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Azotobacter in environment and agriculture: a centennial review of research, current applications, and future perspectives

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ABSTRACT

Azotobacter spp. are free-living, obligately aerobic, Gram-negative diazotrophic bacteria widely distributed in neutral to alkaline soils. Although they generally occur at relatively low abundance in agricultural ecosystems, these microorganisms have attracted considerable scientific and agronomic interest because of their diverse functional traits and significant potential for promoting sustainable agriculture. As non-symbiotic nitrogen-fixing bacteria, *Azotobacter* species convert atmospheric nitrogen into bioavailable forms while enhancing plant growth through a broad range of direct and indirect mechanisms. These include the production of phytohormones, such as indole-3-acetic acid (IAA), gibberellins, and cytokinins; phosphate solubilization; mineral nutrient mobilization; siderophore production; and improved nutrient uptake efficiency. Moreover, several *Azotobacter* strains have been shown to enhance plant tolerance to abiotic stresses, including salinity, drought, and heavy metal toxicity, while suppressing certain soil-borne phytopathogens. Beyond their agricultural applications, members of this genus possess considerable ecological and biotechnological importance. Their capacity to produce extracellular polysaccharides, accumulate polyhydroxyalkanoates, form stress-resistant cysts, and participate in the transformation or immobilization of environmental pollutants has stimulated increasing interest in their use for soil restoration, bioremediation, and environmental management. Furthermore, advances in microbial genetics, genomics, and molecular biology have established *Azotobacter vinelandii* as an important model organism for investigating biological nitrogen fixation, respiratory protection mechanisms, alginate biosynthesis, and microbial adaptation to environmental stress. This review summarizes current knowledge of the taxonomy, evolutionary history, morphology, physiology, ecology, and functional characteristics of the genus *Azotobacter*. Particular emphasis is placed on its plant growth-promoting mechanisms, agricultural applications, and environmental significance. The review also critically examines the major factors limiting the field performance of *Azotobacter*-based inoculants, including environmental constraints, carbon availability, competition with indigenous microbial communities, and formulation-related challenges. Finally, current knowledge gaps and future research priorities are discussed to facilitate the development of more effective inoculants and integrated microbial technologies. A deeper understanding of plant–microbe–soil interactions, together with strain-specific functional diversity, will be essential for improving the reliability, consistency, and large-scale application of *Azotobacter* in sustainable agricultural systems.

Keywords: Biofertilizer, Biological nitrogen fixation, Inoculation, PGPR, Sustainable agriculture.

1. Introduction

The growing demand for sustainable agricultural production has intensified the search for environmentally friendly alternatives to conventional agrochemical inputs. Although synthetic fertilizers have played a crucial role in increasing crop productivity during the past century, their excessive use has contributed to numerous environmental challenges, including soil degradation, nutrient imbalances, contamination of water resources, greenhouse gas emissions, and declines in soil microbial biodive rsity (Asadu et al., 2023; Chen et al., 2018). Consequently, beneficial microorganisms have received increasing attention as biofertilizers and biostimulants capable of supporting sustainable and climate-smart agricultural systems.

Among plant growth-promoting rhizobacteria (PGPR), species of the genus *Azotobacter* have attracted considerable scientific attention for more than a century. Originally described by Beijerinck in 1901, *Azotobacter* comprises free-living, aerobic, diazotrophic bacteria capable of fixing atmospheric nitrogen into biologically available forms without forming symbiotic associations with plants (Beijerinck, 1901). Members of this genus are widely distributed across diverse soil environments worldwide and play important roles in nutrient cycling and ecosystem functioning, despite typically occurring at relatively low population densities (Aasfar et al., 2025).

Numerous studies have demonstrated that *Azotobacter* spp. possess multiple plant growth-promoting mechanisms beyond biological nitrogen fixation (BNF). These include the production of phytohormones such as indole-3-acetic acid, gibberellins, and cytokinins, phosphate solubilization, mobilization of mineral-bound potassium, siderophore production, and enhancement of nutrient uptake efficiency (Bhattacharyya and Jha, 2012;

Wani et al., 2016). In addition, some strains have been reported to suppress plant pathogens and improve plant tolerance to abiotic stresses, including salinity, drought, and heavy metal toxicity (Albdaiwi et al., 2019; Kibret et al., 2024; Teiba et al., 2023).

