



Plant Microbe and Sustainability

Journal homepage: <https://sshapi.hapiacademia.com/PMS>



Type of Paper (Review Article)

Biofertilizers for Improving Nutrient Use Efficiency in Sustainable Agriculture: Emerging Technologies and Future Perspectives

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Abstract

Global application of inorganic fertilizers surpassed 190 million tonnes in 2025; however, crops typically take up less than 50% of the nutrients applied, especially nitrogen, leading to major nutrient losses, deterioration of soil quality, greenhouse gas emissions, eutrophication, and reductions in biodiversity. Consequently, increasing nutrient use efficiency (NUE) without compromising yields has become a central objective for sustainable agriculture. Biofertilizers containing beneficial microorganisms such as nitrogen-fixing bacteria, phosphate- and potassium-solubilizing microbes, arbuscular mycorrhizal fungi (AMF), and plant growth-promoting rhizobacteria (PGPR) have gained attention as environmentally friendly substitutes or supplements to mineral fertilizers because they enhance nutrient availability, nutrient acquisition, and plant tolerance to stress. This review offers a critical synthesis of current understanding of how biofertilizers improve NUE through mechanisms such as biological nitrogen fixation, nutrient solubilization and mobilization, phytohormone synthesis, rhizosphere modification, and regulation of plant-microbe interactions. It also discusses recent progress in microbial consortia, biofilm-based products, nano-biofertilizers, microbiome engineering, and genomics-guided strain improvement that has increased the effectiveness and field performance of microbial inoculants. Nonetheless, issues involving microbial survival, formulation durability, performance across diverse agroecological settings, and scaling up for commercial use still hinder broad adoption. Future work should prioritise the design of climate-smart microbial products via synthetic microbial communities, multi-omics tools, precision agriculture, and artificial intelligence-assisted strain selection to enhance the reliability and effectiveness of biofertilizers. Incorporating biofertilizers into nutrient management strategies can decrease reliance on synthetic fertilizers, restore soil health, improve NUE, and support environmentally resilient and sustainable farming systems. Overall, this review presents a comprehensive and forward-looking analysis of how biofertilizers can contribute to sustainable nutrient management and climate-resilient agriculture.

Keywords: CRISPR; Metagenomics, Microbial consortia; Plant-microbe interactions; Rhizosphere microbiome

1. Introduction

Modern agriculture has achieved remarkable gains in crop productivity largely through the intensive use of chemical fertilizers (Xiang *et al.*, 2008). However, this success has come at a considerable environmental cost. Global agricultural use of inorganic fertilizers exceeded 190 million tonnes in 2025, yet the efficiency with which crops utilize applied nutrients remains relatively low, particularly for nitrogen, resulting in substantial nutrient losses and environmental degradation. Excessive and imbalanced fertilizer application has contributed to soil degradation (Bhattacharyya *et al.*, 2015), nutrient imbalance (Vitousek *et al.*, 2009), loss of soil biodiversity (Krasilnikov *et al.*, 2022), water pollution (Saha *et al.*, 2024), and disruption of natural biogeochemical cycles (Zaman *et al.*, 2025). These challenges have raised serious concerns regarding the long-term sustainability of conventional nutrient management practices.

Improving nutrient use efficiency (NUE) while, maintaining or enhancing crop productivity has become a central objective of sustainable agricultural systems. NUE reflects the ability of crops to acquire, assimilate, and convert applied nutrients into economic yield, making it an important indicator of both agronomic performance and environmental sustainability (Govindasamy *et al.*, 2023). Low NUE results in significant nutrient losses through leaching (Alva *et al.*, 2006), volatilization (Liu *et al.*, 2019), and surface runoff (Liang *et al.*, 2016), thereby increasing production costs while contributing to environmental pollution. Consequently, sustainable nutrient management requires integrated strategies that maximize nutrient availability and uptake while minimizing losses and improving soil health, ecosystem functioning, and crop resilience.

Biofertilizers consist of beneficial microorganisms, including nitrogen-fixing bacteria, phosphate- and potassium-solubilizing microbes, AMF, and PGPR, and have gained prominence as environmentally friendly alternatives or complements to chemical fertilizers (Bhat *et al.*, 2015). Instead of directly supplying nutrients, these microbes increase nutrient availability via biological nitrogen fixation, nutrient solubilization and mobilization, phytohormone synthesis, and enhancement of root system architecture, thereby boosting nutrient uptake efficiency and reinforcing plant resistance to biotic and abiotic stresses. Their capacity to restore soil microbial diversity, improve soil fertility, and optimize rhizosphere processes positions them as key elements of integrated nutrient management strategies (Bomboriya *et al.*, 2018). Recent progress in microbial ecology, genomics, nanotechnology, and biofilm-based technologies has further strengthened the prospects of biofertilizers by enhancing microbial persistence, formulation robustness, and performance under field conditions. Novel approaches such as multifunctional microbial consortia, nano-biofertilizers, and genomics-driven strain improvement provide promising avenues to increase NUE while decreasing reliance on synthetic fertilizers (Rabani *et al.*, 2023).

The accelerated depletion of non-renewable resources, notably rock phosphate and petroleum, presents an additional threat to global food security, with particularly severe implications for developing nations (Clair & Lynch, 2010). Although sustainable agricultural production has been widely recognized as a priority by policymakers, researchers, and industry (Seufert *et al.*, 2012; Wezel *et al.*, 2014), intensive farming systems that depend heavily on synthetic fertilizers, pesticides, and herbicides continue to degrade soil quality, diminish biodiversity, reduce water-holding capacity, and disrupt nutrient cycling (Kahiluoto *et al.*, 2014; Tahat *et al.*, 2020). Organic agriculture offers a viable alternative pathway toward more sustainable production (Reddy, 2013); however, in many production contexts, chemical fertilizers are expected to remain indispensable for satisfying global food demand (Macilwain, 2004). Consequently, enhancing fertilizer use efficiency, rather than aiming for complete substitution, constitutes a more pragmatic and achievable strategy for sustainable intensification.

Biofertilizers offer a promising strategy to enhance nutrient availability, stimulate plant growth, improve stress resilience, and mitigate the environmental impacts associated with intensive fertilizer use (Vessey, 2003; Bertola *et al.*, 2019; Murgese *et al.*, 2020; Fasusi *et al.*, 2021). Recent advances, such as the

discovery of novel nitrogen-fixing bacteria from extreme environments using metagenomic tools (Renganathan *et al.*, 2025), CRISPR-based enhancement of nitrogenase activity (Russell *et al.*, 2024), and co-inoculation approaches that increase nitrogen use efficiency in cereal systems (Galindo *et al.*, 2025), highlight the rapid progress of microbial technologies for sustainable agriculture. However, despite the rapidly expanding body of literature on biofertilizers, most existing review articles have primarily focused on individual microbial taxa, such as PGPR, AMF, or phosphate-solubilizing microorganisms (PSMs), or on specific nutrient transformation processes, with limited integration of recent advances in microbial ecology, microbiome engineering, synthetic microbial communities (SynComs), CRISPR-mediated microbial engineering, nano-enabled formulations, multi-omics technologies, and artificial intelligence-assisted biofertilizer development. Furthermore, many previous reviews emphasize laboratory-scale research and conventional microbial inoculants, while comparatively less attention has been given to critical aspects such as formulation stability, microbial survival, field-scale validation, commercialization, regulatory frameworks, biosafety considerations, and the practical implementation of next-generation biofertilizers under diverse agroecological conditions. In view of these limitations, the present review provides an updated and comprehensive synthesis of the mechanisms through which biofertilizers enhance nutrient use efficiency, critically evaluates recent technological innovations and their agronomic relevance, identifies current knowledge gaps and research challenges, and discusses emerging opportunities for integrating advanced microbial biotechnology with precision agriculture and climate-smart nutrient management. By combining conventional biofertilizer research with the latest developments in microbial engineering and sustainable agricultural technologies, this review offers a broader and more forward-looking perspective than previous reviews (Table 1), thereby providing a valuable framework for the development and large-scale adoption of next-generation biofertilizers for sustainable and climate-resilient agriculture.

Table 1: Comparison of recent review articles with the present review.

Reviews	Main focus	References
Biofertilizers in sustainable agriculture: mechanisms, challenges, and future perspectives	Mechanisms of biofertilizers, nutrient cycling, plant–microbe interactions	Shahzad <i>et al.</i> (2025)
Biomaterial amendments improve nutrient use efficiency and soil health	Biofertilizers, biochar, nanomaterials, nutrient use efficiency	Liu <i>et al.</i> (2025)
Nitrogen use efficiency in agriculture: Integrating biological nitrogen fixation and emerging technologies	NUE, biological nitrogen fixation, microbial technologies	Navarro <i>et al.</i> (2025)
Engineering plant–microbe communication for microbiome engineering	Root microbiome engineering, synthetic communities, rhizosphere engineering	Griffin <i>et al.</i> (2024)
Review of research and innovation on novel fertilizers for responsible plant nutrition	Biofertilizers, nanofertilizers, enhanced-efficiency fertilizers	Maaz <i>et al.</i> (2025)

Present review (2026)	Provides a comprehensive and up-to-date synthesis of conventional and next-generation biofertilizers for improving NUE. Unlike previous reviews, it integrates CRISPR-mediated microbial engineering, multi-omics, metagenomics, microbiome engineering, synthetic microbial communities, artificial intelligence-assisted biofertilizer development, and nano-enabled formulations. The review further includes critical analyses of current challenges, research gaps, field-scale validation, commercialization, biosafety, regulatory frameworks, and farmer adoption, while outlining future research priorities for developing climate-resilient and commercially viable biofertilizers for sustainable agriculture.
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2. Nutrient Use Efficiency (NUE) in Sustainable Agriculture

Nutrient use efficiency (NUE) is a fundamental indicator of sustainable agricultural production, describing the ability of crops to acquire, utilize, and convert available nutrients into economic yield while minimizing nutrient losses to the environment. It reflects the combined efficiency of nutrient availability, plant uptake, assimilation, translocation, and utilization, and is therefore influenced by interactions among soil properties, crop genotype, fertilizer management, climatic conditions, and biological processes within the rhizosphere (Mikkelsen *et al.*, 2012).

NUE is determined by several interacting factors, including fertilizer source, application rate, timing, placement, irrigation practices, soil physicochemical properties, microbial activity, and crop genetics (Barbieri *et al.*, 2008). The widely implemented 4R Nutrient Stewardship framework, applying the right nutrient source at the right rate, at the right time, and in the right place, has been recognized as an effective strategy for improving NUE and reducing nutrient losses. In recent years, biological approaches are increasingly recognized for their role in improving nutrient acquisition, stimulating root development, enhancing nutrient cycling, and boosting plant resilience to environmental stresses through beneficial soil microorganisms. NUE is a multidimensional concept evaluated through various agronomic indices, including agronomic efficiency, physiological efficiency, and apparent recovery efficiency, which assess fertilizer performance and nutrient management strategies (Table 2).

Table 2: Major indices used for evaluating nutrient use efficiency.

NUE Index	Formula	Description	Significance
Agronomic Efficiency (AE)	(Grain yield of fertilized crop – Grain yield of unfertilized crop) / Quantity of fertilizer applied	Increase in grain yield per unit of nutrient applied	Indicates fertilizer effectiveness in increasing crop yield
Physiological Efficiency (PE)	(Biological yield of fertilized crop – Biological yield of unfertilized crop) / (Nutrient uptake by fertilized crop – Nutrient uptake by unfertilized crop)	Efficiency of converting absorbed nutrients into biomass	Reflects the plant's internal nutrient utilization efficiency
Apparent Recovery Efficiency (RE)	(Nutrient uptake by fertilized crop – Nutrient uptake by unfertilized crop) / Quantity of fertilizer applied	Fraction of applied nutrient recovered by the crop	Indicates the effectiveness of nutrient uptake and reduced nutrient losses

3. Significance of Biofertilizers

Biofertilizers are preparations containing live or dormant beneficial microorganisms that promote plant growth by enhancing nutrient availability, nutrient uptake, and soil biological activity, rather than serving as direct nutrient sources. These microbes colonize the rhizosphere or internal plant tissues and

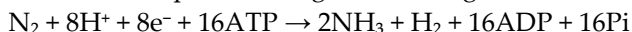
support plant nutrition through biological nitrogen fixation; solubilization and mobilization of phosphorus, potassium, and micronutrients; synthesis of phytohormones; and stimulation of advantageous plant–microbe interactions (Ghamry *et al.*, 2018; Singh *et al.*, 2019). Advances in microbial ecology and biotechnology have broadened the definition of biofertilizers from single-strain inoculants to include microbial consortia, multifunctional bioformulations, and plant growth-promoting microorganisms capable of concurrently improving nutrient uptake, stress resilience, and soil health. Within this context, PGPR have become one of the most intensively investigated groups because they employ multiple direct and indirect mechanisms to enhance crop yields and nutrient use efficiency (Suyal *et al.*, 2016). Biofertilizers are commonly categorized according to their main functional contribution to nutrient transformation and plant growth promotion. Key groups include nitrogen-fixing microorganisms; phosphate-solubilizing microorganisms; potassium-solubilizing microorganisms; sulfur- and micronutrient-mobilizing microorganisms; mycorrhizal fungi; compost-degrading microorganisms; and PGPR (Naveed *et al.*, 2015). Nonetheless, newer biofertilizer products increasingly incorporate combinations of several beneficial microorganisms in the form of microbial consortia to strengthen nutrient acquisition, enhance stress tolerance, and improve performance under diverse agroecological conditions.

3.1. Biological Nitrogen Fixation: Mechanism and Agronomic Significance

Nitrogen (N) is a vital macronutrient for plant growth and development, forming a core constituent of amino acids, proteins, nucleic acids, chlorophyll, and numerous other metabolic compounds. Despite atmospheric dinitrogen (N₂) comprising about 78% of the Earth's atmosphere, it is not directly available to plants because of the strong triple bond linking the two nitrogen atoms. Instead, plants assimilate nitrogen predominantly as ammonium (NH₄⁺) and nitrate (NO₃⁻) ions (Křiška *et al.*, 2023). Consequently, modern agricultural production has relied heavily on synthetic nitrogen fertilizers to satisfy crop nitrogen requirements. However, excessive nitrogen fertilizer application has resulted in low NUE, leading to substantial losses through leaching, volatilization, denitrification, soil acidification, eutrophication of water bodies, and increased emissions of nitrous oxide (N₂O), a potent greenhouse gas.

Biological nitrogen fixation (BNF) provides a sustainable alternative for improving nitrogen availability while reducing dependence on synthetic fertilizers. BNF is a naturally occurring process in which specialized microorganisms; collectively known as diazotrophs, convert atmospheric nitrogen (N₂) into ammonia (NH₃), a plant-available form of nitrogen (Fig. 1). This process is restricted to certain bacteria and archaea, including members of the *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, *Firmicutes*, green sulfur bacteria, and methanogenic archaea, which possess the nitrogenase enzyme complex responsible for nitrogen reduction. Diazotrophic microorganisms exhibit diverse physiological characteristics and include aerobic species such as *Azotobacter*, anaerobic species such as *Clostridium*, facultative anaerobes such as *Klebsiella*, photosynthetic cyanobacteria such as *Anabaena*, and chemolithotrophic bacteria such as *Leptospirillum ferrooxidans*.

The nitrogenase enzyme complex, composed of the molybdenum–iron (MoFe) protein and the iron (Fe) protein, catalyzes the reduction of atmospheric nitrogen according to the following reaction:



The MoFe protein catalyzes the reduction of atmospheric nitrogen to ammonia, whereas the Fe protein transfers electrons required for this energy-intensive process (Dance, 2023; Warmack *et al.*, 2023). Because nitrogenase is highly sensitive to oxygen, diazotrophic microorganisms have evolved protective mechanisms, including the formation of low-oxygen microenvironments within legume root nodules and the production of protective mucilage around bacteroids, thereby maintaining nitrogenase activity under aerobic conditions (Hania *et al.*, 2023; Martin *et al.*, 2023; Reineke & Schlomann, 2023).

