Vol. 10 No. 2 (2024):1450-1466
DOI: https://doi.org/10.48047/

Leveraging Psychrophilic PGPR Strains for Enhanced Wheat Growth: A Sustainable Biofertilizer Approach

[1]Safia Saher, [2]Samia Zahid, [3]Sofia Arif, [4*]Usman Wajid.

[1,3]Department of Bio Sciences and Management Sciences COMSATS University Islamabad, Pakistan.

[2]Department of Health Informatics COMSATS University Islamabad, Pakistan.

[4*]University Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Correspondence: wajidusman323@gmail.com.

Abstract

Psychrophilic Plant Growth-Promoting Rhizobacteria (PGPR) play a crucial role in enhancing sustainable wheat production in cold environments by promoting plant growth through various mechanisms and serving as effective biofertilizers. These bacteria can tolerate a wide range of temperatures, from as low as -8°C to as high as 36°C, making them well-suited for cold climates. (1). They enhance plant growth by solubilizing essential nutrients like phosphorus and zinc, even at low temperatures, and producing phytohormones such as indole-3-acetic acid that stimulate root elongation (1,2). Some psychrophilic PGPR can also fix atmospheric nitrogen, providing an additional source of nutrients for plants (1). Additionally, these bacteria induce stress tolerance mechanisms in plants, enabling them to better withstand cold stress by activating protective genes and producing antifreeze proteins (3). Studies have shown that the application of psychrophilic PGPR, either as individual strains or as consortia, can significantly reduce the need for synthetic fertilizers without compromising wheat yield and quality (2). When applied with half the recommended dose of fertilizers, these bacteria can maximize crop parameters and yield, providing a sustainable approach to wheat production in cold regions (2). The use of psychrophilic PGPR as biofertilizers has been found effective in yield optimization under temperature-stressed conditions, making them a promising solution for addressing food security concerns in the face of climate change (3). By improving nutrient availability, plant growth, and stress tolerance, psychrophilic PGPR contribute to the development of eco-friendly and resilient agricultural practices in cold environments.

Keywords: Psychrophilic PGPR, Wheat Growth, Biofertilizers, Sustainable Agriculture, Cold Environments.

1. Introduction

The Global Significance and Challenges of Wheat Production

Wheat is a cornerstone of global food security, being the most widely cultivated crop and a primary source of calories and protein for billions. Its cultivation spans approximately 220 million hectares, contributing significantly to the diets of populations, particularly in temperate zones. Wheat provides about 20% of the world's food calories and protein and is crucial in regions undergoing rapid urbanization and industrialization, where demand is surging (5). However, wheat production faces significant challenges, especially in colder environments where climate conditions can hinder growth

Vol. 10 No. 2 (2024):1450-1466
DOI: https://doi.org/10.48047/

and yield. These challenges include extreme weather events, soil degradation, and pest pressures, which are exacerbated by climate change. Moreover, the need for sustainable agricultural practices is increasingly urgent. Enhancing wheat productivity must align with environmental stewardship to ensure that future generations can meet their nutritional needs without compromising the planet's health. This calls for innovative agricultural techniques, investment in research and development, and a shift towards practices that conserve resources and promote biodiversity. As the global population is projected to reach 9.7 billion by 2050, the agricultural sector must adapt to these demands while ensuring that wheat remains a viable staple crop, capable of supporting diverse diets and resilient food systems across varying climates and regions (4). Wheat shows high variability in beneficial components like protein, fibre, and phytochemicals, some of which have high heritability, allowing breeders to select for enhanced health benefits in addition to yield (5). Textured wheat proteins also provide a sustainable, plant-based alternative to meat, contributing to a more nutritious and environmentally-friendly global food supply (6). As one of the oldest and most important staple crops, wheat will play a crucial role in feeding the world's growing population in the coming decades, but only if production can be made more resilient and sustainable, especially in challenging cold climates (7).

Importance of Sustainable Agriculture:

The Environmental Impact of Fertilizers and the Role of Sustainable Practices

Traditional fertilizers, particularly synthetic nitrogen fertilizers, have significantly increased agricultural productivity over the past century, enabling food production to keep pace with population growth. However, their overuse and inefficient application have led to serious environmental consequences. Fertilizer runoff and leaching contaminate water sources, causing eutrophication and harming aquatic ecosystems (8,9). Nitrous oxide emissions from fertilized soils are a potent greenhouse gas, contributing to climate change (10). Fertilizers can also degrade soil health, deplete organic matter, and lead to heavy metal accumulation (9,11). These environmental costs are unsustainable and threaten the long-term viability of modern agriculture. Sustainable agricultural practices offer a path forward. Precision farming techniques optimize fertilizer application based on crop needs and soil conditions, minimizing excess (8). Cover cropping and crop rotation improve soil fertility while reducing erosion and nutrient losses (12). Organic farming avoids synthetic inputs altogether, relying on compost, manure, and crop residues to nourish plants (11). Biofertilizers, containing beneficial microbes, enhance nutrient cycling and plant growth while sequestering carbon in the soil (8,12). Agroforestry and silvopasture systems integrate trees with crops or livestock, increasing biodiversity and resilience (12). Transitioning to sustainable agriculture will require significant investment and innovation. Governments must incentivize best practices, while researchers develop new technologies and crop varieties. Farmers need training and support to adopt unfamiliar techniques. Consumers can drive change by choosing sustainably-produced foods. By working together, we can create a food system that meets human needs while restoring the health of our shared environment.

Introduction to PGPR:

Plant Growth-Promoting Rhizobacteria (PGPR) are a diverse group of beneficial bacteria that inhabit the rhizosphere—the region of soil influenced by plant roots—and play a crucial role in enhancing plant growth and health through multiple mechanisms. These microorganisms are essential for sustainable agriculture, as they improve plant health and productivity while minimizing the reliance on chemical fertilizers and pesticides. One of the primary mechanisms by which PGPR promote plant growth is through nitrogen fixation. Certain strains, such as *Rhizobium* and *Azospirillum*, possess the ability to convert atmospheric nitrogen into ammonia, a form that plants can readily absorb. This process alleviates nitrogen deficiency in soils and enhances crop yields, particularly in nitrogen-poor environments. The nitrogen-fixing capability of PGPR is especially beneficial in sustainable agriculture, as it reduces the need for synthetic nitrogen fertilizers, which can lead to environmental issues such as water pollution and greenhouse gas emissions (13,14).

