

# Microbial genetic adaptations to life in extreme cold

## INTRODUCTION:

Understanding life in extreme environments on Earth can tell us a great deal about the potential for life in similar environments on other planets. Understanding the limits of life as we know can also help determine what makes a planet habitable. Astrobiology is a relatively new scientific field, devoted to the scientific study of life in the universe – its origin, distribution, evolution and future. This multidisciplinary field brings together the physical and biological sciences to address some of the most fundamental questions of the natural world: How do living systems emerge? How do habitable worlds form and how do they evolve? Does life exist on worlds other than Earth? How could terrestrial life potentially survive and adapt beyond our home planet?

Scientists now realize that the origin and evolution of life itself cannot be fully understood unless viewed from a larger perspective than just our own Earth. Biologists are working with astronomers to describe the formation of life's chemical precursors, to discover new planets, and to determine their habitability; with chemists to understand the transition from molecular interaction to life itself; with geologists to search for evidence of water and key minerals on other planets; with paleontologists and evolutionary molecular biologists to look for and comprehend the earliest forms of life, as well as with climatologists, planetary scientists, and researchers from nearly every field of science (1).

We began our work in astrobiology as a team of an astronomer (M. Ognjanovic) and molecular biologist (S. Ognjanovic) in collaboration with NASA Astrobiology Institute (NAI) in Hawaii. Our original work, presented at Astrobiology Conference in Iceland 2004, led to discovery of a common gene signature found in seven sequenced hyperthermophile genomes by gene profiling the microorganisms living in extremely hot environments with different additional life-limiting factors (2). But most bodies of our Solar System are "Frozen Worlds" where the prevailing surface temperature remains at or below freezing. Even on Earth there are vast permanently frozen regions of permafrost, polar ice sheets, and glaciers and the deep oceans and deep-sea marine sediments. Oceans make up half of earth surface and they are consistently at a temperature of 1-3 degrees Celsius and more than 80% of biosphere is permanently below 5° C (3)

With the exception of homeothermic animals that maintain a warm body temperature in the cold, most fish and plants, and all invertebrates and microorganisms are at complete thermal equilibrium with their environment. As a result, all components of psychrophiles, cold loving bacteria, must be adapted to the cold to enable an overall level of cellular function that is sufficient for growth and survival. The extent of cellular adaptation is illustrated by studies (particularly in bacteria) that have linked cold adaptation to most structural and functional components of the cell; ranging from the outer membranes (lipid composition) to the inner cellular machines (ribosomes), enzymes and nucleic acids (tRNA) (3)(4).

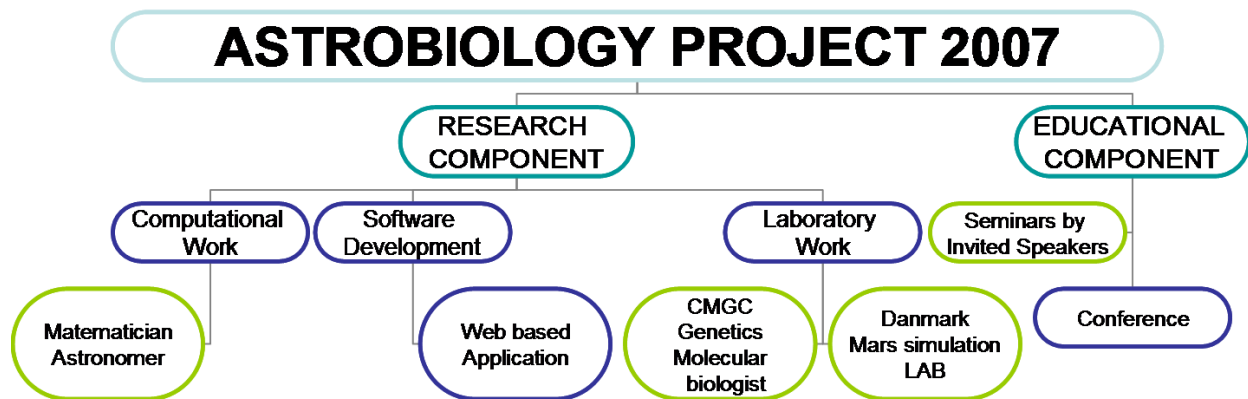
Revelations about extremophiles have invigorated the field of astrobiology 11NP (4). In recent years, the field has also been stimulated by the discoveries of apparently biogenically derived methane on Mars96NP (5), the knowledge that methanogens exist and are active in the cold, and that methanogens can grow and metabolize in Martian-soil simulant97NP (6). Other exciting findings have been the discoveries of live microorganisms in ice cores taken from sea ice23NP (7) and the accretion ice of Lake Vostok, Antarctica98NP (8), and the presence of water in cold environments on Mars and Europa99NP (9). Ice sheets and permafrost of Mars, comets, or the ice/water interfaces or sediments deep beneath the icy crusts of Europa, Callisto,

