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NITROGEN-FIXING BACTERIA IN BIOFERTILIZER PRODUCTION: BENEFITS, LIMITATIONS AND PROSPECTS

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ABSTRACT

Since conventional fertilizers have detrimental effects on both the environment and human health, biofertilizers have gradually taken their place. Agricultural enterprises rely heavily on biofertilizers because they preserve the soil ecosystem and boost crop productivity. Additionally, the environmentally favorable features of bio-based fertilizers support sustainable agriculture. The biofertilizer market is booming, and new strategies are being introduced all the time. The development of liquid, nano-sized, and microbial biofertilizers holds the key to the future of organic fertilizers since they can revolutionize the agricultural sector in an environmentally friendly way. The most popular types of microbial biofertilizers are endophytic *(Pseudomonas, Rahnella*), fungal (*Mycorrhizae*, *Penicillium*), and nitrogenous (*Rhizobacteria*, *Azospirillum*). Gold, silver, iron oxide, and zinc nanoparticles or nanocomposites compose nanobiofertilizers. Regarding liquid biofertilizers, the three most often utilized solutions (solubilizers) are phosphate, potassium, and nitrogen. These are the fertilizers' main ingredients. Most people agree that one of the main elements that limit plant growth is nitrogen. Nitrogen fixation is the term used to describe the biological process that converts molecular nitrogen into ammonia. Numerous phyla within the Bacteria domain comprise a diverse range of nitrogen-fixing bacterial species capable of colonizing the rhizosphere and interacting with plants. By forming a specialized organ called a root nodule on their respective host plants, rhizobia or Frankia can help leguminous and actinorhizal plants get nitrogen. Diverse cereal crops and pasture grasses have been found to have an increasing number of nitrogen-fixing species colonizing their root surfaces and, in certain circumstances, their root interiors (known as nitrogen-fixing endophytes). Other symbiotic connections involve heterocystous cyanobacteria. Biological nitrogen fixation (BNF), phytohormone synthesis, environmental stress alleviation, blocking plant ethylene synthesis, and boosting nutrient availability (e.g., iron) by siderophore production are some of the bacterial processes of plant growth promotion. A growing number of nitrogen-fixing species are colonizing the root surface and, in certain situations, the root interior (known as nitrogen-fixing endophytes) of a range of cereal crops and pasture grasses. Other symbiotic relationships involve heterocystous cyanobacteria. Biological nitrogen fixation (BNF), phytohormone synthesis, environmental stress alleviation, blocking plant ethylene synthesis, and boosting nutrient availability (e.g., iron) by siderophore production are some of the bacterial processes of plant growth promotion. This review provides a thorough understanding of the new types of nitrogen-fixing biofertilizers and how they affect crop productivity and the soil ecosystem. They have characteristics that improve crop output by preserving and/or enhancing the microbial and nutritional balance in the soil. Furthermore, by assisting in the preservation of nature's important elements, these biofertilizers support green agriculture. Even if the next biofertilizers have some limitations discussed in the articles the futuristic approach will also be considered to address the difficulties encountered when using biofertilizers.

KEYWORDS: Nitrogen Fixing Bacteria, Nitrogen Fixing Biofertilizer, Plant Growth Promoter.

1. INTRODUCTION

In the majority of cropping systems, biological nitrogen fixation is one of the most important processes for agricultural productivity since its inputs are the direct source of atmospheric nitrogen and have rotational effects like disease and pest management.[1,2] Further developments in molecular biology methods could open up new avenues for researching the ecology of root nodule bacteria. Additionally, it can enhance the process of choosing the best strains for the inoculation. Improving inoculation technologies requires a basic understanding of the genetic basis of nodulation in pasture legumes, including grains.[3,7]

