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Synthesis of a Species-Defined Microcosm with Protozoa

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ABSTRACT

A species-defined microcosm, consisting of bacteria *Escherichia coli* DH5a, protozoa *Tetrahymena thermophila* B and algae *Euglena gracilis* Z, was synthesized in which all species co-existed for over 130 days. This microcosm was developed as an experimental system to study gene-population interaction in a community.

INTRODUCTION

Protozoa play several important roles in ecosystems, such as regulating bacterial populations by grazing, serving as a source for metazoa (e. g., Porter et al. 1985), and inducing morphological changes of bacteria under protozoan predation (Shikano et al. 1990). Combining these studies suggests the necessity of clarifying the ecological role of protozoa in a community through different interaction levels (e. g., gene-population). For this study, microcosms as model ecosystems (Odum 1969) are useful as an experimental tool. It is important to develop a microcosm in which all components are defined and its maintenance is clarified so that replicability is insured. Organisms which have been analyzed genetically are preferable. Other requirement for analyzing a microcosm are as follows: initial abiotic components should all be known in order to evaluate the changes in the cultural environment, the use of sub-systems to isolate and analyze the interactions of each species and the medium, and the various

combinations of species in order to learn the development mechanisms of a community.

The purpose of this study is to synthesize a species-defined aquatic microcosm, satisfying the conditions required above, in which all species can co-exist for an extended period of time.

MATERIALS AND METHODS

The biotic components of the microcosm were compiled by inoculating bacteria *Escherichia coli* DH5a as a decomposer, protozoa *Tetrahymena thermophila* B as a consumer and algae *Euglena gracilis* Z as a producer into a culture medium. The organisms were chosen from microbes which satisfy the following conditions, 1) axenic, 2) extensively, genetically and physiologically studied, 3) observed commonly in aquatic systems, and 4) except for bacteria, morphologically identifiable under the microscope for quantifying. Ten ml of a half strength of #36 Taub and Doller solution (1/2 #36 solution) (Taub and Doller 1968) without $NaNO_3$ containing a concentration of proteose peptone (Difco laboratory, USA), as a culture medium, was transferred into a test tube with a screw cap. Then the test tube was autoclaved. The organisms were inoculated aseptically into this medium. The prepared concentrations of proteose peptone were 0.0, 10, 50, 100, 500 and 1,000 mg per lL of 1/2 #36 solution.

Test tubes containing culture media and organisms were placed in an incubator with fluorescent lamps under a 2,500 lux and 12-12 LD light regime at 25° C. The test tubes were kept stationary, and were sacrificed on each sampling day.

In order to find the quantity of viable *E. coli*, 0.5 ml samples of the test tube media were transferred to petridishes which were incubated at 25° C for 5 days. The resulting colonies on the broth-agar medium (extract bonito: 3.0 g, polypeptone: 3.0 g, NaCl: 5.0 g, agar powder: 15.0 g, distilled water: 11, pH adjusted to 7.0) were counted. The counting of colonies was carried out for three petridishes and the mean value of these counts was used for the number of *E. coli* per ml.

After shaking well, a one ml sample from one test tube was transferred into a 1 mm deep counting chamber of 50x20 mm^2 meshed with 1 mm^2. All the cells of *T. thermophila* in ten randomly chosen meshes were counted microscopically. Counting, repeated 5 times in this way using the same sample, gave 2.5% of a coefficient deviation of $s/x \times 100$ (s: standard deviation of the sample, x: mean value of sample). *Eu. gracilis* was also counted the same way as *T. thermophila*.