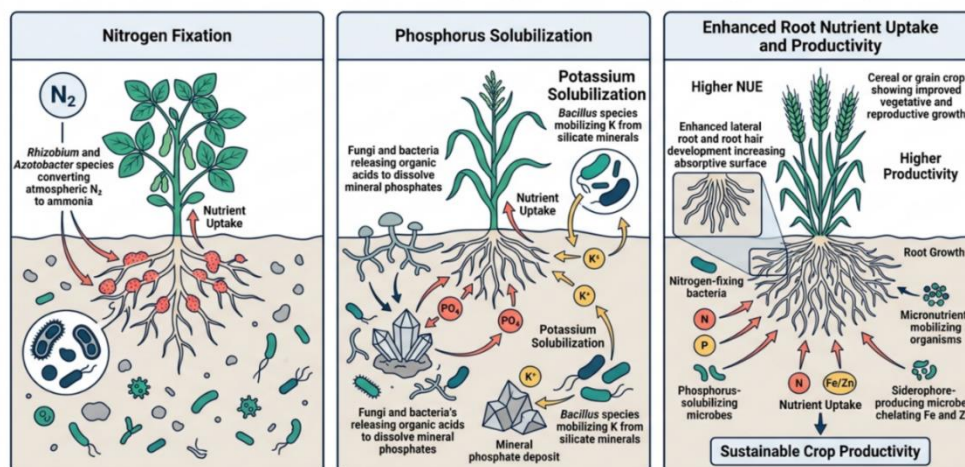


Fig. 1: Biofertilizer-mediated nutrient cycling for sustainable crop productivity.

From an agronomic perspective, BNF is one of the most efficient biological processes for improving NUE. Unlike synthetic fertilizers, which are susceptible to significant environmental losses, biologically fixed nitrogen is released gradually in synchrony with plant demand, thereby enhancing nitrogen recovery, reducing nutrient losses, and improving fertilizer use efficiency. In addition to supplying nitrogen, diazotrophic microorganisms frequently stimulate root growth through phytohormone production, enhance nutrient acquisition, and improve plant tolerance to abiotic stresses, resulting in greater crop productivity and improved soil fertility (Fig. 1). Consequently, nitrogen-fixing biofertilizers represent an essential component of integrated nutrient management and climate-smart agricultural systems. Based on their ecological associations with plants, nitrogen-fixing microorganisms are broadly classified into symbiotic, free-living, and associative (endophytic) diazotrophs, each differing in their mechanisms of nitrogen fixation, host specificity, and contribution to nutrient use efficiency. These groups are summarized in tabular form (Table 3).

Table 3: Classification of nitrogen-fixing biofertilizers and their contribution to nutrient use efficiency.

Nature of Association	Microorganisms	NUE	Plants	Impacts on roots	Overall Impacts	References
Symbiotic	<i>Rhizobium</i> , <i>Bradyrhizobium</i> , <i>Mesorhizobium</i> , <i>Azorhizobium</i> , <i>Sinorhizobium</i> , <i>Frankia</i>	50–300 kg N ha ⁻¹ yr ⁻¹	Soybean, chickpea, pea, lentil, groundnut, alfalfa	Root nodule formation, biological nitrogen fixation through nitrogenase	Host specificity, soil acidity, high mineral N suppresses nodulation	Bourscheidt <i>et al.</i> (2023)
Free-living	<i>Azotobacter</i> , <i>Clostridium</i> , <i>Beijerinckia</i> , <i>Azoarcus</i> , <i>Klebsiella</i> , <i>Cyanobacteria</i>	5–20 kg N ha ⁻¹ yr ⁻¹	Wheat, maize, rice, vegetables	Atmospheric nitrogen fixation, phytohormone production, siderophores	Lower N contribution, competition with native microbes	Schultz <i>et al.</i> (2014)

Associative / Endophytic	<i>Azospirillum</i> , <i>Herbaspirillum</i> , <i>Gluconacetobacter diazotrophicus</i> , <i>Burkholderia</i> , <i>Enterobacter</i>	20–40 kg N ha ⁻¹ yr ⁻¹	Maize, rice, wheat, sugarcane, sorghum	Rhizosphere/endosphere colonization, nif gene expression, root stimulation, ACC deaminase	Performance varies with soil type, crop genotype, and environment	Zilli <i>et al.</i> , (2020)
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3.1.1. Symbiotic Nitrogen-Fixing Microorganisms

Symbiotic nitrogen-fixing bacteria, collectively termed rhizobia, form highly specialized mutualistic associations with leguminous plants and represent the most efficient biological nitrogen fixation (BNF) system in terrestrial ecosystems. The principal genera include *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Azorhizobium*, *Allorhizobium*, and *Sinorhizobium* (currently classified as *Ensifer*) (Sindhu & Dadarwal, 1997). In this partnership, rhizobia invade root hairs and induce the formation of specialized organs called nodules, where atmospheric nitrogen (N₂) is reduced to ammonia (NH₃). The resulting ammonia constitutes an immediately assimilable nitrogen source for the host, while the bacteria receive photosynthetically derived carbon compounds in return (Ahemad & Khan, 2012).

Symbiosis is initiated by a finely tuned molecular dialogue between the host plant and rhizobia. Legume roots exude flavonoids into the rhizosphere, which activate bacterial nodulation (nod) genes. These genes direct the production of lipochitooligosaccharides (LCOs), or Nod factors, that act as key signalling molecules. Perception of LCOs by specific receptors on root hairs triggers root hair curling, infection thread formation, cortical cell division and, ultimately, the development of nitrogen-fixing nodules (Hassen *et al.*, 2020; Rajkumari *et al.*, 2022). In mature nodules, nitrogen fixation is carried out by the nitrogenase enzyme complex, which comprises the molybdenum–iron (MoFe) protein and the iron (Fe) protein (Fig. 2). This complex catalyzes the ATP-dependent reduction of atmospheric nitrogen to ammonia (Dance, 2023; Warmack *et al.*, 2023; Fig. 2). Since, nitrogenase is highly oxygen-labile; efficient nitrogen fixation requires tight regulation of oxygen levels. Legume nodules maintain a low-oxygen microenvironment through diffusion barriers and the presence of leghemoglobin, whereas bacteroids further safeguard nitrogenase via physiological strategies such as mucilage production and elevated respiratory activity (Hania *et al.*, 2023; Martin *et al.*, 2023; Reineke and Schlömann, 2023).

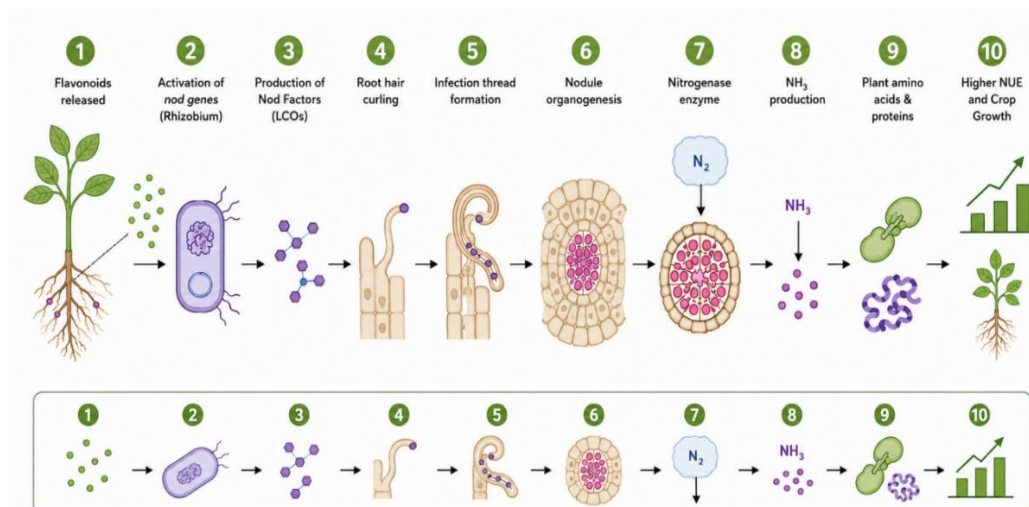


Fig. 2: Mechanistic overview of *Rhizobium*-legume symbiosis showing flavonoid signaling, nod gene activation, Nod factor production, infection thread formation, nodule development, BNF, and the resulting improvement in NUE.

The performance of symbiotic nitrogen fixation is modulated by numerous biotic and abiotic factors, including host–rhizobial compatibility, soil pH, nutrient status, temperature, moisture, salinity, and signalling molecules such as flavonoids, phytohormones, and polysaccharides (Kumawat *et al.*, 2017; Shamseldin, 2022). Environmental stresses and excessive application of mineral nitrogen fertilizers frequently inhibit nodulation and BNF, diminishing the benefits of rhizobial inoculation. By supplying nitrogen directly within the root zone throughout crop growth, symbiotic nitrogen fixation markedly enhances nitrogen recovery efficiency, lessens dependence on synthetic nitrogen fertilizers, and curtails nitrogen losses via leaching, volatilization, and denitrification. Accordingly, rhizobial inoculation is now regarded as a key component of integrated nutrient management in legume-based cropping systems.

3.1.2. Free-Living Nitrogen-Fixing Microorganisms

Free-living diazotrophs are non-symbiotic nitrogen-fixing bacteria that inhabit the rhizosphere, phyllosphere, or bulk soil without forming specialized structures such as root nodules. Although their contribution to biological nitrogen fixation is generally lower than that of symbiotic rhizobia (typically 5–20 kg N ha⁻¹ yr⁻¹), these microorganisms significantly improve nutrient use efficiency (NUE) through multiple plant growth-promoting mechanisms. Besides fixing atmospheric nitrogen, they synthesize phytohormones including indole-3-acetic acid (IAA) and gibberellins, produce siderophores that enhance iron acquisition, solubilize phosphate, and express ACC deaminase, which lowers stress-induced ethylene levels and promotes root development. Enhanced root architecture subsequently improves nutrient and water uptake, thereby indirectly increasing NUE.

Among free-living diazotrophs, *Azotobacter*, *Clostridium*, *Beijerinckia*, and *Azoarcus* are the most extensively studied. *Azotobacter* species are particularly recognized for their multifunctional plant growth-promoting attributes, including phytohormone production, biological control activity, and abiotic stress tolerance. For example, Chaudhary *et al.* (2013) demonstrated that inoculation with the salt-tolerant *Azotobacter* strain ST24 significantly increased nitrogen accumulation, biomass production, and grain yield of wheat grown under saline conditions when combined with 120 kg N ha⁻¹, highlighting its potential for improving fertilizer use efficiency under stress environments (Table 4).

Table 4: Characteristics and agronomic significance of major free-living nitrogen-fixing biofertilizers.

Microorganisms	Approximate N-fixation	Major mechanisms	Plant growth-promoting traits	Major crops	Contribution to NUE	References
<i>Azoarcus</i>	10–20 kg N ha ⁻¹ yr ⁻¹	Endophytic nitrogen fixation	Root colonization, phytohormone production	Rice, grasses	Improves N uptake and root development	Hurek & Hurek (1998)
<i>Beijerinckia</i>	5–15 kg N ha ⁻¹ yr ⁻¹	Biological N fixation	IAA production, phosphate solubilization	Sugarcane, cereals	Improves nutrient acquisition in acidic soils	Kennedy <i>et al.</i> (2004)
<i>Clostridium</i>	5–10 kg N ha ⁻¹ yr ⁻¹	Anaerobic nitrogen fixation	Organic acid production	Rice, wetland crops	Improves N availability under anaerobic conditions	Gaby & Buckley (2014)
<i>Azotobacter</i>	10–20 kg N ha ⁻¹ yr ⁻¹	Biological N fixation	IAA, gibberellins, siderophores, ACC deaminase, phosphate solubilization	Wheat, maize, vegetables	Enhances root growth, nutrient uptake, and fertilizer recovery	Aasfar <i>et al.</i> (2021)

3.1.3. Associative Nitrogen-Fixing Microorganisms

Associative diazotrophs establish loose associations with plant roots or colonize internal tissues without inducing nodule formation. Unlike symbiotic rhizobia, their primary contribution to crop productivity is mediated through stimulation of root growth and enhancement of nutrient acquisition rather than direct nitrogen transfer. These microorganisms colonize the rhizosphere and endosphere, where activation of *nif* genes enables biological nitrogen fixation while simultaneously producing phytohormones, nitric oxide, siderophores, and ACC deaminase that collectively improve root architecture and nutrient uptake. Among associative diazotrophs, *Azospirillum*, *Herbaspirillum*, and *Gluconacetobacter diazotrophicus* are the most widely investigated. *Azospirillum* spp. can contribute approximately 20–40 kg N ha⁻¹ under favourable conditions (Bourscheidt *et al.*, 2023), whereas lower contributions have been reported under nutrient-limited conditions (Pathak *et al.*, 2002). Similar nitrogen contributions have been observed for *Herbaspirillum* in sugarcane (Schultz *et al.*, 2014). Increasing evidence indicates that improvements in crop performance are primarily attributable to enhanced root proliferation, greater nutrient uptake, and improved physiological efficiency rather than direct nitrogen transfer (Steenhoudt & Vanderleyden, 2000). Recent studies demonstrate that inoculation with *A. brasilense* modulates genes involved in nitrogen uptake and assimilation, thereby improving NUE in maize, potato, and cereal cropping systems (Zeffa *et al.*, 2019; Naqqash *et al.*, 2022).

Recent advances have substantially expanded the potential of associative nitrogen-fixing biofertilizers. Metagenomic exploration has enabled the discovery of novel diazotrophic bacteria from saline and degraded ecosystems (Renganathan *et al.*, 2025). In contrast, CRISPR-assisted engineering of nitrogenase-associated genes has improved nitrogen fixation under aerobic conditions (Russell *et al.*, 2024). Furthermore, co-inoculation of *Azospirillum* with *Rhizobium* and AMF has demonstrated synergistic improvements in nitrogen recovery, nutrient uptake, and crop productivity (Galindo *et al.*, 2025). The integration of microbial consortia is therefore emerging as a promising next-generation strategy for enhancing the consistency and field performance of biofertilizers under diverse agroecosystems are summarized in tabular form (Tables 5 & 6).

Table 5. Characteristics and emerging applications of associative nitrogen-fixing microorganisms.

Microorganisms	Typical host crops	Nitrogen fixation	Major mechanisms	Emerging technologies	Contribution to NUE	References
<i>A. brasilense</i>	Maize, wheat, rice, sorghum	20–40 kg N ha ⁻¹ yr ⁻¹	Root colonization, <i>nif</i> gene activation, IAA, ACC deaminase	Co-inoculation, microbial consortia	Improves root growth, nutrient uptake, and N recovery	Bashan & Bashan (2010)
<i>G. diazotrophicus</i>	Sugarcane, rice	20–30 kg N ha ⁻¹ yr ⁻¹	Endophytic N ₂ -fixation, phytohormone production	Genomic improvement	Improves NUE	Kennedy <i>et al.</i> (2004)
<i>Herbaspirillum</i> spp.	Sugarcane, maize	~40 kg N ha ⁻¹ yr ⁻¹	Endophytic colonization, N ₂ -fixation	Multi-strain inoculants	Enhances nutrient acquisition and biomass production	Baldani <i>et al.</i> (2014)
Microbial consortia (<i>Azospirillum</i> + AMF + PGPR)	Cereals and legumes	Higher than single inoculants	Synergistic nutrient acquisition	Precision biofertilizers	Improved NUE, fertilizer recovery and yield stability	Backer <i>et al.</i> (2018)

Novel diazotrophic strains	Multiple crops	Variable	N ₂ -fixation under stress environments	Metagenomics, CRISPR engineering	Development of climate-resilient biofertilizers	Compant <i>et al.</i> (2019)
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Table 6: Nitrogen-fixing bacteria and their associated crops.