Beyond agriculture, this bacterium has attracted increasing attention for environmental applications. Studies have reported its involvement in pollutant biodegradation, heavy-metal biosorption, and remediation of contaminated soils, highlighting a broader ecological significance than previously recognized. These multifunctional properties have stimulated interest in the use of *Azotobacter*-based technologies for sustainable land management and environmental restoration (Basit et al., 2021).

Despite more than a century of research and numerous reports demonstrating beneficial effects under laboratory and greenhouse conditions, the field performance of *Azotobacter*-based inoculants remains variable. Their effectiveness is influenced by soil physicochemical properties, climatic conditions, plant genotype, agricultural management practices, and interactions with indigenous microbial communities. Furthermore, the obligately heterotrophic nature of *this genus* and its dependence on available carbon sources may limit establishment and persistence under field conditions. These factors have contributed to a persistent gap between promising experimental results and large-scale agricultural adoption (Vessey, 2003).

Recent advances in molecular biology, genomics, metagenomics, and microbiome research have provided new opportunities to reassess the biology and applications of *Azotobacter*. These approaches have improved understanding of genetic diversity, stress adaptation mechanisms, ecological interactions, and functional traits, thereby facilitating the development of more effective inoculants and microbial consortia (Panek et al., 2026)

Given the expanding interest in plant growth-promoting microorganisms and the increasing emphasis on sustainable agriculture, a comprehensive reassessment of *Azotobacter* is timely. This review examines the taxonomy, ecology, physiology, agricultural applications, environmental significance, and factors affecting field performance, existing knowledge gaps, and future opportunities for enhancing the agricultural and environmental utilization of *Azotobacter* (Sumbul et al., 2020). In addition, selected findings from studies conducted in Iran are integrated within the broader international context to provide a more comprehensive perspective on the current status and future potential of this important bacterial genus

2. Taxonomic History and Evolution of the Genus *Azotobacter*

2.1. Historical development and scientific importance of *Azotobacter*

Since its discovery by Beijerinck, *Azotobacter* has occupied a prominent position in microbiological research. Beyond its ecological significance as a free-living nitrogen-fixing bacterium, this genus has served as an important experimental model for investigating a wide range of fundamental biological processes.

Members of the genus are distributed across diverse terrestrial environments, ranging from tropical and temperate regions to arctic soils, and exhibit remarkable physiological and metabolic versatility (Sumbul et al., 2020).

Several characteristics have contributed to the scientific significance of *Azotobacter*. These bacteria possess the unique ability to fix atmospheric nitrogen under aerobic conditions through highly efficient respiratory protection mechanisms. In addition, they produce substantial quantities of extracellular polysaccharides, accumulate intracellular carbon-storage compounds such as poly-β-hydroxybutyrate (PHB), and form stress-resistant cysts that enhance their persistence and long-term survival under unfavorable environmental conditions (Gauri et al., 2012; Castillo et al., 2017; Shirinbayan et al., 2019).

Historically, *Azotobacter vinelandii* has emerged as one of the most important model organisms for studying BNF. Research on this species has contributed substantially to our understanding of nitrogenase structure and function, electron transport pathways, metal cofactor biosynthesis, and the genetic regulation of diazotrophy (Kennedy et al., 2015). Owing to its relatively rapid growth, metabolic versatility, and genetic tractability, *A. vinelandii* continues to serve as a valuable model organism in studies of microbial genetics and physiology.

Beyond BNF, investigations involving *A. vinelandii* have advanced knowledge in a wide range of microbiological and biochemical fields, including respiratory protection mechanisms, alginate biosynthesis, iron metabolism, redox regulation, hydrogen metabolism, carbon storage, and microbial adaptation to environmental stress. Consequently, the scientific significance of this bacterium extends far beyond its agricultural applications and continues to contribute to advances in microbial physiology, biotechnology, and environmental microbiology (Poole and Hill, 1997; Setubal et al., 2009; Kennedy et al., 2015).

2.2. Historical changes in genus and species delineation

The taxonomic history of *Azotobacter* genus began with the pioneering work of Beijerinck and described two species, *A. chroococcum* from soil and *A. agilis* from aquatic environments (Beijerinck, 1901). As was common in early bacterial systematics, species delineation was primarily based on morphological appearance, physiological traits, ecological origin, and nitrogen-fixing ability.

