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ORIGINAL RESEARCH ARTICLE

Azotobacter Species as Sustainable Biofertilizers for Crop Productivity, Soil and Plant Health: A Comprehensive Review

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ABSTRACT

Azotobacter spp. are heterotrophic, nonsymbiotic, free-living nitrogen-fixing bacteria that live primarily in neutral or alkaline soils. This review presents the most current literature, following PRISMA flow guidelines, to provide a comprehensive and contemporary view of *Azotobacter* as a multifunctional biofertilizer, including its mechanisms of action on crop yield, soil health, and plant health. By producing growth compounds and affecting plant growth, *Azotobacter* has the potential to be used as microbial inoculants, boosting agricultural crop yields. Azotobacteria are free-living nitrogen-fixing bacteria that produce cytokinins, auxins, and other compounds that are key growth regulators and promoters. It protects plants from phytopathogens, promotes rhizosphere microorganisms, and safeguards plant health. *Azotobacter*-inoculated plants exhibit improved plant health through a variety of methods. For instance, speed up the synthesis of plant hormones like indole-3-acetic acid, remove stressors, fix nitrogen, degrade pesticides and oil globules, and metabolize heavy metals. *Azotobacter* application increased wheat yield by up to 30%, Maize by 20% and tomato by 20% compared with chemical fertilizers. Their application can improve crop yields, soil fertility, and plant health, offering an eco-friendly alternative to chemical fertilizers and supporting long-term soil management. Future research would focus on molecular mechanisms, strain selection, and integration with modern soil genomics to maximize benefits.

ARTICLE HISTORY

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INTRODUCTION

Biofertilizers are materials that include microorganisms. When applied to plant surfaces, soil, or seeds, these substances colonise the interior of the plant or the rhizosphere and increase the supply of primary nutrients to the host plant, which in turn encourages development. Chemical fertilizers continue to increase agricultural yields, but the soil and environment are becoming more polluted and deficient in essential nutrients (Sumbul *et al.*, 2020). When plants are inoculated with biofertilizers, they produce water-soluble vitamins and phytohormones thereby becoming more disease-resistant (Zaib *et al.*, 2023). Following the discovery of *Azotobacter*, blue-green algae, and a host of other microorganisms, Nobbe and Hiltner introduced the "Nitragin," a laboratory culture of rhizobia, in 1895, marking the beginning of the commercial history of biofertilizers. Vesicular-abuscular mycorrhiza (VAM) and *Azospirilla* were only recently identified. The nitrogenase enzyme complex catalyzes a reaction that may convert atmospheric nitrogen (N_2) to ammonia, making it one of the most intriguing non-symbiotic bacteria with enormous potential for the generation of biofertilizers (Mohan *et al.*, 2024). For a century, the Azotobacter genus has been used as a biofertilizer. Martinus Willem Beijerinck, a soil microbiologist and the pioneer of environmental microbiology, originally defined this genus in 1901. Azotobacteria are frequently detected in soil samples and belong to the family Azotobacteraceae. Known species of the genus *Azotobacter* are *Azotobacter vinelandii, Azotobacter chroococcum, Azotobacter beijerinckii, Azotobacter paspali, Azotobacter armeniacus, Azotobacter nigricans* and *Azotobacter salinestris* (Mukungu 2024). Despite decades of study, limited integration of Azotobacter's bioremediation and biofertilizer roles in current sustainable agriculture models warrants a holistic synthesis. Current research involves optimising the use of *Azotobacter* in sustainable agriculture

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and exploring integrated approaches with other biofertilizers (such as phosphate-solubilising bacteria) and organic matter to maximise efficiency. There is also ongoing genetic analysis to understand the mechanisms underlying nitrogen fixation and plant growth stimulation. This research draws attention to a niche where *Azotobacter* is particularly effective, guiding researchers to target crop and edaphic conditions where non symbiotic fixation can be maximized. It also lies in systematically reviewing and synthesizing the most current literature (using PRISMA FLOW 2020 guidelines) to provide a comprehensive and contemporary view of *Azotobacter* as a multifunctional biofertilizer, including its mechanisms of action on crop yield, soil health and plant health

METHODOLOGY

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines were developed to guide the search strategy, eligibility criteria, and synthesis approach (Munir *et al.*, 2025).

Search Strategy

A comprehensive search strategy was used to identify all potentially relevant literature using the keywords *Azotobacter*, biofertilizers, phytohormones, and phytopathogens. A systematic search was conducted across three major electronic databases: Google Scholar, Web of Science, and PubMed, for articles published between 2018 and 2025. Prior to screening, all identified records were exported to a reference management software and duplicate records were systematically identified and removed. The Final Records for Screening was proceeded to the next stage.

Eligibility Criteria

The inclusion/exclusion of these studies was based on a detailed assessment of the remaining articles.