Crops	Nitrogen-Fixing Bacteria	References
Rice (<i>Oryza sativa</i>)	<i>Lysinibacillus sphaericus</i> , <i>Rhizobium</i> spp., <i>Azospirillum</i> spp.	(Pham <i>et al.</i> , 2017; Shabanamol <i>et al.</i> , 2018)
Maize (<i>Zea mays</i>)	<i>Klebsiella</i> spp., <i>Bacillus</i> spp., <i>Enterobacter</i> spp.	(Kuan <i>et al.</i> , 2016; Sandhya <i>et al.</i> , 2017)
Groundnut (<i>Arachis hypogaea</i>)	<i>Bradyrhizobium</i> spp., <i>Enterobacter ludwigii</i>	(Dhole <i>et al.</i> , 2016)
Greengram (<i>Vigna radiata</i>)	<i>Rhizobium</i> spp., <i>Bradyrhizobium</i> spp.	(Hifwe <i>et al.</i> , 2019)
Tomato (<i>Solanum lycopersicum</i>)	<i>Pantoea</i> spp., <i>Acinetobacter</i> spp.	(Castillo <i>et al.</i> , 2019)
Sugarcane (<i>Saccharum officinarum</i>)	<i>Gluconacetobacter diazotrophicus</i> , <i>Kosakonia</i> spp.	(Ali <i>et al.</i> , 2020)

3.1.4. Critical Analysis and Research Gaps

BNF remains one of the most effective biological strategies for improving NUE while reducing dependence on synthetic nitrogen fertilizers. Symbiotic rhizobia are capable of supplying substantial amounts of biologically fixed nitrogen, whereas associative and free-living diazotrophs additionally promote plant growth through phytohormone production, improved root architecture, and enhanced nutrient acquisition. These multifunctional attributes make nitrogen-fixing biofertilizers important components of sustainable nutrient management systems.

Despite these advantages, several limitations continue to restrict their widespread agricultural adoption. Nitrogen fixation efficiency is highly influenced by soil pH, temperature, moisture, salinity, crop genotype, and competition with indigenous microbial populations, often resulting in inconsistent field performance compared with controlled laboratory or greenhouse conditions. Furthermore, the oxygen sensitivity of the nitrogenase enzyme, limited shelf life of microbial formulations, and variability in rhizosphere colonization remain significant constraints. Future research should prioritize engineering stress-tolerant diazotrophic strains, designing stable synthetic microbial consortia, integrating CRISPR and multi-omics approaches, and conducting long-term multi-location field trials to improve the reliability and scalability of nitrogen-fixing biofertilizers.

4. Phosphate Solubilizing/mobilizing Microorganisms

Phosphorus (P), the second most critical macronutrient after nitrogen, is indispensable for energy transfer, macromolecular biosynthesis, and respiration (Khan *et al.*, 2009). Its availability in soil is strongly influenced by the nature of the parent material; for example, carbonate rocks typically contain less P than volcanic substrates, making P dynamics a key consideration for sustainable agricultural production (Ali *et al.*, 2020). Despite its overall abundance in soils, most P occurs in insoluble forms, and plants can utilize it only as orthophosphate ions (HPO_4^{2-} and H_2PO_4^-) (Bhattacharyya & Jha, 2012). Soil microorganisms play a pivotal role in regulating P cycling and bioavailability (Gyaneshwar *et al.*, 2002), stimulating growing interest in microbial approaches to reduce reliance on chemical P fertilizers.

Phosphate-solubilizing microorganisms (PSMs), notably phosphate-solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF), enhance plant P acquisition by transforming inorganic phosphorus into plant-available forms through mineralization, solubilization, and immobilization processes (Mabrouk *et al.*, 2018; Hussain *et al.*, 2019). Recent advances in microbial ecology have led to the identification of highly efficient PSB strains, such as *Bacillus safensis* and *Pseudomonas putida*, which exhibit tolerance to both acidic and calcareous soils (Loredana-Elena *et al.*, 2025). In parallel, the use of nano-clay carriers and alginate-based encapsulation has been shown to improve PSB survival and performance under field conditions (Balla *et al.*, 2025). Multi-omics investigations have further elucidated the molecular basis of phosphate solubilization, highlighting key genes such as *gcd* and *pqqC* involved in gluconic acid-mediated P solubilization, and have revealed synergistic interactions between PSB and AMF in cereal-legume cropping systems (Benmrid *et al.*, 2023).

Inoculation of PSB in chickpea has been reported to significantly enhance nodulation, shoot length, pod number, and grain yield by 23, 13, 17, and 15%, respectively (Ditta *et al.*, 2018). AMF, which naturally establish symbiotic associations with plant roots, further promote phosphorus mobilization and nutrient cycling, particularly in P-deficient soils, and are widely recognized for their beneficial roles in cereals and horticultural crops (Dalpe & Monreal, 2004). Integrated management strategies that combine silicon supplementation with PSB and AMF inoculation have been shown to markedly increase phosphorus availability and plant uptake compared with individual applications, underscoring the potential of such combined approaches for sustainable P management (Etesami *et al.*, 2021). Some PSB present in various leguminous and non-leguminous crops have been summarized in Table 7.

Table 7: Phosphorus-solubilizing bacteria (PSB) in various leguminous and non-leguminous.

Crops	PSB	Major Functions	Contribution to NUE	References
Groundnut (<i>Arachis hypogaea</i>)	<i>Bacillus</i> spp., <i>Pseudomonas</i> spp.	Solubilization of insoluble phosphates, phytohormone production	Enhances phosphorus availability and nodulation	Dhole <i>et al.</i> (2016)
Soybean (<i>Glycine max</i>)	<i>B. megaterium</i> , <i>P. fluorescens</i> , <i>Enterobacter</i> spp.	Phosphate solubilization, organic acid production, IAA production	Improves P uptake, root growth, and phosphorus use efficiency	Sood <i>et al.</i> (2019)
Green gram (<i>Vigna radiata</i>)	<i>Bacillus</i> spp., <i>Rhizobium</i> spp., <i>Pseudomonas</i> spp.	Phosphate solubilization and biological nitrogen fixation	Improves phosphorus uptake and BNF	Hifwe <i>et al.</i> (2019)
Maize (<i>Zea mays</i>)	<i>B. mojavensis</i> , <i>Pseudomonas</i> spp., <i>Enterobacter</i> spp.	Phosphate solubilization and phytohormone production	Increases phosphorus uptake, biomass, and fertilizer use efficiency	Kuan <i>et al.</i> (2016); Akintokun <i>et al.</i> (2019)
Rice (<i>Oryza sativa</i>)	<i>Bacillus</i> spp., <i>Pseudomonas</i> spp., <i>Enterobacter</i> spp.	Solubilization of inorganic phosphate, siderophore production	Improves phosphorus availability and grain yield	Pham <i>et al.</i> (2017); Shabanamol <i>et al.</i> (2018)
Wheat (<i>Triticum aestivum</i>)	<i>B. subtilis</i> , <i>P. putida</i> , <i>Serratia marcescens</i>	Organic acid secretion, phosphatase activity	Enhances phosphorus acquisition, root development, and NUE	Batool & Iqbal (2019)
Tomato (<i>Solanum lycopersicum</i>)	<i>Pantoea</i> spp., <i>Bacillus</i> spp., <i>P. fluorescens</i>	Phosphate solubilization, IAA production	Enhances phosphorus uptake, root growth, and fruit yield	Castillo <i>et al.</i> (2019)
Sugarcane (<i>Saccharum officinarum</i>)	<i>Kosakonia</i> spp., <i>Bacillus</i> spp., <i>Burkholderia</i> spp.	Phosphate solubilization and nutrient mobilization	Improves phosphorus uptake and crop productivity	Ali <i>et al.</i> (2020)

Various leguminous and non-leguminous crops	<i>Bacillus</i> spp., <i>Pseudomonas</i> spp., <i>Enterobacter</i> spp., <i>Burkholderia</i> spp., <i>Rhizobium</i> spp.	Organic acid production, phosphatase secretion, proton extrusion	Enhances phosphorus availability, nutrient uptake, and phosphorus use efficiency	Arora <i>et al.</i> (2020); Sood <i>et al.</i> (2019)
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4.1. Critical Analysis and Research Gaps

Phosphate-solubilizing microorganisms have demonstrated considerable potential for enhancing phosphorus availability through organic acid secretion, phosphatase activity, and synergistic interactions with arbuscular mycorrhizal fungi. Their application can reduce dependence on phosphate fertilizers while improving phosphorus use efficiency and soil biological activity. Nevertheless, phosphate solubilization varies substantially among microbial strains and soil types, particularly in calcareous and alkaline soils where phosphorus rapidly becomes immobilized. Moreover, most available studies are based on short-term greenhouse experiments, whereas evidence from long-term field trials remains limited. Future investigations should focus on developing multifunctional microbial consortia, improving carrier technologies, and validating their performance across diverse agroecological environments.

5. Potassium Solubilizing Microorganisms

Potassium is the third most important macronutrient required by plants after nitrogen and phosphorus (Ding *et al.*, 2021). Although total potassium is abundant in most soils, only a small proportion (1–2%) is present in forms readily available to plants, necessitating a continuous replenishment of potassium in the soil solution to ensure adequate supply for crop growth (Meena *et al.*, 2014; Parmar & Sindhu, 2019). Similar to other essential nutrients, potassium strongly influences plant growth and development; inadequate potassium supply results in stunted growth, poorly developed root systems, and reduced yields (Williams & Pittman, 2010). In addition, potassium plays a central role in key physiological processes, including starch synthesis, root development, and regulation of stomatal movement (Cedillo *et al.*, 2016). Under potassium deficiency, root growth is inhibited and poorly structured, seeds are smaller, and plants exhibit greater susceptibility to diseases, collectively contributing to yield reductions (Troufflard *et al.*, 2010).

PGPR inhabiting the soil and rhizosphere can convert insoluble forms of soil potassium into soluble, plant-available forms. Reported potassium-solubilizing microorganisms (KSMs) include *Acidithiobacillus*, *Enterobacter hormaechei*, *Paenibacillus* spp., *Aminobacter*, *Pseudomonas*, *Paenibacillus glucanolyticus*, *Sphingomonas*, *Bacillus* and *Burkholderia* (Uroz *et al.*, 2007; Parmar & Sindhu, 2019). The secretion of organic acids by these microorganisms promotes the dissolution of mineral potassium, making KSM application a promising strategy to enhance crop productivity (Sindhu *et al.*, 2016). Inoculation studies with potassium-solubilizing bacteria have demonstrated improvements in germination percentage, seedling vigour, nutrient uptake, and overall plant growth and yield (Singh *et al.*, 2010; Sindhu *et al.*, 2016). Beneficial effects of KSM inoculation have been documented in several crops, including pepper and cucumber (Han & Lee, 2006), wheat (Sheng & He, 2006), cotton, and rape (Sheng, 2005). By solubilizing and mobilizing potassium, KSMs provide a sustainable means to reduce dependence on chemical fertilizers, thereby offering an eco-friendly approach to sustainable agriculture (Meena *et al.*, 2016; Kour *et al.*, 2020).

Ding *et al.* (2021) evaluated 50% and 100% of the recommended potassium dose, with or without PSB and 40 kg ha⁻¹ of humic acid (HA), in faba bean grown in horizontal soil. A KUE of 40% was achieved in soils treated with both HA and PSB. The highest growth and yield of faba bean plants were obtained when HA and PSB were applied in combination with 50% of the recommended mineral potassium dose. Relative to the uninoculated control, chlorophyll content and leaf carbohydrates increased by 36% and 50%,

respectively, following HA and PSB application. Supplying half of the faba bean potassium requirements as 40 kg ha⁻¹ HA combined with PSB inoculation resulted in 14% and 19% increases in seed and straw yield, respectively, compared with full mineral fertilization without inoculation. Effects of some phosphate solubilizing bacteria on some crops have been summarized in Table 8.

Table 8: Potassium-solubilizing bacteria across different crops.

Crops	Potassium Solubilizing Bacteria	Major Functions	Contribution to NUE	References
Faba bean (<i>Vicia faba</i>)	<i>Rhizobium</i> spp.	Potassium solubilization, biological nitrogen fixation	Improves K availability, nodulation, nutrient uptake, and crop productivity	Shravanthi <i>et al.</i> (2019)
Maize (<i>Zea mays</i>)	<i>B. cereus</i> , <i>B. mojavensis</i> , <i>Klebsiella oxytoca</i>	Solubilization of mineral-bound potassium, phytohormone production	Enhances potassium uptake, root growth, and fertilizer use efficiency	Akintokun <i>et al.</i> (2019)
Chickpea (<i>Cicer arietinum</i>)	<i>Mesorhizobium ciceri</i> , <i>Pseudomonas jessenii</i>	Potassium mobilization, plant growth promotion	Enhances potassium uptake, nodulation, and nutrient use efficiency	Valverde <i>et al.</i> (2007)
Sorghum (<i>Sorghum bicolor</i>) and Chilli (<i>Capsicum annuum</i>)	<i>Bacillus</i> spp., <i>Pseudomonas</i> spp.	Potassium solubilization, phytohormone production	Improves potassium availability, root development, and crop yield	Archana <i>et al.</i> (2013)

5.1. Critical Analysis and Research Gaps

Potassium-solubilizing microorganisms (KSMs) represent a promising biological approach for enhancing potassium use efficiency by mobilizing insoluble potassium-bearing minerals through the secretion of organic acids, chelating compounds, and extracellular enzymes. Beyond improving potassium availability, many KSMs exhibit multiple plant growth-promoting traits, including phytohormone production, phosphate solubilization, siderophore secretion, and abiotic stress mitigation, making them attractive candidates for integrated nutrient management. Nevertheless, the effectiveness of KSM inoculants remains highly variable under field conditions due to differences in soil mineralogy, pH, moisture availability, crop genotype, climatic conditions, and competition with indigenous microbial communities. Moreover, the majority of published studies have been conducted under controlled laboratory or greenhouse conditions, while evidence from long-term, multi-location field trials remains limited. Additional challenges include poor shelf life, inconsistent rhizosphere colonization, and limited commercialization of highly efficient KSM formulations. Future research should focus on identifying robust native KSM strains adapted to diverse agroecological conditions, developing multifunctional microbial consortia capable of simultaneously mobilizing multiple nutrients, improving carrier materials and formulation technologies to enhance microbial survival, and integrating genomics, metagenomics, and artificial intelligence-based microbial selection to develop climate-resilient and crop-specific potassium-solubilizing biofertilizers with consistent field performance.

6. Sulphur Oxidizing Microorganisms

Sulphur is an essential macronutrient required in relatively large amounts by plants, where it functions as a structural constituent of the amino acids cysteine, cystine, and methionine, and as a key regulator of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, monodehydroascorbate

reductase, dehydroascorbate reductase, and glutathione reductase. Sulphur deficiency is associated with chlorosis, reduced lipid accumulation, and substantial decreases in plant growth and yield (Saha *et al.*, 2018). In soils, sulphur occurs in both organic and inorganic pools, and its transformation into the plant-available sulfate (SO_4^{2-}) form is largely driven by the activity of sulphur-oxidizing bacteria, including genera such as *Xanthobacter*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Thiobacillus* (Riaz *et al.*, 2020).

