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AZOTOBACTER-BASED BIOFERTILIZERS: MECHANISM OF PLANT GROWTH PROMOTION, AGRICULTURAL APPLICATIONS AND FUTURE PERSPECTIVES

Mrs. Pitani Aswini Devi*, Ms. Barre Papa[1], Ms. Rayudu Sravani[2], Mr. Palivela Kishor[3], Ms. Bonthu Varalakshmi[4], Ms. Shaik Raziya Sultana[5]

*Department of Biotechnology, Koringa College of Pharmacy, Korangi, Andhra Pradesh, India.
[1,2,3,4,5]UG. Student, Koringa College of Pharmacy, Korangi, Andhra Pradesh, India.

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***Corresponding Author: Mrs. Pitani Aswini Devi**

Department of Biotechnology, Koringa College of Pharmacy, Korangi, Andhra Pradesh, India.

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ABSTRACT
The increasing use of chemical fertilizers has raised serious environmental concerns, highlighting the need for sustainable and eco-friendly alternatives. Azotobacter spp. are free-living, nitrogen-fixing bacteria that play an important role in improving soil fertility and promoting plant growth. These bacteria convert atmospheric nitrogen into biologically available forms and enhance plant development through the production of phytohormones, phosphate solubilization, siderophore production, and biocontrol activities. This review discusses the classification, structural features, nitrogen fixation process, and plant growth-promoting activities of Azotobacter species. It also discusses their applications as biofertilizers in sustainable agriculture, particularly their ability to improve nutrient availability, support root development, enhance crop productivity, and contribute to soil health. Furthermore, the comparative advantages of Azotobacter over other beneficial bacteria, including Rhizobium, Azospirillum, Bacillus, and Pseudomonas, are highlighted. Beyond agriculture, Azotobacter has attracted attention for its pharmaceutical and industrial applications. Its production of biopolymers such as alginate and polyhydroxybutyrate (PHB) offers potential applications in drug delivery systems, wound dressings, tissue engineering, and biodegradable materials. Other valuable products, including vitamins, melanin, enzymes, and biosurfactants, further demonstrate its biotechnological importance. Despite these promising applications, challenges such as inconsistent field performance, formulation limitations, storage stability, and inadequate farmer awareness restrict its widespread adoption. Future research focusing on genetic improvement, advanced formulation techniques, nano-encapsulation, and microbial co-inoculation may enhance its effectiveness and commercial potential. Overall, Azotobacter represents a multifunctional microorganism with considerable promise for sustainable agriculture, pharmaceutical biotechnology, and environmentally responsible industrial development.

KEYWORDS: Azotobacter spp., Nitrogen fixation, Biofertilizer, Plant growth-promoting rhizobacteria, Sustainable agriculture.

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1. INTRODUCTION

Agriculture is the backbone of global food security however its productivity is largely dependent on nitrogen availability. Although atmospheric nitrogen constitutes about 78 percent of air, plants cannot utilize it directly. Therefore, nitrogen fixation is a crucial biological process.[1]

Initially, chemical fertilizers were introduced to overcome nitrogen deficiency and to increase crop yield. Nevertheless, their indiscriminate use has resulted in soil infertility, water pollution and loss of microbial diversity. Consequently, researchers have shifted their focus towards biofertilizers as a sustainable alternative.[2][3]