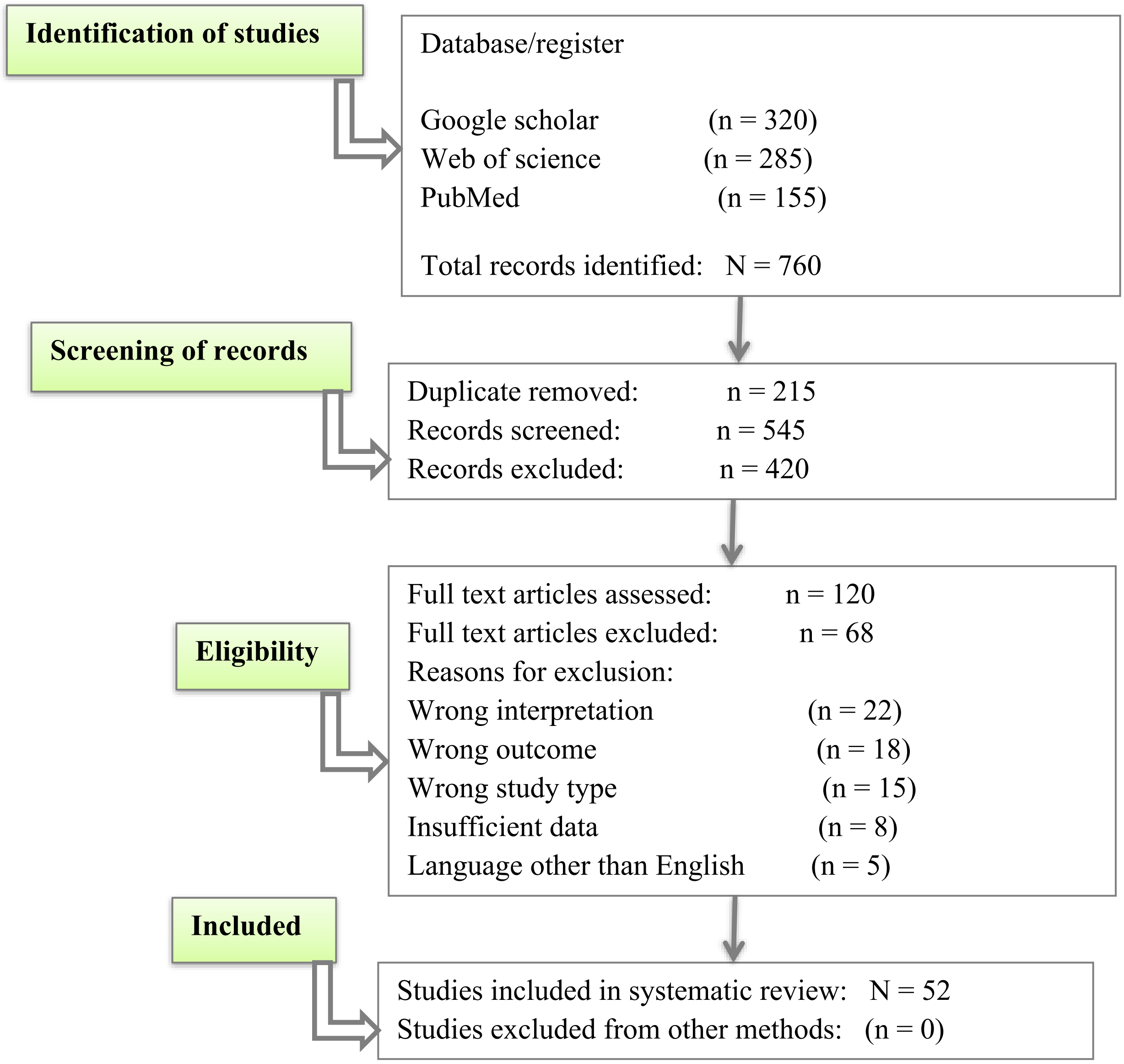

Figure 1: Prisma Flow Diagram

Inclusion Criteria

The original research articles related to biofertilizers, *Azotobacter*, plant health, crop yield, soil health were included in this studies.

Exclusion Criteria

All records that clearly did not match with the relevant literature on *Azotobacter*, biofertilizers, phytohormones, phytopathogens were excluded in this research.

Final Selection

The final count of these studies that form the evidence base for the systematic review, after passing all stages of screening, was 12 articles deemed suitable.

Method of Application of *Azotobacter*

Azotobacter is used as a biofertilizer by applying it directly to seeds (seed treatment), soil (broadcast/furrow), or plant roots (root dipping), effectively converting atmospheric nitrogen into plant-usable forms, producing growth hormones (like IAA, cytokinins), and improving soil health, leading to better crop yields and reduced reliance on chemical fertilizers, often in liquid or powder formulations (Mukungu 2024).