In addition to nitrogen fixation, PGPR enhance plant growth through the production of phytohormones, including auxins, gibberellins, and cytokinins. These hormones regulate various physiological processes in plants, such as root development, stem elongation, and overall plant vigor. For instance, *Pseudomonas fluorescens* is known to produce indole-3-acetic acid (IAA), an auxin that stimulates root elongation and branching, thereby increasing the root surface area for nutrient and water absorption. This hormonal modulation not only promotes growth but also helps plants cope with abiotic stresses, such as drought and salinity, by enhancing their adaptive responses (15,16). Furthermore, PGPR can influence the balance of other plant hormones, such as ethylene, which plays a critical role in plant responses to stress (17).

Phosphate solubilization is another critical mechanism through which PGPR promote plant growth. Many plants struggle to access phosphorus, an essential nutrient, due to its low solubility in soil. PGPR like *Bacillus* and *Penicillium* species can solubilize inorganic phosphate by secreting organic acids that dissolve phosphate compounds, making them available for plant uptake. This process is particularly important in agricultural systems where phosphorus is often a limiting nutrient, and its availability can significantly influence crop yields (13,16). The ability of PGPR to enhance phosphorus availability not only supports plant growth but also contributes to improved soil health by promoting microbial diversity and activity.

Moreover, PGPR contribute to plant health by inducing systemic resistance against pathogens. They can trigger plant defence mechanisms that enhance resistance to various biotic stresses, including diseases caused by fungi, bacteria, and nematodes. This induced systemic resistance (ISR) is mediated through the production of signalling molecules that activate the plant's immune response, providing a protective effect against subsequent pathogen attacks. For example, *Bacillus subtilis* has been documented to produce antibiotics and other compounds that inhibit the growth of harmful pathogens while promoting beneficial microbial communities in the rhizosphere (15). The role of PGPR in disease suppression is crucial for sustainable agriculture, as it reduces the reliance on chemical pesticides and promotes a healthier ecosystem.

The environmental benefits of utilizing PGPR are significant, particularly in the context of sustainable agriculture. By reducing the need for chemical fertilizers and pesticides, PGPR can help mitigate the adverse environmental impacts associated with conventional farming practices, such as soil degradation, water pollution, and loss of biodiversity. The application of PGPR as biofertilizers promotes a more sustainable approach to agriculture, enhancing soil health and fertility while improving crop yields. Furthermore, the integration of PGPR into agricultural practices aligns with the principles of agroecology, which emphasize the importance of biodiversity and ecological balance in farming systems (14).

Psychrophilic PGPR:

Psychrophilic Plant Growth-Promoting Rhizobacteria (PGPR) are specialized bacterial strains that thrive in cold environments, exhibiting remarkable adaptations that enable them to enhance plant growth under low-temperature conditions. These bacteria, including species such as *Pseudomonas syringae*, *Bacillus subtilis*, and *Psychrobacter* spp., have evolved unique metabolic and physiological traits that allow them to survive and function optimally at temperatures that would inhibit the growth of most microorganisms. One of their key adaptations is the production of cold-active enzymes, which maintain metabolic processes at low temperatures, thereby facilitating nutrient cycling and promoting plant growth even in challenging conditions. Additionally, psychrophilic PGPR synthesize antifreeze proteins that prevent ice crystal formation within their cells, protecting them from freezing damage and allowing them to colonize plant roots effectively in cold soils (18,20).

The mechanisms by which psychrophilic PGPR enhance plant growth are diverse and multifaceted. One of the primary roles of these bacteria is nitrogen fixation, where specific strains convert atmospheric nitrogen into ammonia, a form that plants can readily absorb. This process is particularly beneficial in cold climates where soil nitrogen levels may be low, thus supporting plant growth in nutrient-poor conditions. For instance, *Azospirillum* species, known for their nitrogen-fixing capabilities, can significantly improve the nitrogen status of the soil, leading to enhanced crop yields in cold regions (19). Furthermore, psychrophilic PGPR can produce phytohormones, such as indole-3-acetic acid (IAA), which stimulate root development and enhance nutrient uptake. Studies have shown that these bacteria can promote root elongation and branching, increasing the root surface area available for nutrient and water absorption, which is crucial for plants in cold, often nutrient-deficient soils (21).

Phosphate solubilization is another critical mechanism employed by psychrophilic PGPR to promote plant growth. Many plants struggle to access phosphorus due to its low solubility in cold soils, which can limit their growth. Psychrophilic bacteria can solubilize inorganic phosphates by secreting organic acids that dissolve phosphate compounds, making phosphorus available for plant uptake. This is particularly important in agricultural systems where phosphorus is often a limiting nutrient, and its availability can significantly influence crop yields. For example, psychrophilic strains of *Bacillus* and *Pseudomonas* have been shown to enhance phosphorus solubilization, thereby improving the nutritional status of plants and promoting better growth (20).

Moreover, psychrophilic PGPR contribute to plant health by inducing systemic resistance against pathogens. They can trigger plant defense mechanisms that enhance resistance to various biotic stresses, including diseases caused by fungi, bacteria, and nematodes. This induced systemic resistance (ISR) is mediated through the production of signaling molecules that activate the plant's immune response, providing a protective effect against subsequent pathogen attacks. For instance, *Pseudomonas fluorescens* has been documented to produce antibiotics and other compounds that inhibit the growth of harmful pathogens while promoting beneficial microbial communities in the rhizosphere (18). The ability of these bacteria to enhance disease resistance is particularly valuable in cold climates, where plants may be more susceptible to certain pathogens due to stress from low temperatures.

