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<p>The research objectives of the project were to develop genetic and molecular techniques that will permit the detection, isolation, and cloning of genes that are regulated during acetate catabolism in the methanosarcina. These methods should provide a basis for examining the regulation of acetate utilization by the methanogenic <u>Methanosarcina</u>. We have concentrated on three areas in the second year of the contract: 1) refining plating techniques and selecting for auxotrophic mutants of <u>Methanosarcina</u> spp., 2) characterizing plasmid(s) from <u>Methanosarcina</u> spp.; 3) creating gene libraries of <u>Methanosarcina</u> spp. genomic DNA,; 4) creating oligonucleotide and antibody probes to probe the chromosomal library for acetoclastic genes.</p>			
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PROGRESS REPORT

PROGRESS REPORT ON CONTRACT: N00014-86-K-0193

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DATE: 25 June 1988

PRINCIPLE INVESTIGATOR: Dr. Robert P. Gunsalus

CONTRACTOR: UCLA

CONTRACT TITLE: Gene Regulation of Methanogenesis from Acetate in the Acetotrophic Methane-Producing Archaeobacteria.

START DATE: 1 April 1986

PERIOD OF PERFORMANCE: 7/1/87 - 6/30/88

RESEARCH OBJECTIVE: The goals of our research were to characterize the regulation of genes which are involved in acetate catabolism by the acetotrophic archaeobacteria. Our approach was to develop basic techniques for studying molecular biology and genetics of Methanosarcina spp. by developing plating techniques for mutant selection, screening the Methanosarcina for plasmids to be used as gene shuttle vectors, then combining these methods to develop an effective transformation system. Genes from Methanosarcina spp. were then be cloned and characterized by conventional methods in Escherichia coli vectors. The long term goal is to reintroduce these genes into Methanosarcina by transformation to study their regulation. Techniques developed in this study will facilitate other studies involving the other acetate and methylotrophic methanogenic archaeobacteria.

PROGRESS: Progress during the past year have been in three areas of research. A description of each follows.

A. Cell Plating of the acetate utilizing methane bacteria. In the second year of the project cell plating techniques were refined for the acetotroph Methanosarcina thermophila. We developed a procedure to grow this species as colony clones from single cells, a necessary requisite for genetic studies, in contrast to the multicellular aggregates typical of other species. Using the general approach devised by Bertani and Baresi for the hydrogen oxidizing methanogen Methanococcus voltae, we developed a technique for growing M. thermopnila at 50-90% efficiency of viable counts. We also demonstrated that colonies could be effectively replica plated. We have subsequently been able to grow all species of Methanosarcina as single cells instead of as cell aggregates. We refined the plating techniques to obtain high efficiency for growth of these other species on solid medium. Mutagenesis of M. thermophila by UV irradiation has been studied in order to generate reproducible kill curves. The goal is to project was to generate mutants of this strain that are defective

for the use of one or more of the methanogenic substrates.

B. Characterization of Plasmids from the Methanosarcina spp. In the first year of the project we reported the screening of twelve strains of acetotrophic and methylotrophic methanogens for plasmid DNA. Plasmid was detected in three species of marine Methanosarcina and plasmid from one of these species, M. acetivorans, was described in detail (see publication # 2). A procedure was developed for the large scale purification of this plasmid. We have completed the characterization of the plasmid and a manuscript is in press (J. Bacteriol.). The plasmid is the first to be isolated from an acetotrophic methanogen. It has several properties that make it potentially useful for construction of a shuttle vector for genetic purposes. It's relatively small size (5.1 kb) makes it useful for generating a shuttle vector for eventual gene transfer experiments. It has several unique restriction sites (EcoRI, BglII, SmaI, and PstI) that are useful for this purpose. Insertion of pC2A into pBR322 will allow us to amplify the plasmid in E. coli for subsequent characterization.