During the first half of the twentieth century, additional species were described as cultivation techniques improved and interest in free-living

diazotrophic bacteria increased. Among these, *A. vinelandii* gained particular importance because of its physiological versatility and later became a model organism for studies of BNF. Subsequently, *A. beijerinckii*, *A. nigricans*, *A. paspali*, *A. armeniacus*, and *A. salinestris* were proposed based on phenotypic, biochemical, and ecological differences observed among isolates from diverse habitats (Döbereiner, 1966; Thompson and Skerman, 1979; Page and Shivprasad, 1991).

As bacterial taxonomy evolved, the limitations of phenotype-based classification became increasingly evident. Numerical taxonomy, DNA–DNA hybridization, and chemotaxonomic analyses revealed substantial overlap among several described species and raised questions regarding the validity of some taxonomic assignments. Consequently, a number of taxa were reclassified, synonymized, or excluded from the genus following more rigorous systematic evaluation (Becking, 2006).

The introduction of molecular phylogenetic approaches, particularly 16S rRNA gene sequencing, represented a major turning point in the taxonomy of *Azotobacter*. These methods provided a more reliable framework for assessing evolutionary relationships and demonstrated close phylogenetic affinities between *Azotobacter* and certain members of the family Pseudomonadaceae (Kenawy et al., 2005). More recently, whole-genome sequencing, phylogenomic analyses, Average Nucleotide Identity (ANI), and digital DNA–DNA hybridization (dDDH) have further refined species boundaries and improved understanding of genomic diversity within the genus (Abdul Aziz et al., 2025; Khosravi and Dolatabad, 2020).

Overall, the taxonomy of *Azotobacter* has undergone a progressive transition from morphology-based classification to a modern framework integrating phenotypic, phylogenetic, and genomic evidence. This evolution reflects broader developments in bacterial systematics and has significantly improved the accuracy of species delineation within the genus (Lalucat et al., 2020). Table 1 summarizes the major milestones and taxonomic revisions in the history of *Azotobacter* classification.

2.3. Taxonomic position of the genus *Azotobacter*

The taxonomic placement of *Azotobacter* has been revised repeatedly as bacterial classification systems evolved. Early classifications relied largely on phenotypic characteristics such as Gram reaction, motility, cyst formation, pigmentation, metabolic versatility, and aerobic nitrogen fixation. Based on these traits, *Azotobacter* was traditionally assigned to the family Pseudomonadaceae within the order Pseudomonadales in successive editions of Bergey's Manual of Systematic Bacteriology (Garrity et al., 2005).

Currently, the genus is generally regarded as comprising several validly described species, including *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. armeniacus*, *A. nigricans*, and *A. salinestris*. Most species are free-living inhabitants of soil ecosystems characterized by high metabolic flexibility and aerobic nitrogen fixation. In contrast, *A. paspali* exhibits a more specialized ecological association with the rhizosphere of *Paspalum notatum* and related grasses (Garrity et al., 2005; Page and Shivprasad, 2008; Anzai et al., 2000).

The modern taxonomic framework of *Azotobacter* therefore reflects the integration of classical microbiology with contemporary molecular and genomic approaches. This combination has greatly improved understanding of species diversity, evolutionary relationships, and ecological adaptation within the genus. Table 2 presents the historical taxonomic placement of this genus according to Bergey's classification systems, while Table 3 summarizes currently recognized species and their principal ecological characteristics.

3. Morphology and Cultural Characteristics

3.1. Vegetative cells and colony morphology

Azotobacter species are Gram-negative, free-living diazotrophic bacteria characterized by a biphasic life cycle consisting of vegetative cells and stress-resistant cysts. During active growth, vegetative cells exhibit considerable pleomorphism and may appear as rods, ovoids, or nearly spherical forms depending on species, culture conditions, and physiological state. Cell dimensions generally range from approximately 2–7 μm in length and 1.5–3 μm in width, although variations among species and growth conditions have been reported (Kennedy et al., 2015).

