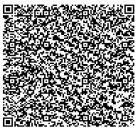

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Diversity and Bioprospecting Potential of Bacteria Isolated from the Arctic: A Preliminary Study

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Abstract

Bioprospecting is a search for new or better bioproducts from biological sources preferably from novel biodiversity. Molecules derived from natural products, particularly those produced by plants and microorganisms, have an excellent record of providing novel chemical structures for development as new pharmaceuticals. Bacteria living under stress are best sources for bioprospecting and also these organisms are least explored. This paper focuses on the bioprospecting and biotechnological potential of bacteria isolated from the Polar Regions. In this context, let us have a glance through the major cold adaptations of psychrophiles and their application potentials with special reference to psychrophilic enzymes. Our lab focus on identifying the biofilm inhibitors against bacterial pathogens and novel molecules of medical importance from bacteria and actinomycetes isolated from Arctic, the North Pole. We are also interested in profiling the diversity, multidrug resistance pattern and the molecular mechanisms involved in bacteria isolated from the pristine environment.

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Introduction

Bioprospecting relies on provision of a bioresource, a supply of novel biodiversity. Many of the world's most successful and valuable pharmaceuticals have been derived directly or indirectly from natural product sources eg. acetylsalicilic acid (aspirin) from willow bark and penicillin from the fungus *Penicillium*. Extreme environments provide microorganisms containing robust enzymes. Such microorganisms that flourish in extremes of temperature, pressure, acidity or alkalinity are sources of extremophilic enzymes that are required by many industrial processes. Bioprospecting for such enzymes involves culturing and isolation of novel extremophiles from polar habitats. Microorganisms represent the largest reservoir of unexplored biodiversity, and hence possess the greatest potential for the discovery of new natural products.

Microbial adaptations to cold environments

Investigations of sea ice and glacial microbial communities have demonstrated the dominance of bacterial populations highly adapted to life at constant low temperature. These bacteria are known as “psychrophiles”. The term "psychrophile" (*psychro*- cold, *phile*- loving) was first coined in 1902 to describe bacteria capable of growth and survival at 0°C [1]. Microbial adaptation to a permanently cold environment includes the optimization of basic cell processes necessary for growth and survival. These can be summarized into three categories; enzyme function, nutrient transport and cell membrane function. A typical example is the protein profiling studies done on the cold adapted bacteria *Methanococcus burtonii [2]*. The adaptations of cellular processes in each of these areas represent potential biotechnology products for exploitation. Two examples are the production of polyunsaturated fatty acids (PUFA) and the production of cold-active enzymes by bacteria from Arctic and Antarctic sea ice.

Protein synthesis, cold-acclimation proteins and antifreeze proteins

Transcription and translation are temperature-sensitive steps and psychrophiles have obviously adapted the process of protein synthesis to low temperatures. It is expected that the enzymatic activities that are involved in protein synthesis have the general traits of cold-adapted enzymes — high activity associated with low stability — but this aspect has not been consistently analysed so far. However, low temperatures strengthen the interactions between DNA strands in the double helix and in the supercoiled state, therefore impairing unwinding and access to RNA polymerase. Low temperatures also promote unfavorable RNA secondary structures, which are likely to interfere with translation. Accordingly, it is expected that nucleic acid binding proteins, which relieve the adverse effects of low temperatures, have a central role in the cold adaptation of psychrophiles [3-5]. In this respect, it is significant that the five unique genes that have been detected in the genome of two cold-adapted Archaea are predicted to encode nucleic acid binding proteins [6]. In an Antarctic archaeon, the gene transcript for an RNA helicase, which possibly removes cold-stabilized secondary structures, is accumulated during growth at 4°C, but is undetectable at 23°C [7]. Moreover, increased post-transcriptional incorporation of dihydrouridine in tRNA from psychrophilic bacteria is thought to improve the conformational flexibility of RNA [8].

Cold-acclimation proteins (CAPs) seem to be another important and general feature of cold-adapted microorganisms [9]. A set of ~20 proteins is permanently synthesized during steady-state growth at low temperatures, but not at milder temperatures [10, 11]. Interestingly, some of the few CAPs that have been identified in cold-adapted bacteria are cold-shock proteins in mesophiles, such as the RNA chaperone *CspA* [12]. It has been proposed that these CAPs are essential for the maintenance of both growth and the cell cycle at low temperatures [9],but their function is still poorly understood. Antifreeze proteins have been widely studied in polar fish [13].These peptides and glycopeptides of various sizes decrease the freezing point of cellular water by binding to ice crystals during formation. Although antifreeze proteins have been

reported in several eukaryotes, there is no supporting evidence for the occurrence of such glycopeptides in psychrophilic prokaryotes.

