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Heterotrophic activities and estimation of bacterial growth rates during a bloom of halobacteria in the Dead Sea

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Abstract

The activity of the community of heterotrophic bacteria in the Dead Sea water column was measured during a bloom of halobacteria in 1980 - 1981. With the development of the bloom, rates of incorporation of amino acids, glycerol and acetate increased. Concomitant with the decline in bacterial numbers, the incorporation rate of amino acids and acetate decreased, while glycerol uptake rates remained high. The thymidine incorporation rate was low even at the peak of the bloom, and became negligible afterwards. The results presented suggest that the bacterial community in the Dead Sea is not subjected to rapid turnover, and may remain static in a state of low activity for prolonged periods.

Introduction

A systematic survey of bacterial community densities in the Dead Sea (salinity around 340 g/l) from 1980 onward (OREN 1983, 1985, 1989) has shown extensive fluctuations in bacterial numbers. A dense bloom of halobacteria (up to 2×10^7 /ml^{-1}) was observed in summer 1980, following a dilution of the upper 10 - 20 m of the water column by winter rain floods, and the development of a bloom of the green algae *Dunaliella*, the main primary producer in the Dead Sea (OREN and SHILO 1982). The algal bloom declined, and from the beginning of 1981 onward the size of the bacterial community remained approximately constant for almost two years. Measurements of phosphate uptake (OREN 1983) suggested an extremely slow phosphate turnover during this period, at a time when phosphate was a limiting nutrient. This result suggested that the Dead Sea halobacteria were present in a state of low activity. With the end of the stratification in December 1982 the bacterial density declined sharply (OREN 1985), and has remained low since, as conditions have not been favorable for *Dunaliella* development (OREN 1989).

To obtain further information on the metabolic state of the bacterial community in the Dead Sea during the halobacterial bloom and after, I performed measurements of incorporation of different radiolabeled substrates, as a probe for heterotrophic activity.

Material and methods

Water samples were collected at different locations in the western half of the northern basin of the Dead Sea. Most samples were taken about 8 km east of Ein Gedi or 3 km off the western shore near Massada. Samples from different depths were collected with Nansen bottles or pumped through a hose.

Subsamples (10 ml) were incubated in the dark at 25 °C in 15-ml screw-capped tubes with labeled substrates as specified below. The incubation time was between 11 and 18 h, and was chosen so that no more than 10 % of the added radiolabel was taken up, and that incorporation increased linearly with time. As far as possible, incorporation rates of different radiolabeled compounds were measured in the presence of saturating concentrations of the compound tested, so that heterotrophic potentials, rather than *in situ* rates were determined. The following labeled substrates were used: (1) 5 µl [U-^{14}C]glycerol (Amersham), yielding a final concentration of 7.8 µM; (2) 10 µl Na-[1-^{14}C]acetate (Amersham), yielding a final concentration of 1.7 µM; (3) 10 µl [U-^{14}C]L-amino acids mixture (New England Nuclear, 100 µCi/ml, 100-450 mCi/mmol of each of 15 amino acids); (4) 5 µl [2-^{14}C]thymidine (Amersham), yielding a final concentration of 0.45 µM. At the end of the incubation the contents of the tubes were filtered through 2 layers of glass fiber filter (Whatman GF/C). The filters were washed thrice with 20 ml cold 1 M HCl in 90 % Dead Sea water, dried, and counted by liquid scintillation counting. In a number of experiments 0.45 µm pore size Millipore filters were used, and/or filters were washed with cold 10 % trichloroacetic acid; results obtained using the different filtration and washing procedures did not differ significantly. Doubling times of the bacterial community were estimated from thymidine incorporation rates, based on the assumptions that: (1) the bacteria present take up thymidine (OREN 1990a); (2) thymidine was added at saturating concentration; (3) 2.1×10^{18} cells are produced/mol of thymidine in purified DNA of 50 % guanine + cytosine content (RIEMANN et al. 1982); (4) 50 % of the thymidine label is found in DNA (no attempt was made to purify the DNA fraction); (5) the bacteria have a normal genome size with 66 % guanine + cytosine.

Results and discussion

A bloom of the unicellular green algae *Dunaliella* enabled the development of a dense community of halobacteria in the summer of 1980 (Fig. 1), with numbers of particles microscopically recognizable as bacteria reaching values of up to 2×10^7/ml, decreasing to about 6×10^6/ml, and remaining constant at this level for almost two years.