Many sulphur-oxidizing microorganisms also display plant growth-promoting traits, and biofertilizers formulated with *T. thiooxidans* and *B. japonicum* have been reported to enhance the growth of cereals, forage crops, and medicinal plants (Hu *et al.*, 2018). (Boroujeni *et al.*, 2021) isolated salt-tolerant, sulphur-oxidizing *Halothiobacillus* spp. from saline habitats in Iran, identifying *H. neapolitanus*, *H. hydrothermalis*, and *H. halophilus*; among these, *H. neapolitanus* strain 119 produced the highest biomass and sulfate at 0.5–1 M NaCl, underscoring its potential to improve sulfate availability in saline soils. Consistently, field experiments conducted by Gilani *et al.* (2021) showed that the combined application of *Thiobacillus* inoculation and sulfur fertilization at 100% of the recommended dose significantly increased chlorophyll content, biological yield, and grain yield in sesame–mungbean intercropping systems, highlighting the promise of integrated sulphur nutrition strategies for improving crop productivity.

6.1. Critical Analysis and Research Gaps

Sulphur-oxidizing microorganisms (SOMs) play an important role in improving sulphur availability by converting elemental sulphur into plant-available sulfate, thereby enhancing sulphur use efficiency and crop productivity. In addition to sulphur oxidation, several SOMs exhibit plant growth-promoting traits that contribute to improved nutrient uptake and stress tolerance. However, their field performance is often influenced by soil pH, salinity, temperature, and the availability of sulphur substrates, resulting in inconsistent responses under different agroecological conditions. Future research should focus on developing stress-tolerant SOM strains, improving formulation technologies, and validating their long-term performance through multi-location field trials to facilitate their large-scale adoption in sustainable nutrient management.

7. Zinc Solubilizing Microorganisms

Among the micronutrients, zinc (Zn) deficiency is the most widespread nutritional disorder constraining crop production (Hafeez *et al.*, 2013). Zinc plays a vital role in chlorophyll synthesis, enzyme activation, protein formation, and diverse metabolic processes (Ali *et al.*, 2008). Deficiency of Zn leads to chlorosis, loss of membrane integrity, reduced leaf size, stunted shoot and root growth, poor pollen formation, lower grain yield, and increased susceptibility to heat, light, and fungal stresses (Tavallali *et al.*, 2010; Kamran *et al.*, 2017). In soils, Zn predominantly occurs in insoluble forms such as ZnO , $\text{Zn}_3(\text{PO}_4)_2$, ZnCO_3 , and metallic Zn, which substantially limit its bioavailability to plants (Ayoub *et al.*, 2022).

Zinc-solubilizing microorganisms (ZSMs) enhance Zn availability by secreting organic acids that lower soil pH and chelate Zn cations, as well as through siderophore production, proton extrusion, and oxidoreductase activity (Kaur *et al.*, 2021). Several plant growths-promoting rhizobacteria, including *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Bacillus* spp., and *B. aryabhatai*, exhibit strong Zn-solubilizing potential. Strains such as *P. aeruginosa*, *G. diazotrophicus*, *P. striata*, *P. fluorescens*, *S. liquefaciens*, *B. cenocepacia*, *S. marcescens*, and *B. thuringiensis* have been reported to improve Zn uptake and nutritional status in crops including maize, soybean, and wheat (Hakim *et al.*, 2021; Ditta *et al.*, 2022).

Microbial inoculation thus represents an effective alternative or complements to conventional Zn fertilization, as a substantial fraction of plant-available Zn is mediated by microbial activity in the rhizosphere (Sindhu *et al.*, 2019). Zinc solubilization is primarily driven by the microbial production of organic acids, chelating ligands, and siderophores, which mobilize Zn from otherwise inaccessible soil

complexes (Saravanan *et al.*, 2011). Prominent Zn-solubilizing microbes include *Pseudomonas* spp., *Rhizobium* spp., *B. aryabhatai*, *T. thiooxidans*, and *Azospirillum* spp. (Ijaz *et al.*, 2019). Inoculation with *B. aryabhatai* has been shown to enhance maize growth (Mumtaz *et al.*, 2017), while *P. pseudoalcaligenes* and *B. pumilus* significantly increased plant height and dry biomass in rice (Jha, 2019).

7.1. Critical Analysis and Research Gaps

Zinc-solubilizing microorganisms (ZSMs) offer a sustainable strategy for improving zinc use efficiency by converting insoluble zinc compounds into plant-available forms through the production of organic acids, siderophores, and other chelating metabolites. Besides enhancing zinc availability, many ZSMs also promote plant growth through phytohormone production and improved nutrient acquisition. However, their effectiveness is strongly influenced by soil properties, native microbial communities, and environmental conditions, often leading to inconsistent performance under field conditions. Furthermore, long-term field validation and commercialization of efficient ZSM formulations remain limited. Future research should focus on identifying robust, stress-tolerant strains, developing stable carrier formulations, and integrating advanced microbial engineering approaches to improve the consistency and large-scale adoption of zinc-solubilizing biofertilizers.

8. Plant-Associated Microbiota and Stress Management

Microbial inoculants are central to sustaining ecosystem functions, yet their performance is frequently constrained by diverse environmental stressors (Qadir *et al.*, 2024). Both biotic (pathogens, weeds, pests) and abiotic (salinity, drought, metal toxicity, extreme temperatures) stresses impair microbial activity and thereby reduce agricultural productivity. In arid and semi-arid regions, drought and nutrient limitations are particularly detrimental to crop yields (Arani *et al.*, 2017). These stressors disrupt soil–plant–microbe interactions and adversely affect plant physiological and metabolic processes (Fig. 3). For example, salinity stress elevates ethylene levels, while drought diminishes root activity and chlorophyll content. Purslane, a common species in such environments, often suffers pronounced injury under combined drought and heat stress, but it also adopts physiological strategies to cope, including enhanced antioxidant activity, accumulation of osmoprotectants, and reduced water loss (Jin *et al.*, 2016; Danyal *et al.*, 2023).

Drought stress impacts plants in multiple, interconnected ways: it limits water availability to roots, decreases transpiration and photosynthetic rates, and intensifies oxidative damage (Fig. 3). These conditions further compromise enzyme activities, ion uptake, and nutrient assimilation, ultimately leading to poor seed germination and substantial economic losses in agriculture (Hasanuzzaman *et al.*, 2012). Beneficial biofertilizers, including rhizospheric and endophytic microbes, mitigate such stresses through various mechanisms, notably by synthesizing phytohormones such as indole-3-acetic acid (IAA) and cytokinins that support plant growth under adverse conditions (Chaudhary *et al.*, 2022). For instance, inoculation of soybean with *P. putida* elevates flavonoid, salicylic acid, and abscisic acid levels, thereby enhancing drought tolerance (Kang *et al.*, 2014), while co-inoculation of wheat with *Azospirillum* and *B. velezensis* improves heat tolerance by reducing oxidative damage (Dinesh *et al.*, 2015). Similarly, *Paraburkholderia phytofirmans* promotes rapid growth in heat-stressed tomato plants through the production of ACC deaminase, a key enzyme in stress mitigation (Esmaeel *et al.*, 2018).

Application of bacterial inoculants also enriches root-associated microbial communities and strengthens their resilience to environmental constraints. For example, inoculating maize with *Rhizophagus irregularis* and *F. mosseae* enhances root nutrient status, respiration rates, and plant height (Cabral *et al.*, 2016). In anaerobic environments, acidophilic microorganisms such as *A. ferrooxidans* contribute to the mineralization of iron and sulfur via aerobic respiration. Phosphorus (P), an essential soil nutrient, occurs

in both organic and inorganic forms (Hayat *et al.*, 2010), and microbial respiration generates carbon dioxide that forms carbonic acid, further improving P solubility (Berg, 2009). AMF are particularly important under water-deficit conditions, as they enhance water uptake and thereby improve plant drought tolerance (Khalvati *et al.*, 2005).

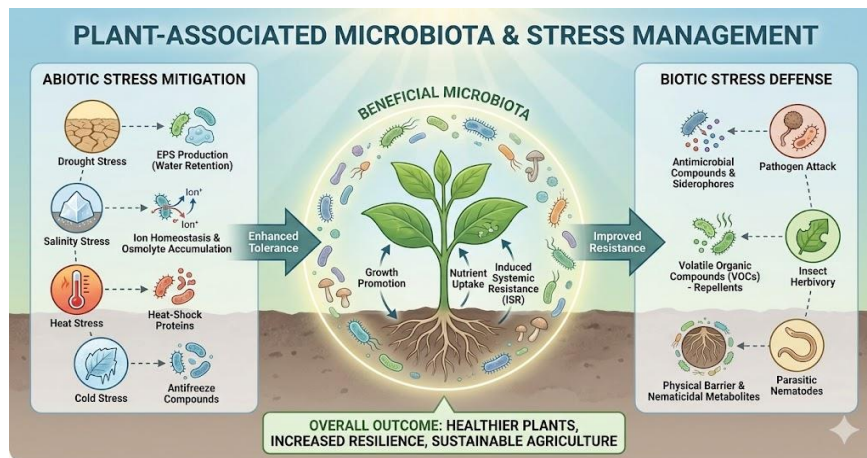


Fig. 3: Plant-associated microbiota and stress management.

8.1. Potential Use of Biofertilizers in Sustainable Crop Production

Biofertilizers are regarded as a viable and sustainable biotechnological approach to increase crop yield, improve and restore soil fertility, promote plant growth, reduce production costs, and minimize the environmental impacts associated with chemical fertilization (Vassilev, 2015; Thomas, 2019). A range of microorganisms are commonly employed as biofertilizers, including free-living nitrogen-fixing bacteria in the soil (e.g., *Azotobacter*, *Rhizobium*), nitrogen-fixing cyanobacteria (e.g., *Anabaena*), and phosphate-solubilizing bacteria. Microorganisms that produce phytohormones (e.g., auxins) and cellulolytic microorganisms are also widely used as biofertilizers. In addition, inoculating plants with beneficial bacteria can support the development of strategies that enhance plant growth under both optimal and stressful environmental conditions.

9. Applications of Biofertilizers

The following are the important applications of biofertilizers.

9.1. Seedling Root-dip

This method is particularly suitable for rice. It is used for transplanted crops. Two packets of the biofertilizer inoculant are mixed in 40 L of water to prepare a suspension. Seedlings required for one acre are immersed in this suspension for 5–10 min (or placed in a water bed containing the biofertilizer for 8–10 hrs) and then transplanted.

9.2. Seed Treatments

To prepare the inoculant slurry, one packet of biofertilizer is mixed with 200 mL of rice gruel. Seeds required for one hectare are thoroughly mixed in the slurry to ensure uniform coating with the inoculant and then dried in the shade for 30 min before sowing.

9.3. Main Field Application

Four packets of inoculant are mixed with 20 kg of well-dried, finely powdered farmyard manure and broadcast uniformly over one hectare of land just before planting.

9.4. Long-Term Field Validation and Large-Scale Implementation of Biofertilizers

Although numerous laboratory and greenhouse studies have demonstrated the effectiveness of biofertilizers in improving nutrient use efficiency, crop productivity, and soil health, their performance under field conditions often remains inconsistent due to variations in soil properties, climatic conditions, crop genotype, indigenous microbial communities, and agronomic management practices. Therefore, greater emphasis should be placed on long-term multi-location field trials to evaluate the persistence, survival, rhizosphere colonization, and agronomic performance of microbial inoculants across diverse agroecological environments. Large-scale validation should also include assessments of yield stability, nutrient recovery efficiency, economic feasibility, compatibility with existing fertilizer management practices, and environmental impacts over multiple cropping seasons. Furthermore, standardized evaluation protocols, quality control measures, and farmer participatory trials are essential for generating reliable data that support commercialization and regulatory approval. Integrating long-term field validation with precision agriculture technologies and digital monitoring systems will improve confidence in biofertilizer performance and facilitate their large-scale adoption as key components of sustainable nutrient management.

10. Recent advancements in biofertilizers

Several recent innovations aim to enhance soil and plant nutrient dynamics to improve crop productivity. These include the use of specialized microorganisms for plant inoculation, optimized application technologies, and the development of suitable carrier materials for inocula. Modern strategies also encompass artificial selection of microbiomes, mixed microbial inoculants, the use of PGPR as biofertilizers, biofilm-based inoculants, nano-biofertilizers, and bioorgano-chemical fertilizers. Collectively, these approaches seek to reduce the cost of biofertilizers while supporting long-term sustainable production.

10.1. PSM Inoculants

Phosphate-solubilizing microorganisms (PSMs) increase phosphorus-use efficiency in agriculture by transforming insoluble phosphates into forms readily available to plants. Strains such as *P. putida*, *P. fluorescens* CHA0, and *P. fluorescens* have been reported to solubilize approximately 51%, 29%, and 62% of phosphorus, respectively. Inoculation with *G. fasciculatum* and *Azotobacter* has been shown to enhance N and K uptake in mulberry, while, *Pseudomonas* and *Bacillus* spp. increased P uptake and grain yield in wheat. PSMs improve soil biological properties and phosphorus availability, particularly when chemical fertilizer inputs are limited. Studies have documented improved growth, yield, and quality in crops such as wheat, rice, maize, soybean, mustard, chilli, potato, sugarcane, legumes, fruits, and oilseeds following PSM application. Effective PSMs include *B. megaterium*, *B. circulans*, *B. subtilis*, and *P. striata*, underscoring their potential as sustainable biofertilizers.

10.2 PGPR as Biofertilizers

PGPR are free-living bacteria originating from the rhizosphere that can colonize plant roots and produce metabolites that stimulate plant growth (Beneduzi *et al.*, 2012). Upon inoculation, PGPR help plants tolerate drought stress (Ilyas *et al.*, 2020), salinity (Mayak *et al.*, 2004), and various biotic stresses (Verma *et al.*, 2016). Applications of PGPR have been reported to enhance seed germination, soil fertility, and overall plant growth through the production of phytohormones such as auxins, ethylene, and gibberellins (Jang *et al.*, 2017; Tahir *et al.*, 2017). Genera commonly included among PGPR comprise *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Azotobacter*, *Acinetobacter*, *Actinoplanes*, *Bacillus*, *Frankia*, *Pseudomonas*, *Rhizobium*,

Micrococcus, *Streptomyces*, *Xanthomonas*, *Enterobacter*, *Cellulomonas*, *Serratia*, *Flavobacterium*, and *Thiobacillus* (Glick and Gamalaro, 2021).

10.3 Nano-biofertilizer

Nano-biofertilizers are hybrid formulations that integrate nanotechnology with biofertilizers to enhance agricultural efficiency and yield. The combined action of nanomaterials and microbial inoculants improves soil moisture retention and facilitates nutrient uptake by plants (Kumari & Singh, 2019; Fig. 4). Conventional fertilizers often suffer from major limitations, including instability under fluctuating environmental conditions (temperature, and pH), short shelf life, reduced viability of microbial strains, short-term effectiveness, and the requirement for large quantities to cover extensive areas, which can ultimately lead to suboptimal growth and yield (Cakmakci, 2019). In contrast, nano-biofertilizers improve field performance, lower input costs, and increase productivity (Mala *et al.*, 2017). They are environmentally friendly and enhanced the efficiency of indigenous microbial populations in utilizing key nutrients such as N, P, and K, and in turn, stimulate microbial enzyme activity and contribute to improved soil fertility.



Fig. 4: Diagrammatic representation of Nano-biofertilizer.