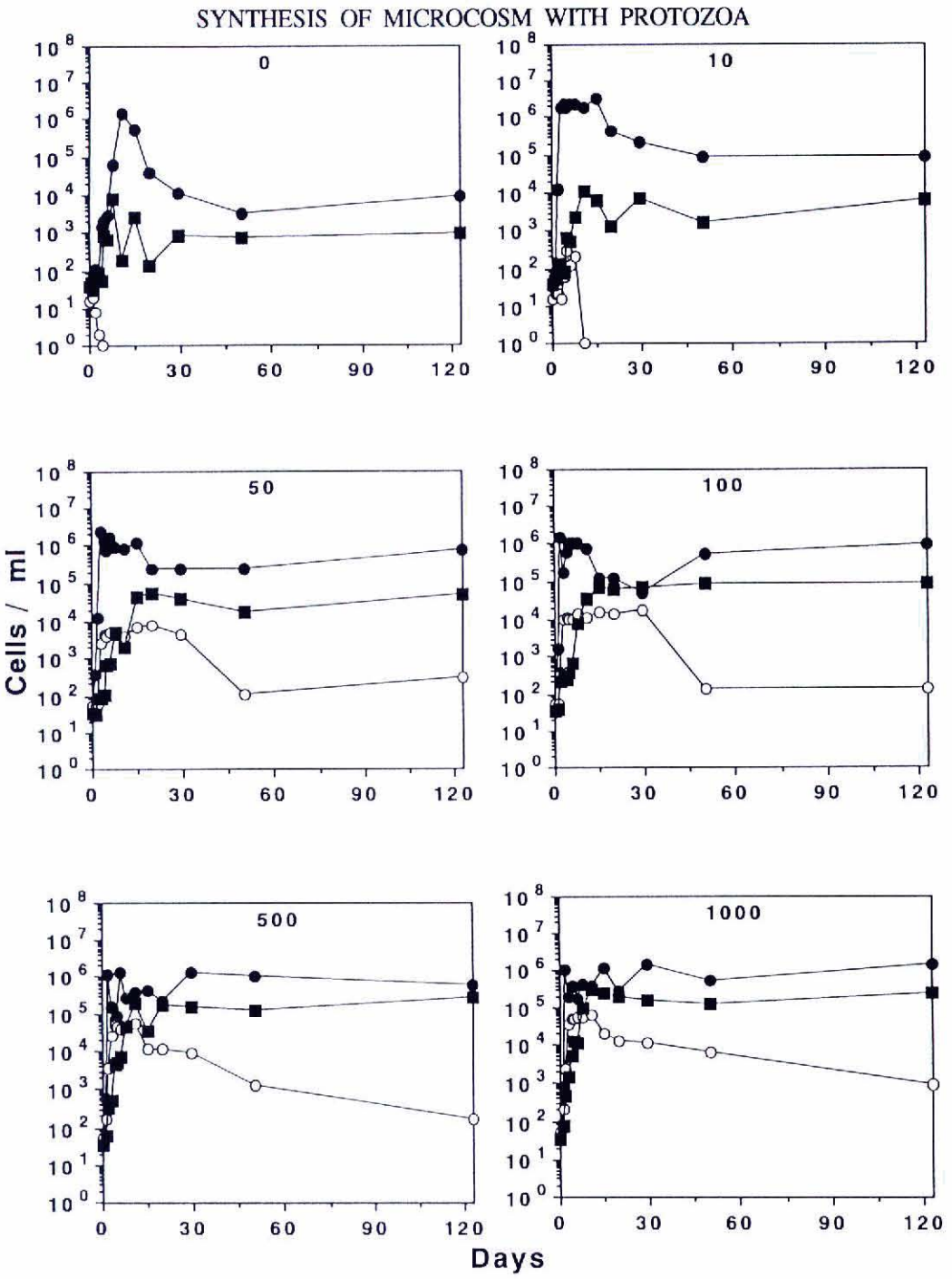

Figure 1. Population changes of each organism (● *E. coli*, ○ *T. thermophila*, ■ *Eu. gracilis*). The numbers in the graph indicate the concentrations of proteose peptone (mg/L).

RESULTS AND DISCUSSION

The population changes of each organism in the mixed culture of the selected three species in the salt solution media containing each concen-

tration of proteose peptone are shown in Fig. 1. Co-existence of the three species was attained for over 130 days in the medium containing more than 50 mg of proteose peptone per 1L of 1/2 #36 solution. Population changes reached a steady state after 50 days. It was considered that in the medium, either with or without 10 mg of polypeptone, the amount of *E. coli* was not enough for *T. thermophila* to grow.

In microbial ecology it has become necessary to develop a microcosm which provides the same experimental system "type community" (Kawabata, 1990), corresponding to a type species in the field of systematics, for those who intend to carry out experiments on a similar system from different points of view.

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