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Soil Microbiome of the Cold Habitats: Trends and Application

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ABSTRACT

Bacterial communities in hard temperate are very important for low temperature diversity of microbes is vital for sustainability of an healthy ecosystem. Study of these microbes is very important as these can be source of various cold tolerant biomolecules. It has been observed that human interference in exclusive niches can disturb natural populations of a cold niche. Undisturbed niches of cold regions can be utilized for bioprospecting new biomolecules and can be a vital resource for new genomes. This review is focused on microbes and their habitat in which they can be studied for the benefit of mankind and shall provide information regarding variation in diversity and composition of bacterial communities. This will also give an idea about the association between bacterial community structures and soil physicochemical variables in cold-desert habitats.

Keywords- Temperate, Community, Soil microbiome and Cold habitat.

I. INTRODUCTION

Microbial communities exist in a wide range of environments, including both normal and extremely harsh conditions, such as those with extreme temperatures, salinity levels, pH levels, and water scarcity. These organisms have developed adaptive properties and produce bioactive compounds and secondary metabolites to survive under such conditions. Extremophiles are able to thrive in some of the harshest environments on Earth, including those with high salinity, alkalinity, and temperatures. Microbial communities in extreme habitats have adapted to various types of stress, including chemical, UV radiation, and changes in acidity levels. Recently, there has been a growing interest in using these communities in different fields such as white and green biotechnology, medicine, and food production. The microbiomes of cold environments are especially important to global ecology, as many terrestrial and aquatic ecosystems are exposed to cold temperatures.

The Earth is predominantly a cold, marine planet with a large portion of the ocean's waters being at temperatures of 5°C or lower. Cold environments such as permafrost soils, glaciers, polar sea ice, and snow cover make up about 20% of the Earth's surface. Microbial communities in these habitats have undergone physiological adaptations to low temperatures and chemical stress. Recent research has focused on the biotechnological potential of these communities, as well as their use as primitive analogues of biomolecules that existed in early Earth environments. Culture-dependent and culture-independent techniques have been used to extensively investigate the microbiomes of cold environments, resulting in the discovery of many novel microbes from Antarctic sub-glacial lakes, permanently ice-covered lakes, cloud droplets, ice cap cores, snow, and ice glaciers. Extremophiles or stress-adaptive microbes are classified as living microbes that can survive or adapt to harsh environmental conditions such as pH levels, pressure, radiation, redox potential, salinity, and temperature. Polyextremophilic microbiomes can survive and grow under two or more harsh conditions.

Cold-adapted microbes are found in various habitats such as permanently ice-covered lakes, cloud glaciers, and hilly regions. The extreme low temperatures in these environments provide unique ecosystems that support novel biodiversity, which has been extensively studied using culture-dependent and culture-independent techniques. These cold environments are known to harbor a diverse range of psychrotrophic, psychrophilic, and psychrotolerant microbiomes. Polar ecosystems, including the lands and oceans of the Arctic and Antarctic, are well-known habitats for cold-adapted microbes, but high-altitude regions such as the Himalayan ranges also harbor these organisms. Unlike polar habitats, hilly terrains show seasonal temperature variations and are surrounded by land masses with relatively higher temperatures that support mesophilic bacterial communities.

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II. RECENT TRENDS

Early estimates suggested that a single gram of soil could potentially contain up to 10 billion microorganisms [14], which could represent up to 10,000 different microbial species [15]. The biodiversity of psychrotrophic microbes that inhabit cold environments has been extensively studied worldwide and has been found to be distributed across various phyla, including Actinobacteria, Gemmatimonadetes, Ascomycota, Acidobacteria, Bacteroidetes, Basidiomycota, Chlamydiae, Chloroflexi, Proteobacteria, Cyanobacteria, Firmicutes, Mucoromycota, Verrucomicrobia, Nitrospirae, Planctomycetes, Spirochaetes, Thaumarchaeota, and Euryarchaeota. In the Himalayan region, 45% of the bacterial strains that were cultured belonged to the Proteobacteria group, with g-Proteobacteria accounting for 39% of these isolates. The Firmicutes class was the second most abundant, accounting for 32% of the total isolates, followed by Actinobacteria (16%) and Bacteroidetes (6%) (Table S12). Among the 18 different genera observed, Bacillus was the most prevalent, accounting for 30% of the strains, followed by Pseudomonas (24%) and Arthrobacter (12%). Other isolated genera included Acinetobacter, Aeromonas, Flavobacterium, and Exiguobacterium. Some other genera were represented by only one or two isolates, including Brevundimonas, Hydrogenophaga, Iodobacter, Paucibacter, Vogesella, Alishwanella, Rheineheimera, Yersinia, Mycobacterium, Rhodococcus, and Streptomyces. Based on their closest match in the database, these strains belonged to 49 different species, with both Pseudomonas and Bacillus showing the highest diversity with 10 different species each, followed by Arthrobacter with seven different species.