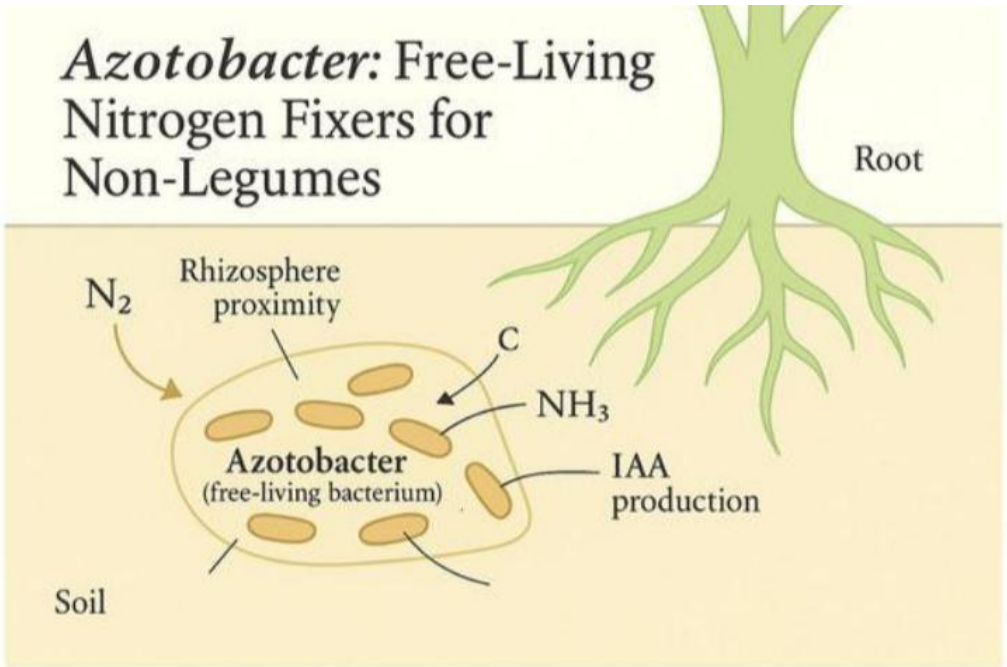
Azotobacter is a free-living, aerobic, gram-negative bacterium belonging to the family Azotobacteriaceae. It was first isolated by the Dutch microbiologist Martinus Beijerinck in 1901. Specifically, species like Azotobacter chroococcum and Azotobacter vinelandii are widely studied for their ability to fix atmospheric nitrogen. Furthermore, these organisms are known to produce various growth-promoting substances.[4][5]

In addition to nitrogen fixation, Azotobacter exhibits plant growth promoting rhizobacteria activity through production of indole acetic acid, gibberellins, cytokinins and phosphate solubilization. Moreover, recent investigations have highlighted its role in production of biodegradable polymers such as alginate and polyhydroxybutyrate which have significant pharmaceutical and industrial applications.[6][7][8]

Therefore, the present review aims to provide a comprehensive overview on taxonomy, mechanism of nitrogen fixation, agricultural benefits and emerging pharmaceutical potential of Azotobacter spp.[9]

1.2 TAXONOMY AND MORPHOLOGICAL CHARACTERISTICS AZOTOBACTER

Azotobacter is classified under the family Azotobacteriaceae. Although it is a free-living diazotroph, it shares certain physiological similarities with Pseudomonadaceae. Therefore, its taxonomic position has been revised several times based on molecular studies.[10]



Initially, classification was based on morphological and biochemical characteristics. However, recent 16S rRNA sequencing has provided more accurate phylogenetic placement. Consequently, Azotobacter is now placed under Gammaproteobacteria.[11][12]

Morphologically, Azotobacter spp. are gram-negative, large, oval to spherical cells. Specifically, their size ranges from 2 to 10 micrometres and they often appear in pairs. Furthermore, they form cysts under unfavourable conditions which helps in survival. In contrast to vegetative cells, cysts are resistant to desiccation and radiation.[13][14]

Major species includes Azotobacter chroococcum, Azotobacter vinelandii, Azotobacter beijerinckii, Azotobacter nigricans and Azotobacter armeniacus. Among these, Azotobacter chroococcum is predominantly found in Indian soils and is extensively used as biofertilizer. Moreover, Azotobacter vinelandii is recognized as model organism for nitrogenase studies due to its high nitrogen fixing efficiency.[15][16]

