

## Microbial Marvels in Cotton Farming: Unveiling the Potential of Plant Beneficial Microbes for Enhanced Growth and Disease Resistance in Pakistan

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Cotton is a crucial cash crop in Pakistan, playing a pivotal role in the country's economy, agricultural sector, and employment opportunities. Its cultivation spans over 3 million hectares, primarily concentrated in the Punjab and Sindh provinces. Cotton production in Pakistan contributes significantly to the country's foreign exchange earnings, accounting for a substantial portion of its exports. However, cotton pest and disease management is always a challenge as their impact change over time. The widespread use of agrochemicals and synthetic pesticides in major field crops poses significant threats to human and animal health, as well as environmental degradation. Use of beneficial microbes for sustainable cotton production can be a natural ally in the field, as they promote the plant growth as well as protect them from disease, insects and pests. They act as a reservoir of beneficial metabolites, enzymes, and nutrients, and they play a crucial role in biological pest control and disease resistance induction. Additionally, PGP rhizobacteria possess bioremediation potential, enabling them to phytoextract and detoxify pollutants and pesticides. Beneficial microorganisms like PGPR hold immense potential to replace and supplement these toxic chemicals, offering promising applications in organic farming and contributing to sustainable agricultural practices. Despite their potential, only a limited number of bio-formulations have been developed using PGPR strains with plant growth promotion, metabolite production, enzyme synthesis, nutrient mobilization, and biocontrol activities. An increasing body of evidence underscores the potential of diverse microorganisms to enhance plant productivity and yield within cropping systems. Unlocking the full benefits of these advantageous microbes requires a profound understanding of their role in promoting growth, specifically in the realms of fertilization and disease control, along with the elucidation of underlying mechanisms. It is imperative to confront challenges associated with the application of plant growth-promoting (PGP) microbes. This review focuses on the application of PGP microbes in the context of cotton production. Given the scarcity of information on beneficial microbes for cotton production, a comprehensive examination of current research becomes crucial, especially given the increasing interest in cotton inoculants, particularly in developing nations. The heightened attention towards PGP applications underscores the importance of advancing sustainable agricultural practices.

**Keywords:** Cotton production, Biocontrol, PGP applications, Beneficial microbes, pest control, disease resistance.

### INTRODUCTION

Cotton is a crucial cash crop in Pakistan, playing a pivotal role in the country's economy, agricultural sector, and employment opportunities. Its cultivation spans over 3

million hectares, primarily concentrated in the Punjab and Sindh province (Muhammad *et al.*, 2022). Over the past years, the cultivation of cotton and its acreage in Pakistan has been consistently diminishing, attributed to the swift spread of pests.

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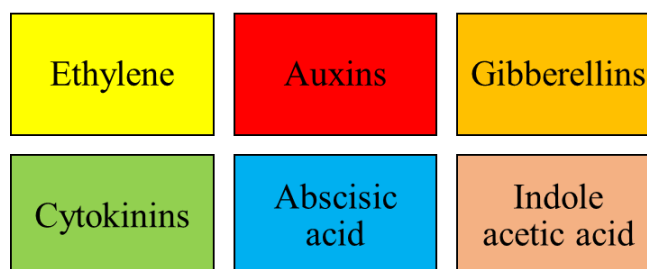
Plant beneficial microbes offer a multitude of benefits for augmenting plant growth. A promising way to manage soil-borne diseases like *Verticillium dahliae* is a use of PGR rhizobacteria which also have the potential to deal with notorious pathogens in organic farming. Researchers have found many entophytic bacteria that can fight against *Verticillium dahliae* (Khursheed *et al.*, 2022). Indole acetic acid (IAA), a phytohormone produced by some of these bacteria also help plants grow better and stronger. IAA helps plants grow more roots, which means they can get more water and nutrients (Lopes *et al.*, 2023)

