Biotechnological perspectives of microorganisms isolated from the Polar Regions

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Polar permanently frozen grounds cover more than 20% of the earth's surface, and about 60% of the Russian territories are permafrost. In the permafrost environments, the combination of low temperature and poor availability of liquid water make these habitats extremely inhospitable for life. To date, both culture-dependent and culture-independent methods have shown that permafrost is a habitat for microorganisms of all three domains: *Bacteria*, *Archaea* and *Eukarya*. An overview of applying psychrophilic and psychrotolerant bacteria and archaea isolated from Arctic and Antarctic permafrost ecosystems in biotechnological processes of wastewater treatment, production of cold-adapted enzymes, etc. is discussed here. The study of existing collections of microorganisms isolated from permanently cold habitats, improved methods of sampling and enrichment will increase the potential biotechnological applications of permafrost bacteria and archaea producing unique biomolecules.

Even though microorganisms were found in the permafrost more than 100 years ago, intensive studies of microbial communities in the Arctic and Antarctic began at the end of the last century. Cold-adapted microorganisms do not only survive at low temperatures, but can show significant growth and metabolic activity (respiration and biosynthesis) at temperatures down to −20°C, and even at −39°C[1,2]. The number of publications describing new prokaryotic taxa isolated from the polar ecosystems has increased by eight times between 1990 and 2015 (Figure 1).

Cold-adapted microorganisms face diverse challenges inherent to living in cold environments: low enzyme activity and low rates of biochemical reactions, altered transport systems, decreased membrane fluidity, and cold denaturation of proteins[3]. To get over these challenges, they have developed remarkable adaptations. Psychrophiles produce higher amounts of unsaturated and methyl-branched fatty acids and shorter acyl-chain fatty acids to increase the membrane fluidity[4]. They also produce cold-shock proteins to aid in different cellular processes as well as antifreeze proteins that inhibit ice crystal growth. Furthermore, all components of their cells must be suitably adapted to cold temperatures. The key adaptation to cold is the synthesis of cold-adapted enzymes that show an improved flexibility of enzyme conformation and maintain high specific activities at low temperatures.

The vast majority of current industrial processes are performed under harsh conditions, including extremely high and low temperatures, acidic or basic pH values, and elevated salinity. Standard enzymes have specific requirements for maximal function and perform optimally in narrow ranges of physical and chemical conditions. Cold-adapted enzymes have a combination of specific

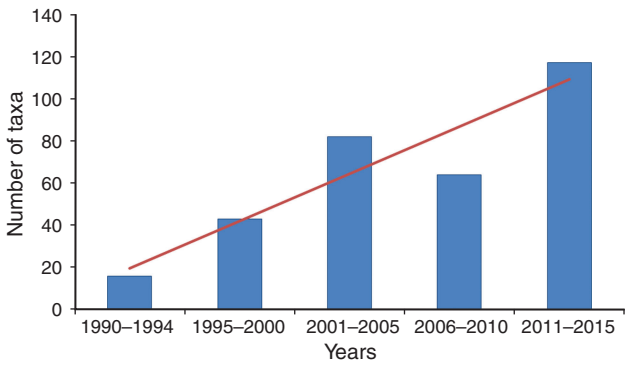

Fig 1. Distribution of the number of new prokaryotic taxa from the Polar regions validated from 1990 to 2015.

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adaptations in their structural features that give them more flexible structures than mesophilic and thermophilic enzymes. This trait allows for high catalytic activity at low temperatures[5] and thermolability. In recent years, scientific and industrial efforts to discover and develop novel cold-adapted enzymes have increased substantially. The intrinsic characteristics of cold-adapted enzymes, such as high activity at low temperatures and thermolability, are extremely valuable for biotechnological applications in a wide variety of industries from molecular biology to food and beverage preparation[6]. Consequently, psychrophilic enzymes are replacing mesophilic enzymes in many different industrial processes. Due to the limitations of cultivating psychrophiles for production of cold-adapted enzymes, the current strategy is to clone and express genes encoding the desired product in mesophilic hosts prior to the operation in a bioreactor[7].