2. Objectives

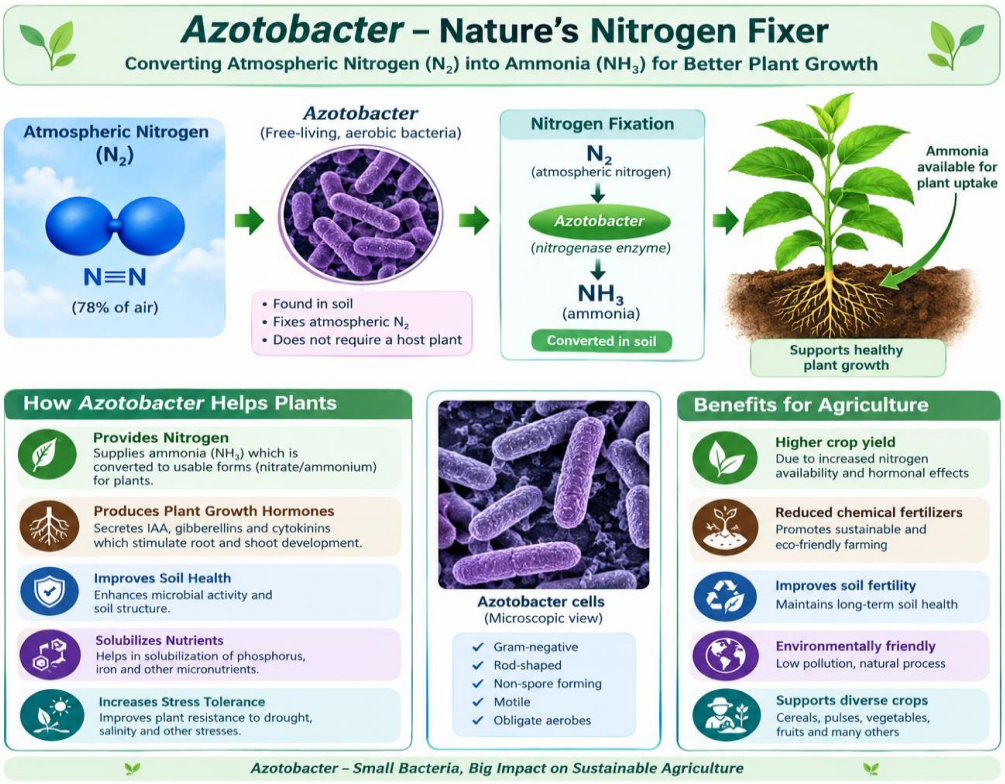
1. To review the role of Azotobacter as a free-living nitrogen-fixing bacterium and its contribution to improving soil fertility and plant growth.

2. To examine the mechanisms through which Azotobacter promotes plant growth, including biological nitrogen fixation, phytohormone production, and nutrient mobilization.
3. To evaluate the effects of Azotobacter-based biofertilizers on seed germination, root development, crop productivity, and nutrient-use efficiency.
4. To assess the influence of different carrier materials, formulation techniques, and storage conditions on the survival and effectiveness of Azotobacter in biofertilizer preparations.
5. To discuss the potential of Azotobacter biofertilizers to reduce dependence on chemical nitrogen fertilizers and support environmentally sustainable agricultural practices.
6. To identify the major challenges associated with the production, application, field performance, and commercialization of Azotobacter-based biofertilizers.
7. To highlight emerging research approaches for developing improved Azotobacter formulations with enhanced microbial viability, nutrient availability, and long-term agricultural benefits.

3. Azotobacter as plant growth promoting rhizobacteria (pgpr)

Plant growth promoting rhizobacteria are a group of beneficial bacteria that colonize rhizosphere. Although several genera are included, Azotobacter is considered as one of the most efficient PGPR. Therefore, its PGPR activity has been extensively investigated.[17]

Initially, PGPR activity of Azotobacter was attributed only to nitrogen fixation. However, recent studies have revealed multiple direct and indirect mechanisms. Specifically, direct mechanisms include nutrient mobilization and phytohormone production. Furthermore, indirect mechanisms involve biocontrol and stress tolerance.[18][19]



Firstly, Azotobacter enhances nutrient availability. It fixes atmospheric nitrogen and contributes up to 20 kg N per hectare per year. In addition, it solubilizes phosphorus and zinc through secretion of organic acids such as gluconic acid. Moreover, production of siderophores facilitates iron uptake by plants under iron deficient conditions.[20][21][22]

Secondly, Azotobacter produces plant growth regulators. For instance, indole acetic acid stimulates root development and increases absorptive surface area. Similarly, gibberellins and cytokinins promote shoot growth and delay senescence. Consequently, treated plants exhibit improved vigour and higher biomass.[23][24]

Another important PGPR trait is induction of systemic resistance. Azotobacter triggers defense enzymes such as phenylalanine ammonia lyase and peroxidase. Therefore, plants become resistant to pathogens. Additionally, it produces antifungal metabolites like anisomycin and hydrogen cyanide that suppress fungal growth. Thus, it acts as natural biocontrol agent.[25][26]

Finally, Azotobacter helps plants to tolerate abiotic stress. Under drought conditions, exopolysaccharide production improves water holding capacity of soil. In contrast, under salinity stress, it reduces ethylene production through ACC deaminase activity. Hence, it ensures crop survival in adverse environments.[27]