Seed Treatment

Normally, 500 g of *Azotobacter* is required to apply to a hectare of field. It is mixed with water and an adhesive material so that the seed coat is not broken, and it is dried for half an hour before sowing. Seeds are coated with a slurry (often sugar/gum solution) containing *Azotobacter* powder before planting, ensuring bacteria are present at germination (Razmjooei *et al.*, 2022)

Soil Application

About 2 kg of *Azotobacter* are mixed with 40-50 kg of decomposed Farm Yard Manure and are broad cast at the time of sowing/prior to sowing.To use *Azotobacter* in soil, you apply it as a seed treatment, soil application, or via drip irrigation, typically by mixing the biofertilizer with water and coating seeds before planting or applying to the root zone, which enhances nitrogen fixation for non-leguminous crops like wheat, paddy, and cotton, improving soil fertility and reducing synthetic fertilizer needs (Razmjooe *et al.*, 2022). The bacterial powder or liquid is mixed into the soil (e.g., in furrows with seeds) or broadcasted for even distribution around roots.

Root Dipping

Seedlings' roots are dipped into an *Azotobacter* suspension before transplanting. This is mainly done in transplanted crops. A slurry of *Azotobacter* biofertilizer is prepared. Seedlings are dipped in this slurry for about 15 minutes, allowed to dry and then transplanted (Razmjooei *et al.*, 2022).

RESULTS AND DISCUSSIONS

Study Selection

Figure 1 shows that a total of 760 records from all sources were identified. After removing 215 duplicate records, 545 records remained for the screening. The title and abstract screening excluded 420 records unrelated to *Azotobacter as* biofertilizers. The 120 full-text articles were successfully retrieved and assessed for detailed eligibility, leading to the exclusion of the remaining 5 articles, which were related to biofertilizers; 68 articles were further excluded due to incorrect interpretation, incorrect study type, or incorrect outcome. After passing all screening and eligibility stages, the remaining 52 articles were included in the systematic review, leading to original research on *Azotobacter*, Biofertilizers, phytohormones, and phytopathogens.

Mechanisms of Action of *Azotobacter* on Crop Yield

Table 1: Yield Improvements in Major Crops with *Azotobacter* Biofertilizer.

Crop	Yield Increase (%)	Citations
Maize	15–20	(Aasfar *et al.*, 2021; Sagar *et al.*, 2022; Mohan *et al.*, 2024)
Wheat	8–30	(Mohan *et al.*, 2024; Gothandapani *et al.*, 2017)
Rice	10	(Mohan *et al.*, 2024)
Vegetables	2–24	(Aasfar *et al.*, 2021; Mohan *et al.*, 2024; Gothandapani *et al.*, 2017)

Table 1 above shows that *Azotobacter* biofertilizers can boost crop yields by 5–40%, depending on the crop, according to field and greenhouse research, with considerable gains in maize, wheat, rice, and vegetables. Using both organic and inorganic fertilisers together increases soil microbial activity, plant growth, and nutrient uptake. *Azotobacter* can be combined with other helpful microorganisms to provide synergistic effects, and it works particularly well in neutral to alkaline soils (Aasfar *et al.*, 2021; Sagar *et al.*, 2022).

Fixation of Nitrogen

One macronutrient that plants require in great amounts is nitrogen. It performs a variety of functions, such as forming base pairs of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), phosphate groups of proteins (like the hemp group of chlorophyll), hormones like cytokines, metal uptake, xylem and phloem transport and as osmo-modifiers (like spinach and lettuce) (Mukungu 2024). In addition, nitrogen is taken up from the soil by fertilisers, rainfall, lightning, biological nitrogen fixation, and the breakdown of organic matter, and is absorbed by plants as ammonium, nitrates, and occasionally urea (Mohan *et al.*, 2024). According to

Zhang *et al.* (2018), biological nitrogen fixation is crucial for preserving soil fertility and raising crop productivity. Azotobacteria are used in nitrogen fixation, thereby helping convert atmospheric nitrogen into ammonia using nitrogenase enzymes (Sumbul *et al.*, 2020). Figure 2 below shows *Azotobacter* interactions in the rhizosphere

