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ANTARCTIC PSYCHROPHILES

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What are psychrophiles?

According to a previous concept, the term "psychrophiles" was used for bacteria, which could grow at 0°C or sub zero to 18-20°C with an optimum growth at 10-12°C, thus differentiating them from "psychrotrophs" which were organisms that could grow at 0°C or below but capable of growing at temperature as high as 30-32 °C. However at present all the cold-tolerant bacteria are called psychrophiles and the term psychrotroph is no longer used. Psychrophiles are found in environment that remains cold throughout the year e.g, oceans, glaciers and snowfields.

Life in Antarctica

The continent of Antarctica is one of the coldest places on the globe. The average annual temperature of the continent ranges between –20°C to –30°C, and in summer, the temperature reaches a maximum of +5°C. The permanent ice-cover, extreme low temperature, strong winds, low humidity and non-availability of water make the continent unique and seemingly uninhabitable. Inspite of these inhospitable conditions, the seas around Antarctica and the lakes and ponds in the continent support several forms of life. This could be attributed to the presence of nutrients in the water. The terrestrial habitats also support a few living forms, such as lichens, algae, fungi and bacteria. All these life forms are unique by virtue of to their ability to survive and propagate under prevailing harsh conditions. Therefore, investigations on bacteria, obtained from Antarctica, can help in the discovery of novel and unique ways of adaptation of life forms to low temperatures.

Scientists have discovered a microbial world hidden deep beneath the frozen Antarctic ice. It could help them learn more about how life can survive under extreme conditions on other planets or their satellites. Scientists believe that ice is a good environment for primitive bacteria. The bacteria need less food as its metabolism slows down at low temperature, somewhat like a hibernating bear's. Scientists have also found signs of bacterial life in the ice core and detected metabolic activity in some of the bacteria by measuring the bacteria's respiration rate during incubation.

Scientists have discovered living creatures in many other places where existence of any form of life was considered impossible earlier. For example, bacteria have been found living in hot springs in Yellowstone National Park where the temperature is 100 °C, the boiling point of water at sea level. Different forms of life have also been found deep in the earth and at the bottom of the ocean where there is no light and pressure is high. Some forms of living creatures are found to occur in places that were once considered too acidic for life. Others live in water, which is so salty that once upon a time it was thought to be fatal to any form of life. The bacteria, that survive in the extreme environmental conditions of temperature, pressure, pH, desiccation and salinity, are collectively known as extremophiles.

Microbiological studies in specific regions of continental Antarctica (such as Victoria dry valleys and McMurdo station area) have indicated that the most dominant bacteria in the soils of these regions are *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, *Planococcus*, *Deinococcus* and *Micrococcus*. Studies performed at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India on oases of continental Antarctica have indicated the presence of both bacteria and yeasts. Bacterial isolates collected by Dr S.Shivaji, Scientist, CCMB, from soil and ice of Antarctica, during his visits to the cold continent (first in 1984-85 and subsequently in 1995), have provided excellent tools for investigations on the mechanism of bacterial cold adaptation (Shivaji et al 1994).

Metabolism of bacteria in Antarctic climate

Bacteria, obtained from Antarctic ice cores and from the deep cores of the accreted ice above sub glacial Lake Vostok , revealed a high diversity of species that were found to be metabolically active when warmed to 3°C. Scientists from Marine Sciences Research Center, New York, also reported the presence of bacterial populations and associated metabolic activity in surface (upper 20cm) snow and fern collected from the South Pole during the austral summer.

Investigations reveal that 200 to 5,000 of bacterial cells ml^{-1} of snowmelt were present in surface snow, sampled from the South Pole in January 2000 . DNA isolated from this snow yielded ribosomal DNA sequences similar to that of several psychrophilic bacteria. One of the isolates aligned closely with members of the genus *Deinococcus*, an ionizing-radiation-and desiccation-resistant genus. Scientists have also obtained evidence of low rates of bacterial DNA and protein synthesis. It indicates that the organisms were metabolizing at ambient, subzero temperatures (-12 to -17°C). Recently, respiratory activities of bacteria obtained from the Arctic sea, have been demonstrated at subzero temperatures using a fluorescent dye (Junge et al 2004).