Motility varies among species and strains. Motile forms possess peritrichous flagella that facilitate movement within soil microenvironments and aqueous habitats, whereas some strains may exhibit reduced motility or appear non-motile under specific physiological conditions (Garrity et al., 2005).

When cultivated on nitrogen-free media such as Ashby or Vinogradsky agar, colonies typically become visible within 24–48 h at approximately 28–30°C. Mature colonies generally range from 2 to 6 mm in diameter after several days of incubation, depending on strain characteristics and nutrient availability. Colonies are usually smooth, convex, opaque, and distinctly mucoid due to the abundant production of extracellular polysaccharides (EPS). This mucoid appearance is one of the most recognizable diagnostic characteristics used in the isolation and identification of *Azotobacter* species (Thompson and Skerman, 1979).

Pigment production represents another important phenotypic characteristic within the genus. Several species produce pigments that vary in color, solubility, and intensity depending on culture conditions and

Table 1. Historical changes of genus *Azotobacter*

Named species	Explorer or researcher	Year
Azotobacter chroococcum	Beijerinck	1901
A. agilis	Beijerinck	1901
A. vinelandii	Lipman	1903
A. beijerinckii	Lipman	1909
A. agilis	Winogradsky	1938
A. indicus	Starkey	1939
A. insolita	Stapp	1940
A. fluorescens	Krasil'nikov	1947
A. nigricans	Krasil'nikov	1949
A. agilis subsp jaktiae	Krasil'nikov	1949
Beijerinckia indica	Starkey and De	1950
B. mobilis	Derx	1950
B. indica subsp alba	Derx	1951
A. insignis	Derx	1951
A. lacticogenes	Kuffman and Tousiaint	1951
Beijerinckia lacticogenes	Tchan	1953
A. beijerinckii subs acid tolerance	Tchan	1953
A. beijerinckii subs achromogenes	Jensen And Peterson	1954
A. macrocyrogenes	Jensen	1955
A. macrocyrogenes	Rubenchik	1959
Beijerinckia derxii	Tchan	1957
B. fluminensis	Dobereiner and Ruschell	1958
Derxia gummosa	Jensen, Peterson, De and Bhattacharya	1960
B. congensis	Hilger	1963
A. agilis ssp armenia	Kirakosyan	1964
A. vitreus ssp armenia	Kirakosyan; Melkonyan	1964
A. miscellus	Pshenin	1964
A. paspali	Dobereiner	1966
A. armeniacus	Thompson , Skerman	1981
A. salinestris	Page , Shivprasad	1991

Table 2. Taxonmy of *Azotobacter* based on Bergey's manual (Kennedy et al., 2015

Levels of Bergey's classification	Position of *Azotobacter*
Domain	Prokaryote
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gamma-proteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	*Azotobacter*
Species	7 (spp.)

Table 3. The species of *Azotobacter* (Kennedy et al., 2015)

Species	Subspecies
A. chroococcum	
A. salinestris	
A. paspali	
A. beijerinkii	
A. armeniacus	
A. vinelandii	
A. nigricans [	nigricans achromogenes

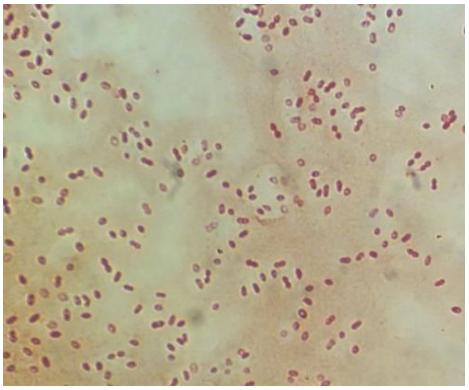

Figure 1. Vegetative cells of *A. salinestris* (Original photograph by the author).

Figure 2. Colony morphology of *A. salinestris* on Vinogradsky medium after 48 hours (Original photograph by the author).