Recent advancements in technology, such as metagenomics, have greatly improved our understanding of the biodiversity and distribution of extremophilic microbiomes living in harsh environments. These approaches have revealed that microorganisms are highly abundant in these environments, and that the true depth of microbial diversity and their functional capacity is much greater than previously thought. However, it is important to note that metagenomic approaches have limitations, as they cannot distinguish between living, dormant, or dead cells in a microbial community. To complement metagenomics, other omic technologies, such as gene expression and proteomic analysis, have been developed to gain further insights into the mechanisms and metabolisms of extremophilic microorganisms. It has also been observed that extreme environments harbor lower levels of microbial diversity compared to more benign ecosystems, likely due to the specific physiological adaptations required for organisms to survive in these conditions. As a result, simplified ecosystems dominated by relatively few taxa are common in extreme environments.

Recent advancements in emerging technologies have led to a better understanding of the role of microbial life in cold environments, such as metagenomics which analyzes collective genomes of microbial communities to determine their functional potential. However, a major limitation of this approach is the uncertainty of the origin of the microbial community DNA, which may come from living, dormant, or dead cells. To complement metagenomics, other -omic technologies like gene expression and proteomic analysis have been developed to provide more insight into the mechanisms and metabolisms of these microbial communities.

Extremophilic microbiomes living in harsh environments have been extensively studied using culturable and metagenomic methods. Early estimations suggested that a gram of soil may harbor up to 10 billion microorganisms, possibly representing as many as 10^4 different microbial species. Thanks to the use of culture-independent methods and omic approaches, we now have a better understanding of the true depth of microbial diversity and their functional capacity. It has been observed through the application of metagenomic approaches that extreme environments tend to have lower levels of microbial diversity, both in terms of species richness and relative abundance, compared to more benign ecosystems. This is due to the specific physiological adaptations required for these organisms to exploit the combination of physical and biochemical stressors, resulting in simplified ecosystems dominated by relatively few taxa.

A large number of microbes in soil cannot be cultured in laboratory conditions, which are referred to as viable but unculturable (VBNC). To analyze these VBNC microbes, a technique that focuses on their biomolecules such as nucleic acids, lipids, and proteins has been developed, and their 16S rRNA is being studied. This technique involves various molecular techniques, which are categorized into two main groups: partial community analysis and whole community analysis. Partial community analysis involves PCR-based strategies, where DNA or RNA samples extracted directly from the soil are used as a template for characterization. PCR products can be analyzed using techniques like clone library method, DNA microarray, and genetic fingerprinting. Whole community analysis, on the other hand, involves the sequencing of the 16S rRNA gene using techniques such as DNA-DNA hybridization, guanine-plus-cytosine content fractionation, whole-microbial-genome sequencing, metagenomics, metatranscriptomics, proteogenomics, and metaproteomics. In a study conducted in the Indian Himalayan region, 247 culturable bacteria were isolated using serial dilution and spread plate methods from different sites. The bacteria were identified using 16S rRNA gene sequencing and BLAST analysis, revealing that they were affiliated with four phyla, namely Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria.

Researchers analyzed the biodiversity of cold-adapted bacteria in the northern hills zone of India. They used serial dilution and spread plate methods to isolate 247 bacteria from various sites in the Indian Himalayan regions, and identified them through 16S rRNA gene sequencing and BLAST analysis. Based on the analysis of the sequences, they determined that the bacteria belonged to four phyla: Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteia.

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III. APPLICATIONS

In recent times, there has been an increasing focus on studying microbial communities (including archaea, bacteria, and fungi) in harsh environments, as they have potential applications in several fields such as biotechnology, medicine, and food production. These extremophilic microbes are believed to have great potential in agriculture, pharmaceuticals, and related sectors. The study of extremophiles can offer tremendous potential for future sustainability, and the enzymes produced by them, known as extremozymes, can play a vital role in important biological processes, including agriculture, pharmaceuticals, and chemistry, and have significant economic and biotechnological potential.

Microbial communities that can adapt to extreme environments have been recognized for their potential to contribute to the sustainability of agro-environmental systems. These stress-adaptive microbes can produce bioactive compounds, including indole acetic acids, gibberellic acids, cytokinin, hydrogen cyanides, ammonia, siderophores, 1-aminocyclopropane-1-carboxylate deaminase, and extracellular microbial hydrolytic enzymes such as amylase, xylanase, pectinase, chitinase, and cellulase. These extremophilic microbiomes have broad applications across several fields, such as agriculture, biodegradation, chemical processing, molecular biology, composting, food and dairy industry, leather industry, paper and cellulose industry, feed additives and feed industry, pharmaceutical industry, and peptide synthesis. Additionally, they are utilized in the food processing industry to produce various bioactive compounds, secondary metabolites, and value-added products like flavors, food ingredients, and vitamins.

Psychrophilic and psychrotolerant microbes are significant due to their ability to produce enzymes that are active at low temperatures. These enzymes have gained interest in various industrial applications as they can potentially reduce energy consumption. Additionally, studying the adaptation of life to low temperatures and the potential for biotechnological exploitation of cold-active enzymes are other reasons why these microbes are important. A lot of research has focused on investigating cold-active enzymes such as amylase, protease, lipase, pectinase, xylanase, cellulase, β-glucosidase, β-galactosidase, and chitinase produced by psychrophilic bacteria.