Mechanism of cold tolerance

Bacteria survive at low temperatures using several strategies. Investigations involving Antarctic bacteria in CCMB revealed the presence of cold-active enzymes in some of them. One of the isolates has been shown to continue transcription and translation at low temperature unlike the mesophilic bacterium *Escherichia coli* . The role of some fatty acids (short chain fatty acids, branched chain fatty acids, anteiso fatty acids) in maintenance of optimum fluidity of the bacterial cell membrane at low temperature is well-known (Chintalapati et al 2004). In two Antarctic strains, some carotenoid pigments have also been postulated to play an important role in homeoviscous adaptation of membrane fluidity at low temperature (Chattopadhyay and Jagannadham 2001). Homologs of *csp A*, which encodes the major cold shock protein in *Escherichia coli*, have been detected in several Antarctic strains. Enhanced activities at low temperature of some enzymes (e.g, protease) involved in biodegradative processes indicate that they might be useful for obtaining nutrients during cold adaptation. Some low molecular weight substances (glycine betaine, proline) have been found to have growth-enhancing effects on an Antarctic isolate at low temperature. Some special structural features have been demonstrated recently in the RNA-degrading machinery of an Antarctic isolate. Investigations with other cold-tolerant bacteria have highlighted role of some heat shock proteins in cold adaptation of bacteria. Accumulating evidences suggest interlink between cold-tolerance and other types of stress adaptation (e.g, barotolerance, radiation resistance) in bacteria. For more details readers are referred to a recent review (Chattopadhyay 2006).

Genetic basis of cold-sensitivity

A cold-sensitive mutant of the Antarctic strain *Pseudomonas syringae* (Lz 4W), isolated and characterized some time back in CCMB, has been shown to have a mutation in the *recD* gene (Regha et al 2005). The gene encodes Rec D protein, which is involved in DNA repair. Some other biochemical mutants of several Antarctic bacteria have been raised subsequently by transposon mutagenesis. A cold-sensitive mutant of the Antarctic strain *P fluorescens* (10 CW) has alteration in a gene which encodes a protein with unknown function in *P putida* . In another investigation, a mutant of the same isolate is found to have mutation in a gene, which encodes a serine protease in *P aeruginosa* (unpublished) . Investigations are in progress with some other biochemical mutants, which grow poorly compared to their wild type counterparts, at low temperature. Detection of the mutated genes is likely to provide insight into the molecular basis of bacterial cold adaptation.

Novel Antarctic isolates

Recently a number of new Antarctic bacterial strains belonging to the genus *Psychrobacter*, *Pseudonocardia* , *Halomonas,* *Sporosarcina, Leifsonia* , *Planococcus* and *Pseudomonas* have been isolated and characterized in CCMB . Further investigations involving these isolates are in progress (reviewed by Chattopadhyay 2006).