Studied have revealed that entophytic bacteria, which can live inside plants, make plants stronger, and produce such chemicals that have the potential to kill fungi. These chemicals, like cellulase and chitinase, break down the walls of fungi, making it harder for them to infect plants (Muthu Narayanan *et al.*, 2022). Scientists measure the amount of these chemicals in bacteria by using a special test called the DNS method (Lu *et al.*, 2023). PGP rhizobacteria also induce systemetic resistance in plants. Induced systemic resistance (ISR) is when plants make changes induced by the bacteria to protect themselves from diseases (Yu *et al.*, 2022). Some of these changes include making special chemicals called enzymes, like polyphenol oxidase (PPO), peroxidase (POD), and phenyl ammonia-lyase (PAL), and producing more phenols. POD and PPO help make plant cell walls stronger, which makes it harder for fungi to get inside and damage the plant (Jha & Mohamed, 2022). *Bacillus subtilis* strain having the ability to produce (APX) ascorbate peroxidase and (PAL) phenylalanine ammonia-lyase reduced the bacterial blight about 30% (Le Thanh *et al.*, 2023). In the last decade, plant growth promoting (PGP) activities of effective microbes have been reviewed and their role in cotton production have been discussed (Pereg *et al.*, 2015) We shall discuss the mechanisms of effective microbes for plant growth promotion as well as their role as a biocontrol agent in recent years.

**Bacterial Strategies for Augmenting Plant Growth:** PGP rhizobacteria and rhizobia employ diverse mechanisms to enhance plant growth, productivity, and survival in varying conditions (Bhat *et al.*, 2023). Positive impacts include the production of plant growth regulators, osmolytes, and enzymes, along with increased nutrient availability, biofilm formation, and systemic tolerance induction (Bouremani *et al.*, 2023). PGPR inoculation under abiotic stress induces responses such as augmented K<sup>+</sup>/Na<sup>+</sup> ratio, expanded root surface area, swift osmotic adjustment, and reduced ethylene levels. These mechanisms highlight the potential of PGPR as sustainable solutions for plant health and resilience in agriculture.

**Phytohormone Production by Plant Beneficial Microbes:** The synthesis of phytohormones, including ethylene, auxins, gibberellins, cytokinins, and abscisic acid by PGPR and rhizobia stimulates plant growth across diverse conditions (Fahad *et al.*, 2015). Indole acetic acid (IAA) biosynthesis

influences root architecture, enhancing nutrient uptake. Cytokinins play a role in mitigating water stress effects, while gibberellic acid contributes to improved productivity. Dual roles of phytohormones and their modulation under environmental conditions showcase the adaptability of PGPRs and rhizobia in promoting plant growth (Qasim & Sciences, 2023) figure 1.



**Figure 1. The Production of Phytohormones by Microbes That Are Beneficial to Plants**

**Osmolyte Production by Beneficial Microbes:** Rhizobacteria exhibit resilience in the rhizosphere by producing osmolytes like sugars, amino acids, and quaternary amines, acting as osmoprotectants. These osmolytes reduce cytoplasmic osmotic potential, maintaining cell turgidity and preventing degenerative processes (Camaille *et al.*, 2021). They also function as molecular chaperones and free-radical scavengers, detoxifying reactive oxygen species. Enhanced proline and trehalose contents in plants demonstrate the role of osmolytes in maintaining growth under water deficit stress (Kosar *et al.*, 2019).

**Enzyme and Antioxidant Production for Stress Mitigation:** Rhizobacteria produce enzymes and antioxidants crucial for scavenging reactive oxygen species (ROS) and mitigating stress-induced ethylene. Rhizobacteria produced enzymatic scavengers and non-enzymatic compounds counteract ROS, ensuring cell viability (Das & Mukherjee, 2021). Studies on *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* link enzyme production with enhanced drought tolerance. ACC-deaminase activity in rhizobia contributes to stress-induced ethylene reduction. The multifaceted enzymatic and antioxidant arsenal of rhizobia showcases their role in promoting plant health (Stringlis *et al.*, 2019).

