PRIVILEGED COMMUNICATION

PROSPECTUS OF PROPOSED RESEARCH

EXTREMELY THERMOPHILIC BACTERIA

SUBMITTED BY MEMBERS OF THE FACULTY OF

THE JOHNS HOPKINS UNIVERSITY

BALTIMORE, MARYLAND

INTRODUCTION

Recent explorations of the seafloor along the tectonic ridges of the East Pacific Rise at 21 degrees North have revealed spectacular 3-10 meter high conical sulfide chimneys or "black smokers" venting 350 C jets of hydrothermal fluid into ambient seawater (Spiess et al., 1980, Science, 207: 1421-1444; Haymon, 1983, Nature, 301: 695-698). The venting fluids contain supersaturated levels of reduced gases (hydrogen sulfide, hydrogen, ammonia, and methane) and metals (iron and manganese), thus providing geothermal sources of energy for chemoautotrophic bacteria which support the extensive and diverse groups of animals living near the vents. No one imagined, however, that bacteria might actually exist in the 350 C vent waters until Dr. John Baross, a microbiologist at Oregon State University, succeeded in culturing complex communities of extreme thermophilic bacteria from relatively pure hydrothermal fluids (Baross et al., 1982, Nature, 298,: 366-368). He and Dr. Jody Deming, currently with the Chesapeake Bay Institute of Johns Hopkins, have now demonstrated in laboratory experiments that one of these cultures can grow with a generation time as short as 40 minutes at unprecedented temperatures of at least 250 C when incubated under the in situ vent pressure of 265 atmospheres (Baross and Deming, 1983, Nature: in press).

This remarkable group of bacteria flourishes entirely at the expense of inorganic sources of carbon (carbon dioxide and methane), nitrogen (ammonia and nitrate), and energy (reduced forms of sulfur, manganese, and iron). Based on the characteristics of the whole community (single strains have not, as yet, been purified), genetic information is available in the culture for a variety of both aerobic and anaerobic processes, including the production of gases (methane, carbon dioxide, carbon monoxide, hydrogen, and nitrous oxide), oxidation of metals (sulfur, manganese, iron, and possibly other trace elements), and oxidation of hydrocarbons (methane). Because of the novelty and uniqueness of these black smoker bacteria, nothing is known about the metabolic pathways responsible for these processes. Nor do we understand how the nucleic acids and proteins involved remain stable at temperatures previously believed to be highly denaturing, although the compensatory effects of elevated pressure appear to be crucial.

We propose to establish at the Johns Hopkins University a center of excellence based on an interdisciplinary effort aimed at unravelling these mysteries and, ultimately utilizing the bacteria, their genes, and enzymes for application in the realm of biotechnology. The research effort will draw together a wide variety of disciplines and expertise to maximize our chances for significant progress. A group of people with backgrounds in microbiology, protein chemistry, molecular biology, and chemical engineering has been assembled with the following stated research objectives:

(1) isolate and purify individual members of the original mixed culture from samples collected at the hydrothermal vents;

(2) fully characterize their chemical and physical requirements for optimal growth as well as for gas production, metal oxidation and extraction, and hydrocarbon oxidation;

(3) determine the molecular basis for the thermostability of nucleic acids and proteins involved in metabolic and oxidative processes; and

(4) develop ways to manipulate the bacteria, their genes and their unusually stable, highly catalytic enzymes for industrial applications using the combined tools of genetic and chemical engineering.

PERSONNEL

The following people and their specializations comprise the interdisciplinary group that will be involved in the research. This group is not exclusive in that additional people may be added to enhance our overall expertise:

Christian B. Anfinsen	Biochemistry, Protein Chemistry
Jody Deming	Marine Microbiology
Dennis A. Powers	Enzymology, Molecular Biology
John Baross	Microbiology, Geochemistry
E.N. Moudriankis	Biochemistry, Molecular Biology
Robert M. Kelly	Chemical and Biochemical Engineering

Associated with the Faculty will be a group of post-doctoral and graduate students who, as a result of this collaboration, will be trained in an interdisciplinary program to become expert in this promising area of research.

It should be evident that the group listed above covers a wide range of expertise. We think that the interaction that will occur in this research will not only result in a fundamental understanding of several characteristics of these extremely thermophilic bacteria but will provide the framework to examine their industrial significance.

RESEARCH PROGRAM

Several elements of the proposed research will be discussed briefly so as to provide some perspective of the scope of the program.

Isolation and Purification of Components of the Mixed Culture

Because of the nature of the environment in which the bacteria were found to exist, i. e. high temperature and high pressure, it will be necessary to develop novel laboratory equipment to culture the microorganisms. For instance, it is desirable to culture the bacteria continuously (at high pressure and high temperature) while monitoring chemical and microbiological activity. Preliminary discussions drawing upon expertise in microbiology, chemical engineering and materials science have yielded a design and set of specifications for such a system. We think this is an improvement on the original system used to document bacterial growth at 250 C and 265 atmospheres. An effort will be made to interface the measurement and control requirements with a microcomputer-based data acquisition system. A similar computer system has already been developed and is being used in an unrelated experimental program.