Proteases are a type of extremozyme that are widely found in various microorganisms, including prokaryotes, eukaryotes, and viruses. They are known to be commercially important enzymes and are commonly used in a variety of industries such as detergents, food, pharmaceuticals, chemicals, leather, and silk production. Proteases also play a role in maintaining ecological balance through the breakdown and recycling of agricultural waste.

The combination of pectinases and xylanases in biotechnological refining is an economical and environmentally friendly option. Microbes, both normal and stress-adaptive, produce amylases that are crucial for starch-related industries, as well as having significant potential in the pharmaceutical and fine chemical industries.

Psychrophilic microbes have potential applications in biodegrading agro-wastes at low temperatures. A microbial consortium comprising *Eupenicillium crustacean, Paceliomyces sp., Bacillus atrophies* and Bacillus sp. was developed by Shukla et al. [43] to degrade agri-residues and convert them into compost for improving soil fertility and reducing environmental pollution caused by burning of agro-wastes. Psychrotrophic microbes produce anti-freezing compounds (AFCs) at low temperatures [1,27], which can be used in cryosurgery and cryopreservation of isolated organs, cell lines, tissues, and whole organisms. In the food industry, anti-freezing proteins (AFPs) can be used to enhance the quality of frozen food. Some studies have shown that improved cold tolerance in fish can be achieved through the direct injection or transgenic expression of AFPs.

IV. SUMMARY

To tackle the negative impact of climate change on agriculture, it is crucial to develop new sustainable and environmentally friendly strategies that can limit the effect of cold stress on crops. As global warming is expected to cause earlier spring-related events in plants, the risk and severity of spring frosts are likely to increase. One potential solution against cold stress is the use of plant-associated microorganisms and their compounds. However, most of the microbial products used in agriculture are derived from mesophilic microorganisms, which are not effective in promoting plant growth under cold conditions.

Many microorganisms isolated from alpine, Arctic, and Antarctic environments have been found to possess plant growth-promoting traits, including the ability to produce indole3-acetic acid, gibberellic acid, 1-aminocyclopropane-1-carboxylate-deaminase, hydrogen cyanide, hydrogen sulfide, ammonia, siderophores, hydrolytic enzymes, phosphate solubilizing products, and nitrogen-fixing activity (Calvo et al. 2010; Sheng et al. 2011; Wang et al. 2016; Yadav et al. 2016; Ogata-Gutierrez et al. 2017; Castellano-Hinojosa et al. 2018; Chumpitaz-Segovia et al. 2020; Ma et al. 2020; Tapia-Vazquez et al. 2020; Ulloa-Munoz et al. 2020; Yang et al. 2020; Nosko, Bliss, and Cook 1994; Sun et al. 1995; Barrientos-Dıaz, Gidekel, and Gutierrez-Moraga 2008; Selvakumar et al. 2011; Berrıos et al. 2013; Peixoto et al. 2016; Yarzabal et al. 2018; Tiryaki, Aydın, and Atıcı 2019; Tistechok et al. 2019; Araya et al. 2020). Some of these traits have also been shown to improve cold stress tolerance in plants, including antioxidant enzymes like superoxide dismutase and peroxidases, osmolytes like trehalose and raffinose, and nutrient production like nitrogen and phosphorus (Acuna-Rodrıguez et al. 2020).

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Most studies have examined the functional role of plant-associated microorganisms from cold environments in promoting plant growth and increasing tolerance to cold stress in controlled settings. However, further research is needed to validate their effects under field conditions for the development of effective agents for protecting plants from cold stress using psychrotolerant plant-growth-promoting bacteria. Microbes can improve soil fertility and crop yield, as well as help plants resist biotic and abiotic stresses, thus reducing the negative impact of chemical fertilizers on the environment. (Kumar et al., 2019b; Rana et al., 2019).

Soil microbes play a crucial role in enhancing crop productivity by regulating plant growth and providing nutrients. They produce hormones and vitamins that promote plant growth and are considered a sustainable and environmentally-friendly solution for improving plant productivity. These microbes, known as plant growth-promoting microbes, are effective biofertilizers and can be classified into nitrogen-fixing bacteria, phosphorus solubilization microorganisms, composting microbiomes, and biopesticides. These microbial groups also exhibit other plant growth-promoting characteristics such as polysaccharide, phytohormone, amino acid, and siderophore production, which contribute to further improvements in crop yield.

Insects and microbial pathogens pose a significant threat to crop production, causing substantial economic losses for farmers. Historically, pesticides were used to combat these pests, but their overuse has resulted in soil degradation and fertility loss. Additionally, the excessive use of chemical products has had negative impacts on human health and soil microbial populations, leading to the development of resistant insect pests and microbial pathogens. As a result, researchers have proposed the use of beneficial soil microbes as an alternative strategy to control pathogen growth, which was first suggested in 1901. This approach aims to promote environmental health and crop production while avoiding the negative consequences associated with chemical pesticides.

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