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Biological Factors Involved in Horizontal Gene Transfer between Bacteria in a Model Aquatic Ecosystem

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I have an ecological view point that biological interactions are involved in horizontal gene transfer as well as matter cycling and energy transfer in ecosystems. To clarify how and how much the biological interactions relate to horizontal gene transfer is essential for understanding the evolution process and ecosystem functioning, as well as for evaluation of possible consequences of the deliberate environmental release of recombinants. In this lecture, I will show the involvement of biological factors affecting horizontal gene flow between differentbacterial species by natural transformation in model aquatic ecosystems.

Outline of Experiments, Results and Discussion

1) Production of dissolved DNA

Dissolved DNA may serve as genetic resources to express genetic information. A gnotobiotic aquatic microcosm, consisting of three species, *Escherichia coli*, (bacteria), *Euglena gracilis* (Euglenophyta) and *Tetrahymena thermophila* (protozoa), in which all species co-existed for over 130 days, was established. Studies on the changes in concentration dissolved DNA in the microcosm revealed that the preypredatory relationship bewtween *E. coli* and *T. thermophila* was responsible for the increase of dissolved DNA. Extrapolation was undertaken from this finding to a hypereutrophic pond by ascertaining if consumption of bacteria by ciliates does contribute to the release of extracellular DNA. Changes in concentration of dissolved DNA in the pond correlated well with abundance of ciliates, rotifers and ciliate community ingestion rate. These results suggest that consumption of bacteria by ciliates is an important reason for the release of dissolved DNA. Comparison of the concentrations of dissolved DNA in the subsystems of microcosm suggested that *E. gracilis* also affected the release of DNA from *E. coli*. This finding led to a hypothesis that algae contribute to production of dissolved DNA. We studied the effects of cocultivation with either *E. gracilis*, *Microcystis aeruginosa* (Cyanophyta), *Chlamydomonas neglecta* (Chlorophyta), or *Carteria inversa* (Chlorophyta) on the production of extracellular plasmid DNA by *E. coli*LE392(pKZ105). Dot blot hybridization analysis showed a significant release of plasmid DNA cocultivation with all the algae tested. Further analysis by electrotransformation confirmed the release of transformable plasmid DNA by cocultivation with either *E. gracilis*, *M. aeruginosa*, or *C. inversa*. These results suggested algal involvement in bacterial horizontal gene transfer by stimulating the release of transformable DNA into aquatic environments.

2) Persistence of dissolved DNA

Dissolved DNA remaining in aquatic environments would serve as a reservoir of extracellular gene sequences for other microorganisms, via transformation. Several studies demonstrated that seeded, radiolabeled extracellular DNA was hydrolyzed and very rapidly incorporated into hetrotrophic microorganisms. Most of these studies of DNA turnover have evaluated only the surface water layers. Thus, dissolved DNA might have different turnover characteristics in different layers of thermally stratified lake environments. The fate of the exogeneous plasmid DNA, pEGFP, was traced in lake water collected from the epilimnion and the hypolimnion during a period of marked thermal stratification. Both gel electrophoresis analysis and dot-blot hybridization analysis demonstrated that the seeded pEGFP was completely degraded in epilimnion water within 170 hours of incubation, while no pEGFP degradation was observed in hypolimnion water. These results suggested the possibility dissolved DNA is less nutritionally important in the hypolimnion. Transformability of the pEGFP was not altered in hypolimnion water during the incubation, which suggested stability of genetic information that gradually decreased in epilimnion water. Dissolved DNA is commonly observed in aquatic environments and is thought to be important nutritional source for microorganisms. However, our results suggest that dissolved DNA in the hypolimnion environment might be important as a genetic pool for further natural transformation.

3) Natural transformation

Tranfromation was studied in a set of experimental tanks named Aquatron. The Aquatron consists of replicated, 70 litters stainless steel tanks in which the inflow and outflow of culture medium can be manipulated separately as can the water temperature gradient, light intensity, and aeration conditions. A boom of *M. aeruginosa* was formed artificially from a pond water containing natural community in triplicate tanks and no bloom in the other triplicate tanks. Fluorescent antibody method and FISH targeted to pT7GFP showed that *E. coli* (pT7GFP) transformed indigenous bacteria with a frequency of 0.1 to 0.8% in the tanks both with and without *M. aeruginosa* bloom.

The involvement of microbial interactions in natural transformation of bacteria was evaluated using an aquatic model systems. For this purpose, the naturally transformable *Bacillus subtilis* was used as the model bacterium which was co-cultivated with the protist *T. thermophila* (a consumer) and/or the photosynthetic alga *E. gracilis* (a producer). Co-cultivation with as few as 10E02 individuals/mL of *T. themophila*lowered the number of transformants to less than the detectable level (less than 1 cell/mL) while co-cultivation with *E. gracilis* did not. Metabolites of *T. thermophila* and *B. subtilis*also decreased the number of transformnats to less than the detectable level, while metabolites from co-culture of *T. thermophila* and *B. subtilis* with *E. gracilis*did not. Thus, the introduction of transformation inhibitory factor(s) by the grazing of *T. thermophila* and the attenuation of this inhibitory factor(s) by *E. gracilis* is indicated. These observations suggest that biological components do affect the natural transformation of *B. subtilis*. The study described is the first to suggest that ecological interactions are responsible not only for the carbon and energy cycles but also for the processes governing horizontal transfer of genes, in microbial ecosystems.

Conclusion

A series of experiments using model aquatic ecosystems suggested that biological factors were involved in horizontal gene transfer between bacteria in natural aquatic ecosystems. By combining your knowledge and ideas, our experimental results, and the references, Iwould like to discuss the possibility of initiative for the gene ecology as a new field of science.

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