Biotechnological applications of psychrophilic and psychrotolerant microorganisms include cold-water detergents, food additives and flavour modifying agents, biosensors, and environmental bioremediation. Cold-active lipases catalyse the hydrolysis of fats and remove fatty acid stains from tissues at low temperatures[8–10]. Recently, high-performance lipase from *Pseudomonas stutzeri* PS59 has been identified and proposed for use. This lipase has its optimal activity at 20°C, pH 8.5, and its activity is not inhibited by various surfactants and oxidants[11]. Despite a number of successes achieved in recent years in the study of cold-active enzymes for the production of detergents, there is a clear need for even more active enzymes from psychroactive microorganisms. Enzymes that can work optimally at low temperatures (15–25°C), but retain high activity over a wide range of temperatures (from 5–60°C), and in the presence of surfactants and at alkaline pH will have future applications in detergent compositions. Enzymes, which possess such universal properties, have already been obtained in the form of recombinant proteins[12]. Proteases are enzymes that catalyse the hydrolysis of peptide bonds in proteins breaking them into shorter fragments. They also play an important role in the washing industry for removal of protein stains. Recent studies have focused on the discovery of new cold-active proteases in psychrophilic microorganisms derived from the Arctic and Antarctic ecosystems[13].

Psychrophilic and psychroactive microorganisms synthesise various molecules to protect the cell from freezing or to minimise the harmful effects of ice crystal formation[3]. Antifreeze proteins (AFPs) are ice-binding proteins that have the ability to change the crystalline structure of ice and suppress ice growth in two directions. For the first time, the presence of a protein leading to thermal hysteresis (TH) in bacteria was demonstrated by Duman and Olsen[14], and the *Moraxella* sp. strain became the first bacterial producer of AFPs[15]. At the beginning of studies of the antifreeze phenomenon, the highest values of TH were found in insects (3–6°C) and fish (0.7–1.5°C). AFPs isolated from plants and microorganisms had a significantly smaller TH: from 0.2 to 0.5°C for plants, less than 0.1°C for bacteria. Later, an AFP with high activity was detected in the Antarctic bacterium *Marinomonas primoryensis*[16], which is able to reduce the freezing point by 2°C. Up until now, the antifreeze activity has been detected in a small number of bacteria, mainly in Antarctic isolates. This fact directly indicates a role for AFPs in bacterial adaptation to cold. To date, antifreeze proteins have been detected in actinobacteria of the genera *Micrococcus* and *Rhodococcus*, γ-proteobacteria of the genera *Colwellia, Marinomonas* and *Pseudomonas* and bacteria belonging to the genera *Flavobacterium* and *Chryseobacterium*[17]. Screening of anaerobic prokaryotes in the All-Russian collection of microorganisms (VKM) showed AFP of a new type in the spore-forming bacterium *Clostridium tagluense* strain 121^T isolated from the Canadian permafrost[18]. Metagenomic analysis of several permafrost Arctic samples of various origins and ages has been carried out[19] and the screening for AFP will be our next step.

Psychrotolerant microorganisms are of great value for bioremediation of hydrocarbon-contaminated soil in Polar Regions, because they can maintain activity under extreme environmental conditions. Several strains of psychrotolerant bacteria have been isolated from the oil-contaminated soil in Antarctica and studied in detail[20,21]. Low-temperature anaerobic digestion (AD) has been successfully applied to treat a vast range of wastewater types, such as phenolic, chlorinated aliphatic, brewery, pharmaceutical and glucose-based wastewaters. The evidence of comparable treatment efficiencies to mesophilic setups has also been recorded, as well as the successful treatment of complex wastewater. Despite successful applications, there is a lack of fundamental knowledge regarding the mechanisms underpinning AD. The future full-scale implementation of AD, and particularly the development of promising new applications, such as low-temperature AD, is severely impaired by this knowledge gap. Methanogenic populations have been the focus of many low-temperature AD studies due to their crucial role in biogas formation and biofilm integrity[22]. As our preliminary tests have shown, permafrost methanogens (Table 1) can be used for the process start-up at moderate and low temperatures. Other possible applications of psychroactive isolates from VKM are also presented in the Table 1.

