Exploring Azotobacter Species as Soil Biological Enhancers for Enhanced Crop Nutrition and Stable Yields: A Review

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Abstract

Abiotic and biotic stresses often impede optimal plant germination by disrupting natural growth and development mechanisms. Gram-negative *Azotobacter*, among crop growth-promoting *rhizobacteria*, emerges as a potent agent for enhancing plant health. *Azotobacter* employs various mechanisms, such as nitrogen fixation, phosphorus solubilization, pesticide and fungicide degradation, siderophore production, and synthesis of growth-promoting hormones, collectively contributing to improved plant vigor. Furthermore, *Azotobacter*-based biofertilizers offer additional benefits for soil fertility enhancement. As a favorable and cost-effective alternative to chemical fertilizers, the utilization of biofertilizers has gained traction. Nonetheless, commercial-scale microbial biofertilizer formulation remains a challenge. This study aims to consolidate the advantageous attributes and effective research endeavors regarding *Azotobacter* biofertilizers for fostering sustainable agroecosystems.

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1. Introduction

Rise in global population, there is an urgent need to enhance agricultural productivity to ensure food security (Harold and

Reetz, 2016; Reetz, 2016). Achieving this objective necessitates the optimization of agricultural lands with essential nutrients (Keane, 2009). Annually, approximately 52.3 billion tons of phosphorus and 0.2% of nitrogen are consumed from chemical fertilizers to meet plant nutritional requirements, with nitrogen-based fertilizers contributing to almost half of the global food production; consequently, a significant increase in consumer demand is anticipated by 2025 (Bindraban et al., 2015). However, nearly 50% of traditional nitrogen fertilizers are lost to the environment and soil, leading to soil acidification, nitrous oxide volatilization, and water eutrophication. Addressing the world's agricultural demands requires sustainable and eco-friendly nitrogen fertilizers (Lescourret et al., 2015).

There is considerable scope for developing novel "food and feed" approaches to meet growing demands with reduced reliance on traditional fertilizers. By conserving natural resources, environmental quality can be maintained through meticulous mineral and biological resource management. Biologically fixed nitrogen poses a challenge in highly mobile nutrient environments within agroecosystems. Noteworthy nitrogen-fixing bacteria such as *Azotobacter*, *Azospirillum, Beijerinckia, Herbaspirillum, Burkholderia,* and *Clostridium* exhibit significant efficacy (Malik et al., 2002; Bhattacherjee and Dey, 2014; Kennedy et al., 2015; Ladha et al., 2016). *Azotobacter*, as a nitrogen fixer, serves as a primary nitrogen source in diverse soil ecosystems lacking Symbiotic Nitrogen Fixation (SNF) (Choudhury and Kennedy, 2004; Das and Saha, 2007). Additionally, *Azotobacter* inoculation increases carbon and sulfur content, reducing metal absorption by roots while enhancing nitrogen through Biological Nitrogen Fixation (BNF) (Velmourougane et al., 2019).

Azotobacter is a free-living, gram-negative, anaerobic nitrogen-fixing bacterium with spherical or oval-shaped morphology and cysts, resilient to unfavorable soil conditions. Some *Azotobacter* species exhibit motility with peritrichous flagella, while others are immotile (Martyniuk and Martyniuk, 2003). Polymorphic in size, *Azotobacter* ranges from 2 to 10 mm in length and 1 to 2 mm in width. Initially identified as a free-living anaerobic nitrogen fixer in 1901 by Dutch microbiologist and botanist Beijerinck and colleagues, *Azotobacter* utilizes atmospheric nitrogen in the soil for protein synthesis, rendering nitrogen accessible to plants. *Azotobacter* offers several advantages for crop growth, including the release of plant pathogen inhibitors, growth-promoting hormones, stimulation of rhizosphere microorganisms, and biological nitrogen fixation (Lenart, 2012). Notably, *Azotobacter* releases various amino acids into the medium when supplemented with nitrogen and carbon sources, crucial for promoting plant growth (Kurrey et al., 2018). A. chroococcum, in particular, enhances crop quality and yield through growth hormone release when employed as a microbial inoculant, as extensively studied in various experimental designs. Besides nitrogen fixation, *Azotobacter* exhibits beneficial mechanisms such as siderophore production, ammonia excretion, synthesis of antifungal and pesticidal substances, along with the release of growth-promoting hormones, vitamins, and regulators.

The production of biofertilizers involves three critical steps: strain development, biomass upscaling, and inoculant preparation. To ensure effective application, moist formulations with high microbial density are prepared by aseptically blending bacterial growth broth with specific carriers such as charcoal, peat, and lignite. While growth, development, and maintenance are conducted in research laboratories, commercial biofertilizer production units face challenges in this process. Nonetheless, several advancements have been made to make soil-, region-, and crop-specific microbial strains readily available to production units. Biofertilizers, being high-concentration microbial formulations, require continuous monitoring of desired microorganism presence and cell count to prevent contamination (Rupela et al., 1997). There is a pressing need to develop procedures for maintaining and marketing biofertilizers in small rural areas, promoting their use as an agribusiness alternative to chemical fertilizers.

This review focuses on research and development concerning *Azotobacter*, summarizing the beneficial effects of *Azotobacter* biofertilizers on different crops over the last decade. It highlights the highly beneficial properties of *Azotobacter* and its potential as an alternative to synthetic nitrogen fertilizers. Additionally, the study provides detailed insights into the production, formulation, and commercialization of *Azotobacter*-based biofertilizers, paving the way for further research on product innovation and market investment, addressing associated challenges.