or Ganymede. Cryopreserved micro-organisms can remain viable (in a deep anabiotic state) for millions of years frozen in permafrost and ice. Psychrophiles proliferate at temperatures 0° – 10°C, metabolize in snow at ice at -20° C, are predicted to metabolize at -40° C and can survive at -45° C (9).

This possibility of extreme cold tolerance and longevity, once again increases the odds of life in the solar system and expands the potential life zone. Mars' polar caps vary between -120°C and -40°C. Theoretical temperatures for Europa's ocean are in the -90°C to -10°C range and Titan at about -180°C may be within range!!!

We propose to investigate a gene signature responsible for adapting microscopic life forms to the life in extreme Earth environments with the goals to: (a) predict what is the range of environmental conditions on the other planets and solar bodies that allows the existence of the basic life forms, and (b) to investigate whether such genes can be used to modify organisms which can be used for terraforming suitable planets.

RESEARCH CIRCLE ORGANIZATION:



The proposed study of genetic adaptations of microbes to extremely cold environments will have a research and educational component (Table 1). The research component will be conducted by multidisciplinary international team including a microbiologist (Denmark), molecular biologist (US), astronomer (Australia), mathematician (US), geneticist (US) and astronomer (US). This team comprises the “inner circle”, which will be responsible for carrying out research project, gathering preliminary data for the next grant application as well as outputting the results in the form of web applications and peer-reviewed publications. The preliminary data for this project, discovery of common gene signature in heat-loving bacteria (hyperthermophiles), serves as proof of principle, that genomic comparison of extremophiles can lead to discovery of a limited number of common genes responsible for adaptation to an extreme environment. In this proposal, we would like to take this a step further to test experimentally whether in silico identified genes are indeed expressed larger group of psychrophiles and thereby confirm the computational approach. The properties of identified proteins will be studied in detail, with special effort in discovering gene families, present in other species. This is very important, since half of the proteins we identified in preliminary study were

in this category, and similar results are obtained by other researchers doing genome comparisons (10). In addition, the groups' monthly meetings, conducted using internet teleconferencing, will also include "outer circle" members, a material scientist and a biologist leading the electron microscopy facility with his own interest in psychrophiles. The material scientist was chosen to be included in these discussions if the future research proposal would benefit from inclusion of analyses of environment and how the physical characteristics of the environment may enhance or limit life of these microbes. Of particular interest are the sophisticated models of ice developed by this group (11). One of the goals of our discussions is to identify which of the 150 psychrophiles in our collaborator's collection would be the best candidate for sequencing of genome and initial criteria were already discussed with Dr Finster. Biomedical Genomics Center (led by Dr Kapur) has recently acquired one of the latest models of sequencing machines, Roche 454 model suitable for these applications, and the funds for sequencing are available at the Department of Energy (DOE). The main purpose of inclusion of the outer team in all the discussions is to determine which would be the most appropriate module to involve environment component in the next grant application and whether the significance of such component for the future research directions. The potential granting institutions identified for our future mutual grant proposal include: NSF and DOE.