4. Emerging pharmaceutical and industrial potential

In recent years, research focus on Azotobacter has expanded beyond agriculture. Although initially recognized solely as biofertilizer, it is now considered as a promising microbial cell factory. Therefore, its pharmaceutical and industrial applications have attracted considerable interest.[28]

Firstly, Azotobacter is a proficient producer of alginate, which is an exopolysaccharide. Specifically, Azotobacter vinelandii synthesizes alginate with high guluronic acid content and superior rheological properties. Furthermore, bacterial alginate is more biocompatible and immunologically inert compared to algal alginate. Consequently, it is widely utilized in wound dressings, controlled drug delivery systems and tissue engineering scaffolds.[29][30]

Secondly, Azotobacter accumulates polyhydroxybutyrate as intracellular carbon reserve. In contrast to synthetic plastics derived from petroleum, PHB is biodegradable, non-toxic and biocompatible. Moreover, PHB based nanoparticles are employed for sustained drug release and implants. Thus, it provides eco-friendly alternative to conventional plastics.[31][32]

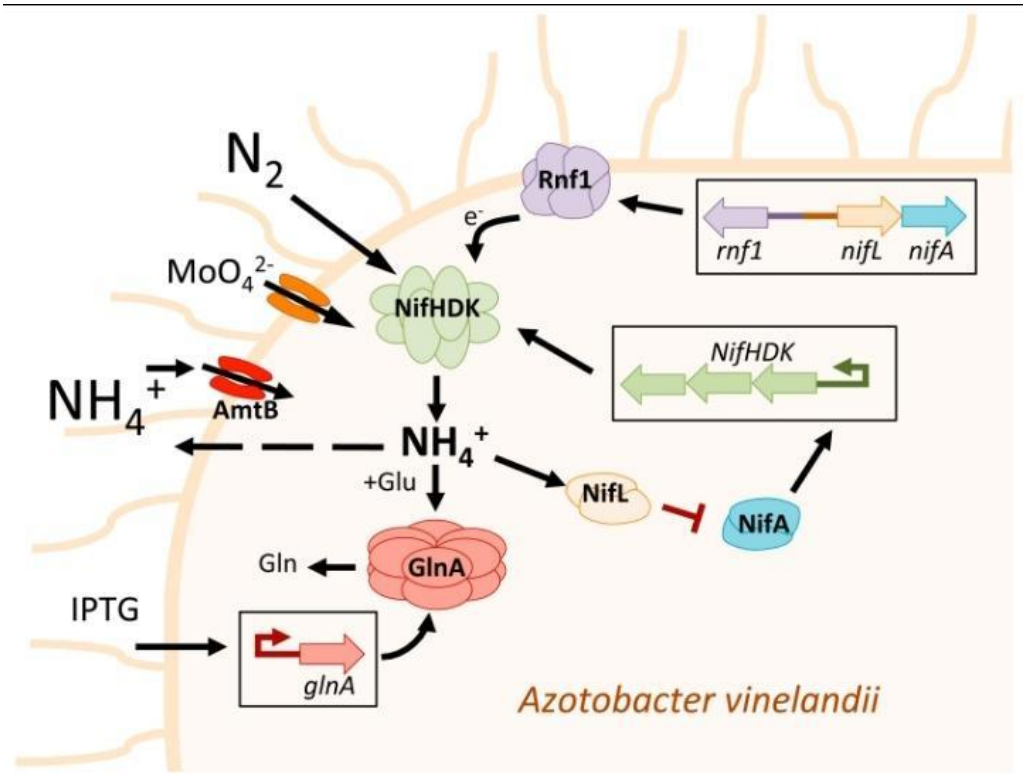
In addition, Azotobacter produces valuable metabolites such as riboflavin, folic acid and poly gamma glutamic acid. Similarly, melanin pigment synthesized by Azotobacter chroococcum exhibits strong antioxidant, radioprotective and antimicrobial properties. Additionally, this melanin has potential application in sunscreens and pharmaceutical formulations.[33][34]

Another emerging application is enzyme and biosurfactant production. Azotobacter secretes enzymes like lipases and proteases which are industrially important. Therefore, it contributes to bioremediation and waste management. Ultimately, these versatile properties position Azotobacter as a dual purpose organism for both agricultural sustainability and pharmaceutical biotechnology.[35][36]