1.1 Nitrogen-Fixing Bacteria

One of the most restricting nutritional elements is nitrogen (N), which is one of the primary macronutrients required for plants to grow and develop properly. As plants cannot digest air N_2, their uptake of N is reliant on the amount found in the soil. For plants to absorb atmospheric N_2, it must first be converted to ammonia (NH_3). Luckily, diazotrophic prokaryotes can convert atmospheric N_2 to NH_3 through the enzymatic complex nitrogenase, a process known as biological nitrogen fixation (BNF). Diazotrophic bacteria can be categorized as either non-nodular or nodule-forming depending on their life strategies.[8] Rhizobia, a group of bacteria that are associated with plants in the Leguminosae (Fabaceae) family, are the primary examples of nodule-forming bacteria. Actinobacteria of the Frankia genus are capable of forming nodules and symbioses with a wide range of plants from 23 genera in eight different families that are members of the orders Rosales, Cucurbitales, and Fagales in addition to rhizobia. Non-nodular bacteria can be found in the rhizosphere free-living, in plant tissues (endophytic), or connected with roots (associative). The genera *Azotobacter, Bacillus, Beijierinckia, Burkholderia, Clostridium, Desulfovibrio, Derxia, Enterobacter, Klebsiella, Paenibacillus,* and *Serratia* are members of the group of soil free-living organisms.[9,10] Phototrophic sulfur bacteria and cyanobacteria are also members of the free-living category. Cyanobacteria can also coexist in symbiosis with plants, such as Nostoc with bryophytes, a few gymnosperms and angiosperms, and Anabaena with the aquatic fern Azolla.[11] This is in addition to the group of cyanobacteria that live freely. On the surface of the roots, rhizospheric-associated bacteria multiply and feed on root exudates. *Acetobacter, Azoarcus, Azospirillum, Burkholderia* and *Herbaspirillum* are the genera of associative diazotrophic bacteria that have been studied the most.[9] However, the genus *Azospirillum* focusing on A. brasilense and A. lipoferum is undoubtedly the most significant agronomically[9,12,13] and is sold as inoculants in numerous countries.

For some part of their life cycle, endophytic bacteria penetrate and multiply quickly within plant components without exhibiting indications of disease.[9] Since nitrogen-fixing endophytic bacteria are shielded inside plant tissues, they have less competition and can directly provide plants with fixed nitrogen. This is thought to give them some advantages over rhizospheric associative bacteria. Furthermore, low oxygen concentrations are necessary for effective biological nitrogen fixing and are readily encountered in plant tissue.[14] One of the earliest endophytic diazotrophic bacteria to be researched, with a focus on sugarcane, was *Acetobacter diazotrophicus*.[15]

Furthermore, Herbaspirillum is typically found in pastures and grasses like sugarcane, rice, and wheat as endophytic diazotrophic bacteria. *A diazotrophic* bacterium model for endophytic interactions is *Herbaspirillum seropedicae*.[16] Thus far, the majority of N_2-fixing endophytes have been identified in isolation from monocots, including kallar grass[17], sugarcane[18], rice[19], maize[20], wheat[19], Sorghum halepense[21], miscanthus[22], and elephant grass.[23] Additionally, certain reports of endophytic diazotrophic bacteria in mosses[24] and conifers[25,26] have been made.

In contrast to monocots, however, relatively little is known regarding their existence in dicots. N_2-fixing endophytes have been identified from sweet potato plants[27] and coffee plants[28] in dicots.

Furthermore, some scientists isolated diazotrophic endophytes from *Arctium lappa*, a member of the Asteraceae family.[29] In contrast, other scientists isolated diatrotrophic endophytes from poplar and willow and tested them in rice.[30] A list of the endophytic diazotrophic bacteria that were isolated together with the crops that they colonized was provided in the work by many researchers.[31,32] By increasing nutrient availability and improving soil fertility, mostly through BNF and other mechanisms like phosphate solubilization or iron sequestration by siderophores, all of these bacteria have the potential to enhance plant performance.[13,33,34] According to a thorough analysis by Thiebaut et al.[35], they also create plant growth regulators, modulate phytohormone and defense responses, manufacture antioxidants, regulate osmotic balance, and help plants tolerate biotic and abiotic challenges.

