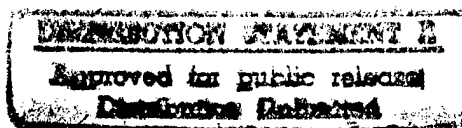


ANNUAL PROGRESS REPORT



GRANT #: N00014-95-1-0606

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GRANT TITLE: The Role of Plasmids in Marine Sediment Bacterial Communities

REPORTING PERIOD: 1 June 1996 - 15 March 1997

AWARD PERIOD: 15 March 1995 - 14 March 1998

OBJECTIVE: To investigate the role of plasmids in determining the structure and functions of marine sediment microbial communities. To use molecular approaches, in addition to classic microbial ecology methods, to determine the distribution and characteristics of naturally occurring plasmids within bacterial populations focusing on assessing the potential for transfer of plasmid-encoded genes within the indigenous microbial community present in marine sediments.

APPROACH: To investigate plasmid distribution and diversity in marine sediment microbial communities bacteria are isolated from coastal California marine environments and characterized with regard to their plasmid content(s). Plasmids are screened using Southern hybridization analysis for homology to ~30 well defined incompatibility groups. Plasmids are also screened for phenotypic traits including antibiotic and heavy metal resistances and for the presence of novel insertion sequences and transposable elements. Microbial community DNA is also analyzed for the presence of plasmid-specific replication and incompatibility determinants from known plasmid groupings.

ACCOMPLISHMENTS: (last 9 months):

During this period we have completed an extensive molecular analysis of more than 300 plasmid-containing heterotrophic bacteria isolated from coastal California salt marsh sediments using incompatibility (*inc*) and replication (*rep*) sequences from ~30 different well defined plasmid groups. The plasmids, ranging in size from 5-kb to more than 250-kb, were isolated from a number of genera including *Vibrio*, *Bacillus*, *Paracoccus*, *Alteromonas* and *Aeromonas*. None of the plasmids share homology to known plasmid groupings indicating that these naturally occurring plasmids contain novel incompatibility and replication (*inc/rep*) control systems. Using the polymerase chain reaction, we did find evidence of a broad-host-range plasmid group, IncP, present at a low level in microbial community DNA isolated from marine sediments. Interestingly, a narrow-host-range group, IncF, thought to be common in enteric bacterial isolates present in sewage contamination was not detected in the community DNA.

We have developed a cesium chloride density gradient centrifugation procedure that enabled us to isolate significant quantities of supercoiled plasmid DNA that has greatly facilitated molecular studies to characterize replication and incompatibility determinants from numerous indigenous plasmids. Concurrent with developing improved marine plasmid DNA isolation procedures, we have also developed a "replicon-rescue" protocol that isolates small (2 to 3-kb) DNA fragments that are replication proficient and suitable for DNA sequencing. Using this approach we have isolated four different broad-host-range plasmid replication fragments that are presently being sequenced. Southern hybridization and preliminary sequence analysis indicates that these replication regions are novel.

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Lastly, we are developing a cell fractionation protocol for isolating supercoiled plasmid DNA from total microbial communities (e.g., culturable and nonculturable bacterial populations). This methodology will aid in the construction of libraries of indigenous plasmids that will facilitate studies aimed at better characterizing plasmids populations and their potential to transfer within naturally occurring marine sediment microbial communities.

SIGNIFICANCE: Little information is known regarding the role of indigenous broad- and narrow-host-range plasmids in promoting gene transfer in marine ecosystems and how plasmid-encoded genes determine the structure and function of bacterial populations. To the best of our knowledge, our attempts to classify plasmids from marine sediment microbial communities using incompatibility and replication regions of well-characterized plasmid groupings are among the first of their kind. Our findings indicate that the culturable marine bacterial populations contain plasmids with novel incompatibility and replication regions or genes and that information regarding the host range of these naturally occurring plasmids will be of use in understanding plasmid transfer dynamics in marine microbial communities. Since the culturable bacterial population represents only an insignificant fraction of the total microbial community it seems likely that the nonculturable populations may contain even greater diversity with respect to their host range and genetic content than the culturable populations. Our attempts to characterize and classify indigenous plasmids will enable us to determine the potential for dissemination of plasmid-encoded genes with the sediment microbial community.

WORK PLAN: (next 12 months): We plan to continue to isolate and sequence indigenous plasmid-specific incompatibility and replication regions from broad-host-range replicons previously isolated from bacterial populations found in coastal salt marsh sediments. We will use the numerous replicon-specific sequences we have recently obtained as DNA probes to further characterize plasmid populations in marine microbial communities. We have also begun to determine the host-range nature of these broad-host-range plasmids and plan to elucidate their potential for genetic exchange with indigenous marine bacterial assemblages. Such characterizations will facilitate the study of plasmid diversity and transfer dynamics in marine microbial communities. Concurrent with our attempts to classify indigenous plasmids based on replication and incompatibility sequences we are developing methodologies that will aid in the construction of libraries of indigenous plasmids (from both culturable and nonculturable communities) to address such questions as plasmid population dynamics, presence of novel plasmid-encoded genes, transposable elements, mobilization sequences and conjugal transfer elements.