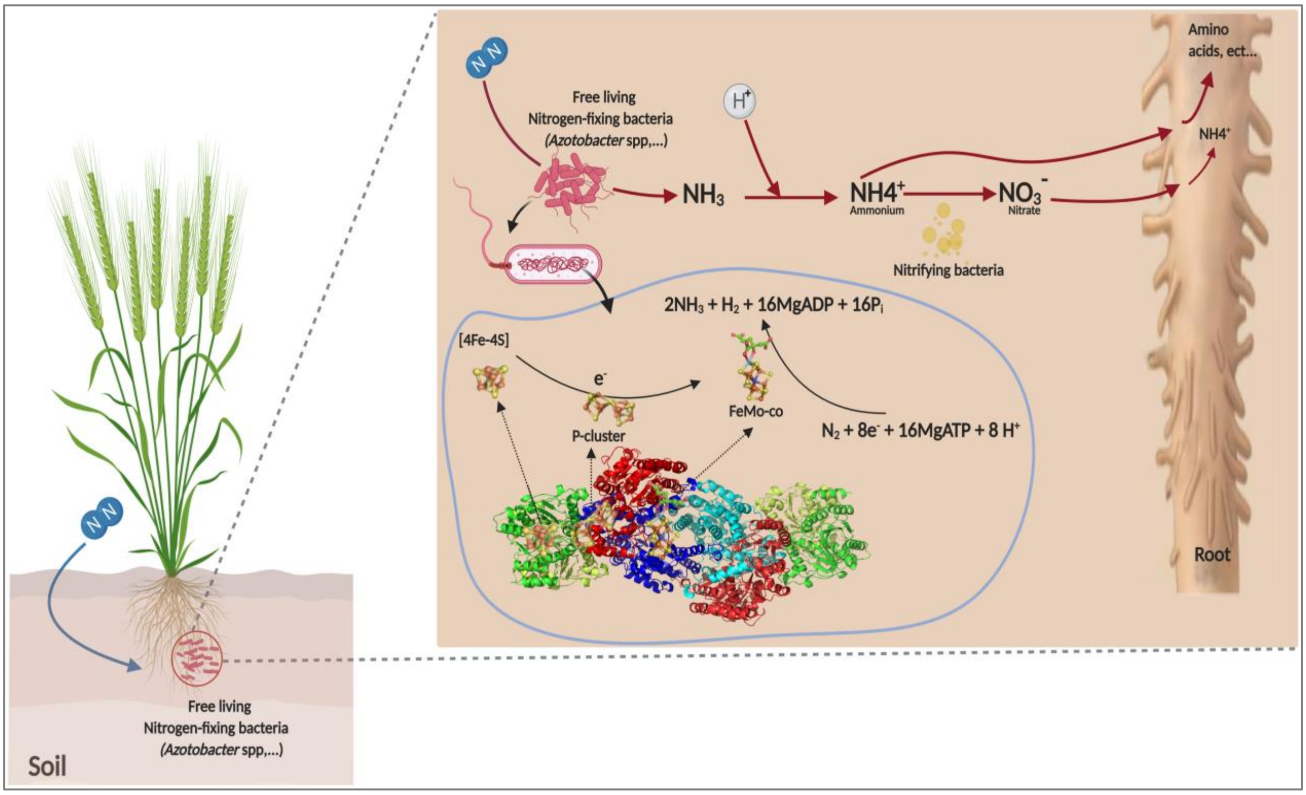

Figure 2: *Azotobacter* Interaction in the Rhizosphere (Aasfar *et al.*, 2021).

Table 2: Bioactive Metabolites by *Azotobacter* and their Plant Growth effects.

Bioactive Metabolites	Effect on Plant Growth	Citations
Auxin (Indole-3-acetic acid (IAA)	Promotes cell division and elongation, leading to increased root and shoot length, enhanced root branching and surface area, and improved nutrient and water absorption	(Jehani *et al.*, 2023; Aasfar *et al.*, 2021)
Gibberellins	Stimulates seed germination, stem elongation, and overall plant height	(Aasfar *et al.*, 2021)
Cytokinins	Promotes cell division, delays senescence (aging), and influences shoot development.	(Aasfar *et al.*, 2021;
Siderophores (e.g., azotobactin, protochelin)	Chelates insoluble iron (Fe^{3+}) in the soil, making it available for plant uptake. This also deprives fungal pathogens of iron, providing a biocontrol effect.	(Timofeeva *et al.*,2022)
Exopolysaccharides (EPS)	Forms a protective biofilm around bacteria and roots, helping maintain cellular hydration during drought and improving soil structure.	(Mohan *et al.*, 2024).
Nitrogenase	Converts atmospheric nitrogen into ammonia, providing a direct nutrient source for the plant.	(Daniel *et al.*, 2022; (Aasfar *et al.*, 2021)
Phosphatase	Increases the availability of phosphorus, a vital macronutrient for energy transfer and growth	(Timofeeva *et al.*,2022; Timofeeva *et al.*,2023)
ACC Deaminase	Reduces inhibitory ethylene levels during stress, preventing growth retardation and increasing stress resistance.	(Timofeeva *et al.*,2023)

Phytohormones Produced by *Azotobacter*

Besides fixing atmospheric nitrogen used by plants, *Azotobacter* generate hormones that promote plant development (Jehani *et al.*, 2023). *Azotobacter vinelandii* & *Azotobacter chroococcum* have been reported to secrete auxins, e.g. Indole acetic acid (IAA), which promotes stem elongation, gibberellins, which help plants to divide, and cytokinins, which improve seed germination (Aasfar *et al.*, 2021).