2. Beneficial Activities of *Azotobacter*

2.1. Plant Growth Promotion

Plant growth-promoting hormones, produced by both plants and microorganisms, exert either inhibitory or stimulatory effects on their biochemical and physiological processes (Ansari and Mahmood, 2019a; Ansari and Mahmood, 2019b). In vitro studies have demonstrated that the presence of tryptophan in the media facilitates the release of Indole-3-acetic acid (IAA), as first reported by Brakel and Hilger in 1965, whereas the absence of tryptophan correlates with the absence of IAA (Hennequin and Blachère, 1966). Quantitative studies have shown that A. chroococcum exhibits the presence of auxins and three gibberellins-like compounds in a single strain (Brown et al., 1968). Cultures of a 14-day-old strain have been reported to contain 0.01-0.1 Ig GA3 equivalent/ml and five cytokines in culture filtrate (Nieto and Frankenberger, 1989). These findings have been further confirmed by field experiments on various crops, demonstrating that *Azotobacter* produces growth-promoting hormones (cytokines, auxins, and gibberellins-like compounds) that play a beneficial role in plant growth.

2.2. Nitrogen Fixation

Nitrogen fixation by microorganisms and the recycling of nitrogen, coupled with the maintenance of biosphere nitrogen homeostasis, enhance soil fertility and productivity, making it one of the most crucial biological activities on Earth (Wani et al., 2016). *Azotobacter* emerges as an effective bioinoculant for studying nitrogen fixation, as it can fix large quantities of nitrogen with swift and rapid growth, thereby making atmospheric nitrogen available to plants and converting it into ammonia (Prajapati et al., 2008). Nitrogen-fixing bacteria exhibit resistance to oxygen for hydrogenase uptake and protection of the nitrogenase enzyme through a switching on-off mechanism (Hakeem et al., 2016). The hydrogen released from nitrogen fixation is metabolized during hydrogenase uptake to enhance the growth and nitrogen-fixing ability of *Azotobacter*, with calcium playing a crucial role as a necessary nutrient, while high levels of nitrogen may suppress the ability of *Azotobacter* (Nosrati and Gooshchi, 2013).

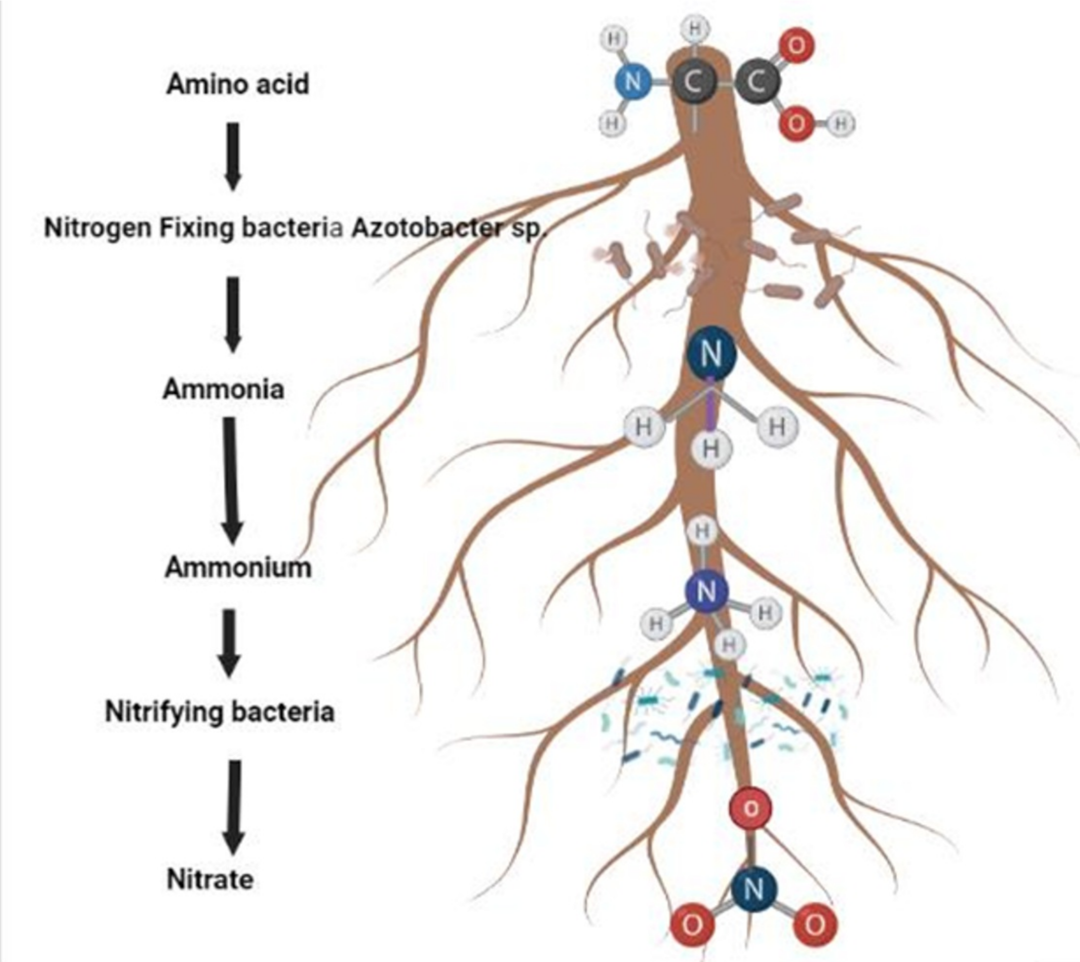

Fig. 1. *Azotobacter* species have a mechanistic role using atmospheric nitrogen in non-symbiotic fixation