2. Nitrogen Fixing Biofertilizers

For intensive agriculture to maximize crop output, N fertilizer application is crucial, coupled with the application of other vital nutrients. Around half of the world's food supply is generally thought to be produced by the use of synthetic N-based fertilizers, and by 2050, the rate of consumption of N fertilizers is expected to rise from 80 to 180 mt.[36] However, conventional N-based fertilizers have a 50% loss rate to the soil and environment after application.[37] This has the potential to significantly harm the environment and economy. For example, nitrous oxide volatilization contributes to emissions of CO_2 equivalent that are about ten times higher than they would otherwise be; it can also lead to soil acidification, the depletion of non-renewable resources, and nitrate leaching into surface and groundwater, which can have disastrous consequences like eutrophication of the water. To fulfill the problems of agriculture sustainability, which include improving crop nutrition and productivity required for the growing global population, it is necessary to sustain the usage of N fertilizers. Most importantly, the soil ecosystem services with safe provision are, undoubtedly, a must for securing agro-ecosystems sustainability.[38] The most crucial requirement for ensuring the sustainability of agro-ecosystems is the safe provision of soil ecosystem services.[38] Without suitable mineral fertilizer supplies and best practices, it will be impossible to meet the urgent and quickly rising need for food, particularly in underdeveloped countries, where resources and crops barely make a dent in efficient crop production. Continuous efforts are required to increase agricultural output in a sustainable way that takes into account the biochemical variety of the entire agro-ecosystem and the ability to lessen the negative effects of diseases, pests, abiotic stresses, low soil fertility, and other factors.[39,40] Under these circumstances, there is an increasing need to think about new, creative methods for intelligent and sustainable "food and feed" production that rely less on traditional fertilizers, particularly N. This is in keeping with addressing both present and future shifts in human requirements within a sustainable framework, which will probably rely on optimal management practices and more prudent use of mineral and biological resources while preserving the environment and protecting natural resources. It is still difficult to provide enough plant N nutrition for such a highly mobile nutrient in soils. In this sense, the primary source of N in agro-ecosystems has been biologically fixed N.

Both soil-root rhizosphere interfaces and plant tissues (such as nodules and roots) are home to atmospheric nitrogen (N_2)-fixing bacteria, which can provide sizable N supplies for plant growth. This is mostly because of the microbial-mediated process known as "biological nitrogen fixation (BNF)," which converts atmospheric N_2 into ammonia (NH_3) through a very sensitive bacterial enzymatic reaction. Mineral N fertilizers can be supplemented or replaced with BNF fertilizers that are acceptable for the environment. The availability of some critical resources, including water, phosphate (P), and molybdenum (Mo), governs this process.[41,42,43,44] The two main ways that BNF is produced are (i) symbiotic N fixation (SNF), which occurs when bacteria live in symbiotic relationships with higher plants and leguminous animals and give carbon to N_2-fixing bacteria in exchange for nitrogen (N), and (ii) non- symbiotic BNF, which occurs when heterotrophic or autotrophic bacteria live in soils, water, rocks, leaf litter, or in conjunction with plants.

The nutrient that primarily restricts plant growth is nitrogen.[45] About 80% of the nitrogen found in the free state is found in the atmosphere, although most plants are unable to use this nitrogen. This nitrogen must be fixed by a certain kind of microbe before the plant can use it. We refer to these microbes as biological nitrogen fixers (BNFs). They convert the inert N_2

into an organic form that plants may use.[46]

Crop yields can rise by 10–50% and 300–400 kg N/ha/yr by nitrogen fixation. Up to 25% of the total nitrogen in plants is obtained through N-fixation. Plant roots release compounds into the soil that help bacteria in the rhizosphere of the plant colonize and fix nitrogen. They can effectively replace chemical fertilizers to varying degrees, lowering the environmental chemical load. A rough estimation of this substitution are divided into three categories: blue-green algae, symbionts like *Rhizobium, Frankia and Azolla*, and free-living bacteria like *Azotobacter* and *Azospirillium*. *Rhizobium, Mesorhizobium, Azorhizobium, Bradyrhizobium, Sinorhizobium* and *Allorhizobium* are among the N_2-fixing bacteria linked to legumes. On the other hand, bacteria associated with non-legumes include *Achromobacter, Alcaligenes, Mycobacterium, Arthrobacter, Desulfovibrio, Acetobacter, Corynebacterium, Azomonas, Beijerinckia, Clostridium, Bacillus, Enterobacter, Erwinia, Rhodo-pseudomonas, Derxia, Campylobacter, Herbaspirillum, Klebsiella, Lignobacter, Rhodospirillum, Xanthobacter, Mycobacterium*, and *Methylosinus.*[47] Even though many genera are isolated from the rhizosphere, a lot of research has been done on the *Azospirillum* and *Azotobacter* genera, in particular, to see if they might boost cereal and legume output in field conditions.[48]