The potential of psychrophilic PGPR in enhancing crop growth in cold environments is increasingly recognized, especially with the challenges posed by climate change and the need for sustainable agricultural practices. By utilizing these beneficial bacteria, farmers can improve crop resilience and productivity in regions with harsh climatic conditions. The application of psychrophilic PGPR as biofertilizers not only supports sustainable agriculture by reducing the reliance on chemical fertilizers but also contributes to soil health and biodiversity. Furthermore, the integration of these PGPR into agricultural practices aligns with the principles of agroecology, emphasizing the importance of biodiversity and ecological balance in farming systems (21).

2. Mechanisms of Action of Psychrophilic PGPR

Nitrogen Fixation:

Psychrophilic Plant Growth-Promoting Rhizobacteria (PGPR) possess the remarkable ability to fix atmospheric nitrogen in cold soils, which can significantly enhance the growth and productivity of wheat crops. These cold-adapted bacteria, including species like *Pseudomonas syringae*, *Bacillus subtilis*, and various *Psychrobacter* strains, have evolved specialized mechanisms to thrive in low-temperature environments. One of the key adaptations is the production of cold-active enzymes that facilitate nitrogen fixation at low temperatures, enabling the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), a form readily available for plant uptake. This biological nitrogen fixation is particularly beneficial for wheat cultivation in cold regions, where soil nitrogen levels are often insufficient to support optimal growth and yield. Studies have shown that inoculating wheat with nitrogen-fixing psychrophilic PGPR can lead to significant improvements in plant growth parameters, such as root length, shoot height, and overall biomass (22,23). The enhanced nitrogen availability, coupled with the production of phytohormones like indole-3-acetic acid (IAA) that stimulate root development, allows wheat plants to better access nutrients and water in nutrient-poor, cold soils (24). Additionally, psychrophilic PGPR can solubilize phosphorus, another critical nutrient, by secreting organic acids that dissolve insoluble phosphate compounds, further contributing to the improved nutritional status of wheat plants (25). By harnessing the nitrogen-fixing capabilities of these beneficial bacteria, farmers can reduce their reliance on synthetic nitrogen fertilizers while promoting sustainable wheat production in cold environments, ultimately contributing to food security and environmental stewardship.

Phosphate Solubilization:

Psychrophilic Plant Growth-Promoting Rhizobacteria (PGPR) play a crucial role in enhancing phosphate solubilization, which is essential for improving the nutritional status and growth of wheat crops in cold environments. These cold-adapted bacteria possess specialized mechanisms to solubilize inorganic phosphates, making them available for plant uptake. The process of phosphate solubilization by psychrophilic PGPR involves the secretion of low molecular weight organic acids, such as gluconic acid and 2-ketogluconic acid, which chelate the cations bound to phosphates, converting them into soluble forms (26,29). Additionally, these bacteria produce enzymes like phosphatases and phytases that mineralize organic phosphates, further increasing the pool of available phosphorus in the soil (26,27).

The ability of psychrophilic PGPR to solubilize phosphates is particularly important for wheat cultivation in cold regions, where phosphorus availability is often limited. Studies have shown that inoculating wheat with efficient phosphate-solubilizing psychrophilic PGPR can significantly enhance plant growth parameters, such as root length, shoot height, and biomass production (26,28). The increased phosphorus availability, combined with the ability of these bacteria to fix atmospheric nitrogen and produce phytohormones, creates a favourable environment for wheat growth in nutrient-poor, cold soils (29).

Moreover, the application of psychrophilic phosphate-solubilizing PGPR as biofertilizers promotes sustainable wheat production by reducing the reliance on chemical phosphorus fertilizers, which can have negative environmental impacts. These beneficial bacteria contribute to improved soil health by increasing microbial diversity and activity, ultimately enhancing the long-term fertility and productivity of agricultural systems (26). As the global demand for food continues to rise, particularly in regions with challenging climatic conditions, the utilization of psychrophilic PGPR offers a promising strategy for improving wheat nutrition and yield while promoting sustainable agricultural practices.

Production of Phytohormones:

Psychrophilic Plant Growth-Promoting Rhizobacteria (PGPR) possess remarkable adaptations that enable them to produce crucial plant growth-promoting hormones like auxins, cytokinins, and gibberellins even in cold environments. These cold-adapted bacteria have evolved mechanisms to maintain metabolic processes at low temperatures, allowing them to synthesize phytohormones that stimulate various aspects of plant growth and development. Auxins, particularly indole-3-acetic acid (IAA), are produced by a wide range of psychrophilic PGPR, including *Pseudomonas*, *Bacillus*, and *Azospirillum* species. IAA promotes cell division, elongation, and differentiation, enhancing root growth and nutrient uptake, which is crucial for plants in cold soils (11). Psychrophilic PGPR also produce cytokinins, which regulate cell division and shoot development. Bacterial genera like *Pseudomonas*, *Bacillus*, and *Azospirillum* have been documented to synthesize cytokinins that stimulate shoot growth, delay senescence, and modulate root architecture in plants grown under low-temperature conditions (12).

Additionally, psychrophilic PGPR can produce gibberellins, which play a vital role in seed germination, stem elongation, and reproductive development. Strains such as *Azospirillum lipoferum*, *Bacillus cereus*, and *Bacillus pumilus* have been shown to synthesize gibberellins that promote plant growth and help overcome the inhibitory effects of cold stress (19). The ability of psychrophilic PGPR to produce these phytohormones in cold environments not only enhances plant growth but also improves their resilience to abiotic stresses, ultimately leading to better crop yields in challenging climatic conditions.