Table 3: Bioremediation Potentials and Pollutant type degraded by *Azotobacter*

Pollutant Category	Examples of Pollutants	Species / Strain	Bioremediation Mechanism	Citations
Hydrocarbons	Crude oil, Motor oil, Diesel, Xylene, Anthracene	*A. vinelandii* *A. chroococcum*,	Assimilation as carbon source; biosurfactant production; stimulation of other hydrocarbon-oxidizers	(Garcha *et al.*, 2024; Aasfar *et al.*, 2021)
Pesticides	Pendimethalin, Chlorpyrifos, Carbendazim, Lindane	*A. vinelandii*, *A. chroococcum*, *A. salinestris*	Metabolism into non-toxic products (up to 90–100% degradation); enzymatic hydrolysis	(Timmusk et al., 2017)
Herbicides	2,4-D (2,4-Dichlorophenoxyacetic acid)	*A. chroococcum*, *A. vinelandii*	Metabolism as a sole carbon source; high resistance even at elevated concentrations	(Aasfar *et al.*, 2021)
Heavy Metals	Cd, Cr, Pb, Ni, Zn, Cu, Co, Hg	*Azotobacter* spp. isolates	Bioaccumulation; binding via extracellular polymeric substances (EPS); plasmid-mediated resistance	(Abbas *et al.*, 2013; Mohan *et al.*, 2024)