5. Comparative analysis

Although several biofertilizers are available commercially, each organism possesses distinct advantages. Therefore, comparative analysis is essential to select appropriate biofertilizer for specific crop and soil. Consequently, Azotobacter is compared with Rhizobium, Azospirillum, Bacillus and Pseudomonas.[37]

Initially, mode of colonization differs significantly. Specifically, Rhizobium is symbiotic and forms nodules only in legumes. In contrast, Azotobacter is free-living and does not require symbiosis. Furthermore, Azospirillum is associative and colonizes mainly cereals. Thus, Azotobacter exhibits broader host range.[38][39]



Secondly, nitrogen fixing efficiency varies. Rhizobium fixes 50 to 200 kg N per hectare but only in legumes. Although Azotobacter fixes comparatively less nitrogen, approximately 20 to 30 kg N per hectare, it is applicable to non-leguminous crops such as wheat, rice and vegetables. Moreover, Azospirillum fixes 20 to 40 kg N per hectare. Hence, for non-legume crops, Azotobacter and Azospirillum are preferable.[40][41]

Another aspect is production of growth promoting substances. Azotobacter produces high amount of indole acetic acid, gibberellins and cytokinins. Similarly, Bacillus is known for phosphate solubilization. However, Azotobacter combines nitrogen fixation, phosphate solubilization, siderophore production and alginate synthesis. Therefore, it offers multifunctional benefits.[42][43]

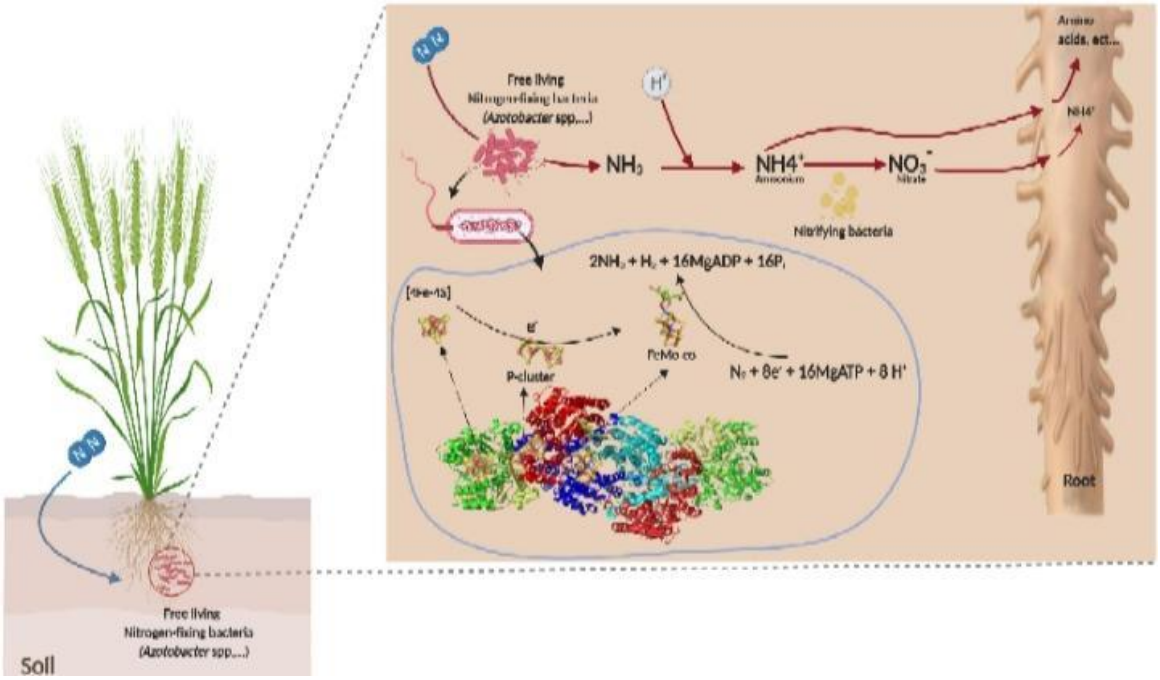
In addition, survival and shelf life are important. Azotobacter forms cysts which enhance survival under adverse conditions. In contrast, Rhizobium and Azospirillum are more sensitive to temperature and pH fluctuations. Consequently, Azotobacter based formulations exhibit longer shelf life.[44]