Nitrogen and phosphorus are major nutrients that play crucial roles in the biochemistry and physiology of plants and microbes. Various insoluble forms of phosphate are present in the soil, such as aluminum phosphate (Al3PO4), tricalcium phosphate (Ca3PO4)2, and iron phosphate (Fe3PO4). Unfortunately, even if the soil contains a surplus of phosphate, plants cannot utilize it in its unprocessed form due to its low mobility and interaction with other soil constituents (Nosrati et al., 2014; Hinsinger, 2001). *Azotobacter* species are efficient members of phosphorus-solubilizing microbes. For example, about 43% of phosphate rock in Egypt was solubilized by A. vinelandii strain (El-Badry et al., 2016), while another study identified A. exopolysaccharides as the primary factor in microbial solubilization of tricalcium phosphate (Yi et al., 2008). *Azotobacter* species undergo mutagenesis in the soil to improve their ability to solubilize phosphate, making them advantageous candidates over the consumption of chemical fertilizers (Nosrati et al., 2014). Although the exact mechanism of phosphate solubilization is not fully understood, solubilization by organic acids has been widely studied and proposed as the main mechanism of phosphate solubilization (Azaroual et al., 2020).

Nitrogen and phosphorus are vital nutrients essential for the biochemical and physiological processes of both plants and microbes. In soil, various forms of phosphate exist in insoluble states, including aluminum phosphate (Al3PO4), tricalcium phosphate (Ca3PO4)2, and iron phosphate (Fe3PO4). Despite the soil's potential surplus of phosphate, plants cannot readily absorb it in its raw form due to its limited mobility and interactions with other soil components (Nosrati et al., 2014;

Hinsinger, 2001). *Azotobacter* species are known for their efficiency in solubilizing phosphorus. For instance, studies have shown that *A. vinelandii* strain solubilized approximately 43% of phosphate rock in Egypt (El-Badry et al., 2016), while A. exopolysaccharides have been identified as key contributors to the microbial solubilization of tricalcium phosphate (Yi et al., 2008). Through mutagenesis in the soil, *Azotobacter* species enhance their capacity to solubilize phosphate, presenting them as favorable alternatives to chemical fertilizers (Nosrati et al., 2014). While the precise mechanism of phosphate solubilization remains unclear, research suggests that solubilization via organic acids is a widely studied and proposed mechanism (Azaroual et al., 2020).

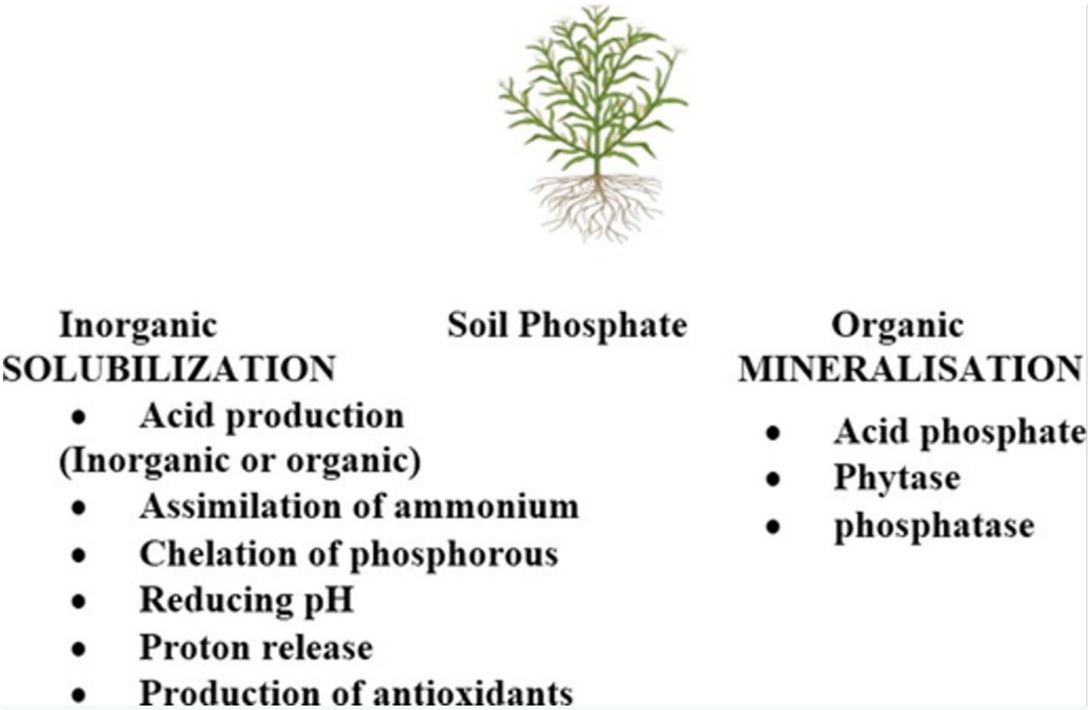

Fig. 2. Representation of the role of Microbial Phosphorus Solubilization (PSM) in Plant growth