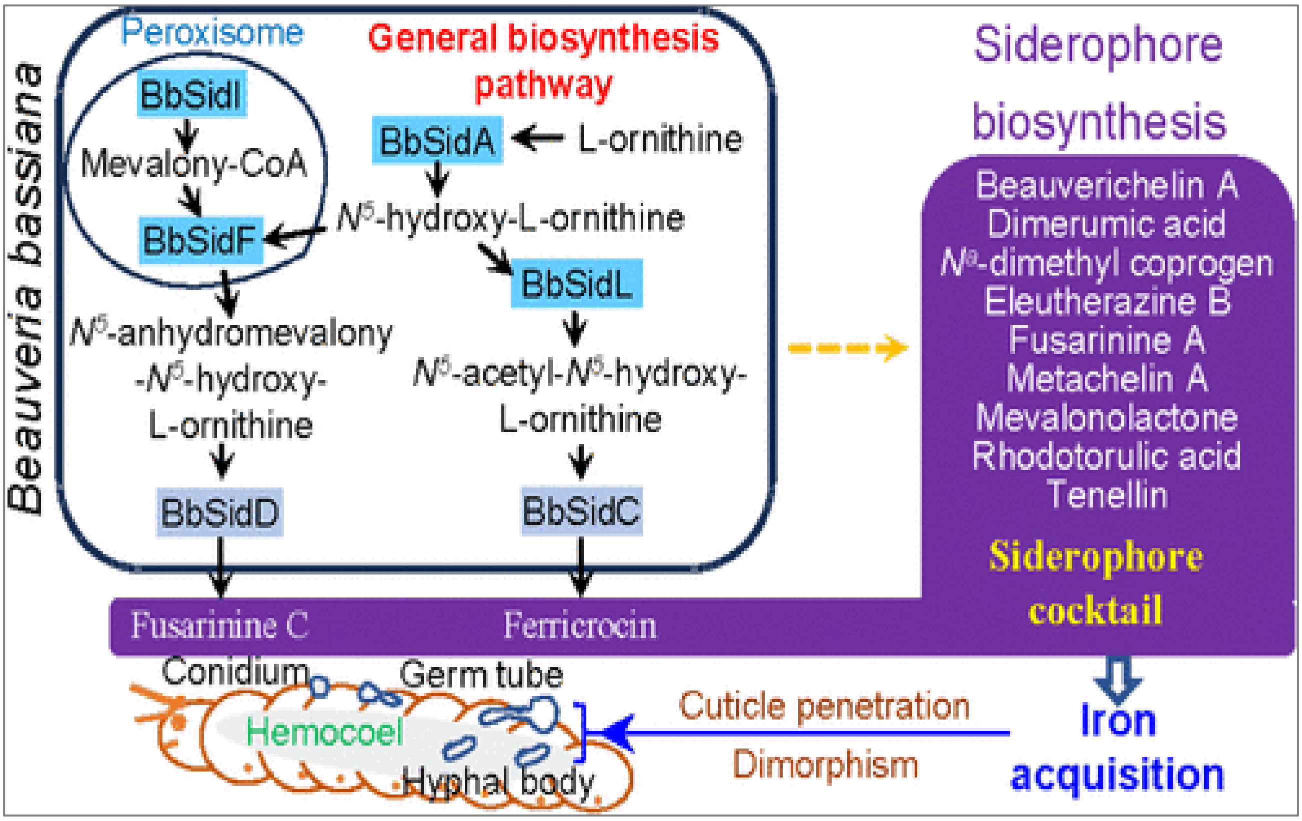

Figure 3: **Siderophore biosynthesis pathways (Ting *et al.*, 2024)**

Azotobacter Solubilize Phosphorus

Phosphorus is essential to plant growth and is required in significant quantities; it promotes legume production and growth, as well as the mass and number of nodules (Mukherjee *et al.*, 2023). Its roles include: phospholipids in membranes; phosphorus in proteins for biological processes; and raising agricultural productivity and quality. It is also crucial to seed development. Analysis showed that it constitutes a significant component of seeds and fruits (Naik *et al.*, 2019). Phosphorus is absorbed by plants as phosphate and is also added to the soil via superphosphate, bone meal, and fertilisers (Aasfar *et al.*, 2021). Insoluble phosphorus in the soil that is necessary for plant growth can be dissolved by azotobacteria (Naik *et al*., 2019).

Mechanisms of Action of A*zotobacter* on Soil Health

Azotobacter species aids in better soil reclamation through the following activities:

Bioremediation

Utilizing biological organisms, primarily microbes, to eliminate contaminants, particularly from contaminated soil or water, is known as bioremediation (Mahan *et al*., 2024). It works well to lessen the quantity of pollutants in the atmosphere. Native soil microbes that can eliminate the pollutants are used in the commonly bioremediation technique. A significant fraction of the soil microbiota is made up of the genus *Azotobacter* (Aasfar *et al*., 2021).

Hydrocarbon Degradation

Using crude oil as its only carbon source, *Azotobacter chroococcum* has been shown to degrade up to 58% of it. Additionally, it creates biosurfactants that emulsify naphthalene, kerosene, and waste motor oil to increase their bioavailability for microbial digestion (Garcha *et al*., 2024; Aasfar *et al*., 2021)

Oil-Contamination Removal

Crude oil pollution alters the physicochemical characteristics of soil, increasing its pH to 8.0 and reducing its available phosphorus (Daniel *et al*., 2022). Since bacteria can absorb hydrocarbons from oil as their sole source of carbon and energy, adding nitrogen-fixing bacteria to soils contaminated with oil speeds up the rate of purification in the contaminated soil (Garcha *et al*., 2024). It has been suggested that nitrogen-fixing bacteria utilise a range of bioactive substances to support rhizosphere microbial development and proliferation. According to Timmusk *et al*. (2017), these bacteria can therefore be employed to promote bioremediation of oil-contaminated soils.

Pesticide Degradation

In contaminated soil, microorganisms are efficient degraders of pesticides. Hexachlorocyclohexane (HCH), also known as linden, is a carcinogenic pesticide that is among the most widely used organochloride pesticides (Timmusk *et al*., 2017). When pesticides are applied to soil, they may decompose and serve as a substrate for microbes. Numerous chlorinated phenols, such as 2-chlorophenol, 4-chlorophenol, 2,4-6-trichlorophenol, and 2,6-dichlorophenol, as well as its derivatives, such as benzoic acid and p-hydroxybenzoic acid, can be broken down by *Azotobacter* (Garcha *et al*., 2024). The only carbon source that *Azotobacter chroococcum* extensively digested was 2,4-dichlorophenoxyacetic acid (2,-4-D) (Kyaw *et al*., 2019). Certain cultures of *Azotobacter chroococcum* have been shown to effectively degrade lindane, both in situ and ex situ, at lower doses, e.g., 10 ppm (Waleed *et al*., 2025). *Azotobacter chroococcum* has been shown by Soleimanzadeh and Gooshchi (2013) to convert the common herbicide pendimethalin into a non-toxic substance, demonstrating that this bacterium is necessary for both environmental harmony and vigorous crop production. *Azotobacter* strains can degrade major agricultural pesticides such as carbendazim and chlorpyrifos by 90% to 100%. According to recent 2025 research, some species can hydrolyze phosphorus-oxygen linkages to degrade pesticides, achieving 81% degradation in just 2 months (Garcha *et al*., 2024).

Tolerance for Heavy Metals

Bacterial exopolysaccharides (EPS) have drawn more attention in recent years. Remarkably, the majority of bacterial polysaccharides contain uronic acid, pentose, polypeptide moieties, or other non-sugar constituents. Members of the genus *Azotobacter* are primarily known as free-living nitrogen fixers, but they possess significant potential for bioremediating diverse environmental pollutants, including hydrocarbons, pesticides, and heavy metals (Garcha *et al*., 2024).

Table 3 below summarizes the types of pollutants degraded or mitigated by *Azotobacter* and the associated mechanisms.

Mode of Action of A*zotobacter* on Plants Health.