Figure 2 shows the result of monitoring heterotrophic potentials in the upper 10 m of the water column during the 1980 summer bloom of halobacteria, and the following year. Concomitantly with the increase in bacterial numbers the incorporation rates of glycerol, amino acids and acetate increased dramatically (Fig. 1). The decrease in the rates in the end of 1980 and in 1981 can be explained by a drop in bacterial numbers and the increase in salinity of the upper water layers, causing a decrease in activity of the bacteria that live in the Dead Sea at supraoptimal salt concentrations. As (due to too high salinities) no *Dunaliella* bloom developed in 1981, bacterial numbers did not increase again in summer 1981, and no peak in acetate and amino acid incorporation was observed. For comparison the incorporation of the same compounds was measured again in

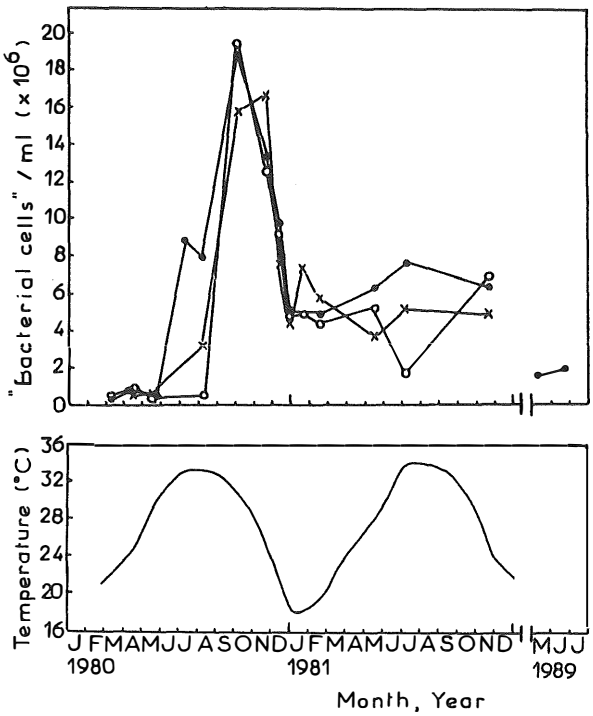

Fig. 1. Bacterial numbers in the Dead Sea during 1980 - 1981 and in the beginning of 1989, at the surface (●), and at 10 m (x) and 15 m (o) depth, as estimated by microscopic enumeration. Data were derived in part from OREN (1983). The lower part of the figure shows the mean water temperature above the pycnocline (derived in part from OREN and SHILO 1982).

June 1989, during a period in which the bacterial community was very small (OREN 1989), and as expected the rates obtained were low.

In the present study only incorporation of ^{14}C labeled substrate, but no respiration was measured, and thus the rates presented underestimate total metabolism of the substrates tested. In a recent study (FENDRICH and SCHINK 1988) the metabolism of ^{14}C-labeled glycerol, acetate, and glucose was measured in the brines (22 % salinity) of the Great Salt Lake (Utah). Both the $^{14}CO_2$ evolved, and the radioactivity left in solution after acidification and centrifugation were determined, enabling the calculation of the extent of incorporation, which turned out to be around 30 % of the amount of substrate metabolized. Using this conversion factor, the incorporation rates measured were: glycerol, about 16 nmol/lxh (compare Dead Sea: up to 100), and acetate, about 5.4 nmol/lxh (Dead Sea: up to 3.8). In NaCl-saturated saltern ponds (about 1.5 x 10^7 bacteria/ml) amino acid incorporation rates of around 6 nmol/lxh were measured (at 35 °C, compare 1.5 nmol/lxh in the Dead Sea at 25 °C) (OREN 1990b).

The rate of glycerol incorporation in Dead Sea surface water followed a pattern different from that observed with acetate and amino acids (Fig. 3), and maximal rates were observed in 1981, when both total bacterial numbers and acetate and