**Nutrient Solubilization and Availability:** Bio-fertilizers, including PGPR, play a crucial role in increasing both macro and micronutrient availability in crops (Kumar *et al.*, 2017). PGPR enhance N, P, and K uptake, reducing reliance on chemical fertilizers. Rhizobia solubilize phosphorus and produce siderophores, influencing iron availability. The ability to optimize fertilizer doses with PGPR supplementation underscores their potential in sustainable agriculture (Miteu *et al.*, 2023).

**Biofilm Formation and Exopolysaccharides Production:** Plant beneficial microbes capability to produce



exopolysaccharides (EPS) contributes to drought stress alleviation and soil structure enhancement. EPS-producing rhizobia enhance crop tolerance and yield (Latif *et al.*, 2022). Biofilm formation on roots facilitates rhizobia colonization, stimulating root exudation and promoting biological activity. Bacterial adaptation to environmental stresses involves changes in LPS structure and osmotic stress-induced alterations (Rowlett *et al.*, 2017).

#### **Induced Tolerance to Abiotic Stress and Biocontrol:**

Rhizobacteria induce systemic tolerance in plants through the production of osmoprotectants, phytohormones, and EPS, enhancing survival under drought. Volatile Organic compound producing bacteria induce drought tolerance, while ACC-deaminase-producing bacteria regulate stress ethylene, contributing to resilience. Rhizobia serve as biocontrol agents by producing antibiotics, siderophores, and chitinase, inhibiting pathogenic bacteria, fungi, and nematodes. The multifaceted activities of rhizobia make them valuable contributors to plant health and stress resilience (Abdelaziz *et al.*, 2023).

#### **Growth Promotion Activities of Beneficial Microbes in Cotton:**

Bacteria are essential for promoting cotton growth, enhancing nutrient solubilization, and acting as chemical fertilizers (Ahmad *et al.*, 2023). *Bacillus subtilis* and *Bacillus aryabhattai* increase boll numbers, seed cotton yield, lint yield, and antioxidant activities. They also improve water retention and nitrate nitrogen retention in saline-alkali soil. Bacte-solubilizing bacteria like *Bacillus* sp. increase seed cotton yield and soil available phosphorus. *Rhizobium* sp. B02, a phosphate-solubilizing bacteria, enhances the efficient use of low-solubility fertilizers like rock phosphate (Sharath

*et al.*, 2021). This suggests the potential application of *Rhizobium* genus as phosphate-solubilizing bacteria in improving cotton cultivation and optimizing phosphorus use (Zhou *et al.*, 2023).

In a single experiment, *Rhizoctonia solani*-caused wilting of cotton seedlings was treated using *Pseudomonas aureofaciens* as a biocontrol agent. Sahu *et al.* (2018) looked at the efficacy of cyanobacteria-based compost formulations as PGP and biocontrol agents in cotton. Furthermore, the mechanisms by which PGPR promotes plant development, produces systemic resistance, and controls infections biologically were investigated. According to Prasanna *et al.* (2016), *Anabaena* sp. and *Calothrix* sp. have shown promise as PGP and biocontrol agents in cotton. *Bacillus subtilis* Bbv57 has antagonistic properties against plant diseases, according to Thiruvengadam *et al.* (2022), suggesting that it could be useful as a biocontrol agent in the context of sustainable agriculture. Zebelo *et al.* (2016) evaluated the effects of PGPR (plant growth-promoting rhizobacteria) treatment on cotton plants in their investigation. Plant resistance to the beet armyworm was found to be enhanced by PGPR treatment, which also boosted gossypol levels and encouraged the expression of genes required for its manufacture (Table 1). Scientists are very interested in plant growth-promoting rhizobacteria (PGPR) because of its ability to improve plant development and productivity in nutrient-deficient environments and to potentially reduce the use of dangerous agricultural pesticides. PGPR employed a wide range of tactics, including substrate competition, antibiotic production, lytic enzymes, and induction of systemic resistance, to prevent infections and promote plant growth. Understanding