Once the individual members of the mixed culture have been isolated and purified, attention will be focused on the growth patterns of each component. Experimental data will be used to develop mathematical models to describe growth rates and the effect of the environment on these rates. These models will be useful in elucidating the mechanistic features of bacterial growth and will serve as the basis for any engineering extrapolation that may be necessary.

Protein and Nucleic Acid Chemistry

Because of the unusually severe conditions at which these extremely thermophilic bacteria thrive and their simple nutritional requirements, they must contain a unique set of macromolecules. These macromolecules will be required for cell structure and enzymatic catalysis as well as for the regulated synthesis of genetic material and cell multiplication.

The "melting" of double-stranded DNA in most eukaryotic cells and "normal" bacteria occurs in the region of 80 C. In such organisms the GC:AT ratio is on the order of 1. Thermophiles isolated from hot springs at 85-95 C exhibit ratios of about 2:1, the higher GC content presumably being responsible for the greater heat resistance. The deepsea thermophiles must contain a much higher G+C content, unless an entirely novel DNA stabilization system is operative. This possibility exists because even if a DNA molecule was entirely a G+C homopolymer (a useless genetic material), it would melt by 110 C under physiological ionic strength (0.14M NaCl). A study of the DNA chemistry in various homogeneous populations of cells will be of great interest and will also form a foundation on which to begin investigations of the genetic and mutational possibilities. It would be extremely interesting to search for a novel DNA stabilization system, perhaps consisting of new, more hydrophobic peptides and/or heavy-metal ions. This could lead to a better understanding of basic genetics and aid us in developing ways towards better controlling environmentally induced mutagenesis since a more compact, more dehydrated DNA will also be a less chemically vulnerable DNA.

Metabolic Characteristics and Membrane Properties

There is no doubt that these bacteria must possess some unusual metabolic pathways and membrane properties. Work to elucidate and understand these features will begin once pure cultures have been obtained. The metabolism of these bacteria is responsible for the uptake of inorganic compounds to satisfy nutritional requirements with the subsequent excretion of end products like methane, hydrogen, and metal oxides. To study the intermediate metabolism in pure cultures, radio-labeled precursors will be used to trace these unique pathways by classical methods and radio-labeled metabolic intermediates will be isolated and characterized. In addition, appropriate enzymes will be purified and characterized physically and chemically in relation to temperature, pressure and pH.

Membrane properties are also interesting and important. All living systems depend on microcompartmentalization of their cytoplasm for the regulated execution of their intermediary metabolism and energy exchanges. The cornerstone of such compartmentalization is based on the differentiation of specialized lipoprotein systems in the form of biological membranes. For energy metabolism, these membranes have evolved a complex electron-transport system that transduces the free energy changes of oxidation-reduction reactions to a proton-motive potential which is utilized by a special multienzyme complex called coupling factor to generate ATP. The function of such a system depends on the presence of closed, relatively impermeable membranous compartments which for conventional biomembranes collapse their permeability barriers at temperatures between 60-70 C at best.

In studying the cell membrane structure of the extremely thermophilic bacteria, we should find either new types of hydrophobic molecules present in their membranes, or new types of molecular interactions between membrane components that stabilize these membrane vesicles and enable their enzymatic systems to operate at temperatures of at least 250 C. Indeed, in another system of thermophilic bacterium it has been found that the conventional lipid bilayer backbone of biomembranes has been replaced by a novel lipid which looks like two lipid molecules covalently held together tail-to-tail through a di-ether linkage. This is understood to increase the stability of such membranes.

From these studies, we expect to increase our knowledge of structure-function relation in biological membranes as well as to isolate new enzymatic systems which could function at greater rates and/or under more adverse conditions than conventional enzymes.

Potential Biotechnological Applications

There are many possible uses of the information gained in this work. Not only are there several fundamental questions that will be answered but possible applications will be identified and analyzed in engineering studies. Of particular interest is the ability of these microorganisms to produce gases of considerable energy value from inorganic sources. To evaluate their potential along these lines, the continuous culturing system designed to be used for cell growth studies will be used. We will try to understand the quantity and composition of excreted gaseous chemicals in terms of the information gained through the study of intermediate metabolism and enzyme biochemistry. This information will also be viewed in terms of growth models developed for pure and mixed culture systems.

In another part of this study, cultures will be exposed to various mutagens and subsequently screened for metabolic genetic variants. Particular emphasis will be given to "superproducers" of methane, hydrogen and metal oxides. These "superproducers" will be analyzed biochemically to identify the responsible enzyme(s). The proteins responsible for increased production of end product chemicals will be cloned and amplified within bacterial species by standard genetic engineering methods. The approach should lead to the development of bacteria that will produce desired chemicals at enhanced rates as well as being the source of highly catalytic enzymes that may, in themselves, be valuable in reactions of industrial significance.