10.4. Biofilm Biofertilizer

Biofilm-based biofertilizers represent a recent breakthrough in biofertilizer development (Fig. 5). Biofilms are structured microbial communities attached to biotic or abiotic surfaces and embedded in an extracellular matrix that supports their structural integrity and long-term stability (Junaid & Khan, 2018). Within these communities, microorganisms communicate through quorum sensing. The main objective of developing biofilm-based biofertilizers is to overcome constraints associated with biotic and abiotic soil factors and to enhance the resilience of indigenous microbial populations to diseases and environmental inhibitors (Parween *et al.*, 2017).

Biofilms can form on plant surfaces or within plant tissues and are particularly beneficial for PGPR-mediated nitrogen fixation and overall crop productivity (Fig. 5 & Fig. 6). They also contribute to zinc solubilization and the synthesis of chelating agents (Igiehon & Babalola, 2017). By facilitating the exchange of genetic material and organic resources among community members, biofilms create a cooperative environment that improves microbe survival and function. Multispecies (*e.g.*, bacterial-fungal) biofilms are generally more resilient and sustainable than single-species biofilms, exhibiting superior nutrient uptake and greater resistance to environmental stress compared with planktonic or non-biofilm-forming microorganisms (Velmourougane *et al.*, 2017).

Biofilms formulated with microbial consortia, such as *Anabaena* sp., and *Pseudomonas* have shown strong potential for NUE in rice production. For instance, the agronomic use efficiency of nitrogen (AUEN) was significantly increased when 75% of the recommended nitrogen dose (RDN) was applied in combination

with the *Anabaena–Pseudomonas* (An–Ps) biofilm formulation. This improvement was observed across different rice establishment methods, with particularly pronounced effects under the System of Rice Intensification (SRI) and puddled transplanted rice (PTR) systems. These findings align with previous reports that biofilms can reduce nitrogen losses by improving nitrogen retention and cycling within the biofilm matrix, thereby increasing its availability for plant uptake (Kumar *et al.*, 2020).

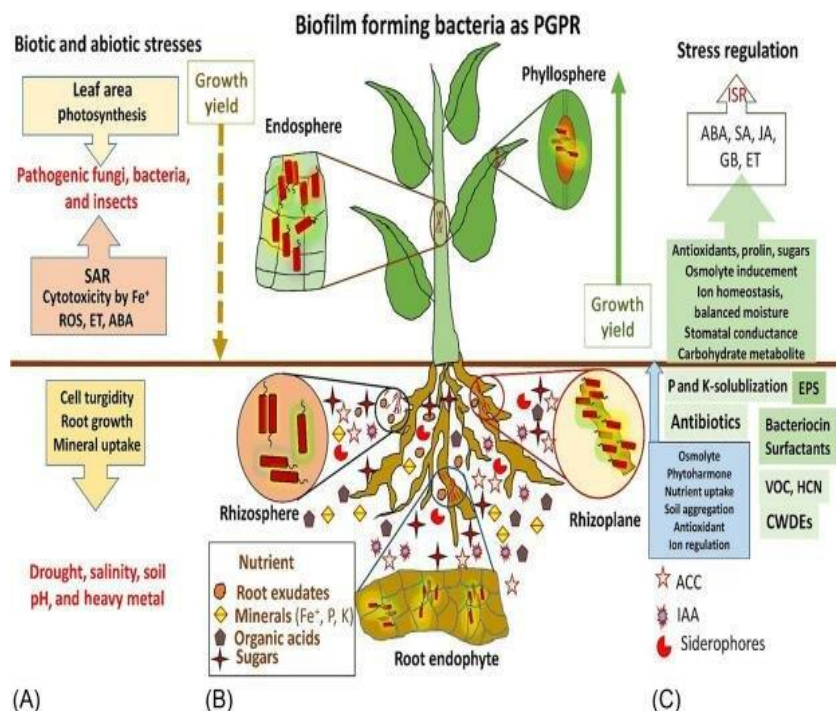


Fig. 5: Formation of biofilm associated with plants and soil (Solanki *et al.*, 2020)

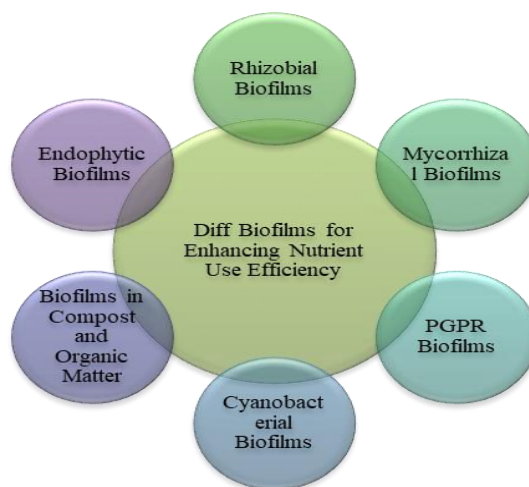


Fig. 6: Different types of biofilms for enhancing nutrient use efficiency.

10.5. CRISPR-Engineered Biofertilizers: Towards Next-Generation Microbial Inoculants

The emergence of the CRISPR-Cas genome-editing system has revolutionized microbial biotechnology by enabling precise, rapid, and cost-effective modification of beneficial microorganisms. Unlike conventional

mutagenesis, CRISPR technologies allow targeted editing, activation, or repression of genes involved in nutrient acquisition, stress adaptation, and plant–microbe interactions, thereby facilitating the development of next-generation biofertilizers with improved efficacy and field performance. Recent advances have expanded CRISPR applications beyond crop improvement to include engineering of plant-associated microbiomes and microbial inoculants for sustainable agriculture (Russell *et al.*, 2024).

One of the major targets of CRISPR engineering is the nitrogen fixation (*nif*) gene cluster, which regulates the synthesis and activity of the nitrogenase enzyme complex. Nitrogenase is highly sensitive to oxygen and tightly regulated by multiple genetic networks, limiting biological nitrogen fixation under field conditions. CRISPR-mediated editing or CRISPR interference (CRISPRi) enables precise regulation of *nif*, *naf*, and *rnf* genes in diazotrophic bacteria such as *Azotobacter vinelandii*, providing a powerful platform to investigate and optimize nitrogen fixation pathways. Recent work by (Russell *et al.*, 2024) demonstrated the first integrated CRISPRi system in *A. vinelandii*, achieving targeted repression of nitrogen-fixation genes and providing a versatile genetic tool for engineering biological nitrogen fixation (Herrero *et al.*, 2025). Table 9 summarizes the some emerging applications of CRISPR technology in next generation biofertilizer development.

Table 9: Emerging applications of CRISPR technology in next generation biofertilizer development.

Targets	CRISPR Applications	Agronomic Benefits	Current Status	References
Nitrogen fixation (<i>nif</i> genes)	Regulation of nitrogenase-associated genes using CRISPR/CRISPRi	Improved biological nitrogen fixation and nitrogen use efficiency	Experimental	Mus <i>et al.</i> (2016)
Nitrogenase optimization	Editing regulatory pathways controlling oxygen sensitivity and enzyme expression	Enhanced nitrogenase stability and activity	Experimental	Bennett <i>et al.</i> (2023)
Phosphorus solubilization	Engineering genes involved in organic acid production and phosphate mobilization	Improved P availability and PUE	Early-stage research	Sharma <i>et al.</i> (2013)
Stress tolerance	Editing genes related to oxidative stress, salinity, drought, and heat tolerance	Greater microbial survival and field performance	Experimental	Backer <i>et al.</i> (2013)
Synthetic microbial consortia	Engineering compatible microbial strains with complementary functions	Stable rhizosphere colonization and enhanced NUE	Emerging research	Großkopf & Soyer (2014)
Precision biofertilizers	Integration of CRISPR with AI, metagenomics, and synthetic biology	Crop-specific, climate-smart microbial inoculants	Future prospect	Singh <i>et al.</i> (2020)

10.6. Multi-Omics Approaches for Next-Generation Biofertilizer Development

The rapid advancement of high-throughput sequencing and systems biology has transformed biofertilizer research from conventional microbial screening to multi-omics-driven microbial engineering. Multi-omics integrates genomics, transcriptomics, proteomics, metabolomics, phenomics, and interactomics to provide a comprehensive understanding of plant–microbe interactions, nutrient cycling, and rhizosphere functioning (Table 10). Unlike single-omics studies, integrated multi-omics revealed how microbial genes are expressed, translated into functional proteins, produce metabolites, and ultimately influence plant phenotype under diverse environmental conditions. This systems-level understanding enables the rational design of efficient microbial inoculants with improved NUE, stress tolerance, and field performance (Jain *et al.*, 2024).

Table 10: Multi-omics technologies in next-generation biofertilizer research (Younas *et al.*, 2025).

Omics	Primary Objectives	Application in Biofertilizer Research	Contribution to NUE
Genomics	Identification of functional genes	Discovery of <i>nif</i> , <i>pqq</i> , <i>gcd</i> , siderophore and stress-responsive genes	Selection and engineering of superior microbial strains
Transcriptomics	Analysis of gene expression	Identification of genes involved in nitrogen fixation, phosphate solubilization, biofilm formation and stress responses	Improved understanding of microbial adaptation and nutrient acquisition
Proteomics	Characterization of functional proteins	Detection of nitrogenase, phosphatases, transport proteins and stress-related enzymes	Functional validation of nutrient cycling mechanisms
Metabolomics	Profiling metabolites and root exudates	Identification of signaling molecules, organic acids, phytohormones and microbial metabolites	Improved nutrient mobilization and rhizosphere signaling
Phenomics	High-throughput plant trait analysis	Assessment of root architecture, biomass, chlorophyll, nutrient uptake and growth	Evaluation of microbial inoculant performance under field conditions
Interactomics	Analysis of molecular interaction networks	Plant-microbe, microbe-microbe and protein interaction studies	Optimization of microbial compatibility and synthetic microbial communities
Systems Biology	Integration of multi-omics datasets	Network modelling, pathway analysis and predictive biology	Rational design of next-generation precision biofertilizers

10.7. Metagenomics and Microbiome Engineering for Next-Generation Biofertilizers

The advent of metagenomics has revolutionized the discovery and development of biofertilizers by enabling comprehensive analysis of microbial communities without the need for cultivation. Conventional culture-dependent methods recover less than 1% of soil microorganisms, leaving the vast majority of microbial diversity unexplored. Metagenomic approaches overcome this limitation by directly sequencing DNA extracted from environmental samples, thereby revealing the taxonomic composition, functional genes, and metabolic potential of entire microbial communities. These technologies have significantly enhanced our understanding of nutrient cycling, plant-microbe interactions, and rhizosphere ecology, providing unprecedented opportunities for designing next-generation biofertilizers with improved NUE. Recent advances in microbiome engineering further enable the rational manipulation of beneficial microbial communities to enhance crop productivity, nutrient acquisition, and resilience to environmental stresses (Berg *et al.*, 2020; Table 11).

Table 11. Metagenomic approaches and their applications in next generation biofertilizer development (Quince *et al.*, 2017).

Technology	Principle	Major applications	Contribution to NUE
16S rRNA sequencing	Marker gene sequencing	Microbial diversity profiling	Identification of beneficial rhizosphere microorganisms
Shotgun metagenomics	Whole-community DNA sequencing	Functional gene discovery (<i>nif</i> , <i>pqq</i> , <i>gcd</i> , <i>acdS</i>)	Selection of elite biofertilizer strains
Functional metagenomics	Screening cloned environmental DNA	Discovery of novel nutrient-cycling enzymes	Improved nutrient mobilization
Comparative metagenomics	Comparison among microbial communities	Identification of stress-adapted microorganisms	Climate-smart biofertilizer development

Metagenome-guided microbiome engineering	Reconstruction of microbial communities	Design of synthetic microbial consortia	Enhanced nutrient acquisition and fertilizer efficiency
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10.8. AI-Assisted Biofertilizer Development

Artificial intelligence (AI) and machine learning (ML) are emerging as transformative tools for accelerating the development of next-generation biofertilizers by integrating large-scale genomic, metagenomic, environmental, and agronomic datasets. AI-driven algorithms enable rapid identification of beneficial microbial strains, prediction of plant–microbe compatibility, optimization of microbial formulations, and rational design of synthetic microbial communities (SynComs) tailored to specific crops and environmental conditions. In addition, AI-powered decision-support systems integrated with precision agriculture technologies facilitate real-time nutrient management by predicting crop nutrient requirements and recommending crop-specific microbial inoculants, thereby improving nutrient use efficiency (NUE) while reducing fertilizer inputs. The convergence of AI with multi-omics and microbiome engineering is expected to enhance the accuracy, scalability, and field performance of biofertilizers, paving the way for climate-smart and sustainable agricultural systems. Nevertheless, the development of robust predictive models requires standardized datasets, extensive field validation, and interdisciplinary collaboration to ensure reliable deployment under diverse agroecological conditions (Wang *et al.*, 2026; Yadav *et al.*, 2026)

10.9. Synthetic Microbial Communities (SynComs)

Recent advances have shifted the development of synthetic microbial communities (SynComs) from empirical microbial mixtures to rationally designed consortia based on ecological principles, functional complementarity, and systems biology approaches. The integration of metagenomics, multi-omics, and computational tools has enabled the identification of keystone microorganisms and prediction of microbial interactions, facilitating the assembly of stable and resilient SynComs with enhanced rhizosphere colonization and nutrient cycling. Compared with single-strain inoculants, SynComs exhibit improved functional stability, greater adaptability to environmental fluctuations, and superior performance in enhancing nutrient use efficiency (NUE), plant growth, and stress tolerance. Despite these advances, challenges remain in maintaining community stability under field conditions, understanding interspecies interactions, and developing standardized protocols for large-scale production and commercialization. Future research should focus on AI-assisted SynCom design, long-term field validation, and crop-specific microbial consortia to maximize sustainable nutrient management and agricultural productivity (Singh *et al.*, 2025)

10.10. CRISPR-Mediated Engineering of Biofertilizer Microorganisms

Recent advances in CRISPR technology have expanded beyond the conventional CRISPR-Cas9 system to include CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), and the Cas12 and Cas13 nuclease systems, enabling more precise regulation and editing of microbial genomes. These next-generation genome-editing platforms facilitate targeted modification of plant growth-promoting rhizobacteria (PGPR) and other beneficial microorganisms to enhance nitrogen fixation, phosphorus solubilization, phytohormone production, stress tolerance, and rhizosphere colonization. In addition, CRISPR-based transcriptional regulation allows modulation of microbial metabolic pathways without permanent genome alteration, improving the safety and flexibility of engineered biofertilizers. Despite these promising developments, challenges related to off-target effects, delivery systems, regulatory approval, and large-scale field validation remain to be addressed before widespread agricultural application. Continued advances in CRISPR technologies, integrated with synthetic biology and microbiome

engineering, are expected to accelerate the development of highly efficient, climate-resilient, and next-generation biofertilizers for sustainable agriculture (Dudeja *et al.*, 2025)

10.11. Critical Analysis and Research Gaps

CRISPR-based microbial engineering, multi-omics technologies, metagenomics, and microbiome engineering have collectively transformed biofertilizer research by enabling the identification, characterization, and rational design of highly efficient microbial inoculants with enhanced nutrient use efficiency (NUE). These approaches facilitate the discovery of elite functional microorganisms, optimization of nutrient-cycling pathways, and development of crop-specific microbial consortia with improved stress tolerance and rhizosphere competence. Despite their enormous potential, most applications remain at the experimental or proof-of-concept stage, with limited validation under diverse field conditions. Challenges related to regulatory approval, biosafety, high implementation costs, data integration, and the complexity of plant-microbiome interactions continue to impede their large-scale agricultural deployment. Future research should focus on integrating these advanced technologies with synthetic microbial communities, artificial intelligence, and long-term multi-location field trials to develop robust, climate-resilient, and commercially viable next-generation biofertilizers for sustainable agriculture.