PUBLICATIONS AND ABSTRACTS

1. Sobecky, P.A., T.J. Mincer, M.C. Chang, and D.R. Helinski. 1997. Plasmids isolated from marine sediment microbial communities contain replication and incompatibility regions unrelated to those of known plasmid groups. *Appl. Environ. Microbiol.* volume 63, in press.
2. Sobecky, P.A., T.J. Mincer, M.C. Chang, and D.R. Helinski. 1997. Characterization of plasmid populations in marine microbial communities. Abstracts of the American Society of Limnology and Oceanography Aquatic Sciences Meeting, Santa Fe, NM, February 10-14.
3. Sobecky, P.A., T.J. Mincer, M.C. Chang, and D.R. Helinski. 1997. Characterization of plasmid replication sequences from marine bacteria for use as molecular probes. Abstracts of the 97th General Meeting, American Society for Microbiology, Miami Beach, FL, May 4-8.

4. Sobecky, P.A., T.J. Mincer, and D.R. Helinski. Isolation and sequence comparison of novel broad-host-range plasmid replicons from natural marine bacterial assemblages. In progress.

REPORT DOCUMENTATION PAGE

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13. ABSTRACT (Maximum 200 words) Plasmid-encoded genes represent a pool of mobile DNA that is likely to play a key role in the genetic adaptation of microbial communities. However, few studies have attempted to characterize plasmids from natural bacterial assemblages by such properties as host range, incompatibility and conjugal transmission. By examining plasmids, at the molecular level, from marine bacterial populations we can determine the distribution and diversity of naturally occurring replicons. Our findings indicate that plasmids from culturable marine bacterial isolates lack sequence homology to numerous well characterized broad- and narrow-host-range plasmids identified in bacteria normally found in animals and the soil. DNA extracted directly from the sediment microbial community (culturable and non-culturable microbes) has also been analyzed for the presence of incompatibility regions from several known plasmid groupings. Only one known plasmid (IncP) group was detected in the marine microbial community DNA. Because of this general lack of homology with known plasmid groups that we have observed for more than 300 plasmid-containing isolates we have developed an approach to isolate and characterize "environmentally-based" replicon probes for examining plasmid diversity and dynamics within marine bacterial communities. We have successfully isolated four different and previously unidentified broad-host-range replicon sequences from selected marine bacteria.				
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D.R. Helinski, UCSD; 1997

The Role of Plasmids in Marine Sediment Bacterial Communities

Objectives

- Investigate the role of plasmids in determining the structure and function of marine sediment microbial communities
- Use molecular tools to characterize naturally occurring plasmids
- Assess the potential for plasmid-encoded gene transfer in marine sediment bacterial populations

Accomplishments

- Developed a cesium chloride gradient centrifugation procedure for isolating significant quantities of plasmid DNA from diverse marine bacteria
- Developed a "replicon-rescue" procedure for isolating replication-proficient fragments from indigenous plasmids suitable for DNA sequence analysis
- Obtained DNA sequences from plasmid-specific replication regions which validate their novel and unique nature
- Genetically tagged several naturally occurring plasmids with a marker gene suitable for monitoring plasmid transfer dynamics under *in situ* environmental conditions

Significance

- Naturally occurring plasmids isolated from coastal marine sediment microbial communities are a source of novel incompatibility and replication sequences; these plasmids have a broad-host-range nature which suggests that they may be important in mediating genetic exchange in natural environments and such plasmids could be useful in biomedical and biotechnological applications
- Developed an effective approach to isolating and characterizing plasmid-specific replication sequences from indigenous plasmids in marine microbial communities
- Developing practical methods and approaches to assess plasmid diversity and transfer dynamics in natural microbial communities

ANNUAL REPORT QUESTIONNAIRE
(for ONR use only)

Principal Investigator: Donald R. Helinski
Institution: University of California, San Diego
Project Title: The Role of Plasmids in Marine Sediment Bacterial Communities

Number of ONR supported

Papers published in refereed journals: 1
Papers or reports in non-refereed publications: 2
Books or book chapters published: 0

Number of ONR supported patents/inventions

Filed: 0
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Patent name(s) and number(s): _____

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	TOTAL	FEMALE	MINORITY	NON-US CITIZEN
No. Grad. Students:	--			
No. Postdoctorals:	1	X		
No. Undergraduates:	1	X	X	

AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP
(please describe):

none

Equipment purchased on grant (number and description of items costing >\$1,500):

Alpha Innotech Gel Documentation System (1); used for DNA gel electrophoresis photodocumentation and image analysis