#### EDUCATIONAL COMPONENT

Educational component plays an important role in this proposal. Currently there is no astrobiology research carried out at the UMN and one of the missions of our proposed project is to change this. We will start with monthly newsletters focused on specific psychrophile, but also each tackling one of the major concepts in astrobiology and summary of the latest achievements. In addition, the leaders of each of the 12 NAI institutions will be asked to contribute a short article covering the NAI sponsored research carried out at their institution. We have collaborated closely with Dr Karen Meech at the University of Hawaii and her help in pursuing this goal will be valuable. In addition, every leader of the 12 NAI institutions will be invited to join our research circle (see list of invited circle members). In addition to newsletter, which will be distributed at major colleges of interest (Astronomy, biology, computer sciences, physics, geology), the inner circle members will be responsible to organize a series of seminars focused on psychrophiles and include the latest results of our project. Likewise, the international inner circle members will be asked to give a presentation of their research, at the time of our organizational conference at UMN. Two members of the inner circle will be selected to present the preliminary data our research at the Bioastronomy conference in Puerto Rico.

The discussions will be held monthly using internet teleconferences including all participants listed on this proposal. In addition, e-mail correspondence will be used for communications between team members who conduct the research activities.

Research project: specific aims:

- 1) Computational identification of genes responsible for adaptation to cold.
- 2) Construction of microarrays containing these genes and use them for screening of 20 psychrophiles from Danish collection of these microbes and
- 3) Construction of Atlas of planetary conditions potentially suitable for life and development of web application where conditions of an Earth extreme environment of interest could be compared to the closest resembling counterpart in space.

Preliminary data:

The approach of extremophile genome comparisons was first developed for hyperthermophilic microorganisms, organisms which grow at 90°C or higher and have the highest growth temperatures known for life. This study was carried out in collaboration with NASA Astrobiology

Institute in Hawaii. We have developed a software which incorporated features of BLAST genome and BLAST protein and existing databases for several sequenced hyperthermophiles and were analyzed using COGs (clusters of orthology) as a bases for comparisons. The first step was exclusion of all genes present in bacteria which do not live in extreme environments. We used E. coli K12, a common laboratory strain, for these purposes. Therefore, the first step of comparisons was between P. Abyssus (one of the seven hyperthermophiles analyzed) and E.coli, which eliminated all the common COGs and only remaining COGs (around 400) were used for search of the COGs common to hyperthermophiles. Our analyses focused on 7 hyperthermophiles with well defined COGs available in the public domain. The 6 archaeal genomes were chosen to represent a wide variety of hyperthermal habitats. This approach significantly reduced the number of common genes which would be found among the Archaea more closely related, since our goal was the search for the minimal common genes to all hyperthermophiles. This goal was limited by the number of currently sequenced hyperthermophile genomes and the annotations of those which were sequenced. The purpose of these analyses was the identification of genes involved in adaptation to extreme environment, in this case extreme temperatures (over 90C) and extreme pressure. We identified 29 proteins common to all 7 hyperthermophiles which we grouped according to function into 4 categories: hypothetical proteins (15 of identified proteins had unknown function and were annotated as hypothetical), enzymes (11), membrane and ribosomal proteins (2) and regulators of gene expression (2). At such extreme heat, as found in these environments, proteins are expected to lose their tertiary structure, denature, due to coagulation. The other challenge is sustaining plasma membrane in semi-liquid state. Yet another is the functioning of enzymes at these temperature. The categories of proteins we identified correspond to these issues.

#### 1) Computational methods:

Background: Comparative genomic analyses has the potential to generate hypotheses regarding the importance of specific genes and molecular characteristics for life in extremely cold environment, such as the permafrost (15). The first psychrophile genome sequenced was Psychrobacter strain 273-4. It contains a 2.64 Mbp genome with 2,147 ORFs. The approach of identification of cold adaptation genes involved comparison of 2 psychrophile genomes (Psychrobacter 273-4 and Exiguobacterium 255-15) with the strains of these bacteria that live in warm waters and can grow at temperatures up to 42 degrees C (15). Using this approach, Ponder et al. identified one extremely large hypothetical protein (6,715 amino acids) and four histone-like proteins potentially involved in cold adaptation (15). However, comparing the genomes of 2 psychrophiles sequenced at that time, showed 75% of ORFs in Exiguobacterium encode for putative protein homologues in Psychrobacter...