2.3. *Siderophore* Production

Siderophores constitute a group of iron-chelating molecules that alter iron availability in the extracellular matrix by outcompeting other ligands (Wichard et al., 2009). Microbes utilize siderophores to access iron-rich areas or sources in the environment. While around five hundred different *siderophores* have been reported, only certain moieties are utilized to capture iron. *Azotobacter* species absorb soluble iron from the environment through membrane-bound receptors in the form of Fe-siderophore complexes (Palanché et al., 2004). These complexes exhibit anti-pathogenic activity by competing with other microorganisms, thereby aiding in plant growth and protection (Hayat et al., 2010). Additionally, *A. vinelandii* possesses the advantageous property of uptaking metals other than iron, including toxic heavy metals, as its *siderophores* can bind to Vanadium (V) and molybdenum (Mo), crucial for nitrogenase activity (Bellenger et al., 2008). *A. chroococcum* is reported to produce cochelins, a novel family of *siderophores*, along with *amphibactins* and *vibroferrin*. Despite its significant agricultural importance, the structure of *siderophores* and the mechanism of iron uptake remain unclear, warranting further investigation into these parameters (McRose et al., 2018).

2.4. Removal of Oil Contamination

Certain species of *Azotobacter* have been investigated for their ability to metabolize various organic substances, including benzoic acid, mannitol, phenolic compounds, and organic acids, serving as carbon and energy sources. Consequently, these bacteria demonstrate efficacy in mitigating oil contamination.

2.5. Pesticide Degradation

Microorganisms play a crucial role in pesticide degradation, with some utilizing pesticides themselves as substrates for degradation (Abo-Amer, 2011). Azotobacter species are known to degrade aromatic compounds and their derivatives, including p-hydroxybenzoate, benzoate, 2,4-D, 2,4,6-trichlorophenol, and protocatechuic acid. They are also capable of degrading several chlorinated phenols such as 4-Chlorophenol, 2-Chlorophenol, 2,4,6-trichlorophenol, and 2,6-Dichlorophenol. *A. chroococcum*, in particular, degrades 2,4-dichlorophenoxyacetic acid as the primary carbon source. Even at low concentrations (around 10 ppm), *A. chroococcum* can degrade lindane both in situ and ex situ (Anupama and Paul, 2009). However, higher concentrations of lindane hinder and reduce the efficiency of degradation, possibly due to the production of inhibitors for bacterial growth (Ergüder et al., 2003). These bacteria not only benefit crop growth and protection but also contribute to environmental harmony.

2.6. Heavy Metal Tolerance

The presence of toxic heavy metals and organic particles from sludge and wastewater exerts pressure on soil microbial communities, altering their activities and diversity and ultimately affecting soil fertility. While some heavy metals are required for microbial growth at low concentrations, high concentrations disrupt essential ecological processes, creating a toxic environment for microorganisms (Afef et al., 2011). The accumulation of heavy metals in the soil indicates the presence of heavy metal-tolerant microbes. These microbes play an essential role in the bioremediation of heavy metal-contaminated environments through mechanisms such as detoxification and resistance (Abo-Amer et al., 2013). Several studies have shown that ten strains of *Azotobacter* from metal-contaminated soil exhibit resistance to certain heavy metals such as Zn2+, Co2+, Cu2+, and Ni2+ (Abo-Amer et al., 2014).

2.7. Survival in Saline Environments

Salinity is a major abiotic stress that adversely affects plant well-being and health by impeding plant physiology, growth, and morphology, ultimately leading to plant death through disturbances in ionic and water movement within plant cells (Maggio et al., 2007). Natural environmental processes and anthropogenic activities contribute to soil salinization (Rengasamy, 2002). To overcome abiotic stresses, microorganisms play a vital role in improving plant growth and biochemical pathways, producing organic compounds that enable plants to tolerate abiotic stresses.

2.8. Disease Management

In addition to their growth-promoting activities, Azotobacter is associated with controlling pathogenic plant diseases. Various studies have demonstrated the ability of different species of *Azotobacter* to suppress diseases, such as the wheat rhizosphere strain of *A. chroococcum*, TRA2, which improves plant growth and exhibits an antagonistic relationship with root rot fungi like *Macrophomina phaseolina* and *F. oxyporum* (Maheshwari et al., 2012). Another study found that the application of *A. chroococcum* on chickpea plants reduced root knot nematode (Meloidogyne incognita) disease (Akram et al., 2016). Disease management mechanisms adopted by microbes include the release of antimicrobial substances, production of *siderophores*, and various growth hormones, all of which depend on environmental conditions, bacterial strains, and the type of pathogen. Previous studies have demonstrated the in vitro production of several antifungal and antimicrobial substances by *A. chroococcum*.

2.9. Stress Tolerance

Azotobacter species are subjected to various abiotic stresses, including temperature, pH, soil moisture, and organic matter levels. Salt concentration can affect the growth-promoting activities of Azotobacter, although tolerance to 10% NaCl has been observed in some species such as *A. salinestris*. *Azotobacter* is a mesophilic microbe requiring an optimal temperature of 25-30°C for activity, with cysts forming at 45-48°C and germinating later under favorable conditions.

3. Current Trends in the Utilization of *Azotobacter* as an Effective Biofertilizer

Azotobacter, a non-parasitic and free-living microorganism, has gained considerable attention due to its ability to significantly enhance plant growth when used either independently or in conjunction with other biofertilizers. When employed in a consortium with other microorganisms, *Azotobacter* demonstrates an amplified effect on plant growth, either by directly providing enhanced nutrients or by synergistically stimulating the action of other biofertilizers.