**Table 1. The activities of beneficial microbes in cotton that promote growth and development.**

Microbial inoculant	Experimental system	Effects	References
<i>Paenibacillus polymyxa</i> IA7 + <i>Bacillus aryabhattai</i> IA20	Pot experiment	Improved the number of bolls, lint yield and seed cotton yield	(Ahmad <i>et al.</i> , 2023)
<i>Bacillus velezensis</i>	Greenhouse	Increased biomass; IAA, Siderphore production	(Su <i>et al.</i> , 2023)
<i>Bacillus subtilis</i>	Pot planting	Improved cotton yield and growth under salt stress conditions.	(Zhou <i>et al.</i> , 2023)
<i>Bacillus subtilis</i> IA6 + <i>Paenibacillus polymyxa</i> IA7	Jar experiment	Enhanced the growth of cotton seedlings	(Ahmad <i>et al.</i> , 2021)
<i>Acinetobacter</i> sp.	Pot experiment	Number of bolls were increase	(Sharath <i>et al.</i> , 2021)
<i>Rhizobium</i> sp.	Glasshouse study	Significantly promoted growth, photosynthetic rate, stomatal conductance	(Zhou <i>et al.</i> , 2023)
<i>Rhizobacteria</i>	A field experiment	Improved the of the Leaf area index, photosynthetic rate, total number of bolls per plant, and seed cotton yield	(Majid <i>et al.</i> , 2020)
<i>Bacillus subtilis</i> strain Q3 and <i>Paenibacillus</i> sp. strain	Jar Experiment	Root and shoot fresh & dry weight	(Ahmad <i>et al.</i> , 2018)
<i>Azotobacter chroococcum</i>	Glasshouse study	To improve cotton growth by decreasing the N fertilizer dose	(Romero-Perdomo <i>et al.</i> , 2017)
<i>Cyanobacteria</i>	Glasshouse study	Enhanced germination, fresh weight, and microbiological activity in cotton plants.	(Prasanna <i>et al.</i> , 2015)
<i>Bacillus fusiformis</i> S10	Field trials	Enhanced growth and yield with abridged application of chemical fertilizer	(Yasmin <i>et al.</i> , 2013)
<i>Pseudomonas aeruginosa</i> Z5 þ <i>Raoultella planticola</i>	Pot trials	Improved seed germination, plant height and weight	(Wu <i>et al.</i> , 2012)



how they function, how they can colonize new areas, and how they are formulated can help them advance as dependable parts of sustainable agricultural systems (Murthy *et al.*, 2021). In a separate study, Prasanna *et al.* (2016) investigated the effect of microbial inoculants on cotton seedling mortality in soil contaminated with *Rhizoctonia solani*. They found significant decreases in mortality and changes in bacterial populations in the rhizosphere. Numerous microbial inoculants have been shown to improve production, indicating that these compounds have the ability to both promote plant growth and prevent illness. The study's goal was to create biocomposts that would lessen cotton seedling damping-off by using talc and bentonite granules as carriers for *Pseudomonas fluorescens* strains. According to Ardakani *et al.* (2010), bioformulations were more effective than traditional fungicide therapy. This shows that both organic and inorganic carriers can improve the stability and efficacy of biocontrol-active bacteria, which are used to control plant diseases.

A family of soil bacteria known as PGP rhizobacteria (PGPR) improves the uptake of nutrients by plants, promotes seed germination, develops roots, and guards against many plant diseases. The potential of PGPR as effective biocontrol agents targeting plant diseases has been greatly enhanced by the recent advancements in their diversity, methods of action, and applications. According to Siddiqui (2006), biofertilization and disease prevention are two possible applications of this technology in agriculture. Of the eleven bacterial isolates linked to whiteflies that were examined in this study, *Erwinia persicinus*, *Bacillus pumilus*, and *Exiguobacterium acetylicum* were the most successful at reducing *Bemisia tabaci*, a variation of the fly. Given their proven effectiveness as biological control agents, these bacteria may be able to lessen the negative effects of whiteflies without the need for traditional pesticides. In light of the findings, more investigation into these bacterial isolates' potential as biopesticides to lower whitefly populations with a minimum of dependence on dangerous chemicals might be undertaken (Ateyyat *et al.*, 2009).