2.1 Rhizobium

The most excellent example of symbiotic nitrogen fixation is Rhizobium, a member of the bacterial family *Rhizobiaceae*. It is capable of fixing N_2 in both non-legume and legume crops. In several legume crops, rhizobium has been demonstrated to fix up to 300 kg N/ha/year.[49] The bacteria cause nodules in the legume root where they break down molecular nitrogen to ammonia, which the plant uses to make proteins, vitamins, and other nitrogen-containing substances. As a result, these root nodules produce ammonia-like factories.[50] Through modifying root form and growth physiology, rhizobium species enhance non-legume growth. Through improvements in plant height, seed germination, leaf chlorophyll, and N content, the Rhizobium spray boosted crop growth.[51] Different strains of Rhizobium inoculated into rice seeds at varying N concentrations enhanced the yield of straw by 4% to 19% and rice grain by 8% to 22%.[52] Rhizobia are *Rhizobium, Bradyshzodium, Sinorhizobium, Azorhizobium*, and *Mesorhizobium* together. They can function in three different ways: directly by affecting plant hormones or fixing nitrogen; indirectly, by lessening the pathogens' inhibitory effects.[53] Since symbiosis accounts for around 80% of biologically fixed nitrogen, rhizobium is frequently utilized in agronomic operations to guarantee enough nitrogen and has the potential to replace chemical nitrogen fertilizers.[54] Higher crop yields and soil fertility are both maintained by rhizobium.[55]

2.2 Azotobacter

Because of its numerous metabolic activities, *Azotobacter* is a free-living, nitrogen-fixing, diazotrophic bacteria that is crucial to the nitrogen cycle.[57] Vitamins like thiamine and riboflavin can be produced by *Azotobacter*.[58] It is utilized as a biofertilizer for all non-leguminous plants, including rice, cotton, vegetables, sugarcane, sweet potatoes, and sweet sorghum. It is a member of the *Azotobacteriaceae* family. After Azotobacter inoculation, researchers found that a notable increase in seed yield in mustard and rapeseed.[59] It is mostly used commercially for sugarcane since it raises the crop's yield by 25–50 tons/hectare and its sugar content by 10-15%. It fixes nearly 30 kg/N/year. Acidic and alkaline soils both contain Azotobacter. The most common species in the soil is *A. chroococcum*, although other species are also present, including *A. macrocytogenes*, *A. beijerinckii*, *A. insignis*, and *A. vinelandii*.[60] The presence of *A. chroococcum* in the rhizosphere of tomato and cucumber was linked to higher seedling germination and growth, as shown by many scientists.[61] *A. chroococcum* inoculation resulted in a noticeably higher rate of plant development as compared to the control, according to another study.[62] Additionally, *Azotobacter* generates antibiotics and antifungal substances that assist stop seedling mortality by preventing the growth of several harmful fungi in the root zone.[63,64] The primary issue impeding Azotobacter's growth is the decreased level of organic matter in the soils; as a result, there are no Azotobacter cells in the rhizoplane.[53,65]

2.3 Azospirillum

This bacterium fixes a significant amount of nitrogen in the soil, which makes it indispensable as well. It is found in the rhizosphere and fixes 20–40 kg N/ha in non-leguminous plants, including cotton, sorghum, millets, grains, and oilseeds.[66] With plants, it primarily develop a symbiotic relationship. *Azospirillum* has been found in several studies to have the capacity to boost crops.[67,70]