3.1. *Azotobacter* Consortium with Various Biocontrol Fungi

Studies have shown that when *Azotobacter* is combined with mycorrhizal fungi, known for their phosphorus-solubilizing capabilities, there is a notable enhancement in plant growth characteristics akin to fungal biofertilizers (Behl et al., 2003). The symbiotic relationship between *Azotobacter* and arbuscular mycorrhiza, which are nitrogen-fixing fungi, has been observed to be particularly synergistic (Ishac et al., 1986; Akram et al., 2016). Research has indicated a significant increase in bacterial population, including actinomycetes, when both *Azotobacter chroococcum* and *Glomus fasciculatum* are inoculated in the tomato rhizosphere compared to single inoculum scenarios. Furthermore, the presence of *Glomus fasciculatum* has been found to augment the population of *Azotobacter chroococcum* in the tomato rhizosphere, maintaining it for an extended duration.

3.2. Bacterial Consortium Development

Various experiments conducted in laboratories, fields, and greenhouses have demonstrated positive responses to the coinoculation of Rhizobium and *Azotobacter* (Wani and Gopalakrishnan, 2019). Azotobacter's symbiotic behavior, which includes the production of auxin and gibberellins, enhances root growth, thereby increasing the root area available for infection and subsequently enhancing nitrogen fixation, nodulation, and crop yield (Verma et al., 2014). Additionally, positive reports have been documented for the synergistic behavior of *Azospirillum* and *Azotobacter* when applied to various crops, including *Cicer arietinum* (Parmar and Dadarwal, 1999), *Brassica napus L* (Yasari et al., 2009), *Brassica juncea* (Tilak and Sharma, 2007), and *Capsicum annum L* (Khan et al., 2012).

3.3. Nutrient Use Efficiency Enhanced in Response to Azotobacter Inoculation

Field trials and laboratory experiments have consistently revealed *Azotobacter* as the superior strain for microbial inoculation as a nitrogen biofertilizer, leading to growth and production rate increases of up to 15-20% in maize and 40% in cauliflower compared to standard fertilizers (Bhattacherjee and Dey, 2014). This enhancement is attributed to the production of biologically effective materials by *Azotobacter*, which activate rhizospheric microbial populations and increase the availability of essential nutrients such as nitrogen, phosphorus, and carbon through biological nitrogen fixation and mineralization of biological residues in the soil (Lévai et al. 2008; Lenart 2012). The presence of *Azotobacter* during crop cultivation has been associated with improved seed germination rates, increased nutrient absorption capacity, enhanced root development, leaf expansion, and augmented biomass production (Wani et al., 2016). Studies have also highlighted the positive impact of *Azotobacter*, either alone or in combination with*Azospirillum* or phosphorus-solubilizing organisms, on crop quality, including protein content and fruit yield. Over the past decade, numerous *Azotobacter*-based biofertilizers tailored for various crops have been developed and cataloged, as shown in Table 1

Table 1. Summary of *Azotobacter* based biofertilizers for various crops in the last decade

Sr. No.	Strain/Organism	Crop/ target	Findings	%Yield enhancement	Reference
1.	Azotobacter	Onion (Allium cepa)	The use of fly ash as a carrier to*Azotobacter* inefficient capacity in the presence of a high concentration of heavy metals. In terms of growth, parameters fly ash can be used successfully to enhance the yield.	Chlorophyll 13% carotene 3% and NRA 10%	(Deepti and Mishra 2014)
2.	Azotobacter, Anabaena variabilis, Chlorella vulgaris	Rice (Oryza sativa)	The finding showed that ZOB-1 remained the most efficient consortium with the presence of *Anabaena variabilis*, *Chlorella Vulgaris* and *Azotobacter* sp. As a biofertiliser and simulator, it upgraded the rice growth quality.	(Length of the rice plant sprout) 27%	(Zayadan et al. 2014)
3.	Azotobacter, PSB (Phosphate Solubilizing Bacteria)	Wheat (Triticum aestivum L.)	Liquid or carrier based inoculants of *Azotobacter* along with phospha te solubilising bacteria enhanced the fertility of the soil and provided optimum possible yield.	9.1%	(Khandare et al. 2015)
4.	Azotobacter, vermicompost	Corn/Maize, (Zea mays)	vermicompost and Azotobacter The combination of bio and chemical fertilisers simultaneously not only simultaneously increases the consumption of nutrients but also enhances the various positive traits and yield in maise.	69.4%	(Shirkhani and Nasrolahzadeh 2016)
5.	*Azotobacter chrocoocum, Pseudomonas putida*	Wheat (*Triticum aestivum* L.)	The shortage of water decrease in total chlorophyll and other essential content in the plants. Thus, by the use of biofertilisers based on *Azotobacter chroococcum* and *Pseudomonas putida* the quality and yield of wheat can be improved and increased even with limited availability of water.	17.40%	(Babaei et al. 2017)
	Azotobacter, PSB (Phosphate	Wheat	*Azotobacter* and liquid form of PSB (Phosphate Solubilizing Bacteria) for		(McCarty et al.