The effects of sooty fungus on cotton were studied using bacteria. According to reports, *Bacillus cereus* YUPP-10 significantly suppresses *Fusarium* wilt in cotton (Zhou *et al.*, 2023). *Streptomyces rochei* 42561, another biocontrol bacterium, has been shown by Huang *et al.* (2023) to have remarkable preventative properties against cotton blight and cotton greensickness. Furthermore, it was found that when exposed to sooty mold, *Pseudomonas chlororaphis* MT5 and *Bacillus velezensis* MT9 performed effectively as biocontrol agents. These bacteria also produced water-soluble antifungal and antibacterial chemicals, as well as volatile organic compounds (VOCs) having antifungal characteristics. The promise of endophytic bacteria for the biological treatment of cotton diseases is further supported by their identification, antibiosis ability against *Fusarium oxysporium* f.sp. and

*Rhizoctonia solani* kuhn, and their isolation from cotton cultivars (Ming *et al.*, 2004). Specifically, *Bacillus* sp. As such, the application of these bacteria as biocontrol agents presents promising opportunities for the prevention and management of cotton sooty mold.

*Pseudomonas chlororaphis* and protegen strains are able to manage plant diseases and eradicate insects through the production of virulence factors. Although these bacteria are commonly found in soil and roots, it is still unclear where they live and how they interact with arthropods (Hofte, 2021). Scientists have isolated *P. chlororaphis* strains and *P. protegens* from a variety of sources, including earth, myriapods, grassland soil, agricultural field soil, and healthy insect roots. Next, a thorough assessment of the isolates' capacity to eradicate insects, avert illnesses, and settle on hosts was conducted. Remarkably, the findings showed that the degree of insecticidal action was not significantly influenced by the isolation's origin or phylogenetic location. While *P. chlororaphis* strains showed significant diversity, *P. protegens* strains showed consistent phylogenetic, biocontrol, and insecticidal features. A thorough behavioral and genomic investigation revealed that the genes encoding different insecticidal components differed amongst closely related *P. chlororaphis* isolates with differing levels of insecticidal activity. These genetic alterations may be the cause of the decreased insecticidal efficiency seen in some isolates. *Bacillus subtilis* strain HMB28948 is useful in avoiding cotton damping-off. The strain demonstrated inhibitory effects against various diseases, including verticillium wilt and botrytis cinerea, Guo *et al.* (2021) (Table 2)

#### **Considerations for PGP Microbes in Cotton Production:**

Translating microbial benefits from controlled environments to field trials presents challenges, necessitating a comprehensive understanding of influencing factors (Highmore *et al.*, 2022). Factors like plant species, soil type, local microbial communities, and environmental variables impact microbial efficacy, emphasizing the need for careful selection and dual-action capabilities (Mitra *et al.*, 2022). Challenges in arid soils and economic considerations, including mass production and shelf life, require attention for successful microbial commercialization (Naorem *et al.*, 2023). The potential of co-inoculation strategies in mitigating field trial variability further supports the adoption of PGP microbes in cotton production (Ahmad *et al.*, 2023). The advantages of reduced environmental impact and compatibility with pest management position microbial inoculants favorably in comparison to chemical inputs (Elnahal *et al.*, 2022). The microbial inoculants should be tested in field condition extensively for evaluation their potential and improvement as well.