Wheat seedlings vaccinated against *Azospirillum* showed good hydration status; fresh weight from vaccinated seeds was greater than that from non-inoculated seeds. According to Somers et al.[71], *A. brasilense* is capable of producing phenylacetic acid (PAA), a chemical that resembles auxin and has antimicrobial properties. Research shows that co-inoculation with

A. lipoferum and *B. megaterium* gave the plant balanced nutrition with nitrogen and phosphorus and resulted in a higher yield compared to inoculation with *Azospirillum* alone.[72]

2.4 Anabaena Azollae

This symbiotic bacterium fixes atmospheric nitrogen, primarily in rice.[73,74] It is invariably connected to the Azolla or floating fern. Azolla leaves swiftly breakdown and supply nitrogen to the plant, containing 4-5% nitrogen (on a dry weight basis) and 0.2–0.4% nitrogen (on a wet weight basis). The Azolla-Anabaena system adds 1.1 kg N/ha/day; in 20–25 days, a single Azolla crop might supply 20–40 kg N/ha to the rice crop.[75] Many nations, including Vietnam, China, Thailand, and the Philippines, use azolla as a biofertilizer.[76,78] This biofertilizer's capacity to withstand metals makes it advantageous to use in areas contaminated by heavy metals.[79]

2.5 Blue-Green Algae (Cyanobacteria)

Cyanobacteria are the most ubiquitous N_2 fixers on Earth. *Nostoc*, *Anabaena*, *Oscillatoria*, *Aulosira* and *Lyngbya* are among the varied group of prokaryotes known as cyanobacteria, or blue-green algae.[80] In addition to providing vitamin B complex and growth-promoting compounds like auxins, indole acetic acid, and gibberellic acid, which hasten plant growth, they are crucial in providing nitrogen to the soil. In submerged rice fields, they fix 20–30 kg/N/ha and, when sprayed at 10 kg/ha, boost crop production by 10–15%. It has been reported that the use of cyanobacteria in agriculture, especially in rice fields, enhanced the amount of nitrogen available to plants.[81,82] Cyanobacteria have been demonstrated to improve wheat and rice yield, shoot and root growth, and seed germination. Blue-green algae can fix 25–30 kg N/ha/season in rice fields.[83] Helianthus annuus and Sorghum durra growth parameters were examined in a study to determine the impact of cyanobacterial strain exudates. In comparison to the control, shoot length rose to roughly 120–242%, with additional beneficial effects.[84] Potential for the release of beneficial substances was demonstrated by strains, which also improved plant development and production. In a different investigation, rice inoculation using cyanobacteria isolated from rice fields showed favourable impacts on soil and rice plant characteristics at the same time.[85] Farmers with limited resources who cannot afford to purchase pricey chemical fertilizers can benefit from applying cyanobacteria as biofertilizers. Terrestrial, rainforest, and desert biomes can all benefit from the use of cyanobacterial biofertilizers.[86,87]

3. Microbes in plant growth

Many microorganisms can stimulate plant growth, and microbial products that do so have found commercial use. It has been shown that bacteria originating from the rhizosphere of plants are advantageous to the roots and general growth of plants. These bacteria are known as rhizobacteria that promote plant growth (PGPR). These rhizobacteria have a major positive impact on plant growth through both direct and indirect processes. The synthesis of substances that promote plant development and lessen stress is one of the direct approaches.[88] Plant growth and the decrease of biotic and abiotic stressors are positively impacted by PGPR, which also show a strong contact with plant roots. The induction of antibiosis, competitive omission, systemic resistance, and other processes all promote plant development.[89] *Bacillus subtilis* demonstrates a dual biocontrol mechanism, utilizing direct and indirect means, to inhibit pathogen-induced illness. The direct method helps the plant defend itself against pathogen attack by synthesizing a variety of secondary metabolites, hormones, enzymes that break down cell walls, and antioxidants. The induction of acquired systemic resistance and the promotion of plant growth are examples of the indirect process. Additionally, *Bacillus subtilis* can improve nitrogen fixation, solubilize soil P, and create siderophores that both stimulate and inhibit the growth of pathogens. Bacillus subtilis increases the expression of stress-response genes, phytohormones, and stress-related metabolites to improve stress tolerance in its plant hosts. The activity of *B. subtilis* in the rhizosphere, its function as a root colonizer, its potential for biocontrol, the related mechanisms of biocontrol, and its capacity to boost crop output in the face of biotic and abiotic stress are all covered in this review.