6.	Solubilizing Bacteria)	(*Triticum aestivum* L.)	wheat crop remained the most suitable combination for better grain yield.	46%	2017)
7.	*Azotobacter chroococcum, Azospirillum brasilense,* *Pseudomonas putida*	Devil's-pepper *(Rauwolfia serpentina)*	Generally, biofertilisers can enhance the nitrogen content of the soil. The microbial combinations in the case of the medicinal plant *Rauwolfia* increases the yield and product quality.	37.87%	(Rai et al. 2017)
8.	*Azotobacter*	Lettuce *(Lactuca sativa)* Mustard (*Brassica rapa* L. var. *rapa)*	The *Azotobacter* based biofertilisers increase the bioavailability of nitrogen and manuring *serpentina* effect surges the nitrogen fixation.	5.0%.	(Ramadhan et al. 2018)
9.	*Azotobacter beijerinckii* CHB 461*,* *Azotobacter chroococcum* CHB 846 *Azotobacter chroococcum* CHB 869, PBS (Phosphate Solubilizing Bacteria)	Rice (*Oryza sativa*)	The diverse profile of individual *Azotobacter* strains for the capability to utilise the carbon source. Thus, in this study the plant growth promoting trait was enhanced for the cultivation of rice and providing base for biofertiliser development and formulation.	17.64%	(Chen et al. 2018)
10.	*Azotobacter niger*	Calabash Gourd (*Lagenaria siceraria*) Okra (*Abelmoschus esculentu) s*	The Azotobacter *niger* strain during the field trails along with*L. siceraria* and *A. esculentus* were selected for biofertiliser trails. Moreover, the combination of phosphorus solubilising and *A. niger* had enhanced the overall quality and yield.	2.97% 38.88%	(Din et al. 2019)
11.	*Azotobacter*	Cauliflower (*Brassica oleracea* L. var. *botrytis*)	*Azotobacter* with 25% and 50% Nitrogen on Cauliflower (*Brassica oleracea* L. var. *botrytis*) as biofertiliser improves morphological characters and yield.	29.80%	(ASM 2019)
12.	*Azotobacter* Manure and inorganic fertiliser (N.P.K.).	Wheat (*Triticum aestivum* L.) var. gautam *Abelmoschus esculentus*	The *Azotobacter* with manure and inorganic fertiliser (N.P.K.) for wheat as biofertiliser resulted in product yield.	16.5%–19.42%	(Mahato and Kafle 2018)

13.	Azotobacter PBS (Phosphate Solubilizing Bacteria)	Wheat (*Triticum aestivum* L.)	The *Azotobacter* and PBS (Phosphate Solubilizing Bacteria) with 75% and 100% N for making carrier and liquid formulations of biofertilisers which enhances the yield and nutrients uptake and soil biological properties.	10.8%	(Khandare et al. 2020)
14.	*Azotobacter spp.* *Streptomyces badius*	Wheat (*Triticum aestivum* L.)	Biofertilisers are comprised of proficient microorganisms that can fix nitrogen and solubilise phosphate. The infield experiments show 87.5% N + 87.5% P with *Azotobacter* spp. and *Streptomyces badius* (Stand 75% N, 75% P + *Azotobacter*	35.12 %	(Kumar et al. 2021)
15.	*Azotobacter* PBS (Phosphate Solubilizing Bacteria) 100%RDF	Sesame (*Sesamum indicum* L.)	The *Azotobacter*, PBS (Phosphate Solubilizing Bacteria) and RDF were found to be effective biofertilisers for Sesame (*Sesamum indicum* L.) for increasing quality, productivity and yield.	20.55%	(Aglawe et al. 2021)
16.	*Azotobacter* 75%R.D.N.	Cabbage (*Brassica oleracea)* var. *capitata* L.	*Azotobacter* along with 75% R.D.N. as biofertiliser for Cabbage *Brassica oleracea.* var. *capitata* L. for better yield quality of the plant.	75.13%	(Anushruti et al. 2022)
17.	*Azotobacter* Azospirillum	Cherry Tomato (*Solanum lycopersicum*)	*Azotobacter* and *Azospirillum* were recorded in cherry Tomato (*Solanum Lycopersicum*) as biofertilisers. Under different salinity levels, the qualitative and quantitative attributes of plants significantly improve.	60.41%	(El-Beltagi et al. 2022)
18.	*Azotobacter Rhizophagus*	Yam (*Dioscorea alata*)	The nitrogen-fixing *Azotobacter* and *Rhizophagus* provide significant amount of nutrients to get a decent yield of nutrient of yam. This biofertiliser treatment improves the nutrient quality and traits yield.	40.88 %	(Kumar et al. 2022)

4. Production of biofertilizers involves a critical aspect

The implementation of quality control procedures to ensure product quality, competency, and compliance with standards (Arora et al., 2016). The assurance of quality and purity of the inoculum is paramount and is achieved through several steps, including screening and competency assessment at the laboratory level, formulation preparation, assembly, and storage at the industrial level, all conducted in accordance with established standards (see Fig. 1). Unfortunately, many biofertilizer units fail to adhere to these standards and protocols due to a lack of knowledge and technical expertise.

