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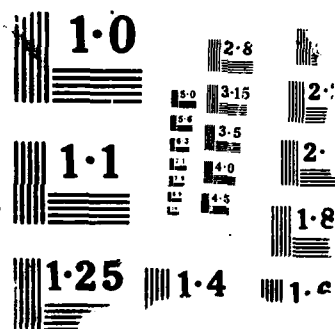
PHYLOGENETIC ANALYSIS OF MARINE PICOPLANKTON USING  
RIBOSOMAL RNA SEQUENCES (U) INDIANA UNIV AT BLOOMINGTON  
DEPT OF BIOLOGY 20 JUN 88 N00014-87-K-0813

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18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Marine picoplankton/16S rRNA phylogeny/recombinant DNA/nucleic acid hybridization/nucleotide sequence analysis.

19. ABSTRACT (Continue on reverse if necessary and identify by block number) The purpose of the program is to analyze phylogenetically the dominant constituents of marine picoplankton in selected Atlantic and Pacific Oceans oligotrophic sites using 16S ribosomal RNA gene cloning and sequencing procedures. Additional goals are the development of rRNA-based hybridization probes for the phylogenetic analysis of single cells and the continued accumulation of 16S rRNA reference sequences. Recent efforts have resulted in the establishment of a recombinant DNA library of Sargasso Sea picoplankton, the development of fluorescence-based, phylogenetic group-specific hybridization probes, and the completion of a comprehensive phylogenetic survey of cultivated cyanobacteria.

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Progress Report Contract N00014-87-K-0813

Principal Investigator: Norman R. Pace

Title: Phylogenetic Analysis of Marine Picoplankton Using Ribosomal RNA Sequencing

Project Goals:

The main goal of the program is the phylogenetic characterization of the dominant constituents among marine picoplankton using 16S ribosomal RNA (rRNA) gene cloning and sequencing. The analysis uses DNA extracted from the natural population, so it sidesteps the necessity of cultivating resident lifeforms. A second goal is the development of rRNA-based oligonucleotide hybridization probes suitable for the phylogenetic analysis of single cells, microscopically. A third goal is the continued accumulation of 16S rRNA sequences from cultivated organisms that are potentially related to picoplankton, in order to provide a reference data base of sequences for phylogenetic analyses.

Progress (Year 1):

A tangential flow filtration system employing 0.1 $\mu$ m pore concentration filters and a 10 $\mu$ m pore intake filter was used to collect mixed picoplankton from an oligotrophic site in the Sargasso Sea. 24 hours of pumping yielded several hundred milligrams of biomass. Mixed DNA was isolated and cloned in phage  $\lambda$  (EMBL3). Several rRNA gene-containing clones have been identified, but not yet analyzed (see below). The bulk Sargasso picoplankton DNA also was inspected for general phylogenetic makeup with regard to the primary lineages, eukaryotes, archaeobacteria and eubacteria, using oligonucleotide hybridization probes specific for those groups. Only eubacteria were detected.

Good progress has been made toward devising hybridization probes for the phylogenetic analysis of single cells. These probes are synthetic oligodeoxynucleotides that are complementary to phylogenetic group-specific sequences in the 16S rRNA; hence there is a sufficient number of targets in each cell that probe binding to a single cell can be visualized by fluorescence microscopy. Oligonucleotides are synthesized to contain a 5'-amino group so that fluor isothiocyanates can be coupled to them. We have had good success using fluorescein- and rhodamine-labeled probes that readily distinguish eukaryotes, archaeobacteria and eubacteria from one another in test mixtures; and several species-specific probes have been shown to distinguish their targets from close relatives. We are confident that these "phylogenetic stains" will prove broadly applicable, however, there currently is difficulty in achieving sufficient probe binding to organisms in natural populations, probably because of low ribosome contents (lower growth rates than laboratory cultures). Efforts are underway to improve the fluorescence yield of probes.

A fairly comprehensive, 16S rRNA-based phylogenetic survey of diverse cyanobacteria (29 new sequences) was recently completed in the effort to expand the data base of reference sequences for the picoplankton analysis. Significant new findings are that the cyanobacteria are a relatively young group of eubacteria, that many diverse forms of cyanobacteria diverged within a short span of evolutionary time, and that the green chloroplasts are a subgroup within the cyanobacteria, not a sister lineage as previously was thought. The phylogenetic analysis points out substantial problems with the traditional, morphology-based taxonomy of cyanobacteria.

Work Plan (Year 2):

Analysis of rRNA clones derived from the Sargasso picoplankton collection will continue. Effort will be invested in streamlining the sequence analysis, to avoid extensive subcloning, by using "polymerase chain reaction" to amplify rRNA sequences in rRNA gene-containing clones. Oligonucleotide hybridization probes based on the sequences will be synthesized in order to identify the morphotypes in the population that correspond to the rRNA sequences.

An analysis of oligotrophic Pacific Ocean picoplankton will be initiated. Collections probably will be made at a newly-established station north of Hawaii (ALOHA site).

Further effort will be invested toward increasing the fluorescence yield of single cell, phylogenetic group-specific hybridization probes. Methods will focus on addition of multiple fluorescent groups to the oligonucleotide probes, using chemical couplings (e.g. by attaching multiple aliphatic amino groups to the oligonucleotides) as well as "sandwich" methods (e.g. using biotinylated probes and staining with avidin-fluor conjugates).

Publications:

Giovannoni, S. J., S. Turner, G. J. Olsen, S. Barns, D. J. Lane and N. R. Pace. Evolutionary relationships among cyanobacteria and green chloroplasts. J. Bacteriol., in press.

Turner, S., E. F. DeLong, S. Giovannoni, G. J. Olsen and N. R. Pae. Phylogenetic analysis of microorganisms and natural populations using ribosomal RNA sequences. In "Microbial Mats: Ecological Physiology of Benthic Microbial Communities," Y. Cohen and E. Rosenberg, eds. (ASM, 1988) in press.

Students Supported:

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Thomas M. Schmidt



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