11. Challenges and Limitations of Biofertilizers

Despite their considerable potential to improve nutrient use efficiency (NUE) and reduce dependence on synthetic fertilizers, the widespread adoption of biofertilizers remains constrained by several scientific, technological, economic, and regulatory challenges. One of the major limitations is the inconsistent performance of microbial inoculants under field conditions. Unlike controlled laboratory or greenhouse environments, field performance is strongly influenced by soil physicochemical properties, climatic conditions, crop genotype, indigenous microbial communities, and agricultural management practices, often resulting in variable efficacy across locations and seasons. The summary of challenges is given in Table 12.

Although numerous studies have demonstrated the effectiveness of biofertilizers under laboratory, greenhouse, and small-scale field conditions, relatively few have evaluated their performance through long-term, multi-location field trials across diverse agroecological regions. Variations in soil physicochemical properties, climatic conditions, crop genotype, indigenous microbial communities, and agricultural management practices often result in inconsistent field responses, limiting farmer confidence and large-scale commercialization. Therefore, future research should prioritize coordinated multi-season and multi-location validation studies using standardized evaluation protocols to assess the stability, persistence, nutrient use efficiency, and economic viability of biofertilizer formulations under practical farming conditions. Such large-scale validation will be essential for generating robust scientific evidence, supporting regulatory approval, and promoting wider adoption of biofertilizers in sustainable agricultural systems.

Table 12. Major challenges limiting the large-scale adoption of biofertilizers.

Challenges	Major Issues	Impact on Biofertilizer Performance	Possible solution
Environmental variability	Soil type, climate, pH	Inconsistent field performance	Region-specific microbial strains
Poor microbial survival	Competition with native microbes	Low rhizosphere colonization	Advanced carrier technologies

Short shelf life	Loss of microbial viability	Reduced inoculum quality	Alginate, hydrogel, biochar carriers
Farmer adoption	Limited awareness	Low commercial utilization	Extension services and demonstrations
Regulatory issues	Lack of quality standards	Variable product quality	International certification systems
Biosafety concerns	CRISPR-engineered microbes	Regulatory restrictions	Risk assessment and biosafety guidelines
Limited field-scale validation	Few long-term multi-location trials	Reduced confidence and inconsistent recommendations	Multi-season, multi-location validation using standardized protocols

12. Future Perspectives

The future of biofertilizers lies in the integration of microbial biotechnology, systems biology, artificial intelligence, and precision agriculture to develop highly efficient, crop-specific, and climate-resilient microbial inoculants. Advances in metagenomics, genome sequencing, and multi-omics technologies are rapidly improving our understanding of plant–microbe interactions, enabling the identification of elite microbial strains with enhanced nutrient-solubilizing capacity, stress tolerance, and rhizosphere competence. Synthetic microbial communities (SynComs) represent one of the most promising approaches for overcoming the inconsistent performance of single-strain inoculants. Carefully designed microbial consortia combining nitrogen-fixing bacteria, phosphate- and potassium-solubilizing microorganisms, arbuscular mycorrhizal fungi, and plant growth-promoting rhizobacteria can provide complementary functions, resulting in improved nutrient acquisition, enhanced microbial stability, and greater resilience under diverse agroecological conditions. Genome-editing technologies such as CRISPR-Cas systems are expected to facilitate the development of next-generation microbial inoculants with enhanced nitrogen fixation, nutrient solubilization, phytohormone production, and abiotic stress tolerance. Coupling genome editing with metagenomics and synthetic biology will accelerate the rational engineering of beneficial microorganisms while enabling the development of customized biofertilizers for specific crops, soils, and climatic regions. Artificial intelligence and machine learning will further transform biofertilizer development by integrating multi-omics datasets, predicting plant–microbe compatibility, identifying novel functional microorganisms, and optimizing microbial consortia. Integration of biofertilizers with remote sensing, IoT-based soil monitoring, digital agriculture, and decision-support systems will facilitate precision application of microbial inoculants, thereby maximize nutrient use efficiency while minimize fertilizer inputs.

Despite remarkable technological advances, the successful translation of next-generation biofertilizers from laboratory research to commercial agriculture will depend on overcoming several scientific, regulatory, and socioeconomic challenges. Commercialization requires the development of cost-effective production technologies, standardized quality control protocols, improved carrier formulations, and extended shelf-life to ensure consistent product performance under diverse agroecological conditions. In addition, harmonized regulatory frameworks are essential to establish quality standards, evaluate the safety and efficacy of microbial inoculants, and facilitate their approval and international commercialization. Particular attention should be given to biosafety assessment of genetically engineered microorganisms, including CRISPR-modified strains, to minimize potential ecological risks and ensure responsible deployment. Furthermore, increasing farmer awareness through extension services, demonstration trials, capacity-building programmes, and supportive government policies will be critical for improving the adoption of biofertilizer technologies. Future research should therefore combine

technological innovation with multidisciplinary efforts involving microbiologists, agronomists, policymakers, industry, and farmers to accelerate the large-scale implementation of safe, effective, and climate-resilient biofertilizers for sustainable agriculture.

13. Conclusion

Biofertilizers have emerged as indispensable components of sustainable nutrient management owing to their ability to enhance nutrient use efficiency through biological nitrogen fixation, nutrient solubilization and mobilization, phytohormone production, and improved plant-microbe interactions. Beyond improving crop nutrition, beneficial microorganisms contribute to soil health restoration, increased microbial diversity, enhanced stress tolerance, and reduced environmental impacts associated with excessive chemical fertilizer use. Recent advances in microbial ecology, synthetic microbial communities, CRISPR-mediated genome editing, multi-omics technologies, metagenomics, microbiome engineering, nanotechnology, and precision agriculture have transformed biofertilizer research from conventional inoculant development to intelligent, systems-based microbial engineering. These innovations provide unprecedented opportunities to develop next-generation biofertilizers with improved field consistency, enhanced nutrient recovery, and greater resilience under changing climatic conditions. Despite these advances, several challenges, including inconsistent field performance, limited microbial survival, formulation constraints, regulatory barriers, and commercialization issues, must be addressed before biofertilizers can achieve widespread agricultural adoption. Continued investment in interdisciplinary research, advanced carrier technologies, quality assurance systems, and evidence-based policy frameworks will be essential to overcome these limitations.

Future research should prioritize long-term multi-location field validation, development of climate-resilient microbial consortia, standardized quality control protocols, and the integration of artificial intelligence, multi-omics, and precision agriculture for designing crop- and region-specific biofertilizers. Equally important are the establishment of robust regulatory frameworks, commercialization strategies, and farmer-oriented extension programmes to facilitate the large-scale adoption of biofertilizers. Strengthening collaboration among researchers, industry, policymakers, and farming communities will be essential for translating laboratory innovations into practical, economically viable, and environmentally sustainable nutrient management solutions that contribute to global food security and climate-resilient agriculture.

Author Contributions

M. R. Khan and S. A. Bhat conceptualized the review and defined its scope. S.A. Bhat coordinated the manuscript preparation and served as the corresponding author. A. A. Wani, M. S. Bhat, and N. Nissar contributed to literature collection, data interpretation, and drafting of specific sections. R. R. Parrray assisted in critical analysis of recent advancements and figure conceptualization. T. Bhat and S. Majeed contributed to manuscript drafting and reference management. All authors reviewed, edited, and approved the final manuscript.

Ethics, Consent to Participate, and Consent to Publish

This article is a review based exclusively on previously published studies. It does not report any original research involving human participants or animals performed by the authors. Therefore, ethical approval and consent to participate are not applicable.

Consent to Publish

Not applicable. This manuscript does not contain any person's data in any form.

Clinical Trial Registration

Clinical trial number: Not applicable. This manuscript is a review article and does not report any clinical trial or interventional study involving human participants.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Declaration

This article received no external funding.

Conflict of Interest

There is no conflict of interest amongst the authors.

References

- 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.
- Ahemad, M., & Khan, M. S. 2012. Effects of pesticides on plant growth promoting traits of *Mesorhizobium* strain MRC4. *Journal of the Saudi Society of Agricultural Sciences*, 11, 63–71.
- Akintokun, A., Ezaka, E., Akintokun, P., Shittu, O., & Taiwo, L. 2019. Isolation, screening and response of maize to plant growth promoting rhizobacteria inoculants. *Scientia Agriculturae Bohemica*, 50, 181–90.
- Ali, M. A., Ajaz, M. M., Rizwan, M., Qayyum, M. F., & Arshad, M. 2020. Effect of biochar and phosphate solubilizing bacteria on growth and phosphorus uptake by maize in an aridisol. *Arabian Journal of Geosciences*, 13, 333.
- Ali, S., Riaz, A. K., Ghazal, M., Arif, M., Fida, M., & Saiqa, B. 2008. Assessment of different crop nutrient management practices for yield improvement. *Australian Journal of Crop Science*, 2, 150–157.
- Alva, A. K., Paramasivam, S., Fares, A., Delgado, J. A., Mattos Jr, D. & Sajwan, K. 2006. Nitrogen and irrigation management practices to improve nitrogen uptake efficiency and minimize leaching losses. *Journal of Crop Improvement*, 15(2), 369–420.
- Archana, D., Nandish, M. S., Savalagi, V. P., & Alagawadi, A. R. 2013. Characterization of potassium solubilizing bacteria (KSB) from rhizosphere soil. *Bioinfolet*, 10, 248–257.
- Arora, N. K., Fatima, T., Mishra, J., Mishra, I., & Verma, S. 2020. Halo-tolerant plant growth promoting rhizobacteria for improving productivity and remediation of saline soils. *Journal of Advanced Research*, 26, 69–82.
- Ayoub, I., Kumar, V., Abolhassani, R., Sehgal, R., & Sharma V. 2022. Advances in ZnO: Manipulation of defects for enhancing their technological potentials. *Nanotechnology Reviews*, 11, 575–619.
- 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, 402666.
- Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & 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.
- Arani, B. A., Modarres-Sanavy, S. A. M., Mashhadi-Akbar-Boojari, M., Mokhtassi-Bidgoli, A. 2017. Towards improving the agronomic performance, chlorophyll fluorescence parameters and

- pigments in Fenugreek using zeolite and vermicompost under deficit water stress. *Industrial Crops and Products*, 109, 346–357.
- Baldani, J. I., Rouws, L., Cruz, L. M., Olivares, F. L., Schmid, M., & Hartmann, A. (2014). The family oxalobacteraceae. In: Rosenberg, E., DeLong, E. F., Lory, S., Stackebrandt, E., & Thompson, F. (Eds.), *The Prokaryotes*. Berlin, Heidelberg: Springer, pp. 919-974.
- Balla, A., Silini, A., Cherif-Silini, H., Mapelli, F., & Borin, S. 2025. Root colonization dynamics of alginate encapsulated rhizobacteria: implications for *Arabidopsis thaliana* root growth and Durum wheat performance. *AIMS Microbiology*, 11(1), 87–125.
- Barbieri, P., Echeverría, H. E., Saínz Rozas, H. R., & Andrade, F. H. 2008. Nitrogen use efficiency in maize as affected by nitrogen availability and row spacing. *Agronomy Journal*, 100, 1094-1100.
- Bashan, Y., & Bashan, L. E. D. (2010). How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—A critical assessment. *Advances in Agronomy*, 108, 77–136.
- Batool, S., & Iqbal, A. 2019. Phosphate solubilizing rhizobacteria as alternative of chemical fertilizer for growth and yield of *Triticum aestivum* var. galaxy. *Saudi Journal of Biological Sciences*, 26, 1400-1410.
- 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, 1044–1051.
- Benmrid, B., Ghoulam, C., Zeroual, Y., Kouisni, L., & Bargaz, A. 2023. Bioinoculants as a means of increasing crop tolerance to drought and phosphorus deficiency in legume-cereal intercropping systems. *Communication in Biology*, 6(1), 1016.
- Bennett, E. M., Murray, J. W., & Isalan, M. (2023). Engineering nitrogenases for synthetic nitrogen fixation: From pathway engineering to directed evolution. *Biodesign Research*, 5, 0005.
- Berg, G. 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology*, 84(1), 11–18.
- Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., & Schlöter, M. (2020). Microbiome definition revisited: Old concepts and new challenges. *Microbiome*, 8(1), 103.
- Bertola, M., Mattarozzi, M., Sanangelantoni, A. M., Careri, M., & Visioli, G. J. 2019. PGPR colonizing three-year biochar-amended soil: towards biochar-mediated biofertilization. *Journal of Soil Science and Plant Nutrition*, 19, 41–50.
- Bhat, T. A., Ahmad, L., Ganai, M. A., & Khan, O. A. 2015. Nitrogen fixing biofertilizers; mechanism and growth promotion: a review. *Journal of Pure Applied Microbiology*, 9(2), 1675-1690.
- Bhattacharyya, P. N., & Jha, D. K. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 28(4), 1327–1350.
- Bhattacharyya, R., Ghosh, B. N., Mishra, P. K., Mandal, B., Rao, C. S., Sarkar, D., & Franzluebbers, A. J. 2015. Soil degradation in India: Challenges and potential solutions. *Sustainability*, 7(4), 3528-3570.
- Bomboriya, S. M., Bomboriya, J. S., & Shaniti, 2018. Role of biofertilizers in agriculture-a review. *International Journal of Recent Scientific Research*, 9, 27727-27732.
- Boroujeni, S.M., Kalbasi, M., Asgharzadeh, A., & Baharlouei, J. 2021. Evaluating the potential of *Halothiobacillus* bacteria for sulfur oxidation and biomass production under saline soil. *Geomicrobiology Journal*, 38, 57–65.
- Bourscheidt, M. L. B., Gomes, F. J., Pedreira, C. G. S., Boote, K. J., Hoogenboom, G., Pereira, D. H., & Pedreira, B. C. 2023. Highlighting the benefits of biological nitrogen fixation on agronomic, physiological, and nutritive value traits of Brachiariagrass. *European Journal of Agronomy*, 143, 126730.
- Cabral, M., Cheng, X., Singh, S., & Ivessa, A. S. 2016. Absence of non-histone protein complexes at natural chromosomal pause sites results in reduced replication pausing in aging yeast cells. *Cell Reproduction*, 17, 1747–1754.