C. Isolation of Chromosomal DNA from M. thermophila: The preparation of large molecular weight DNA from the methanosarcina has been a major technical barrier to performing gene cloning experiments in these species because the harsh mechanical methods needed to rupture the cells also shear the nucleic acids. In our last progress report we reported a technique to obtain intact DNA from Methanosarcina acetivorans. We have since discovered that all the Methanosarcina spp. in our culture collection can be adapted to grow in media that contain marine salt concentrations. Concomitant with this adaption, strains ceased to grow as multicellular aggregates and exist solely as single cells. These cells lack the typical heteropolysaccharide outer layer which make them susceptible to gentle lysis with detergent. We have isolated large molecular weight DNA from M. thermophila, Methanosarcina barkeri, Methanosarcina mazei, and Methanosarcina strain UBS. The development of this technique has accelerated our progress in the project because we are no longer restricted to working with the marine species M. acetivorans as proposed in the original contract. Using DNA from M. thermophila, we have generated genomic libraries for subsequent gene cloning experiments.

We have made significant progress in cloning genes from DNA libraries of the M. thermophila strain as originally planned. Using CO dehydrogenase enzyme from M. thermophila provided by J.G. Ferry (VPI) we have proceeded to generate antibody probes as well as oligonucleotide probes to screen gene libraries and have isolated a number of positive clones for the genes of this enzyme. Characterization of these clones is in progress.

CONCLUSIONS: The following goals have been achieved in the course of this project.

1. Techniques were developed for efficiently growing all Methanosarcina spp. on solid medium. A major limitation for conducting genetics on the Methanosarcina was eliminated by the discovery of medium conditions that would induce cell aggregates

to grow as single cells, a condition necessary for performing genetic studies. Conditions for obtaining mutants for genetic studies were defined.

2. Acetotrophic methanogens were screened for plasmids. Three strains were found to contain plasmids. One of these, designated pC2A, was characterized and found to have properties that should make it useful for construction of a shuttle vector.
3. Techniques were developed to obtain intact large molecular weight DNA from Methanosarcina spp. Gene libraries were constructed for two species and oligonucleotide and antisera probes for regulated genes were produced. Portions of the CO dehydrogenase genes were cloned. The procedure for growing M. thermophila as single cells has been published and another manuscript, describing the ability to do this with all other Methanosarcina species, is in preparation .

INVENTIONS: There are no inventions resulting from this contract.

PUBLICATIONS AND REPORTS

Published Articles:

1. Sowers, K.R. and R.P. Gunsalus. 1988. Adaptation for growth at various saline concentrations by the archaeobacterium Methanosarcina thermophila. J. Bacteriol. 170:998-1002.
2. Sowers, K.R., and R.P. Gunsalus. 1988. Plasmid DNA from the acetotrophic methanogen Methanosarcina acetivorans. J. Bacteriol. (in press).

Manuscripts in Preparation: The following papers are in preparation and are the result of studies carried out during the last year of the Contract.

3. Gunsalus, R. P., S. Sahota, K.S. Sowers, and J. Boone. 1988. Single cell plating of the Methanosarcina spp. (in preparation)
4. Sowers, K.R., and R.P. Gunsalus. 1988. Interconversion between clumped and single cell growth in the Methanosarcina spp. (in preparation).

Abstracts and Research Presentations:

1. Sowers, K.R. and R.P. Gunsalus. 1987. Ultrastructure of unicellular forms of Methanosarcina. Gordon Research Conference: Molecular Aspects of Methanogenesis, New Hampton.
2. Sowers, K.R. and R.P. Gunsalus. 1987. A unicellular phenotype exhibited by Methanosarcina thermophila. West Coast Bacterial

Physiologists Conference, Asilomar.

3. Sowers, K.R. and R.P. Gunsalus. 1988. Adaptation for growth at various saline concentrations by Methanosarcina spp. Abstr. Ann. Mtg. Amer. Soc. Microbiol., 122, p. 18
4. Gunsalus, R.P., and K.R. Sowers. 1988. Genetics and Molecular Biology of the Acetate Utilizing Methanobacteria. Woods Hole Oceanographic Institution and the Marine Biological Laboratories. Woods Hole, MA. June 27, 1988.

TRAINING ACTIVITIES:

Dr. Kevin Sowers, a Postdoctoral Fellow, was supported in part by this contract. He was responsible for the plasmid screening and characterization, chromosomal DNA isolation, gene library construction, and the gene cloning work.

Dr. Surinder Sahota, a Postdoctoral Fellow, was also involved in these studies. He was trained in the anaerobic cell plating aspects of the project and performed much of the cell plating experiments.

Women or minorities - 1
Non-citizens - 1 (citizen of England)

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