CHEMOAUTOTROPHIC PRODUCTION IN A THERMO-MINERAL SULFUROUS CAVE

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Samples from the microbiota living in the "Peştera de la Movile" Cave, southern Dobrogea, Romania, were incubated with ^{14}C labeled CO_2 in order to determine if they were chemoautotrophic, using H_2S as a source of energy. The results of this experiment are presented.

INTRODUCTION

A very rich microbial community was recently discovered in the "Peştera de la Movile" Cave[1] in southern Dobrogea, Romania (Sârbu, 1990). Thick microbial mats cover the water surface and the limestone walls of some air bells in the submerged cave passage. The lower level of this cave is flooded by a thermomineral stream that contains large concentrations of H_2S (7—25 mg/l). Small populations of bacteria are also present in the water column (Popa and Sârbu, 1991).

Preliminary investigations suggested that the microbes living in the cave are chemoautotrophic using H_2S as a source of energy (Sârbu, 1991). This could explain the richness of the aquatic and terrestrial troglobitic communities in a cave with no apparent input of food from the surface, and the presence of a fauna with very little similarities to epigeean forms (Lascu, 1989).

The purpose of this study was to determine if carbon fixation takes place in the cave's microbiota. Future investigations will focus on the ecophysiology of this microbial community, in an effort to characterize the ecosystem associated with the Dobrogean thermomineral sulfurous waters.

MATERIAL AND METHODS

Floating microbial mats were collected in December 1990 from an air bell in the lower level of the Movile Cave. The mats were homogenized thoroughly and mixed with sulfurous water from the underground stream. Samples of 12 ml of the resulting broth were taken up in 5 ml plastic syringes, every sample containing approx. 69 mg biomass. Eighty µl of

[1] The "Peştera de la Movile" Cave was discovered by an artificial shaft dug near the town of Mangalia (Constantinescu, 1989). It is developed in Sarmatian (13 milion years old) limestones and it has no natural entrance. Very unusual faunas, both terrestrial and aquatic have been discovered in this cave (Boghean, 1989; Georgescu, 1989; Grossu and Negrea, 1989; Gruia, 1989; Negoescu, 1989; Pleşa, 1989; Tabacaru and Boghean, 1989; Sârbu, 1990; 1991; Popa and Sârbu, 1991). Preliminary studies suggest that terrestrial troglobites colonized the cave at the end of the Miocene (5.2 milion years ago) (Lascu, 1989). The aquatic fauna invaded the cave more recently (Sârbu, 1991).

^{14}C labeled-Na bicarbonate solution were added, representing 0.7207 μ Ci/ml of sample. The incubation was done in the cave at 20.50° C in total darkness for 0, 0.5, 2, 4, and 12 hours.

Ten replicates were used for each treatment. After the incubation, total lipids were extracted with chloroform — methanol (B l i g h and D y e r, 1959). The amount of labeled CO_2 fixed in the microbes was determined by counting the number of DPM in the total lipids extracted from the incubated samples by scintillation spectrometry (M c K i n l e y et al., 1982). The microbial biomass was determined by analysis of the phospholipid component of the extracted lipids (F i n d l a y et al., 1989). The ratio between the radioactive CO_2 incorporated in the microbial lipids and the biomass was used to characterize the chemoautotrophic microbial activity.

RESULTS AND DISCUSSION

The large number of replicates offered a rich set of data to be interpreted. The results of our investigations indicated a significant correlation between the increase of fixed labeled CO_2 and the length of incubation: from a mean value of 0.216 DPM/nmol of lipid phosphate for an incubation time of zero minutes, the radioactivity increases to a mean value of 4.264 DPM/nmol of lipid phosphate after an incubation time of 4 hours. Figure 1 presents the mean values of the ten replicates for each treatment. The error bars represent one standard deviation.

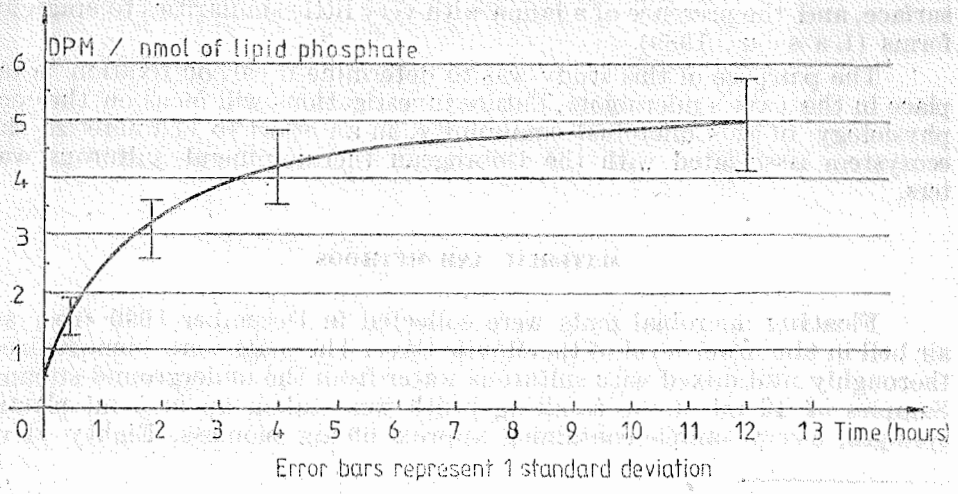

Fig. 1

The rate of CO_2 uptake was high at the beginning of the experiment but decreased with time. A possible explanation for this may be the depletion in the energy source of the chemosynthetic reactions (i.e., H_2S) or an autoinhibition in the incubation syringes. Future experiments will be designed to offer the microbes a constant energy source throughout the incubation.

CONCLUSION

Carbon fixation takes place in the microbiota discovered in the lower level of the Movile Cave. The chemoautotrophic microbes probably use H_2S as a source of energy. The presence of this "in situ" source of organic carbon (food) explains the existence of the very rich and unusual aquatic and terrestrial faunas discovered in this cave. The "Peştera de la Movile" Cave contains the first known chemoautotrophically based subterranean ecosystem.

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