Azotobacter is recognised for reducing plant pathogenic diseases and for having positive effects on plant growth (Emami *et al*., 2020). According to Maheshwari *et al*. (2012), the wheat rhizosphere isolated with the *Azotobacter chroococcum* TRA2 strain exhibits potent anti-*Macrophomina phaseolina* and anti-*Fusarium oxysporum* activity, providing plants with exceptional protection through active colonisation of wheat roots. According to Akram *et al*. (2016), the root-knot nematode's incidence was decreased in chickpea plants when *Azotobacter chroococcum* was applied. Ferric acid carriers, hydrogen cyanide (HCN), and polyhydroxybutyrate (PHB) are all produced by *Azotobacter*. Alginic acid and antifungal substances are produced on a wide scale using PHB. Ferric is a low-molecular-weight iron (Fe) chelating molecule produced and utilised by numerous bacterial and fungal groups. HCN can prevent root colonisation infections in plant roots. These substances are produced by different types of bacteria in response to the iron deficit that typically arises in neutral to alkaline pH soils because iron is poorly soluble at high pH (Bharti et *al*., 2016).

Siderophores are released by *Azotobacter* when the iron supply is low. It has been documented that *A. vinelandii* can release at least five distinct siderophores, which are basically antibiotics. These consist of aminokerin, azotokerin, protokerin, siderophore 2,3-dihydroxybenzoic acid, and azotobactin. In biotechnology, siderophores are primarily used as antimicrobial agents, drug delivery agents, and soil-cleaning agents (Barrera and Soto 2010). Products made by Siderophore-Azotobacter, which isolate Fe^{3+} near the roots, can stop the growth of harmful microbes. The siderophores of different bacteria can serve as an iron source for several plants (Aasfar *et al.*, 2021). Around the colony, *A. salinestris* produces an orange-yellow to golden hue, with UV light revealing the colour's intensity. Azotobacter species can produce a variety of siderophores; *Azotobacter salinestris* produces siderophores with a diameter of 2-4 mm (Aasfar *et al.*, 2021).

Azotobacter and a saline environment

Among the various abiotic stresses, salinity is considered a major abiotic stressor that undermines plant health and well-being (Emami *et al.*, 2020). Salinity causes significant disruption of water and ionic movement in plant cells, hindering plant growth, morphology, physiology, and other activities, leading to plant death (Baars *et al.*, 2016). *Azotobacter* spp. have been found to improve plant health by alleviating various biotic and abiotic stresses (Ansari and Mahmood 2019a, b). There is an implication of various mechanisms occurring at the soil plant-microbe interfaces that regulate the plant growth and yield performance. Application of *Azotobacter* spp. is considered very important in improving plant health by alleviating stress in hostile environments. *Azotobacter* induces increased plant tolerance to both biotic and abiotic stresses (Arora *et al.*, 2018).