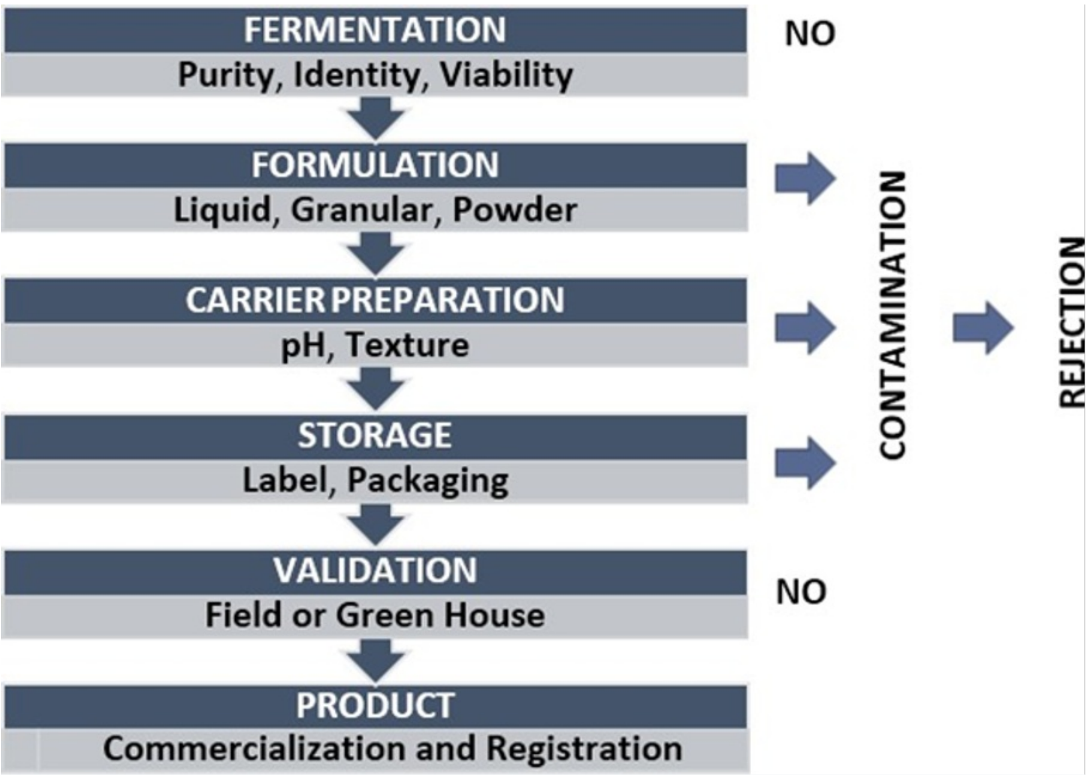

Fig. 3. Bio fertilization Manufacturing is six stage process Quality Control is acquired at a very stage before passing to the next and the project can be rejected at any stage where quality control is compromised where till commercialization

4.1. Strain Identification Techniques

Currently, there is a lack of standardized quality control procedures and regulations at the international level for assessing bacterial activity and growth during inoculum preparation and formulation. However, various proficiency testing methods, such as the spread plate method and Most Probable Number (MPN) count method, are employed to determine viability counts, each with its own advantages and limitations. While these enumeration methods are effective for assessing population levels, they may not be specific to individual strains and can be influenced by contaminants, thus limiting their accuracy in strain identification. To address this limitation, molecular biology techniques are utilized for precise and accurate assessment of microbial populations in the rhizosphere, soil, and commercially used inoculums. Specifically, SCAR (Sequence Characterized Amplified Region) marker and qPCR (Quantitative Polymerase Chain Reaction) methods are employed for assessing inoculant cell load gram/ml and fingerprinting, as demonstrated in Fig. 2 (Reddypriya et al., 2019). Through DNA analysis, bacterial strains like *B. megaterium, A. brasilense,* and *A. chroococcum* in the rhizosphere of biofertilizer-inoculated crops, such as maize, can be identified based on their specific DNA lengths (375bp, 584bp, and 299bp, respectively). Additionally, for farmer satisfaction and quality assurance, SCAR markers are targeted using RTPCR (Reverse Transcription Polymerase Chain Reaction) and Multiplex PCR methods. Immunoblotting procedures are also employed for the detection of specific strains like *Citrobacter freundii* in carrier media, whether sterile or unsterile, in commercial products (Rodríguez-Couto et al., 2009). Furthermore, recent studies have explored the use of Next Generation Sequencing (NGS) techniques for comprehensive identification and enumeration of microbes (Abbasian et al., 2018).

5. Formulation

A high-quality formulation is characterized by simplicity, cost-effectiveness, and efficient delivery to plants, incorporating biocontrol and biofertilizer strains that can be readily applied to crops. The shelf life and application methods of the formulation are contingent upon the physical state, whether solid or liquid, of the strain applied to crops (Mercado-Blanco and JJ Lugtenberg, 2014). In the selection of the method, factors such as the efficacy of microbial biomass, adherence, coverage, sustainability, and the presence of microbial cells at the targeted site after application are crucial. Bacterial inoculants are available in both solid forms (such as dust and wettable powders, granules, or microgranules) and liquids (including water, oil, or emulsions) (Schisler et al., 2004). Gram-positive spore-producing bacteria are commonly utilized in bioformulation production, with some treatments exhibiting high resistance to spores. Similarly, sporulating fungi can be effectively utilized in dry formulations, such as powders and granules (Kaur et al., 2011; Woo et al., 2014). Furthermore, gram-negative bacterial strains are sensitive to extreme environmental conditions such as heat and drought (Kamilova et al., 2015). The efficacy of the formulation may be compromised when contaminated with undesirable cells, which can alter or deactivate the strain's properties. The cost of formulation increases when production occurs under sterile conditions; however, a simple or cost-effective process is often preferred and desirable (Arora and Mishra, 2016).