Numerous bacterial species, such as *Bacillus, Acinetobacter, Pseudomonas, Enterobacter, and Sinorhizobium* have been isolated from the rhizosphere and soil.[90] According to a different investigation, soybean roots were linked to *Bacillus, Mycobacterium, Cellulosimicrobium, Enterobacter, Arthrobacter* and *Pseudomonas.*[91] The rhizobacterial strain that was present was not negatively impacted by the application of PGPR, but plants that were infected with *P. putida*, *Pseudomonas fluorescens* or a *Bacillus sp.* showed improvements in root nodulation, enzyme synthesis, and plant development in comparison to plants that were not.[92] Plant growth hormones (JA, GA3, IAA, and ABA) are reportedly synthesized by endophytic diazotrophic bacteria in the roots of the halophyte shrub *Prosopis strombulifera.*[93]

4. Limitations in the Production of Nitrogen Fixing Biofertilizers

Over the past 50 years, nitrogen-fixing biofertilizers have demonstrated a great deal of promise in agriculture; yet, several obstacles have made it difficult for these fertilizers to be widely used. Important restrictions that can reduce the efficacy of bioinoculants include competition with native soil flora, less-than-ideal soil conditions, and the existence of soil and environmental contaminants. The effectiveness of these biofertilizers is significantly diminished by extreme weather conditions. Another major problem is finding and creating appropriate microbial strains and transport materials. The production units frequently struggle with a lack of qualified and experienced workers, and their ability to produce is further hampered by inadequate money and equipment from the public and private sectors. The lack of adequate facilities for transportation and storage has an impact on the availability and quality of biofertilizers. Furthermore, there are major marketing obstacles that make it difficult to guarantee that the relevant strains will be available at the right time and location. Many farmers are often unaware of the advantages and effective application of nitrogen-fixing biofertilizers. The lack of uniform production norms can result in inconsistent quality, and the use and reach of biofertilizers are restricted by inadequate distribution and promotion networks. All of these elements practically influence how well nitrogen-fixing biofertilizers work in the long run. To fully realize the potential of this technology in sustainable agriculture, it is imperative to address these limits through focused recommendations and deliberate efforts.

5. CONCLUSIONS AND PROSPECTS

Biofertilizers that fix nitrogen are a potentially effective and environmentally responsible way to increase crop yields. These biofertilizers have been more well-known in recent years due to their capacity to provide plants with critical nitrogen, greatly increasing yields. They are better than hazardous chemical fertilizers since they are more affordable, environmentally friendly, and provide plants with a natural growing environment. Biofertilizers that fix nitrogen also strengthen plants' defensive mechanisms and shield them from adverse environmental factors like acidic soil and dryness.

Biofertilizers that fix nitrogen have advantages that go beyond just providing nitrogen. The main microorganisms and their modes of action utilized in nitrogen-fixing biofertilizers are highlighted in this review. It has been observed that compared to using a single biofertilizer or chemical fertilizers alone, the combination inoculation of various types of biofertilizers can result in more notable yield improvements. An increase in the market for nitrogen-fixing biofertilizers suggests that agricultural methods are becoming more sustainable and environmentally friendly.

However, a thorough understanding of soil qualities, field settings, and the host specificity of microbial strains is necessary for the efficient manufacture and implementation of nitrogen-fixing biofertilizers. The development of nitrogen-fixing biofertilizers with increased efficiency, competitive ability, and numerous functions has been made possible by developments in molecular biology, biotechnology, genetic engineering, microbial taxonomy, and nanotechnology.

In the future, nitrogen-fixing biofertilizers could sustain crop yields while having a negligible influence on the environment, offering a viable substitute for chemical fertilizers. To find strains unique to a certain soil, learn more about the composition of biofertilizers, and improve current strains via biotechnological advancements, more study is necessary. Agricultural practices that are more efficient and sustainable will be made possible by this ongoing research.

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