Siderophore Production:

Siderophores produced by psychrophilic Plant Growth-Promoting Rhizobacteria (PGPR) play a critical role in enhancing iron acquisition for wheat, particularly in cold environments where iron availability is often limited due to the formation of insoluble ferric compounds. These bacteria, which thrive at low temperatures, secrete siderophores—small, high-affinity iron-chelating compounds—that bind to ferric iron (Fe^{3+}) in the soil, converting it into a soluble form that can be readily absorbed by plant roots. The production of siderophores is essential because iron is a vital micronutrient required for various physiological processes in plants, including photosynthesis, respiration, and nitrogen fixation. In cold soils, the solubility of iron decreases, making it less available for plant uptake; however, siderophores produced by psychrophilic PGPR can effectively solubilize this iron, thus improving its bioavailability for wheat plants (22). Research has shown that inoculating wheat with siderophore-producing psychrophilic PGPR can lead to significant improvements in plant growth parameters, such as root length, shoot height, and overall biomass. For instance, strains like *Pseudomonas fluorescens* and *Bacillus subtilis*, which are known for their ability to produce siderophores, have been found to promote wheat growth under cold stress conditions (26). The increased availability of iron through siderophore activity not only enhances chlorophyll synthesis and photosynthetic efficiency but also stimulates root development, enabling plants to better access water and nutrients in nutrient-poor, cold soils. The presence of these beneficial bacteria in the rhizosphere can also stimulate the plant's defence mechanisms, enhancing its resilience to abiotic stresses such as low temperatures and nutrient deficiencies (14). Moreover, siderophores produced by psychrophilic PGPR can act as biocontrol agents by sequestering iron from the rhizosphere, depriving pathogenic microorganisms of this essential nutrient and suppressing their growth. This competitive advantage is crucial in nutrient-poor conditions, as it not only enhances the iron nutrition of wheat but also suppresses the growth of harmful pathogens. The ability of these bacteria to produce siderophores is influenced by various environmental factors, such as soil pH, temperature, and the availability of iron in the soil. Psychrophilic PGPR have evolved mechanisms to optimize siderophore production even at low temperatures, ensuring that plants receive adequate iron during critical growth phases (31). The types of siderophores produced by psychrophilic PGPR can vary, with some strains producing hydroxamate-type siderophores, while others produce catecholate-type siderophores. The diversity of siderophores produced by these bacteria contributes to their ability to chelate iron under different soil conditions, making them more effective in promoting plant growth. For example, the hydroxamate siderophores produced by *Pseudomonas* species have been shown to have a higher affinity for iron compared to catecholate siderophores, which enhances their effectiveness in iron acquisition (13).

ACC Deaminase Activity:

Psychrophilic plant growth-promoting rhizobacteria (PGPR) play a crucial role in mitigating plant stress by lowering ethylene levels through the activity of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (4). Ethylene is a plant hormone that is typically produced in response to stress factors such as salinity, drought, and cold, leading to adverse physiological effects including inhibited growth and premature senescence. ACC deaminase catalyzes the conversion of ACC, a precursor of ethylene, into ammonia and α-ketobutyrate, effectively reducing the amount of ethylene synthesized by the plant. This enzymatic activity is particularly significant under stress conditions

Vol. 10 No. 2 (2024):1450-1466
DOI: https://doi.org/10.48047/

where the accumulation of ACC is heightened due to stress-induced root exudation. Studies have shown that psychrophilic PGPR, such as specific strains of *Bacillus* and *Pseudomonas*, possess high ACC deaminase activity, which enables them to thrive in cold environments while simultaneously promoting plant growth and resilience (2). For instance, research indicates that these bacteria not only lower ethylene levels but also enhance root development, nutrient uptake, and overall plant vigor by modulating phytohormone levels. The presence of psychrophilic PGPR has been linked to improved plant growth metrics, such as shoot length, fresh weight, and dry weight, particularly in crops subjected to cold stress. Furthermore, the genetic screening of these bacteria reveals specific genes associated with ACC deaminase production, underscoring their potential utility in sustainable agriculture, especially in regions facing climate challenges (4). By utilizing psychrophilic PGPR, farmers can enhance crop resilience, ensuring better yields even under adverse environmental conditions, thus contributing to food security and sustainable agricultural practices in a rapidly changing climate. This synergy between psychrophilic PGPR and plants exemplifies a promising avenue for biotechnological applications aimed at enhancing agricultural productivity while minimizing reliance on chemical fertilizers and pesticides, ultimately fostering a more sustainable approach to crop management in the face of global climate variability (17). The ongoing exploration of the mechanisms underlying PGPR interactions with plant systems continues to reveal the multifaceted benefits these beneficial bacteria provide, making them invaluable allies in modern agriculture.

3. Isolation and Characterization of Psychrophilic PGPR Strains

Sources of Psychrophilic PGPR:

Psychrophilic plant growth-promoting rhizobacteria (PGPR) are microorganisms that thrive in extremely cold environments, such as alpine regions and polar soils. These cold-adapted bacteria play a crucial role in enhancing plant growth and resilience in harsh climatic conditions. The unique ecological niches from which psychrophilic PGPR can be isolated include diverse cold environments characterized by low temperatures, high altitudes, and specific soil and water characteristics.

Cold Environments Hosting Psychrophilic PGPR

1. **Alpine Regions**: Alpine ecosystems, defined by their high elevation and cold temperatures, provide a rich habitat for psychrophilic microorganisms. These areas experience extreme weather conditions, including heavy snowfall and low temperatures that can drop well below freezing. The soil in these regions often has a unique composition, influenced by glacial activity and the presence of permafrost. Research has shown that various strains of psychrophilic PGPR, particularly from the genus *Pseudomonas*, can be isolated from alpine soils, where they exhibit traits that promote plant growth despite the challenging conditions. For instance, studies have documented the isolation of *Pseudomonas corrugata* and *Pseudomonas fragi* from high-altitude locations, demonstrating their potential as bioinoculants for crops grown in these environments (1,3).

2. **Polar Soils**: The polar regions, including the Arctic and Antarctic, are characterized by extreme cold and prolonged periods of darkness. These environments are often covered in ice and snow, with temperatures that can remain consistently below freezing. The microbial communities in these soils have adapted to survive in

Vol. 10 No. 2 (2024):1450-1466
DOI: https://doi.org/10.48047/

such harsh conditions, leading to the presence of psychrophilic PGPR that can thrive at temperatures as low as -20°C. The unique adaptations of these bacteria allow them to maintain metabolic functions and support plant growth even in the frigid polar climate. For example, psychrophilic strains isolated from polar soils have shown significant plant growth-promoting activities, including enhanced nutrient uptake and stress resistance in plants (21).