Biotechnological importance of cold-tolerant bacteria

- Psychrophiles are the source of cold-active enzymes (Feller and Gerday 2003), which may have several applications in biotechnological and industrial process like food industry. The use of cold-adapted enzymes in food processing would allow product modification at low temperatures, which restrain microbial growth.
- A number of strains belonging to the genera *Pseudomonas, Erwinia and Xanthomonas sp.* cause damage to leaves and flowers by triggering ice crystal formation through the action of ice nucleating proteins at subzero temperatures (-2°C to 5°C) . The *ina* genes encode the ice nucleating proteins. The engineered ice minus bacteria, that lack *ina* genes, have potential application as frost protectants for sensitive plants because of their ability to inhibit the growth of naturally occurring ice nucleating pathogens (Russell 1998). The ice nucleating bacteria have also been used for producing and maintaining the ice surface for ice sports and ice-skating at relatively higher temperatures.
- Marine psychrophiles contain polyunsaturated fatty acids (PUFAs) and are potential source for PUFAs in human food, aquaculture and livestock. Recent evidences indicate that intake of the longer chain PUFA's helps in preventing diseases like arteriosclerosis (Russell 1998).
- Inclusion body formation is a major problem during overexpression of proteins. This problem can be minimized by lowering the growth temperature of *E coli* or any other mesophilic host. Using a psychrotropic or psychrophilic host for overexpression may help to solve this problem further, since the strains can be grown at lower temperatures. It is also possible to achieve low-temperature expression of cold-active proteins since they can be produced in vivo within an intracellular environment, that is thermally appropriate for the folding pathways (Russell 1998). • The high activity of psychrophilic enzymes at low and moderate temperatures offers potential economic benefits through substantial energy savings in large-scale processes that require the expensive heating accessories. A typical example is the industrial 'peeling' of leather by proteases. It can be performed at temperatures of tap water using cold active-enzymes instead of maintaining the temperature at 37°C for the process, performed by the mesophilic enzymes.
- Psychrophilic enzymes can also be useful for domestic purposes. For instance, washing clothes at low temperatures can protect the colors of the fabrics and reduce energy consumption. Enzymes that are used to remove stains (e.g, subtilisin, lipase and glycosidase) are poorly active at temperatures of tap water. They can also be substituted by psychrophilic enzymes.
- Cold-active enzymes are useful also in the food industry in various other ways. For example, lactose intolerance is a problem for approximately two thirds of the population of the world. A process for the removal of lactose from milk by a psychrophilic b-galactosidase has recently been patented. Cold active pectinases can help to reduce viscosity and clarify fruit juices at low temperatures.
- The heat liability of these enzymes also ensures their fast, efficient and selective inactivation in complex mixtures. The use of a heat- labile alkaline phosphatase in molecular biology is probably the first biotechnological application proposed for a psychrophilic enzyme. Glycosidases, often used in baking industry, retain residual activity after cooking. It alters the structure of the final product during storage. This problem can be avoided by the use of psychrophilic glycosidases.
- Psychrophilic microorganisms are potential candidates for bioremediation of polluted soils and wastewaters in extreme cold environments (polar regions, glaciers), where low temperatures impair the

degradative capacity of endogenous microflora. It was demonstrated some time back (Ferrer et al 2003) that the mesophilic bacterium *Escherichia coli* could be grown at low temperatures by expressing two genes, which encode two chaperonins (proteins that help in the folding of other cellular proteins). These genes were obtained from an Antarctic isolate. Similar strategies may be used to engineer cold-tolerant bacteria with high degradative capacity.

Notwithstanding having such potential applications in biotechnology, psychrophilic enzymes remain under-used, mainly because of the difficulties in getting the strains with high productivity and also due to the cost involved in large-scale production of these biocatalysts.

Psychrophiles as models for exobiology

The possibility of existence of life in the other planets and their satellites of the solar system is a very attractive area of investigation for the scientists. It is generally assumed that the availability of water molecules in the liquid state is a prerequisite for the development of life. Though it is expected that the main body of water on Mars will be found as ice trapped in the permafrost soils and in the polar caps, water may also exist as brine veins between ice crystals, protected from sublimation or evaporation in the atmosphere. Such liquid veins arise from the concentrations of salts outside crystals of pure water. The salts depress the freezing point of liquid water. Hence any microorganism living in this particular niche should show strong adaptive characteristics for survival at high salt concentrations and low temperatures. Indeed, living and metabolically active bacteria have been detected in the liquid brine veins of sea ice at –20°C. However, these microorganisms and the physicochemical properties of their macromolecules remain uncharacterized so far. Interestingly, the closest model of Martian environment on Earth is the polar sea ice. It is therefore important to improve the state of our knowledge of such halo-psychrophilic microorganisms in order to explore the possibility of existence of life in Mars.

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