Nature provides a vast source of biocatalysts. However, the probability of finding the right enzymatic activity for a particular application relies on the available technical capabilities to efficiently assess this large microbial diversity. This capability is mainly

Table 1. Psychrophilic and psychroactive prokaryotes from Arctic and Antarctic permafrost in VKM.

Species	Source of isolation	Growth temperature (optimum)	Application
Bacteria			
Clostridium algoriphilum 14D1^T VKM B-2271^T	Cryopeg in permafrost, Arctic	0–20°C (5–6°C)^A	Low temperature waste treatment
'*Clostridium frigoriphilum*' 14F^T VKM B-2368^T	Cryopeg in permafrost, Arctic	0–15°C (6°C)^A	Low temperature waste treatment
Clostridium tagluense 121 VKM B-2271^T	Permafrost, Arctic	0–28°C (15°C)^A	Low temperature waste treatment, AFP producing
'*Clostridium deceptionensis*' G4 VKM B-2784^T	Volcano ground, Antarctica	0–15°C (6–7°C)^A	Low temperature waste treatment
Celerinatantimonas yamalensis C7^T VKM B-2511^T	Cryopeg in permafrost, Arctic	0–34°C (18–22°C)^A	Nitrogen-fixing at near zero temperature, cold-adapted enzymes
Sphaerochaeta associata GLS2^T VKM B-2742^T	Permafrost, Arctic	5–37°C (30–34°C)	Low temperature waste treatment
'*Psychrobacter murincola*' 1pS VKM B-2269	Cryopeg in permafrost, Arctic	2–37°C (18–20°C)^A	Cold-adapted enzyme
'*Psychrobacter murincola*' 2pS^T VKM B-2270^T	Cryopeg in permafrost, Arctic	2–37°C (16–18°C)^A	Cold-adapted enzyme
Psychrobacter arcticus VKM B-2377^T	Permafrost, Arctic	0–28°C (22°C)^A	Cold-adapted lipase and esterase
Psychrobacter cryohalolentis VKM B-2378^T	Cryopeg in permafrost, Arctic	0–30°C (22°C)^A	Cold-adapted lipases
'*Desulfovibrio algoritolerans*' K3S^T VKM B-2877^T	Cryopeg in permafrost, Arctic	0–36°C (26°C)^A	Low temperature precipitation of heavy metals
Desulfovibrio arcticus B15^T VKM B-2367^T	Cryopeg in permafrost, Arctic	0–28°C (24°C)^A	Low temperature precipitation of heavy metals
Archaea			
'*Methanosarcina gilichinskiia*' JL01^T VKM B-2370^T	Permafrost, Arctic	10–37°C (25–28°C)^A	Low temperature biogas production
Methanobacterium arcticum M2^T VKM B-2371^T	Permafrost, Arctic	10–42°C (37°C)^A	Low temperature biogas production
Methanobacterium veterum MK4^T VKM B-2440^T	Permafrost, Arctic	5–37°C (30°C)^A	Low temperature biogas production

^{A}Capable of subzero growth.

mediated by technologies, such as metagenomic screenings, genome mining, and direct enzymatic exploration[23,24]. Metagenomic screenings and genome mining require that the search for a novel enzyme is based on genetic sequence homology to already described enzymes. But the discovery of new enzymes in this way does not always give accurate information, especially for less studied organisms like psychrophiles.

An alternative method is presented in the direct exploration of cold-adapted enzymes and AFPs based on functional screenings of enzymatic activities in large collections of microorganisms, such as VKM. Studies of microbial communities in cold ecosystems indicate that psychroactive prokaryotes represent a large variety of novel and understudied microorganisms. Research of existing collections of microorganisms isolated from permanently cold habitats, the

development of improved methods of sampling and enrichment will increase the potential biotechnological application of permafrost bacteria and archaea producing unique biomolecules.

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