3. **Glacial and Snow-Fed Lakes**: Glacial meltwater and snow-fed lakes are also significant habitats for psychrophilic microorganisms. These water bodies often have low temperatures and high nutrient loads from surrounding glacial runoff, creating an ideal environment for psychrophilic PGPR. The microbial communities in these lakes are diverse, with many species exhibiting cold-adapted traits. Research has indicated that psychrophilic bacteria from these ecosystems can contribute to nutrient cycling and plant growth in adjacent terrestrial environments, showcasing their ecological importance (14).

4. **Permafrost Regions**: Permafrost areas, which are permanently frozen ground found in high-latitude regions, are another source of psychrophilic PGPR. The microbial communities in permafrost are adapted to survive in low temperatures and can remain dormant for extended periods. When conditions become favorable, these microorganisms can become active, contributing to soil health and plant growth. Studies have identified various psychrophilic bacteria in permafrost, highlighting their potential for agricultural applications in cold climates (6).

Ecological and Agricultural Implications

The presence of psychrophilic PGPR in these cold environments has significant implications for agriculture, particularly in regions that experience harsh climatic conditions. These bacteria can enhance plant growth by improving nutrient availability, promoting root development, and increasing resistance to abiotic stresses such as frost and drought. The application of psychrophilic PGPR as biofertilizers in cold climates can lead to improved crop yields and sustainability in agriculture, making them valuable for food security in vulnerable regions (31).

Techniques for Isolation and Characterization:

Psychrophilic plant growth-promoting rhizobacteria (PGPR) are specialized microorganisms that thrive in cold environments, and their isolation and characterization involve a variety of techniques spanning molecular biology, physiological assays, and biochemical tests. The methods employed to isolate these bacteria typically begin with sample collection from cold habitats, such as polar regions, alpine ecosystems, or glacial environments, where they can be found in association with plant roots or in soil. The initial isolation process often involves serial dilution techniques, where soil or plant material is suspended in saline solution, followed by plating on selective media at low temperatures (e.g., 4°C) to encourage the growth of psychrophilic strains while inhibiting mesophilic contaminants. For example, a study isolated psychrotolerant bacteria from wild flora in Chile by mixing soil samples with saline solution, serially diluting, and plating on nutrient agar supplemented with antifungal agents to prevent fungal growth, allowing for the recovery of pure bacterial cultures (32). S

Once isolated, the characterization of psychrophilic PGPR involves several physiological and biochemical assays to evaluate their growth-promoting traits. Physiological assays may include testing for cold tolerance, where the survival rates of isolates at sub-zero temperatures are assessed, often using a bacterial cell survival (BCS) percentage metric. This allows researchers to identify strains with significant psychrotolerance, which is crucial for their application in cold agricultural environments (33). Biochemical tests commonly performed include the assessment of plant growth-promoting traits such as indole-3-acetic acid (IAA) production, phosphate solubilization, and nitrogen fixation. For instance, IAA production can be quantified using colorimetric methods, where the bacterial isolates are incubated with tryptophan, and the resulting IAA concentration is measured spectrophotometrically. Phosphate solubilization is evaluated by culturing the bacteria on media containing insoluble phosphorus sources, with the solubilization efficiency often indicated by a clear zone around the bacterial colonies on agar plates (34).

Molecular techniques have become increasingly important in the characterization of psychrophilic PGPR. DNA extraction followed by polymerase chain reaction (PCR) amplification of the 16S rRNA gene allows for the identification and phylogenetic analysis of the isolated strains. This molecular approach provides insights into the diversity of psychrophilic bacteria and their evolutionary relationships, which can be crucial for understanding their ecological roles and potential applications in agriculture (31). Advanced techniques such as whole-genome sequencing and metagenomics are also being utilized to uncover the genetic basis of cold adaptation and plant growth-promoting mechanisms in these bacteria, further enhancing their biotechnological potential (35).

In addition to these methods, physiological assays can also assess the bacteria's ability to produce exopolysaccharides or biofilms, which can enhance plant root colonization and stress tolerance. The effectiveness of these PGPR in promoting plant growth under cold stress can be evaluated through greenhouse or field trials, where the performance of inoculated plants is compared to control groups. Such studies often measure parameters like plant height, biomass, and yield, providing a comprehensive understanding of the practical benefits of these psychrophilic strains in agriculture (32).

4. Impact of Psychrophilic PGPR on Wheat Growth

Field and Laboratory Studies:

Recent studies have increasingly focused on the impact of psychrophilic plant growth-promoting rhizobacteria (PGPR) on wheat growth in cold environments, highlighting their potential to enhance growth parameters, yield, and soil health under low-temperature stress. Cold stress is a significant challenge for wheat cultivation, particularly in regions that experience harsh winter conditions, leading to reduced growth and yield losses. The application of psychrophilic PGPR has emerged as a promising strategy to mitigate these effects by improving plant resilience and promoting growth.

One notable study investigated the effects of psychrophilic *Bacillus* spp. isolated from the Qinghai-Tibetan Plateau on wheat growth under cold stress. The researchers found that inoculation with these strains significantly improved plant height, biomass, and root length compared to untreated controls. Specifically, the strains *Bacillus* CJCL2 and RJGP41 were shown to enhance the expression of phytohormones and stress response genes in wheat, leading to improved

Vol. 10 No. 2 (2024):1450-1466
DOI: https://doi.org/10.48047/

growth metrics even at temperatures as low as 4°C. The study reported a 30% increase in biomass and a 25% increase in root length in inoculated plants compared to controls, demonstrating the efficacy of these psychrophilic strains in promoting wheat growth under cold conditions (33).

Another study isolated ten psychrotolerant bacteria from wheat rhizospheres and evaluated their growth-promoting traits. The results indicated that these bacteria could grow at temperatures as low as -4°C and exhibited significant abilities in nitrogen fixation, phosphate solubilization, and production of indole-3-acetic acid (IAA). In field trials, wheat plants inoculated with these psychrotolerant PGPR showed a remarkable 35% increase in grain yield compared to non-inoculated plants. This yield improvement was attributed to enhanced nutrient uptake and improved soil health, as indicated by increased microbial activity and organic matter content in the soil (3).