Biotechnological applications of psychrophilic enzymes

The high activity of psychrophilic enzymes at low and moderate temperatures offers potential economic benefits [3, 14-18]through substantial energy savings in large-scale processes that would not require the expensive heating of reactors. A typical example is the industrial 'peeling' of leather by proteases, which can be done at the temperature of tap water by cold-active enzymes instead of heating to 37°C for the process to be performed by mesophilic enzymes. Psychrophilic enzymes can also be useful in domestic processes. For instance, washing clothes at low temperatures can protect the colours of fabrics (and reduce energy consumption), but enzymes that are added to detergents to hydrolyse macromolecular stains -for example, subtilisin, lipase and glycosidases etc are poorly active at the temperature of tap water; they can be substituted by psychrophilic enzymes. In the food industry, their properties allow the transformation or refinement of heat-sensitive products. Lactose intolerance is a problem for approximately two-thirds of the world's population. The removal of lactose from milk by a psychrophilic β-galactosidase during cold storage has recently been patented.As another example, cold-active pectinases can help to reduce viscosity and clarify fruit juices at low temperatures. The heat-lability 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 (which does not interfere with end labeling by polynucleotide kinase after heat treatment) is probably the first biotechnological application proposed for a psychrophilic enzyme [19]. Glycosidases are often used in the baking industry, but can retain residual activity after cooking that alters the structure of the final product during storage; this can be avoided by the use of psychrophilic glycosidases. However, despite such powerful biotechnological potential, psychrophilic enzymes remain under-used, partly because the cost of production and processing at low temperatures is higher than for the commercial enzymes that are presently in use. Psychrophilic microorganisms have also been proposed for the bioremediation of polluted soils and waste waters during the winter in temperate

countries, when the degradative capacity of the endogenous microflora is impaired by low temperatures. Finally, an important achievement in the field has been the construction of a host–vector system that allows the over expression of genes in psychrophilic bacteria [20]: expression at low temperatures prevents the formation of inclusion bodies and protects heat-sensitive gene products.

Indian Arctic Expedition

The Arctic and Antarctic extreme environments are the least explored areas on the Earth. These areas harbour unusual flora, fauna and microbes, which can be of great benefit to mankind. That is why many countries are interested in having their research base in the Polar Regions. Arctic expedition marks a beginning of long term scientific research by Indian scientists in yet another arena of global scientific collaborative research in the difficult Polar Regions, since the first Indian scientific expedition landed in Antarctica in 1981. Currently, Norway, Germany, France, Britain, Italy, Japan, the Republic of Korea and China have their research stations in Ny-Alesund for Arctic research. Ny-Alesund in Arctic has the outstanding and unique feature of being a global research platform to study the potential of psychrophilic microbes useful to the mankind. India is one among the few countries having their own research bases at both the poles. The Indian Arctic Expedition is organized by the National Centre for Antarctic and Ocean Research, Goa, under the Ministry of Earth Science, Govt. of India. In 2009 summer expedition, the authors went there as part of this scientific expedition. The mission of Rajiv Gandhi Centre for Biotechnology team was to collect microbes capable of producing commercially important psychrophilic enzymes and other biomolecules of biotechnological and medical importance. Our lab has a culture collection of around 300 isolates from the Arctic which were collected from different representative biotopes of the Kongsfjorden, Ny-Alesund. Molecular identification and enzyme profiling have shown a widely diverse collection.

Bacterial diversity in Arctic

Table 1: Bacterial diversity in Ny-Alesund, Arctic

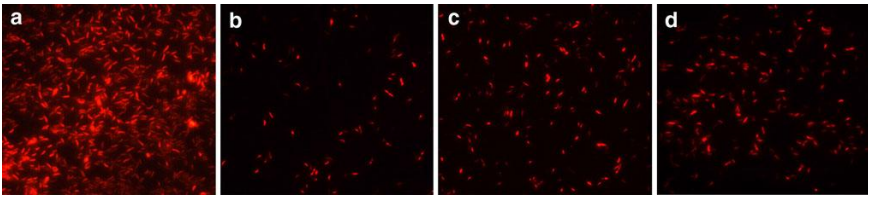
Strain no.	Identified organism
Phylum *Proteobacteria*	
A1	*Pseudomonas* spp. (JN390949)
A8	*Pseudomonas migulae (*JN390950)
A73	*Pseudomonas antarctica* (JN390958)
A74	*Pseudomonas migulae* (JN390959)
A76	*Pseudomonas cannabina* (JN390960)
A52	*Janthinobacterium lividum* (JN390955)
Phylum *Actinobacteria*	
A27	*Corynebacterium casei* (JN390951)
A40	*Arthrobacter koreensis* (JN390952)
A46	*Arthrobacter koreensis* (JN390953)
A51	*Arthrobacter koreensis* (JN390954)
A62	*Arthrobacter* spp. (JN390956)
A71	*Kocuria* spp. (JN390957)
A73	*Nocardiopsis* spp. (JF903933)
A73	*Streptomyces* spp.(JF903932)
A74	*Streptomyces* spp.(JF903931)
Phylum *Bacteriodetes*	
A78	*Flavobacterium* spp. (JN390961)