6. Genetic Engineering of *Azotobacter*

For the large-scale production of *Azotobacter*, enhancing its capacity and growth in the fermentation process while maintaining contamination-free conditions is essential to improve various nutritional and cultural parameters (Gomare et al., 2013). Genetic engineering techniques involving the insertion or deletion of targeted gene(s) can effectively boost the capabilities of *Azotobacter*. For instance, in the nitrogen fixation mechanism of *Azotobacter*, the nif-A gene acts as an activator, while nif-L functions as an inhibitor. In the presence of oxygen, the inhibitor and activator form complexes that hinder function but are associated with increased levels of ammonium release (Das, 2019). Genetic modification involving the disruption of a portion or the complete nif-L gene results in the release of a higher amount of ammonium compared to the wild strain (Ortiz-Marquez et al., 2012). In another study, the HKD15 strain of *A. chroococcum* was developed through the deletion of a nif-L negative regulatory gene, which was successfully utilized as an alternative to urea fertilizer, exhibiting a 60% increase in wheat yield (Bageshwar et al., 2017). Furthermore, besides employing genetic engineering for nitrogen fixation, phosphate solubilization genes can also be modified to improve formulation, produce more resilient cysts, and increase shelf life to withstand harsh environments.

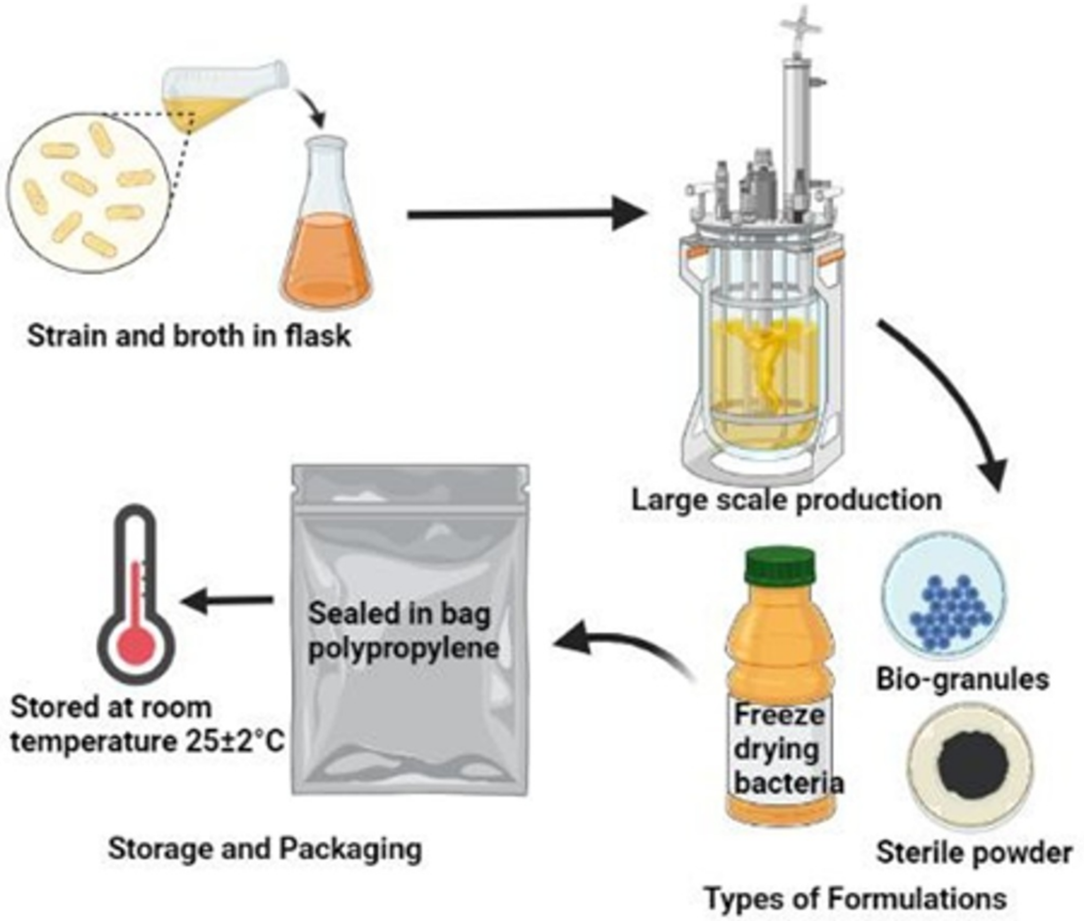

Fig. 4. Schematic illustration for biofertilizer production

7. Prospects and Commercialization of *Azotobacter* Biofertilizer

Azotobacter stands out as one of the most advantageous microbes for enhancing crop productivity as a biofertilizer. Its versatility lies not only in nitrogen fixation and phosphorus solubilization but also in its ability to produce growth hormones, siderophores, and pesticides, ultimately contributing to soil health improvement. A comprehensive understanding of each of these facets of *Azotobacter* holds the promise of advancing crop enhancement in the future (Kyaw et al., 2019). Nevertheless, unraveling the molecular mechanisms involved necessitates research efforts geared towards developing advanced screening methods and characterizing essential pesticide and growth-promoting compounds derived from *Azotobacter* (Verma et al., 2010). Moreover, delving into soil genomics could unveil Azotobacter's efficiency in enhancing soil fertility. To fully exploit the potential of biofertilizers, detailed studies are imperative to identify host plants compatible with each strain of *Azotobacter* (Wani et al., 2013).

Conflict of Interests

The authors have no conflict of interest.

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