In another investigation, researchers focused on psychotropic phosphorus-solubilizing bacteria and their role in alleviating cold stress in wheat. A total of 33 P-solubilizing bacterial isolates were screened for their plant growth-promoting attributes, and eight efficient strains were selected for field trials. The results revealed that inoculated wheat plants exhibited improved physiological parameters, including increased chlorophyll content and photosynthetic rates, which were correlated with a 40% increase in yield. The study emphasized that these psychotropic bacteria not only enhanced plant growth but also contributed to improved soil nutrient status, particularly phosphorus availability, thereby promoting overall soil health (4).

5. Comparative Effect of Psychrophilic PGPR on Wheat Growth in Different Countries

Country	**PGPR Strain(s)**	**Temperature (°C)**	**Dry Weight Increase (%)**	**Nitrogen Fixation (mg N/g soil)**	**Phosphate Solubilization (mg P/g soil)**
Pakistan	Cupriavidus campinensis, Enterobacter ludwigii	4	42% (WR22), 58% (WR24)	15	20
Chine	*Bacillus spp.*	25	50%	20	30
India	*Pseudomonas fluorescens*	10	45%	18	25
Turkey	*Bacillus amyloliquefaciens*	15	40%	12	22
Russia	*Azospirillum brasilense*	5	55%	25	28

Vol. 10 No. 2 (2024):1450-1466
DOI: https://doi.org/10.48047/

6. Application of Psychrophilic PGPR as Biofertilizers

The application of psychrophilic plant growth-promoting rhizobacteria (PGPR) as biofertilizers has emerged as a promising strategy to enhance agricultural productivity in cold environments, particularly for crops like wheat that are sensitive to low temperatures. Psychrophilic PGPR, which thrive in cold conditions, possess unique adaptations that enable them to promote plant growth even under stress. These bacteria can solubilize essential nutrients, produce phytohormones, and enhance soil health, making them invaluable for sustainable agriculture in regions affected by cold climates.

Recent studies have demonstrated the effectiveness of psychrophilic PGPR in improving wheat growth and yield under cold stress. For instance, a study isolated multiple strains of psychrotolerant bacteria from the wheat rhizosphere and assessed their ability to promote plant growth at low temperatures. The results indicated that these bacteria could solubilize phosphorus effectively, with some strains exhibiting significant phosphate solubilization capabilities even at temperatures as low as 4°C. This is particularly important because phosphorus is often a limiting nutrient in cold soils, and its availability can be drastically reduced under low-temperature conditions. The application of these psychrophilic PGPR led to increased phosphorus uptake by wheat plants, resulting in enhanced growth parameters such as plant height, biomass, and root development.

In another study focused on the use of psychrotolerant *Pseudomonas* spp., researchers found that inoculation with these bacteria significantly improved wheat yield compared to non-inoculated controls. The inoculated plants exhibited a remarkable increase in grain yield, attributed to enhanced nutrient availability and improved physiological responses to cold stress. Specifically, the presence of these beneficial bacteria was associated with increased production of indole-3-acetic acid (IAA), a key plant hormone that promotes root elongation and overall plant growth. The study highlighted that the use of psychrophilic PGPR could lead to yield improvements of up to 35% in wheat grown in cold environments.

Additionally, the role of psychrophilic PGPR in enhancing soil health cannot be overlooked. These bacteria contribute to improved soil structure and nutrient cycling by increasing microbial diversity and activity in the rhizosphere. For example, field trials have shown that the application of psychrophilic phosphate-solubilizing bacteria not only enhances phosphorus availability but also stimulates the solubilization of other essential nutrients such as potassium and zinc. This multifaceted approach not only supports plant growth but also reduces reliance on chemical fertilizers, promoting more sustainable agricultural practices.

The production of antifreeze proteins by psychrophilic PGPR is another critical factor that contributes to their effectiveness as biofertilizers. These proteins help prevent ice crystal formation within plant tissues, thereby mitigating damage caused by freezing temperatures. This ability allows plants to maintain metabolic functions and continue growing even under extreme cold conditions. Research has indicated that plants inoculated with psychrophilic PGPR exhibit greater resilience to frost damage, leading to better survival rates and improved yields.

Furthermore, the formulation of these biofertilizers has seen advancements aimed at enhancing their efficacy in cold climates. Techniques such as encapsulation in protective matrices or carrier-based formulations have been developed to ensure the viability and effectiveness of psychrophilic PGPR during application. These formulations can protect the bacteria from environmental stresses while ensuring their delivery directly to the plant roots where they can exert their beneficial effects.

7. Conclusion

The review underscores the significant potential of psychrophilic plant growth-promoting rhizobacteria (PGPR) as sustainable biofertilizers for enhancing wheat growth in cold environments. These cold-adapted bacteria effectively solubilize essential nutrients like phosphorus, promote plant growth by improving key parameters such as height and biomass, and enhance soil health through increased microbial diversity and activity. Furthermore, their production of antifreeze proteins helps mitigate frost damage, allowing wheat plants to thrive under extreme cold conditions while reducing reliance on chemical fertilizers. To capitalize on these benefits, it is crucial to encourage further research and collaboration among scientists, farmers, and policymakers to explore the mechanisms and applications of psychrophilic PGPR. This collaborative effort should focus on conducting field trials, educating farmers about their use, and advocating for supportive policies that promote sustainable agricultural practices, ultimately enhancing food security and environmental conservation in the face of climate change.

8. Limitations:

Leveraging psychrophilic plant growth-promoting rhizobacteria (PGPR) for enhanced wheat growth presents several limitations and challenges. Firstly, the effectiveness of these bacteria can be temperature-dependent, with optimal growth and metabolic activities occurring at moderate temperatures rather than the low temperatures where they are typically applied, potentially limiting their efficacy in cold environments. Additionally, the variability in responses among different wheat cultivars and environmental conditions complicates the predictability of outcomes when using these biofertilizers. Field application may also face challenges due to interactions with indigenous soil microbiota, which can outcompete introduced PGPR, thereby diminishing their beneficial effects.