The permanently frozen regions of the Arctic (North Pole) and Antarctic (South Pole) are really unexplored areas of the world. The sediment and water samples were serially diluted and plated on nutrient and marine agar plates and incubated at 10°C for 3-4 days. Isolated bacteria are identified by 16S rRNA sequencing and they are listed in Table 1. *Janthinobacterium lividum* and *Kocuria* spp. were reported for the first time from the Arctic. Arctic isolates producing industrially important enzymes such as lipase, amylase, protease and β-galactosidase at lower temperatures were also reported.

Antibiofilm activity of Arctic Actinomycetes

The aim of this study was to identify novel biofilm inhibitors from actinomycetes isolated from the Arctic against *Vibrio cholerae*, the

causative agent of cholera. Studies conducted in our lab revealed that Arctic Actinomycetes (*Streptomyces* spp. and *Nocardiopsis* spp.) are good source of biofilm inhibitors against *Vibrio cholera* [21].The biofilm inhibitory activity of actinomycetes was assessed using biofilm assay and was confirmed using air–liquid interphase coverslip assay (Fig. 1). The coverslip assay results showed the reduction in adhesion of the cells on the coverslip surface confirming the biofilm inhibitory activity of the extracts.



Ref: Nimmy *et al*., 2012

Fig 1: Air- Liquid interphase coverslip assay. a) *V. cholerae* biofilm (positive control); b, c and d) Biofilm treated with supernatant of isolates A731, A733 and A745.

Antibacterial activity of Arctic actinomycetes

Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The worldwide spread of multidrug resistant bacteria is an alarming issue as it renders antibiotics ineffective in treating infections. Hence our lab focuses on discovering novel antibiotics isolated from Arctic. In the present investigation, Actinomycete isolates 726, 732, 734, 740 and 743 showed antibacterial activity towards *Staphylococcus aureus* and *Vibrio cholerae*. Further characterizations of the active fractions are in progress.

Emergence of multidrug resistance in Arctic

The emergence of multi-drug resistance in clinical and environmental strains is becoming a major problem faced by the clinicians and the scientific community. However, the emergence of multi-drug resistant bacteria in the polar ice caps has only been recognized recently. All isolates were tested against a set of

antibiotics using disc diffusion method. Results showed that majority of the isolates showed MDR pattern (Fig 2). All the bacterial isolates analysed were resistant to most of the antibiotics tested except a few like chloramphenicol, ciprofloxacin and polymyxin B. Multi drug resistant and *AmpC* producing bacteria were identified from the Arctic environment and study of its molecular mechanism of drug resistance is in progress.

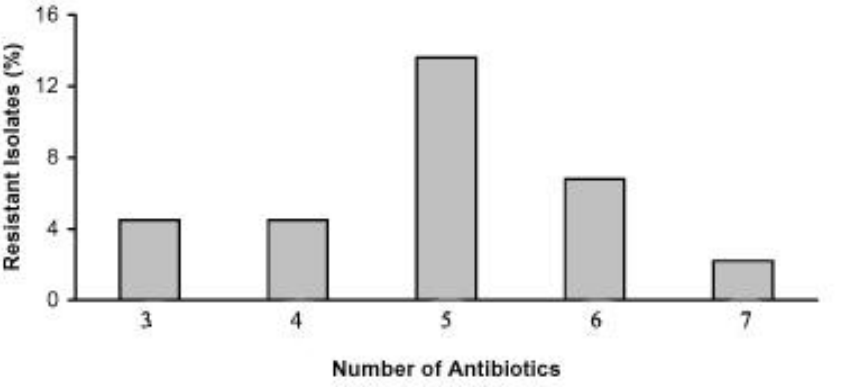


Fig 2: MDR profile of the bacteria isolated from Ny-Alesund, Arctic

To summarize, though several studies have been conducted on the molecular basis of cold adaptation and their enzymes of psychrophiles, owing to their huge diversity innumerable number of cold adaptive mechanisms would have evolved which is really unexplored. Also, fundamental questions on the role of cold induced proteins in psychrophilic bacteria inhabiting these unique cold environments, play at low temperature is waiting to be elucidated. Last but not least, most biotechnological applications of psychrophiles are environment friendly and contribute to energy saving, both aspects being of increasing significance. In this context, it is suggested to conduct in-depth molecular studies on bacteria isolated from Polar Regions.

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