- Cakmakci, R. 2019. A review of biological fertilizers current use, new approaches, and future perspective. *International Journal of Innovative Studies in Sciences and Engineering Technology*, 5(7), 83-92.
- Chaudhary, D., Narula, N., Sindhu S. S., & Behl, R. K. 2013. Plant growth stimulation of wheat (*Triticum aestivum* L.) by inoculation of salinity tolerant *Azotobacter* strains. *Physiology and Molecular Biology of Plants*, 19, 515-519.
- Chaudhary, P., Singh, S., Chaudhary, A., Sharma, A., & Kumar, G. 2022. Overview of biofertilizers in crop production and stress management for sustainable agriculture. *Frontiers in Plant Science*, 13, 930340.
- Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, 19, 29-37.
- Clair, S. S. B., & Lynch, J. P. 2010. The opening of Pandora's Box: climate change impacts on soil fertility and crop nutrition in developing countries. *Plant and Soil* 335, 101-115
- Castillo, A. R., Gerding, M., Oyarzua, P., Zagal, E., Gerding, J., & Fischer S. 2019. Plant growth-promoting rhizobacteria able to improve NPK availability: selection, identification and effects on tomato growth. *Chilean Journal of Agricultural Research* 79, 473-485.
- Cedillo, G. V. M., Urrestarazu, M., & Alvaro, J. E. 2016. Influence of salinity on transport of nitrates and potassium by means of the xylem sap content between roots and shoots in young tomato plants. *Journal of Soil Science and Plant Nutrition* 16(4), 991-998.
- Dalpe, Y., & Monreal, M. 2004. Arbuscular mycorrhiza inoculum to support sustainable cropping systems. *Crop Management*, 3, 1-11.
- Dance, I. 2023. The binding of reducible N₂ in the reaction domain of nitrogenase. *Dalton Transactions*, 52, 2013-2026.
- Danyal, Y., Mahmood, K., Ullah, S., Rahim, A., & Raheem, G. 2023. Phytoremediation of industrial effluents assisted by plant growth promoting bacteria. *Environmental Science and Pollution Research*, 30, 5296-5311.
- Dhole, A., Shelat, H., Vyas, R., Jhala, Y., & Bhange, M. 2016. Endophytic occupation of legume root nodules by Nif H-positive non-rhizobial bacteria, and their efficacy in the groundnut (*Arachis hypogaea*). *Annals of Microbiology*, 66, 1397-1407.
- Dinesh, R., Anandaraj, M., Kumar, A. M., Bini, Y. K., Subila, K. P., & Aravind, R. 2015. Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. *Microbiological Research*, 173, 34-43.
- Ding, Z., Ali, E. F., Almaroai, Y. A., Eissa, M. A., & Abeed, A. H. A. 2021. Effect of potassium solubilizing bacteria and humic acid on faba bean (*Vicia faba* L.) plants grown on sandy loam soils. *Journal of Soil Science and Plant Nutrition*, 21, 791-800.
- Ditta, A., Imtiaz M., Mehmood S., Rizwan, M. S., Mubeen, F., Aziz, O., Qian, Z., Ijaz, R., & Tu, S. 2018. Rock phosphate-enriched organic fertilizer with phosphate-solubilizing microorganisms improves nodulation, growth, and yield of legumes. *Communications in Soil Science and Plant Analysis*, 49(21), 2715-2725.
- Ditta, A., Ullah, N., Imtiaz, M., Li X., & Jan, A. U. 2022. Zn biofortification in crops through Zn-solubilizing plant growth-promoting rhizobacteria in sustainable plant nutrition under contaminated environments. In: Mahmood, Q. (Ed.), *Sustainable Plant Nutrition under Contaminated Environments. Sustainable Plant Nutrition in a Changing World*. Cham: Springer, pp. 115-133.
- Dudeja, C., Mishra, A., Ali, A., Singh, P. P., & Jaiswal, A. K. (2025). Microbial genome editing with CRISPR-Cas9: Recent advances and emerging applications across sectors. *Fermentation*, 11(7), 410.
- Esmaeel, Q., Miotto, L., Rondeau, M., Leclere, V., & Clément, C. 2018. *Paraburkholderia phytofirmans* PsJN-plants interaction: from perception to the induced mechanisms. *Frontiers in Microbiology* 9, 2093.

- Etesami, H., Jeong, B. R., & Glick, B. R. 2021. Contribution of arbuscular mycorrhizal fungi, phosphate solubilizing bacteria and silicon to P uptake by plant. *Frontiers in Plant Sciences*, 12, 699618.
- Fasusi, O., Cruz, C., & Babalola, O. 2021. Agricultural Sustainability: Microbial Biofertilizers in Rhizosphere Management. *Agriculture*, 11, 163.
- Gaby, J. C., & Buckley, D. H. 2014. A comprehensive aligned *nifH* gene database: A multipurpose tool for studies of nitrogen-fixing bacteria. *Database*, 2014, bau001
- Gilani, A., Abbasdokht, H., & Gholami, A. 2021. Effects of *Thiobacillus* and different levels of sulfur fertilizer on growth and physiological indices in intercropping of sesame and mung bean (*Vigna radiata* L.). *Gesunde Pflanzen* 73, 317–333.
- Glick, B. R., & Gamalaro, E., 2021. Recent developments in the study of plant microbiomes. *Microorganisms*, 9, 15-33.
- Ghamry, E. A., Mosa, A. A., Alshaal, T., & El-Ramady, H. 2018. Nanofertilizers vs. biofertilizers: new insights. *Environment, Biodiversity and Soil Security*, 2, 51–72.
- Govindasamy, P., Muthusamy, S. K., Bagavathiannan, M., Mowrer, J., Jagannadham, P. T. K., Maity, A., & Tiwari, G. 2023. Nitrogen use efficiency—a key to enhance crop productivity under a changing climate. *Frontiers in Plant Science*, 14, 112, 10-73.
- Griffin, C., Oz, M. T., & Demirer, G. S. (2024). Engineering plant–microbe communication for plant nutrient use efficiency. *Current Opinion in Biotechnology*, 87, 103150.
- Großkopf, T., & Soyer, O. S. (2014). Synthetic microbial communities. *Current Opinion in Microbiology*, 18, 72–77.
- Gyaneshwar, P., Kumar, N. G., Parekh, L., & Poole, P. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil*, 245, 83–93.
- Hafeez, B., Khanif, Y. M., & Saleem, M. 2013. Role of zinc in plant nutrition—a review. *American Journal of Experimental Agriculture*, 3(2), 374–391.
- Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., & Siddique, M. J. 2021. Rhizosphere engineering with plant growth-promoting microorganism isms for agriculture and ecological sustainability. *Frontiers in Sustainable Food System*, 5, 617157.
- Han, H. S., & Lee, K. 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil and Environment*, 52, 130-137.
- Hania, A., Lopez-Adams, R., Pracil, O., & Eichner, M. 2023. Protection of nitrogenase from photosynthetic O₂ evolution in trichodesmium: methodological pitfalls and advances over 30 years of research. *Photosynthetica*, 61, 58–72.
- Hasanuzzaman, M., Gill, S. S., & Fujita, M. 2012. Physiological role of nitric oxide in plants grown under adverse environmental conditions. In: Tuteja, N., & Singh Gill, S. (Eds.), *Plant Acclimation to Environmental Stress*. New York, NY: Springer, pp. 269–322.
- Hassen, A. I., Lamprecht, S. C., & Bopape, F. L. 2020. Emergence of β -rhizobia as new root nodulating bacteria in legumes and current status of the legume–rhizobium host specificity dogma. *World Journal of Microbiology and Biotechnology* 36, 1–13.
- Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology* 60, 579–598.
- Herrero, J., Ramírez-Santos, A., Díaz-Santos, E., & Torres-Cortés, G. (2025). Biofertilizers for enhanced nitrogen use efficiency: mechanisms, innovations, and challenges. *Nitrogen*, 6(4), 111.
- Htwe, A. Z., Moh, S. M., Soe, K. M., Moe, K., & Yamakawa, T. 2019. Effects of biofertilizer produced from *Bradyrhizobium* and streptomyces *Griseoflavus* on plant growth, nodulation, nitrogen fixation, nutrient uptake, and seed yield of mung bean, cowpea, and soybean. *Agronomy*, 9, 77.

- Hu, L., Robert, C. A., Cadot, S., & Zhang X, Ye. M. 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications*, 9, 27-38.
- Hussain, A., Adnan, M., Iqbal, S., Fahad, S., & Saeed, M. 2019. Combining phosphorus (P) with phosphate solubilizing bacteria (PSB) improved wheat yield and P uptake in alkaline soil. *Pure and Applied Biology*, 8, 1809–1817.
- Igiehon, N. O., & Babalola, O. O. 2017. Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. *Applied Microbiology and Biotechnology* 101(12), 4871-4881.
- Ijaz, M., Ali, Q., Ashraf, S., Kamran, M., & Rehman, A. 2019. Development of future bioformulations for sustainable agriculture. In: Kumar, V., Prasad, R., Kumar, M., & Choudhary, D. (Eds.), *Microbiome in Plant Health and Disease*. Singapore: Springer, pp. 421–446.
- Ilyas, N., Mumtaz, K., Akhtar, N., Yasmin, H., Sayyed, R., Khan, W., Enshasy, H. A. E., Dailin, D. J., Elsayed, E. A., & Ali, Z. 2020. Exopolysaccharides producing bacteria for the amelioration of drought stress in wheat. *J. Sustainability*, 12, 8876.
- Jain, A., Sarsaiya, S., Singh, R., Gong, Q., Wu, Q., & Shi, J. (2024). Omics approaches in understanding the benefits of plant-microbe interactions. *Frontiers in Microbiology*, 15, 1391059.
- Jang, J. H., Woo, S. Y., Kim, S. H., Khaine, I., Kwak, M. J., Lee, H. K., Lee, T. Y., & Lee, W.Y. 2017. Effects of increased soil fertility and plant growth-promoting rhizobacteria inoculation on biomass yield, energy value, and physiological response of poplar in short-rotation coppices in a reclaimed tideland: A case study in the Saemangeum area of Korea. *Biomass and Bioenergy*, 107, 29–38.
- Jha, Y. 2019. The importance of zinc-mobilizing rhizosphere bacteria to the enhancement of physiology and growth parameters for paddy under salt-stress conditions. *Jordan Journal of Biological Sciences*, 12(2), 167–173.
- Jin, R., Wang, Y., Liu, R., Gou, J., & Chan, Z. 2016. Physiological and metabolic changes of purslane (*Portulaca Oleracea* L.) in response to drought, heat, and combined stresses. *Frontiers in Plant Science* 6, 1123.
- Junaid, P. M., & Khan, F. 2018. Biofilm: A future generation biofertilizer. *Indian Journal of Applied and Pure Biology*, 33(2), 131-134.
- Kahiluoto, H., Kuisma, M., Kuokkanen, A., Mikkila, M., & Linnanen, L. 2014. Taking planetary nutrient boundaries seriously: can we feed the people. *Global Food Security*, 3(1), 16-21.
- Kamran, S., Shahid, I., Baig, D. N., Rizwan, M., Malik, K. A., & Mehnaz, S. 2017. Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. *Frontiers in Microbiology*, 8, 25-93.
- Kaur, T., Devi, R., Kour, D., Yadav, A., Yadav, A. N., Dikilitas, M., Abdel-Azeem, A. M., Ahluwalia, A. S., & Saxena, A. K. 2021. Plant growth promoting soil microbiomes and their potential implications for agricultural and environmental sustainability. *Biologia*, 76, 2687 –2709.
- Kennedy, I. R., Choudhury, A. T. M. A., & Kecskés, M. L. (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: Can their potential for plant growth promotion be better exploited? *Soil Biology and Biochemistry*, 36(8), 1229–1244.
- Kennedy, I. R., Choudhury, A. T. M. A., & Kecskés, M. L. (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited?. *Soil Biology and Biochemistry*, 36(8), 1229-1244.
- Khalvati, M. A., Hu Y, Mozafar, A., & Schmidhalter, U. 2005. Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. *Plant Biology*, 7(6), 706–712.

- Khan, M. S., Zaidi, A., Wani, P. A., & Oves, M. 2009. Role of plant growth-promoting rhizobacteria in the remediation of metal-contaminated soils. *Environmental Chemistry Letters*, 7, 1–19.
- Kour, D., Rana, K. L., Kaur, T., Yadav, N., Halder, S. K., Yadav, A. N., Sachan, S. G., & Saxena, A. K. 2020. Potassium solubilizing and mobilizing microbes: biodiversity, mechanisms of solubilization, and biotechnological implications for alleviation of abiotic stress. In: Rastegari, A. A., Yadav, A. N., & Yadav, N. (Eds.), *New and Future Developments in Microbial Biotechnology and Bioengineering*. Elsevier, pp. 177–202.
- Krasilnikov, P., Taboada, M. A., & Amanullah. 2022. Fertilizer use, soil health and agricultural sustainability. *Agriculture*, 12(4), 462.
- Kriska, T., Skarpa, P., & Antosovsky J. 2023. Effect of natural liquid hydroabsorbents on ammonia emission from liquid nitrogen fertilizers and plant growth of maize (*Zea Mays* L.) under drought conditions. *Plants*, 12, 728.
- Kuan, K. B., Othman, R., Khan, A. R., & Shamsuddin, Z. 2016. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. *PLoS One* 11, e0152478.
- Kumar, A., Singh, M., Dhiman, R., & Sharma P. 2020. The role of extracellular polymeric substances (EPS) in biofilm formation and nutrient retention. *Current Opinion in Environmental Science and Health*, 15, 79–86.
- Kumari, R., & Singh, D. P. 2019. Nano-biofertilizer: An emerging eco-friendly approach for sustainable agriculture. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 90, 733–741.
- Kumawat, N., Kumar, R., Kumar, S., & Meena, V. S. 2017. Nutrient solubilizing microbes (NSMs): their role in sustainable crop production. In: Meena, V., Mishra, P., Bisht, J., & Pattanayak, A. (Eds.), *Agriculturally Important Microbes for Sustainable Agriculture*. Singapore: Springer, pp. 25–61.
- Liang, X., Zhang, H., He, M., Yuan, J., Xu, L., & Tian, G. 2016. No-tillage effects on grain yield, N use efficiency, and nutrient runoff losses in paddy fields. *Environmental Science and Pollution Research*, 23(21), 21451–21459.
- Liu, S., Wang, X., Yin, X., Savoy, H. J., McClure, A., & Essington, M. E. 2019. Ammonia volatilization loss and corn nitrogen nutrition and productivity with efficiency-enhanced UAN and urea under no-tillage. *Scientific Reports* 9(1), 6610.
- Liu, Y., Manzoor, N., Han, M., Zhu, K., & Wang, G. (2025). Biomaterial amendments improve nutrient use efficiency and plant growth. *Frontiers of Agricultural Science and Engineering*, 12(1), 81–103.
- Loredana-Elena, M., El-Sabeh, A., Marius, M., & Stefan, M. 2025. *Bacillus safensis* P1.5S exhibits phosphorus-solubilizing activity under abiotic stress. *Horticulturae*, 11(4), 388.
- Maaz, T. M., Dobermann, A., Lyons, S. E., & Thomson, A. M. 2025. Review of research and innovation on novel fertilizers for crop nutrition. *npj Sustainable Agriculture*, 3, 25.
- Mabrouk, Y., Hemissi, I., Salem, I. B., Mejri, S., Saidi, M., & Belhadj, O. 2018. Potential of rhizobia in improving nitrogen fixation and yields of legumes. *Symbiosis* 107, 1–16.
- Macilwain, C. 2004. Organic: is it the future of farming? *Nature*, 428, 792–794.
- Mala, R., Selvaraj, C. A., Sundaram, B. V., Rajan, B. S. S., & Gurusamy, M. U. 2017. Evaluation of nano-structured slow-release fertilizer on the soil fertility, yield and nutritional profile of *Vigna radiata*. *Recent Patents on Nanotechnology* 11(1), 50–62.
- Marschner, P., & Rengel, Z. 2007. Contributions of rhizosphere interactions to soil biological fertility. In: Abbott, L. K., & Murphy, D.V. (Eds.), *Soil Biological Fertility*. Dordrecht: Springer, pp. 81–98.