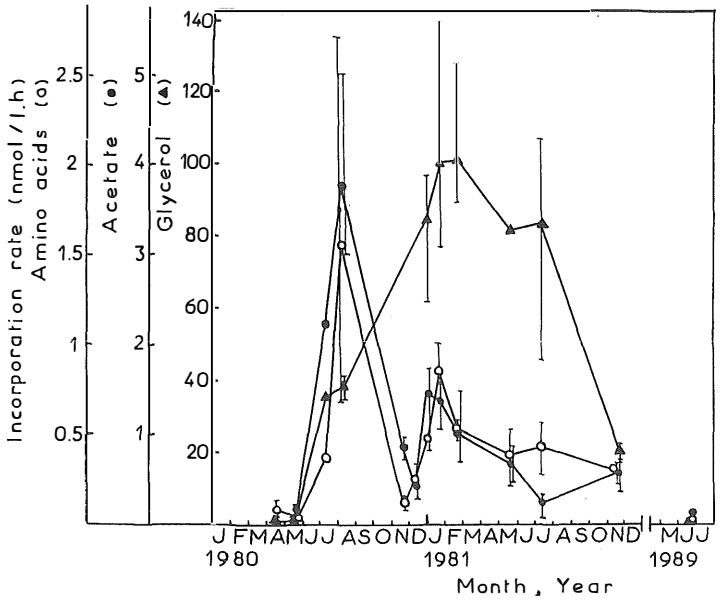

Fig. 2. Incorporation of radiolabeled amino acids (o), acetate (●) and glycerol (▲) in the upper 10 m of the Dead Sea water column, 1980 - 1981, and beginning of 1989. Mean values of the incorporation rates at 25 °C are plotted, together with the range of values measured.

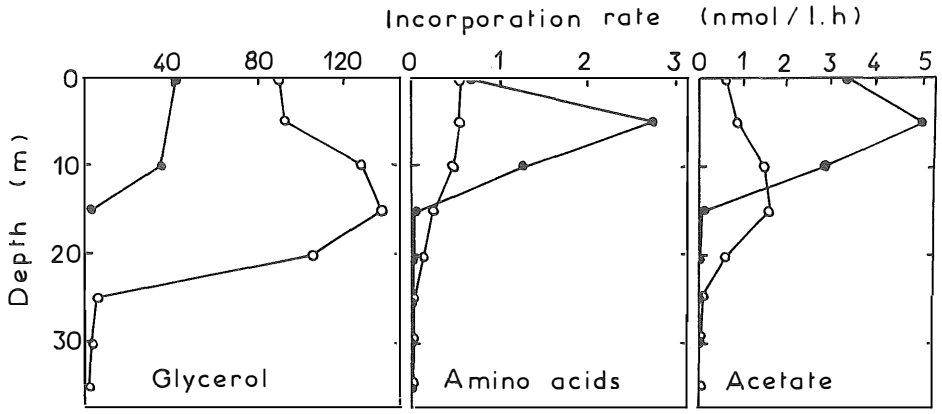

Fig. 3. Vertical distribution of heterotrophic activity, as measured by incorporation of glycerol, amino acids and acetate at 25 °C on August 7, 1980 (●), and February 25, 1981 (o).

amino acid incorporation were relatively low. This observation can only be explained by qualitative changes in the bacterial community. Though no direct evi-

dence is available, it can be speculated that in an environment in which primary producers were lacking, purple membrane-containing bacteria (such as *Halobacterium sodomense)* may have become dominant, due to their ability to use light energy for maintenance purposes (OREN 1983).

The vertical profiles of heterotrophic activities closely reflected the stratification pattern of the water column (OREN 1983, OREN and SHILO 1982): in August 1980 a pycnocline was found at a depth of about 10 m, which had reached a depth of 20-25 m in February 1981. Activity was high (though not completely uniform) in all depths above the pycnocline. Below the pycnocline uptake rates were very low (as were bacterial numbers) (OREN 1983).

Thymidine incorporation was measured in some samples at the height of the 1980 bloom and during its decline. On the basis of the rates measured, the assumptions specified above, and the bacterial numbers (Fig. 1), growth rates were estimated. Doubling times calculated were between 2 and 10 days during the bloom, and 50 - 100 days and more during the decline period. It is thus suggested that during the period following the bloom the bacterial community existed in a state of little activity.

It should be noted here that more satisfactory techniques for measuring bacterial growth than used in this study were developed only after the completion of this work (FUHRMAN and AZAM 1982, RIEMANN et al. 1982). It should also be stated here that [2-^{14}C]thymidine was used, and not [methyl-^{3}H]thymidine, which would have allowed a higher specificity of the labeling.

Even if this taken into account, our results do suggest that the bacterial community remaining after the peak of the bloom existed in a state of very slow growth. A similar conclusion was obtained earlier, based on the study of phosphate turnover (OREN 1983). Protozoa or other secondary producers are absent (or at least do not play a significant role) in the Dead Sea ecosystem. Thus, all evidence leads to the conclusion that the bacterial community in the Dead Sea does not turnover rapidly, and (with the exception of short periods of activity when conditions become favorable) exists almost in a state of static equilibrium, in which cells are viable, but growth is extremely slow.

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