Finally, with regard to industrial potential, Azotobacter is superior. Although other biofertilizers produce limited biopolymers, Azotobacter produces alginate and polyhydroxybutyrate on large scale. Thus, it has dual agricultural and pharmaceutical value. Ultimately, comparative analysis reveals that Azotobacter is more versatile for sustainable agriculture.[45][46]

6. Mechanism of nitrogen fixation

Nitrogen fixation is the process by which atmospheric nitrogen is converted into biologically available ammonia. Although nitrogenase enzyme catalyzes this reaction, it is highly sensitive to oxygen. Therefore, Azotobacter has developed unique protective mechanisms.[47]

Initially, nitrogenase complex consists of two components, dinitrogenase and dinitrogenase reductase. Specifically, molybdenum-iron protein and iron protein work together to transfer electrons. Furthermore, this process requires high energy in the form of 16 ATP molecules for each molecule of nitrogen reduced.[48][49]



No through three strategies. Firstly, through high respiratory rate that maintains low intracellular oxygen. Secondly, by conformational protection through FeSII protein. Moreover, it produces alginate capsule and forms cysts to limit oxygen diffusion.[50][51]

In addition, Azotobacter possesses three types of nitrogenase, Mo-dependent, V-dependent and Fe-dependent. In contrast to Mo-nitrogenase, alternative nitrogenases are expressed when molybdenum is deficient. Consequently, this metabolic flexibility ensures nitrogen fixation under varied soil conditions.[52][53]

Atmospheric N2
↓
Nitrogenase (16 ATP)
↓
NH3
↓
Glutamine
↓
Plant Growth

7. Agricultural applications of azotobacter as biofertilizer

In recent decades, the agricultural sector has witnessed a paradigm shift towards sustainable practices. Although chemical fertilizers initially boosted crop production, their prolonged use has degraded soil health. Therefore, biofertilizers have emerged as an eco-friendly alternative.[54]

Specifically, Azotobacter contributes to plant growth through multiple mechanisms. Firstly, it fixes atmospheric nitrogen and makes it available to plants, thereby reducing the requirement of urea by 20 to 30 percent. Furthermore, it solubilizes insoluble phosphates through production of organic acids. In addition, it produces siderophores that chelate iron and enhance its uptake.[55][56]

Moreover, Azotobacter synthesizes phytohormones such as indole acetic acid, gibberellins and cytokinins. Consequently, these hormones promote root elongation, seed germination and overall plant vigour. For example, the application of Azotobacter chroococcum as a microbial inoculant has been reported to improve the yield of wheat, rice, and vegetables by approximately 15–25%. Similarly, it improves quality parameters in horticultural crops.[57][58][59]

Another significant aspect is its biocontrol activity. Azotobacter produces antifungal compounds and inhibits phytopathogens such as Fusarium and Alternaria. Therefore, it reduces dependency on chemical pesticides. Additionally, it enhances soil organic matter and improves soil structure through exopolysaccharide production.[60][61]

Nevertheless, its efficacy depends on soil pH, organic carbon and temperature. Hence, carrier-based formulations with lignite or charcoal are recommended for better survival. Thus, Azotobacter represents a cost-effective and sustainable solution for modern agriculture.[62]