- Martín del Campo, J. S., Rigsbee, J., Bueno, B. M., Mus, F., & Rubio, L. M. 2023. Overview of physiological, biochemical, and regulatory aspects of nitrogen fixation in *Azotobacter vinelandii*. *Critical Reviews in Biochemistry and Molecular Biology*, 57(5–6), 492–538.
- Mayak, S., Tirosh, T., & Glick, B. R. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry*, 42, 565–572.
- Meena, V. S., Bahadur, I., Maurya, B. R., Kumar, A., & Meena, R. K. 2016. Potassium-solubilizing microorganism in evergreen agriculture: an overview. In: Meena, V., Maurya, B., Verma, J., & Meena, R. (Eds.), *Potassium Solubilizing Microorganisms for Sustainable Agriculture*. New Delhi: Springer, pp. 1–20.
- Meena, V. S., Maurya, B., & Verma, J. P. 2014. Does a rhizospheric microorganism enhance K⁺ availability in agricultural soils? *Microbiological Research*, 169, 337–347.
- Mikkelsen, R., Jensen, T. L., Snyder, C., & Bruulsema, T. W. 2012. Nutrient management planning and accountability. In: Bruulsema, T. W., Fixen, P. E. & Sulewski, G.D. (Eds.), *A Manual for Improving the Management of Plant Nutrition*, International Plant Nutrition Institute, Norcross, USA: CA.
- Mumtaz, M. Z., Ahmad, M., Jamil, M., & Hussain, T. 2017. Zinc-solubilizing *Bacillus* spp. potential candidates for biofortification in maize. *Microbiology Research*, 202, 51–60.
- Murgese, P., Santamaria, P., Leoni, B., & Crecchio, C. 2020. Ameliorative effects of PGPB on yield, physiological parameters, and nutrient transporter genes expression in Barattiere (*Cucumis Melo* L.). *Journal of Soil Science and Plant Nutrition*, 20, 784–793.
- Mus, F., Crook, M. B., Garcia, K., Garcia Costas, A., Geddes, B. A., Kouri, E. D., Paramasivan, P., Ryu, M. H., Oldroyd, G. E. D., Poole, P. S., Udvardi, M. K., Voigt, C. A., & Ané, J. M. (2016). Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Applied and Environmental Microbiology*, 82(13), 3698–3710.
- Naqqash, T., Malik, K. A., Imran, A., Hameed, S., Shahid, M., Hanif, M. K., Majeed, A., Iqbal, M. J., Qaisrani, M. M., & van Elsas, J. D. 2022. Inoculation with *Azospirillum* spp. acts as the liming source for improving growth and nitrogen use efficiency of potato. *Frontiers in Plant Science*, 13, 929114.
- Navarro, B. B., Machado, M. J., & Figueira, A. (2025). Nitrogen use efficiency in agriculture: Integrating biotechnology, microbiology, and novel delivery systems for sustainable agriculture. *Plants*, 14(19), 2974.
- Naveed, M., Mehboob, I., Shaker, M. A., Hussain, M. B., & Farooq, M. 2015. Biofertilizers in Pakistan: initiatives and limitations. *International Journal of Agriculture, Biology and Fertility of Soils*, 17, 411–420.
- Parween, T., Bhandari, P., Siddiqui, Z. H., Jan, S., Fatma, T., & Patanjali, P. K. 2017. Biofilm: a next-generation biofertilizer. In: Prasad, R. (Eds.), *Mycoremediation and Environmental Sustainability*, Cham: Springer, pp. 39–51.
- Pathak, D., Kumar, V., & Sharma, P. 2002. Nitrate tolerance, nitrogen fixation and plant growth promotion by *Azospirillum*—A review. *Agricultural Review*, 23, 31–38.
- Pham, V. T., Rediers, H., Ghequire, M. G., Nguyen, H. H., & De Mot, R. 2017. The plant growth-promoting effect of the nitrogen-fixing endophyte *Pseudomonas stutzeri* A15. *Archives of Microbiology*, 199, 513–517.
- Qadir, M., Hussain, A., Iqbal, A., Shah, F., Wu, W. & Cai, H. 2024. Microbial utilization to nurture robust agroecosystems for food security. *Agronomy* 14(9), 1891.
- Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*, 35(9), 833–844.

- Rabani, M. S., Hameed, I., Gupta, M. K., Wani, B. A., Fayaz, M., Hussain, H., & Ahad, M. B. 2023. Introduction of biofertilizers in agriculture with emphasis on nitrogen fixers and phosphate solubilizers. In: Dar, G. H., Bhat, R. A., Mehmood, M. A. (Eds.), *Microbiomes for the Management of Agricultural Sustainability*, Cham: Springer, pp. 71-93.
- Rajkumari, J., Katiyar, P., Dheeman, S., Pandey, P., & Maheshwari, D. K. 2022. The changing paradigm of rhizobial taxonomy and its systematic growth up to postgenomic technologies. *World Journal of Microbiology and Biotechnology*, 38, 206.
- Reddy, B. S. 2013. Soil health: Issues and concerns-A review. Research Unit for Livelihoods and Natural Resources, Centre for Economic and Social Studies, Begumpet, Hyderabad, India, p. 44.
- Reineke, W., & Schlomann M. 2023. *The Microbial Nitrogen Cycle in Environmental Microbiology* (Eds.), Belin, Germany: Springer, pp. 291-305.
- Hurek, B. R., & Hurek, T. 1998. Life in grasses: Diazotrophic endophytes. *Trends in Microbiology*, 6(4), 139-144.
- Riaz, U., Mehdi, S. M., Iqbal, S., Khalid, H. I., Qadir, A. A., Anum, W., Ahmad, M. & Murtaza, G. 2020. Bio-fertilizers: Eco-Friendly approach for plant and soil environment. In: Hakeem, K. R., Bhat, R. A., & Qadri, H. (Eds.), *Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation*. Cham: Springer, pp. 189-213.
- Rozier, C., Hamzaoui, J., Lemoine, D., Czarnes, S.; & Legendre, L. 2017. Field-based assessment of the mechanism of maize yield enhancement by *Azospirillum lipoferum* CRT1. *Scientific Reports* 7, 7416.
- Russell, S. J., Garcia, A. K., & Kaçar, B. 2024. A CRISPR interference system for engineering biological nitrogen fixation. *mSystems*, 9(3), e0015524.
- Saha, B., Fatima, A., Saha, S., Sahoo, S. K., & Poddar, P. 2024. Environmental pollution due to improper use of chemical fertilizers and their remediation. In: Ganguly, P., Mandal, J., Paramsivam, M., & Patra, S. (Eds.), *Environmental Contaminants*. Apple Academic Press, USA: Boca Raton, pp. 203-219.
- Saha, B., Saha, S., Roy, P.D., Padhan, D., Pati, S., & Hazra, G. C. 2018. Microbial transformation of sulphur: An approach to combat the sulphur deficiencies in agricultural soils. In: Meena, V. (Eds.). *Role of Rhizospheric Microbes in Soil*. Singapore: Springer, pp. 77-97.
- Sandhya, V., Shrivastava, M., Ali, S. Z., & Prasad, S. S. K. 2017. Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. *Russian Agricultural Science*, 43, 22-34.
- Saravanan, V. S., Kumar, M.R., & Sa, T. M. 2011. Microbial zinc solubilization and their role on plants. In: Maheshwari, D. (Eds.), *Bacteria in Agrobiolgy: Plant Nutrient Management*. Berlin, Heidelberg: Springer, pp. 47-63.
- Schultz, N., da Silva, J. A., Sousa, J. S., Monteiro, R. C., Oliveira, R. P., Chaves, V. A., Pereira, W., da Silva, M. F., Baldani, J. I., & Boddey, R. M. 2014. Inoculação de bactérias diazotróficas na cultura de cana-de-açúcar. *Revista Brasileira de Ciencia do Solo*, 38, 407-414.
- Seufert, V., Ramankutty, N., & Foley, J. A. 2012. Comparing the yields of organic and conventional agriculture. *Nature*, 485(7397), 229-232.
- Shabanamol, S., Divya, K., George, T. K., Rishad, K., Sreekumar, T., & Jisha, M. 2018. Characterization and in planta nitrogen fixation of plant growth promoting endophytic diazotrophic *Lysinibacillus sphaericus* isolated from rice (*Oryza sativa*). *Physiology and Molecular Plant Pathology*, 102, 46-54.
- Shahzad, M., Hayat, R., Mujtaba, G., Rehman, W. U., & Nadeem, M. 2025. Biofertilizers in sustainable agriculture: mechanisms, applications, and future prospects. *Discover Agriculture*, 3, 224.
- Shamseldin A. 2022. Future outlook of transferring biological nitrogen fixation (BNF) to cereals and challenges to retard achieving this dream. *Current Microbiology*, 79, 171.

- Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus*, 2, 587.
- Sheng, X. 2005. Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. *Soil Biology and Biochemistry*, 37, 1918–1922.
- Sheng, X. F., & He, L. Y. 2006. Solubilization of potassium-bearing minerals by a wild-type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian Journal of Microbiology*, 52, 66–72.
- Shravanthi, G. V., Panchatcharam, P. A., & Ambikapathy, V. J. 2019, Screening of potassium solubilizing bacteria and their growth promoters. *Journal of Pharmacognosy and Phytochemistry*, 8, 661–664.
- Sindhu, S. S., & Dadarwal, K. R. 1997. *Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production* (Eds.). Scientific Publishers, Rajasthan: Jodhpur, pp. 39-69.
- Sindhu, S. S., Parmar, P., Phour, M., & Sehrawat, A. 2016. Potassium solubilizing microorganisms (KSMs) and its effect on plant growth improvement. In: Meena, V., Maurya, B., Verma, J., & Meena, R. (Eds.), *Potassium Solubilizing Microorganisms for Sustainable Agriculture*. New Delhi: Springer, pp. 171–185.
- Sindhu, S.S., Sharma, R., Sindhu, S., & Phour, M. 2019. Plant nutrient management through inoculation of zinc solubilizing bacteria for sustainable agriculture. In: Giri, B., Prasad, R., Wu, Q. S., & Varma, A. (Eds.), *Biofertilizers for Sustainable Agriculture and Environment*. Soil Biology, vol. 55, Cham: Springer, pp. 173–201.
- Singh, B. K., Trivedi, P., Egidi, E., Macdonald, C. A., & Delgado-Baquerizo, M. 2020. Crop microbiome engineering for sustainable agriculture. *Nature Reviews Microbiology*, 18(11), 601-602.
- Singh, G., Biswas, D., & Marwaha, T. 2010. Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (*Zea mays*) and wheat (*Triticum aestivum* L.): a hydroponics study under phytotron growth chamber. *Journal of Plant Nutrition*, 33, 1236–1251.
- Singh, M., Jha, S., Pathak, D., & Maisnam, G. (2025). Advancing biofertilizers: The evolution from single-strain formulations to synthetic microbial communities (*SynCom*) for sustainable agriculture. *Discover Plants*, 2(1), 226.
- Singh, M., Singh, D., Gupta, A., Pandey, K. D., Singh, P., & Kumar A. 2019. Plant growth promoting rhizobacteria: application in biofertilizers and biocontrol of phytopathogens. In: Singh, A. K., Kumar, A., & Singh, P. K. (Eds.), *PGPR Amelioration in Sustainable Agriculture: Food Security and Environmental Management*. UK: Woodhead Publishing, pp. 41–66.
- Solanki, M. K., Solanki, A. C., Kumari, B., Kashyap, B. K., & Singh, B. K. 2020. Plant and soil-associated biofilm-forming bacteria: Their role in green agriculture. In: Yadav, M. K., & Singh, B. P. (Eds.), *New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Biofilms*. UK: Elsevier, pp. 151-164.
- Sood, G., Kaushal, R., Panwar, G., & Dhiman, M. 2019. Effect of Indigenous plant growth-promoting rhizobacteria on wheat (*Triticum aestivum* L.) productivity and soil nutrients. *Communications in Soil Science and Plant Analysis*, 50, 141–152.
- Steenhoudt, O., & Vanderleyden, J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: Genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews*, 24, 487–506.
- Suyal, D. C., Soni, R., Sai, S., & Goel, R. 2016. Microbial inoculants as biofertilizer. In: Singh, D., Singh, H., Prabha, R. (Eds.) *Microbial Inoculants in Sustainable Agricultural Productivity*. New Delhi, Springer, pp. 311-318.

- Tahat, M. M., Alananbeh, K. M., Othman, Y. A., & Leskovar, D. I. 2020. Soil health and sustainable agriculture. *Sustainability* 12, 4859.
- Tahir, H. A., Gu, Q., Wu, H., Raza, W., Hanif, A., Wu, L., Colman, M.V., & Gao, X., 2017. Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. *Journal of Frontier Microbiology*, 8, 171.
- Tavallali, V., Rahemi, M., Eshghi, S., Kholdebarin, B., Ramezani, A. 2010. Zinc alleviates salt stress and increases antioxidant enzyme activity in the leaves of pistachio (*Pistacia vera* L. 'Badami') seedlings. *Turkish Journal of Agriculture* 34, 349–359.
- Thomas, L., & Singh, I. 2019. Microbial biofertilizers: types and applications. In: Giri, B., Prasad, R., Wu, Q. S., & Varma, A. (Eds.), *Biofertilizers for Sustainable Agriculture and Environment*. Soil Biology, vol. 55, Cham: Springer, pp. 1-19.
- Troufflard, S., Mullen, W., Larson, T. R., Graham, I. A., Crozier, A., Amtmann, A., & Armengaud, P. 2010. Potassium deficiency induces the biosynthesis of oxylipins and glucosinolates in *Arabidopsis thaliana*. *BMC Plant Biology*, 10, 172.
- Valverde, A., Burgos, A., Fiscella, T., Rivas, R., Velazquez, E., Barrueco, C. R., Cervantes, E., Chamber, M., & Igual, J. M. 2007. Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. In: Velázquez, E., & Rodríguez-Barrueco, C. (Eds.), *First International Meeting on Microbial Phosphate Solubilization: Developments in Plant and Soil Sciences*, vol. 102. Dordrecht: Springer, pp. 43–50.
- Vassilev, N., Vassileva, M., Lopez, A., Martos, V., Reyes, A., & Maksimovic, I. 2015. Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. *Applied Microbiology and Biotechnology*, 99, 49 83-96.
- Velmourougane, K., Prasanna, R., & Saxena, A. K. 2017. Agriculturally important microbial biofilms: present status and future prospects. *Journal of Basic Microbiology*, 57(7), 548-573.
- Verma, P., Yadav, A. N., Khannam, K. S., Kumar, S., Saxena, A.K., & Suman, A. 2016. Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro- ecological zones of India. *Journal of Basic Microbiology*, 56, 44–58.
- Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255(2), 571-586.
- Vitousek, P. M., Naylor, R., Crews, T., David, M. B., Drinkwater, L. E., Holland, E. & Zhang, F. S. 2009. Nutrient imbalances in agricultural development. *Science*, 324(5934), 1519-1520.
- Wang, X. W., Wang, T., & Liu, Y. Y. (2026). Artificial intelligence for microbiology and microbiome research. *Cell Systems*, 17(2), 101531.
- Warmack, R. A., Maggiolo, A. O., Orta, A., Wenke, B. B., Howard, J. B., & Rees, D. C. 2023. Structural consequences of turnover-induced homocitrate loss in nitrogenase. *Nature Communications*, 14, 1091.
- Wezel, A., Casagrande, M., Celette, F., Vian, J. F., Ferrer, A. & Peigne, J. 2014. Agroecological practices for sustainable agriculture. A review. *Agronomy for Sustainable Development*, 34(1), 1-20.
- Xiang, Y. A. N. & Ping, H. E. 2008. Recent advances on the technologies to increase fertilizer use efficiency. *Agricultural Sciences in China*, 7(4), 469-479.
- Yadav, J., Gehlot, P., Soni, P., & Jain, T. 2026. Plant microbiome engineering: from inoculation to genome editing. *Frontiers in Microbiology*, 17, 1781381.
- Younas, M. U., Zuo, S., Qasim, M., Ahmad, I., Feng, Z., Korai, S. K., & Malik, T. 2025. Multi-omics approaches in plant biology: decoding agronomic traits for sustainable agriculture. *Plant Stress*, 18, 101118.

- Zaman, W., Ayaz, A., & Puppe, D. 2025. Biogeochemical cycles in plant–soil systems: significance for agriculture, interconnections, and anthropogenic disruptions. *Biology*, 14(4), 433.
- Zeffa, D. M., Perini, L., Silva, M. B., de Sousa, N.V., Scapim, C.A., De Oliveira, A. L. M., Do Amaral, A. T. & Goncalves, L. S. A. 2019. *Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes. *PLoS One* 14, e0215332.