**Conclusion and Future Directions:** Despite facing challenges, there is a growing exploration of diverse microorganisms with potent attributes for enhancing the growth of cotton plants. These microorganisms are





**Table 2. Biocontrol agent for different pathogens.**

Biocontrol agent	Pathogen/Pest/Insect	References
<i>Bacillus</i> sp. T6	<i>Verticillium dahliae</i>	(Zhang <i>et al.</i> , 2023)
<i>Bacillus</i> spp	<i>Xanthomonas citri</i> pv. <i>malvacearum</i>	(Sampathkumar <i>et al.</i> , 2023)
<i>Bacillus velezensis</i>	<i>R. solani</i>	(Su <i>et al.</i> , 2023)
<i>Bacillus thuringiensis</i>	<i>Spodoptera frugiperda</i> and <i>Anticarsia gemmatilis</i>	(Gomis-Cebolla & Berry, 2023)
<i>Beauveria bassiana</i>	<i>Spodoptera litura</i> (Fabricius)	(Islam <i>et al.</i> , 2023)
<i>Bacillus</i> RZ141 and <i>Streptomyces</i> HC658	<i>Fusarium oxysporum</i> f.sp. <i>vasinfectum</i> race 4 (Fov4),	(Hofte, 2021)
<i>Bacillus thuringiensis</i>	<i>Spodoptera frugiperda</i>	(da Costa <i>et al.</i> , 2020)
<i>Bacillus velezensis</i> strain AL7	<i>Verticillium dahlia</i>	(Liu <i>et al.</i> , 2020)
<i>Bacillus amyloliquefaciens</i> , <i>Bacillus velezensis</i> , and <i>Paenibacillus lentimorbus</i>	<i>Ramulosis</i> ( <i>Colletotrichum gossypii</i> var. <i>cephalosporioides</i> )	(Ferro <i>et al.</i> , 2020)
<i>B. velezensis</i> strains Bve2 and Bve12, <i>B. mojavensis</i> strain Bmo3, and Mixture 2	<i>M. incognita</i>	(Xiang <i>et al.</i> , 2017)
<i>Pseudomonas</i> spp., <i>Bacillus</i> spp., and <i>Burkholderia</i> sp.	Cotton leaf curl virus disease	(Ramzan <i>et al.</i> , 2016)

continuously being discovered and tested in real-world conditions, resulting in an increasing number of success stories. The positive outcomes of this research yield substantial financial benefits, manifested in reduced reliance on chemical fertilizers and pesticides, along with heightened productivity through increased crop yields. Additional advantages include the diminished presence of toxins in agricultural lands and a reduction in environmental pollution. Successful outcomes often depend on the synergistic application of inoculants endowed with complementary beneficial traits. For instance, the collaborative action of biofertilizers enhancing nutrient availability around the roots, combined with mycorrhizal fungi improving the root system, optimizes nutrient absorption by cotton plants. Indigenous microbes frequently prove most effective, seamlessly adapting to the specific environmental conditions of the targeted cropping system. However, in the case of biocontrol agents, these indigenous microbes must successfully compete with pathogens for resources efficiently to suppress pathogens.

Moreover, the identification and isolation of these advantageous microbial agents for augmentation highlight the importance of directing additional research efforts towards cultivating cropping practices that promote the safety of both indigenous and introduced beneficial microbes. This entails careful regulation of chemical inputs to prevent over application, which could hinder beneficial activity and inadvertently promote harmful microorganisms. Collaborative efforts are vital for developing technology aimed at screening and identifying microbes with advantageous traits, assessing their impact on cotton plants, testing strains for commercial viability, optimizing formulations for effective inoculant delivery, this includes the examination of soil microbial communities, the assessment of the impact of soil inoculation on soil health, and a comprehensive understanding of the wider repercussions of agricultural practices on particular microbial communities.

Pathogen-suppressive microbes can be isolated from pathogen buried in the soil propagules. Microbes that regulate pathogen populations through competitive colonization, particularly in sterilized soil, prove effective in excluding the growth of other harmful organisms. In conclusion, the ongoing pursuit of novel biocontrol microbes is of paramount importance in addressing issues of pathogenic resistance, especially considering the increasing global demand for cotton crops. Acknowledging the necessity for continuous innovation, interdisciplinary collaborations is the need of the hour and a more thorough understanding of plant microbe interaction in the soil.

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