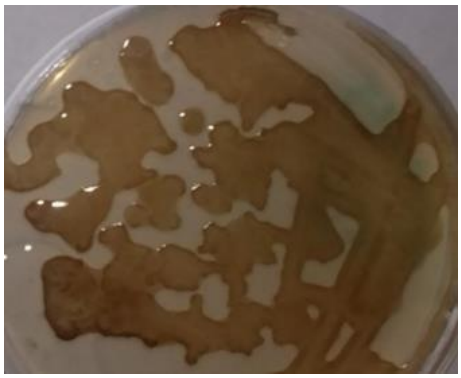

Figure 3. Pigmented colony of *A. chroococcum* on Vinogradsky medium after 7 days of incubation at 30°C (Original photograph by the author).

physiological age. For example, mature colonies of *A. chroococcum* frequently develop a characteristic brown to dark-brown pigment during late growth stages. Such pigmentation patterns have traditionally been used as supplementary taxonomic markers, although molecular approaches now provide more reliable species identification. In addition to their taxonomic value, EPS production contributes significantly to the ecological success of this bacterium. These polymers improve cell aggregation, biofilm formation, moisture retention, and protection against environmental stresses, thereby enhancing persistence in soil ecosystems (Kennedy et al., 2015).

Figures 1–3 illustrate representative vegetative cells, colony morphology, and pigment production patterns of selected *Azotobacter* species under laboratory cultivation conditions (Khosravi, 2009).

3.2. Cyst formation

Unlike endospore-forming bacteria, *Azotobacter* spp. survive unfavorable environmental conditions through differentiation into specialized cysts rather than true spores. Cyst formation is induced primarily by nutrient limitation, desiccation, and other environmental stresses and represents one of the most distinctive adaptive features of the genus (Garrity et al., 2005).

The transformation from vegetative cell to cyst involves extensive morphological, physiological, and biochemical changes. During encystment, cells become spherical and develop a multilayered protective envelope surrounding a central body. A characteristic feature of this process is the accumulation of intracellular poly-β-hydroxybutyrate (PHB), which serves as an important carbon and energy reserve. Simultaneously, substantial amounts of extracellular polysaccharides, particularly alginate and related polymers, contribute to the formation of the cyst coat and exine layers.

The mature cyst exhibits markedly reduced metabolic activity and enhanced tolerance to environmental stresses. Compared with vegetative cells, cysts demonstrate greater resistance to desiccation, ultraviolet radiation, gamma irradiation, oxidative stress, and mechanical disruption. However, unlike bacterial endospores, they do not possess high resistance to prolonged exposure to elevated temperatures. Under favorable environmental conditions, cysts undergo germination and return to vegetative growth. During this process, the central body expands, the cyst envelope ruptures, and a metabolically active cell emerges and resumes normal growth and cell division.

Table 4. Population density of *A. chroococcum* in selected agricultural regions of Iran (Khosravi 1998)

Region	Population density (number of cells per gram of soil)		
	Maximum	Minimum	Average of the area ($*10^3$)
Karaj	7533	233	1.9
Shahriar and Robat-Karim	3733	233	1.3
Qazvin	6433	200	2.7
Varamin	3633	33	1.5
Mean	7533	33	1.5

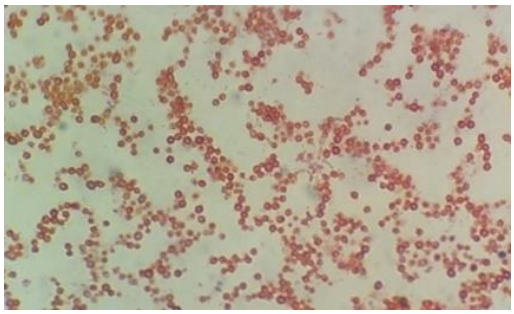

Figure 4. Cyst of *A. salinestris* (Original photograph by the author).

The ability to form cysts is considered a major ecological adaptation contributing to the long-term persistence of *Azotobacter* populations in soil ecosystems. Through this survival strategy, cells can withstand periods of environmental stress and rapidly re-establish active populations when conditions become favorable (Thompson and Skerman, 1979). Figure 4 Illustrates a representative cyst structure of *A. salinestris* observed under laboratory conditions (Khosravi, 2009). where root exudates provide a favorable carbon and energy source for growth (Page and Shivprasad, 2008).