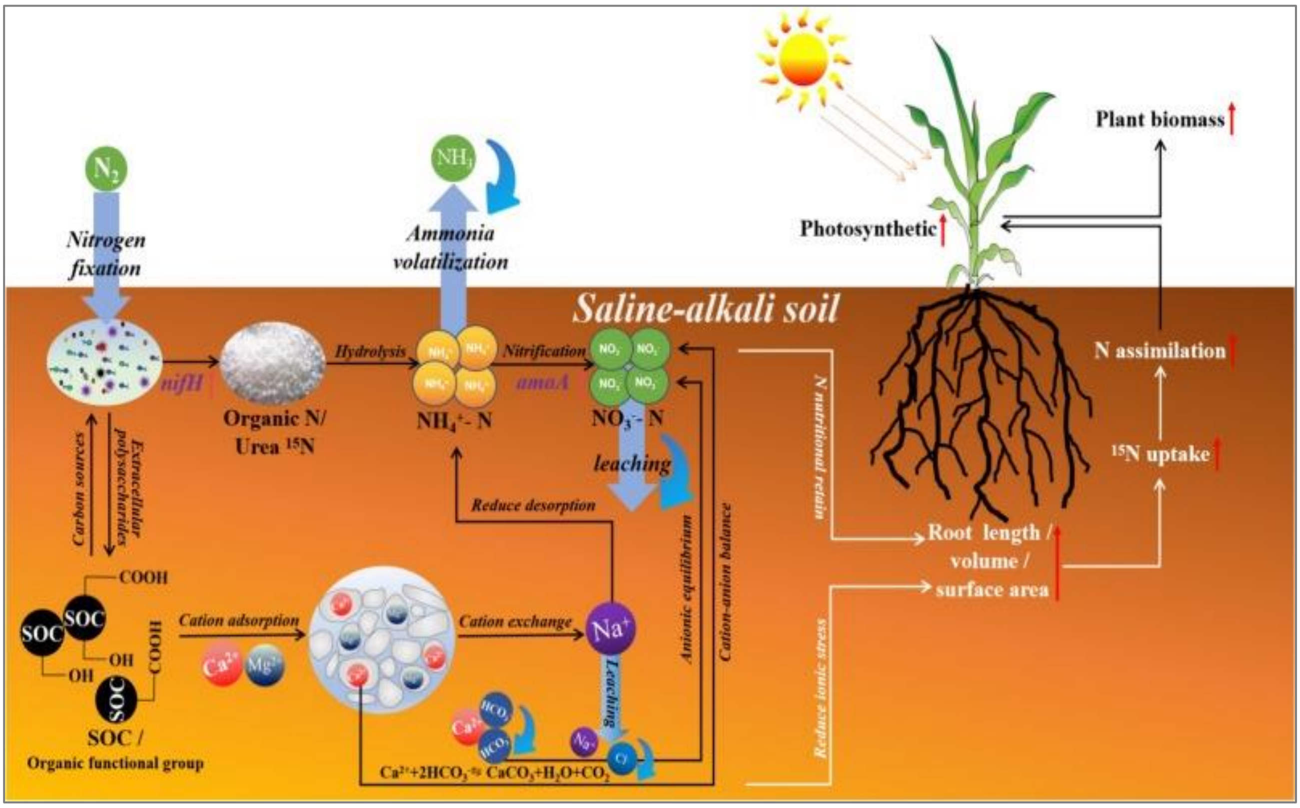

Figure 4: **Mechanistic model linking N-fixation, hormone production, and stress tolerance of *Azotobacter* (Arora *et al.*, 2018).**

Insights into the Genome of *Azotobacter*

Azotobacter's ability to convert nitrogen into ammonia is the most important property that enables these soil bacteria to be categorised as biofertilizers. For the benefit of plants, *Azotobacter* convert nitrogen into ammonia with the aid of nitrogenase enzymes, which comprise dinitrogenase and dinitrogenase reductase (Daniel *et al.*, 2022). *Azotobacter* encodes a wide array of plant growth-promoting genes, including nitrogen fixation and phosphate solubilization (Roy *et al.*, 2025). The genome possesses the complete pathway for nitrogen fixation, including nitrogenase molybdenum-iron protein (nifDK) and dinitrogenase reductase (nifH), as well as single copies of PhoB, PhoR, PstA, PstB and PstS for phosphorous metabolism. Phosphate-specific transporters and phosphate-import permeases play a major role in the solubilization of inorganic phosphate and can make it bioavailable to plants (Soumare *et al.*, 2020). *Azotobacter* can convert nitrate to ammonia via NasAB and NasBDE. It also encodes for anthranilate synthase and anthranilate phosphoribosyltransferase, crucial for tryptophan biosynthesis and linked to auxin production. The presence of chemotaxis proteins (CheA and CheY) and methyl-accepting chemotaxis proteins can regulate the phosphorylation process of the basal body and play a role in flagellar movement. The genome also contains XerC and XerD, which play a putative role in rhizosphere colonization, along with 4-hydroxybenzoate transporter (PcaK). Aminodeoxychorismate lyase, crucial for folate biosynthesis, is present in the genome, indicating its importance towards plant growth (Roy *et al.*, 2025). The bacterium can also biosynthesise trehalose via the maltooligosyl trehalose synthase enzyme, which can help combat drought stress (Roy *et al*., 2025). The rapid use of pesticides and fertilisers is a source of heavy metals, disrupting the functioning of the soil microbiome, fertility, and ecological balance. *Azotobacter* spp. maintains soil stability, increases food crop production and is pivotal in facilitating availability of nutrients required by plants (Roy *et al*., 2025).

LIMITATIONS

The limitations on the use of *Azotobacter* as biofertilizer include; Marketing constraints, Short shelf life, Lack of proper storage facilities, Consumer illiteracy, Low awareness amongst consumers, Inadequate guidelines to consumers, Inadequate production, Seasonal conditions (high temperature), unfavourable Soil pH and incompatibility of biofertilizer with fungicide or insecticide coated on the seed

CHALLENGES AND FUTURE DIRECTIONS

Commercial *Azotobacter* formulations encounter difficulties despite their demonstrated advantages, including strain compatibility with specific crops, susceptibility to high salt and acidic pH conditions, and the need for improved screening and formulation methods. To maximise benefits, future studies should focus on molecular processes, strain selection, and integration with contemporary soil genomics (Sumbul *et al*., 2020; Aasfar *et al*., 2021; Hindersah *et al*., 2020).

CONCLUSION

Azotobacter species are promising biofertilizers that enhance crop yield, soil fertility, and plant health through nitrogen fixation and other growth-promoting activities. Utilising nitrogen-fixing bacteria in crop production has demonstrated their value for plant nutrition and soil fertility. By producing growth compounds and their effects on plants, *Azotobacter* has the potential to be used as microbial inoculants, which would greatly boost agricultural crop yields. In the presence of appropriate strains, the microbiome of the roots is also altered, which is thought to be highly advantageous for enhancing plant health. *Azotobacter* has been shown to alleviate plant stress of various origins and has been recommended for use in a variety of field crops. While their benefits are well documented, optimising their use across diverse agricultural systems requires further research and improved commercial formulations.

CONFLICT OF INTEREST

According to the authors, there is no conflict of interest.

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