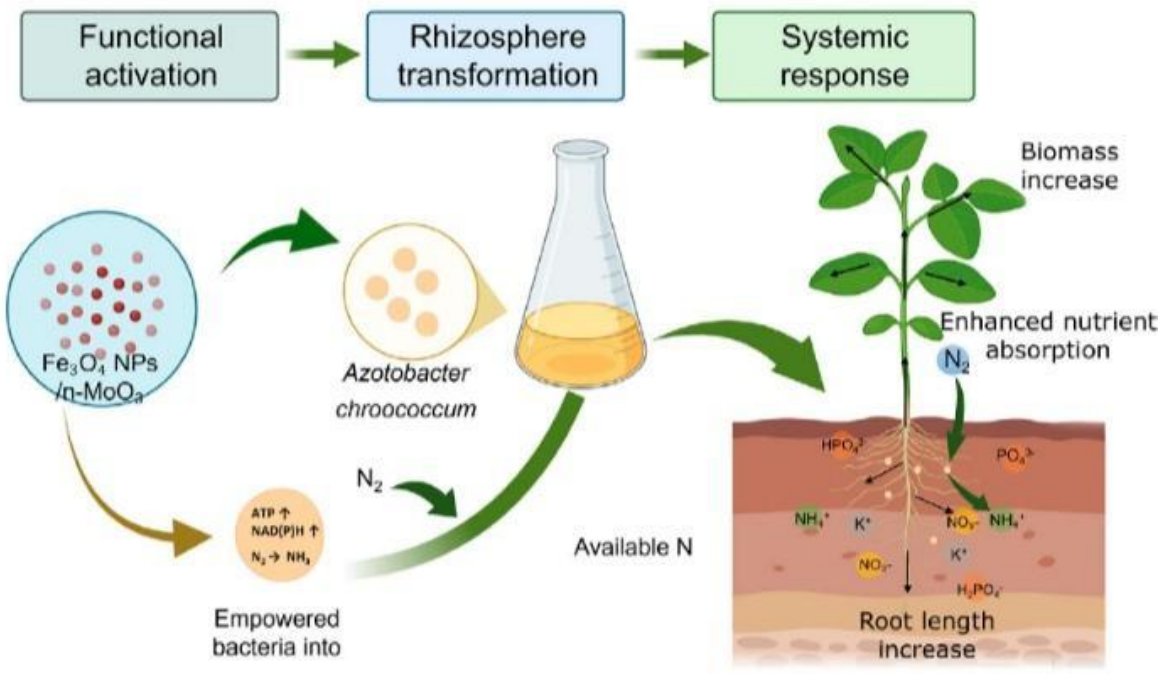
8. Challenges and future aspects

Despite its potential, several factors restrict the large-scale use of Azotobacter.

Therefore, addressing these constraints is essential for commercial success. Consequently, future research should focus on strain improvement and formulation technology.[63]

Initially, field performance of Azotobacter is inconsistent. Specifically, its survival is affected by soil pH, temperature, moisture and native microbial competition. Furthermore, low organic carbon in Indian soils reduces its establishment. Thus, efficacy under laboratory conditions often does not translate to field conditions.[64][65]

Secondly, formulation and shelf life remain major bottlenecks. Carrier based formulations using lignite or charcoal have limited viability of 6 months. In contrast, liquid formulations offer better shelf life but require cold chain storage. Moreover, lack of quality control and contamination during storage reduces effectiveness.[66][67]



Another challenge is limited farmer awareness. Although chemical fertilizers show immediate results, biofertilizers exhibit slow but sustainable effects. Therefore, farmers often hesitate to adopt. Additionally, inadequate regulatory framework and lack of subsidies further hinder market growth.[68]

Nevertheless, future prospects are promising. Advances in genetic engineering can enhance nitrogen fixing efficiency and stress tolerance. Similarly, genome editing tools such as CRISPR can improve alginate and PHB yield. Furthermore, co-inoculation with other PGPR such as Rhizobium and Pseudomonas can produce synergistic effects.[69][70]

Nano-encapsulation can enhance delivery efficiency and extend shelf life. Ultimately, integration of Azotobacter with organic farming and precision agriculture will pave the way for sustainable agriculture and green biotechnology.[71][72]

9. CONCLUSION

In conclusion, Azotobacter represents a versatile and multifunctional microorganism with significant implications for both agriculture and biotechnology. Initially recognized as a free-living nitrogen fixer, its role has expanded considerably beyond conventional biofertilizer application.

Although chemical fertilizers have increased crop productivity, their indiscriminate use has resulted in severe environmental degradation. Therefore, Azotobacter offers an eco-friendly and cost-effective alternative. Specifically, through nitrogen fixation, phosphate solubilization, phytohormone production and biocontrol activity, it enhances plant growth and soil health.

Furthermore, its emerging pharmaceutical and industrial potential, particularly in the production of alginate and polyhydroxybutyrate, highlights its value as a microbial cell factory. Consequently, Azotobacter serves dual purpose in sustainable agriculture and green biotechnology.

Nevertheless, challenges related to field inconsistency, formulation and farmer awareness must be addressed. Thus, future research focusing on genetic improvement, nano-formulation and co-inoculation strategies will further enhance its commercial viability.

Ultimately, integration of Azotobacter based technologies into modern agricultural practices will contribute significantly towards sustainable food security and environmental protection.

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