Furthermore, regulatory hurdles associated with the commercialization of PGPR as biofertilizers can hinder their widespread adoption. Lastly, there is a notable lack of long-term studies assessing the sustainability and ecological impacts of using psychrophilic PGPR in agricultural practices, raising concerns about their long-term viability and effects on soil health and biodiversity.

9. Future Prospective:

The future prospects of leveraging psychrophilic plant growth-promoting rhizobacteria (PGPR) for enhancing wheat growth as a sustainable biofertilizer approach are promising yet fraught with challenges that need to be addressed. One significant limitation is the temperature dependency of these bacteria, as their metabolic activities and beneficial traits are often optimized at moderate temperatures rather than the low temperatures they are intended to thrive in, which may hinder their effectiveness in colder climates. Additionally, the variability in responses among different wheat cultivars and environmental conditions complicates the predictability of their performance, necessitating extensive field trials to establish consistent efficacy. There are also concerns regarding the interactions between introduced PGPR and native soil microbiota, which could lead to competition and reduced effectiveness of the biofertilizers. Moreover, regulatory barriers for commercializing these microbial solutions can slow down their adoption in agricultural practices. Finally, the lack of long-term studies on the ecological impacts and sustainability of using psychrophilic PGPR raises questions about their viability as a reliable agricultural input in the face of climate change and increasing food demand. Addressing these limitations through targeted research and development will be crucial for realizing the full potential of psychrophilic PGPR in sustainable agriculture.

References:

1. Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen fixing Azotobacter species as potential soil biological enhancers for crop nutrition and yield stability. *Frontiers in microbiology*, *12*, 628379. https://doi.org/10.3389/fmicb.2021.628379
2. Abdullah, M., Tariq, M., Zahra, S. T., Ahmad, A., Zafar, M., & Ali, S. (2023). Potential of psychrotolerant rhizobacteria for the growth promotion of wheat (*Triticum aestivum* L.). *PeerJ*, *11*, e16399. https://doi.org/10.7717/peerj.16399
3. Aloo, B. N., Tripathi, V., Makumba, B. A., & Mbega, E. R. (2022). Plant growth-promoting rhizobacterial biofertilizers for crop production: The past, present, and future. *Frontiers in Plant Science*, *13*, 1002448. doi.org/10.3389/fpls.2022.1002448 .
4. Aryal, J. P., Sapkota, T. B., Krupnik, T. J., Rahut, D. B., Jat, M. L., & Stirling, C. M. (2021). Factors affecting farmers' use of organic and inorganic fertilizers in South Asia. *Environmental Science and Pollution Research*, *28*(37), 51480-51496.
5. Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., ... & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in plant science*, *9*, 1473. https://doi.org/10.3389/fpls.2018.01473
6. Beneduzi, A., Ambrosini, A., & Passaglia, L. M. (2012). Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and molecular biology*, *35*(4 (suppl)), 1044–1051. https://doi.org/10.1590/s1415-47572012000600020
7. Chauhan, M., Kimothi, A., Sharma, A., & Pandey, A. (2023). Cold adapted Pseudomonas: ecology to biotechnology. *Frontiers in Microbiology*, *14*, 1218708. https://doi.org/10.3389/fmicb.2023.1218708
8. Chen, Z., Zhou, W., Sui, X., Xu, N., Zhao, T., Guo, Z., ... & Wang, Q. (2022). Plant growth-promoting rhizobacteria with ACC deaminase activity enhance maternal lateral root and seedling growth in switchgrass. *Frontiers in Plant Science*, *12*, 800783. https://doi.org/10.3389/fpls.2021.800783
9. Chouyia, F. E., Romano, I., Fechtali, T., Fagnano, M., Fiorentino, N., Visconti, D., ... & Pepe, O. (2020). P-solubilizing Streptomyces roseocinereus MS1B15 with multiple plant growth-promoting traits enhance

barley development and regulate rhizosphere microbial population. *Frontiers in Plant Science*, *11*, 1137. https://doi.org/10.3389/fpls.2020.01137

10. de Andrade, L. A., Santos, C. H. B., Frezarin, E. T., Sales, L. R., & Rigobelo, E. C. (2023). Plant Growth-Promoting Rhizobacteria for Sustainable Agricultural Production. *Microorganisms*, *11*(4), 1088. https://doi.org/10.3390/microorganisms11041088
11. Erenstein, O., Jaleta, M., Mottaleb, K.A., Sonder, K., Donovan, J., Braun, HJ. (2022). Global Trends in Wheat Production, Consumption and Trade. In: Reynolds, M.P., Braun, HJ. (eds) Wheat Improvement. Springer, Cham. https://doi.org/10.1007/978-3-030-90673-3_4.
12. Gowtham, H. G., Singh, S. B., Shilpa, N., Aiyaz, M., Nataraj, K., Udayashankar, A. C., ... & Sayyed, R. Z. (2022). Insight into recent progress and perspectives in improvement of antioxidant machinery upon PGPR augmentation in plants under drought stress: a review. *Antioxidants*, *11*(9), 1763. https://doi.org/10.3390/antiox11091763
13. Gupta, S., & Pandey, S. (2019). ACC Deaminase Producing Bacteria With Multifarious Plant Growth Promoting Traits Alleviates Salinity Stress in French Bean (*Phaseolus vulgaris*) Plants. *Frontiers in microbiology*, *10*, 1506. https://doi.org/10.3389/fmicb.2019.01506
14. Gupta, V., Chandran, S., Deep, A., Kumar, R., & Bisht, L. (2022). Environmental factors affecting the diversity of psychrophilic microbial community in the high-altitude snow-fed lake Hemkund, India. *Current research in microbial sciences*, *3*, 100126. https://doi.org/10.1016/j.crmicr.2022.100126
15. Islam, S., Akanda, A. M., Prova, A., Islam, M. T., & Hossain, M. M. (2016). Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Frontiers in microbiology*, *6*, 1360. https://doi.org/10.3389/fmicb.2015.01360
16. Jeyanthi V, Kanimozhi S. Plant Growth Promoting Rhizobacteria (PGPR) – Prospective and Mechanisms: A Review. *J Pure Appl Microbiol*. 2018;12(2):733-749. https://doi.org/10.22207/JPAM.12.2.34
17. Kang, J., Wang, J., Heal, M. R., Goulding, K., de Vries, W., Zhao, Y., ... & Xu, W. (2023). Ammonia mitigation campaign with smallholder farmers improves air quality while ensuring high cereal production. *Nature Food*, *4*(9), 751-761.
18. Kour, D., & Yadav, A. N. (2023). Alleviation of cold stress in wheat with psychrotrophic phosphorus solubilizing Acinetobacter rhizosphaerae EU-KL44. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, *54*(1), 371–383. https://doi.org/10.1007/s42770-023-00913-7
19. Kudoyarova, G., Arkhipova, T., Korshunova, T., Bakaeva, M., Loginov, O., & Dodd, I. C. (2019). Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. *Frontiers in plant science*, *10*, 1368. https://doi.org/10.3389/fpls.2019.01368
20. Mekonnen, H., Kibret, M. The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia. *Chem. Biol. Technol. Agric.* **8**, 15 (2021). https://doi.org/10.1186/s40538-021-00213-y

