DeBoy RT, Wister J, Khouri H, Weidman J, Walsh DA, Papke RT, Sanchez Perez G, Sharma AK, Nesbø CL, MacLeod D, Bapteste E, Doolittle WF, Charlebois RL, Legault B, Rodríguez-Valera F (2005) The genome of *Salinibacter ruber*: convergence and gene exchange among hyperhalophilic bacteria and archaea. Proc Natl Acad Sci USA 102:18147–18152

Oren A (1990) Estimation of the contribution of halobacteria to the bacterial biomass and activity in solar salterns by the use of bile salts. FEMS Microbiol Ecol 73:41–48

Oren A (1993) Availability, uptake, and turnover of glycerol in hypersaline environments. FEMS Microbiol Ecol 12:15–23

Oren A, Gurevich P (1994) Production of D-lactate, acetate, and pyruvate from glycerol in communities of halophilic archaea in the Dead Sea and in saltern crystallizer ponds. FEMS Microbiol Ecol 14:147–156

Oren A, Rodríguez-Valera F (2001) The contribution of *Salinibacter* species to the red coloration of saltern crystallizer ponds. FEMS Microbiol Ecol 36:123–130

Oren A, Duker S, Ritter S (1996) The polar lipid composition of Walsby's square bacterium. FEMS Microbiol Lett 138:135–140

Prust C, Hoffmeister M, Liesegang H, Wiezer A, Fricke WF, Ehrenreich A, Gottschalk G, Deppenmeier U (2005) Complete genome sequence of the acetic acid bacterium *Gluconobacter oxydans*. Nature Biotechnol 23:195–200

Rosselló-Mora R, Lee N, Antón J, Wagner M (2003) Substrate uptake in extremely halophilic microbial communities revealed by microautoradiography and fluorescence in situ hybridization. Extremophiles 7:409–413

Sher J, Elevi R, Mana L, Oren A (2004) Glycerol metabolism in the extremely halophilic bacterium *Salinibacter ruber*. FEMS Microbiol Lett 232:211–215

Tomlinson GA, Hochstein LI (1972) Studies on acid production during carbohydrate metabolism by extremely halophilic bacteria. Can J Microbiol 18:1973–1976

Tomlinson GA, Koch TK, Hochstein LI (1974) The metabolism of carbohydrates by extremely halophilic bacteria: glucose metabolism via a modified Entner Doudoroff pathway. Can J Microbiol 20:1085–1091

Widdel F, Kohring G-W, Mayer F (1983) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov. Arch Microbiol 134:286–294