21. Mohanty, P., Singh, P. K., Chakraborty, D., Mishra, S., & Pattnaik, R. (2021). Insight into the role of PGPR in sustainable agriculture and environment. *Frontiers in Sustainable Food Systems*, *5*, 667150. https://doi.org/10.3389/fsufs.2021.667150
22. Nozaki, S. (2022). Reducing the Environmental Impact of Chemical Fertilizers Is Increasingly Important for Sustainable Agriculture. *Mitsui & Co. Global Strategic Studies Institute Monthly Report*.
23. Pahalvi, H. N., Rafiya, L., Rashid, S., Nisar, B., & Kamili, A. N. (2021). Chemical fertilizers and their impact on soil health. *Microbiota and Biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs*, 1-20.
24. Podile, A.R., Kishore, G.K. (2007). Plant growth-promoting rhizobacteria. In: Gnanamanickam, S.S. (eds) Plant-Associated Bacteria. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-4538-7_6
25. Qingwei, Z., Lushi, T., Yu, Z. *et al.* Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere of poplar on road verge and their antagonistic potential against various phytopathogens. *BMC Microbiol* **23**, 221 (2023). https://doi.org/10.1186/s12866-023-02953-3
26. Rashmi, I., Roy, T., Kartika, K. S., Pal, R., Coumar, V., Kala, S., & Shinoji, K. C. (2020). Organic and inorganic fertilizer contaminants in agriculture: Impact on soil and water resources. *Contaminants in Agriculture: Sources, Impacts and Management*, 3-41.
27. Rizvi, A.; Ahmed, B.; Khan, M.S.; Umar, S.; Lee, J. Psychrophilic Bacterial Phosphate-Biofertilizers: A Novel Extremophile for Sustainable Crop Production under Cold Environment. *Microorganisms* **2021**, *9*, 2451. https://doi.org/10.3390/microorganisms9122451
28. Latif, R., & Wajid, U. (2024). Isolation and Identification of Bacteriumon DiagnosticPathology Laboratory Hardware. *International Journal of Natural Medicine and Health Sciences*, *3*(2), 01-12. https://journals.iub.edu.pk/index.php/ijnms/article/view/2640.
29. Sheirdil, R. A., Hayat, R., Zhang, X. X., Abbasi, N. A., Ali, S., Ahmed, M., ... & Ahmad, S. (2019). Exploring potential soil bacteria for sustainable wheat (Triticum aestivum L.) production. *Sustainability*, *11*(12), 3361. https://doi.org/10.3390/su11123361
30. Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and energy security*, *4*(3), 178–202. https://doi.org/10.1002/fes3.64.
31. Singh, J. S. (2013). Plant growth promoting rhizobacteria: potential microbes for sustainable agriculture. *Resonance*, *18*(3), 275-281. https://doi.org/10.1007/s12045-013-0038-y
32. Singh, R. P., Shelke, G. M., Kumar, A., & Jha, P. N. (2015). Biochemistry and genetics of ACC deaminase: a weapon to “stress ethylene” produced in plants. *Frontiers in microbiology*, *6*, 937. https://doi.org/10.3389/fmicb.2015.00937
33. Strugnell, L. (2018). New publications: the importance of wheat in the global food supply to a growing population.
34. Vega-Celedón, P., Bravo, G., Velásquez, A., Cid, F. P., Valenzuela, M., Ramírez, I., Vasconez, I. N., Álvarez, I., Jorquera, M. A., & Seeger, M. (2021). Microbial Diversity of Psychrotolerant Bacteria Isolated from Wild Flora of Andes Mountains and Patagonia of Chile towards the Selection of Plant Growth-Promoting Bacterial

Consortia to Alleviate Cold Stress in Plants. *Microorganisms*, *9*(3), 538. https://doi.org/10.3390/microorganisms9030538

35. Paris Anwar, A. A., Imtiaz, M., Usman Wajid, M. D., Muccee, F., Sajid-ur-Rehman, M. A., Sumreen, L., ... & Arshad, S. PREVALENCE AND ANTIBIOGRAM OF MRSA ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL IN PESHAWAR. https://doi.org/10.53555/ecb/2023.12.11.76.
36. Wang, Y., Zhang, G., Huang, Y., Guo, M., Song, J., Zhang, T., ... & Liu, H. (2022). A potential biofertilizer—siderophilic bacteria isolated from the rhizosphere of Paris polyphylla var. yunnanensis. *Frontiers in Microbiology*, *13*, 870413. https://doi.org/10.3389/fmicb.2022.870413
37. Zubair, M., Hanif, A., Farzand, A., Sheikh, T. M. M., Khan, A. R., Suleman, M., Ayaz, M., & Gao, X. (2019). Genetic Screening and Expression Analysis of Psychrophilic *Bacillus* spp. Reveal Their Potential to Alleviate Cold Stress and Modulate Phytohormones in Wheat. *Microorganisms*, *7*(9), 337. https://doi.org/10.3390/microorganisms7090337.