4. Environmental Factors Affecting *Azotobacter* Populations

The abundance of *Azotobacter* in soil ecosystems is generally low compared with many other heterotrophic bacteria and shows considerable spatial and temporal variability depending on environmental and edaphic conditions. Numerous studies have shown that population densities of *Azotobacter* are strongly influenced by soil organic matter content, aeration status, moisture, pH, and agricultural management practices. In most natural and agricultural soils, *Azotobacter* populations are typically reported at levels ranging from approximately 10^3 to 10^4 CFU per gram of soil, and in many cases may fall below detectable limits under unfavorable conditions (Becking, 2006; Aquilanti et al. 2004) Aasfar et al. 2021). The relatively low abundance of this genus is mainly attributed to its sensitivity to soil acidity, competition with other microbial populations, and dependence on readily available organic carbon sources, despite its ability to fix atmospheric nitrogen under aerobic conditions (Kennedy and Smith, 1995; Wani and Lee, 2002). However, higher populations have occasionally been reported in soils rich in organic amendments or in the rhizosphere of certain plants. Studies conducted in different agricultural regions of Iran reported average populations of *A. chroococcum* of approximately 1.5×10^3 cells g^{-1} soil (Table 4) (Khosravi, 1998).

4.1. Soil organic carbon

As obligately heterotrophic microorganisms, *Azotobacter* spp. rely on organic carbon as both an energy source and a fundamental component for biomass synthesis. Carbon substrates are derived primarily from root exudates, decomposing plant residues, and soil organic matter. Consequently, soils with higher organic carbon content generally support larger and more stable the genus populations. The rhizosphere provides a particularly favorable habitat, as the continuous release of carbon compounds by plant roots stimulates bacterial growth and metabolic activity. Nevertheless, population expansion may ultimately be limited by the depletion of available substrates as well as competition with other soil microorganisms for resources. (Becking, 2006; Kennedy and Smith, 1995; Wani and Lee, 2002).

4.2. Soil moisture

Soil moisture strongly influences the survival, activity, and nitrogen-fixing capacity of *Azotobacter*. Optimal population densities are commonly observed under moderately moist conditions, typically between 50 and 70% of field capacity. Water deficit reduces nutrient diffusion, restricts metabolic activity, and increases physiological stress, resulting in population decline. Conversely, excessive soil moisture may impair growth by reducing oxygen availability and increasing carbon dioxide accumulation, thereby limiting aerobic respiration and BNF ((Subba Rao, 1986; Wani and Lee, 2002; Page and Shivprasad, 2008).

4.3. Soil Aeration

Adequate aeration is essential for *Azotobacter* because all

recognized species are obligately aerobic. High respiratory activity not only supports growth but also contributes to the protection of nitrogenase from oxygen damage. Well-structured soils with efficient gas exchange generally support larger populations than compacted or poorly drained soils. The decline in bacterial abundance with increasing soil depth further illustrates the importance of oxygen availability in determining habitat suitability. Kennedy and Smith, 1995; Wani and Lee, 2002; Page and Shivprasad, 2008).

4.4. Soil pH

Among soil chemical properties, pH exerts one of the strongest influences on the occurrence and diversity of this genus. Most species grow optimally under neutral to slightly alkaline conditions (pH 7.0–7.5), whereas strongly acidic soils generally support only small populations or none at all. Acidic conditions adversely affect cellular metabolism, nutrient availability, and nitrogen fixation efficiency. As a result, species composition and abundance are often closely associated with soil pH gradients (Subba Rao, 1986; Kennedy et al., 2015).

4.5. Additional environmental factors

In addition to the factors discussed above, temperature, salinity, nutrient status, cropping system, and agricultural management practices can significantly influence *Azotobacter* populations. Organic amendments, crop rotations, and reduced soil disturbance frequently promote population development, whereas prolonged fallow periods, nutrient depletion, and adverse environmental conditions may reduce abundance. Consequently, the ecological success of this bacterium in agricultural systems depends on the combined effects of multiple environmental variables rather than any single factor acting independently.

5. Species Diversity and Phylogeny

Although this genus contains a relatively limited number of recognized species, considerable diversity exists in morphology, physiology, pigmentation, ecological adaptation, and metabolic capabilities. Advances in molecular phylogenetics and genome-based taxonomy have substantially improved understanding of species relationships and evolutionary divergence within the genus.

5.1. *Azotobacter chroococcum*

A. chroococcum is the most widely distributed and agriculturally important species within the genus. It occurs predominantly in cultivated soils and has been extensively investigated for its nitrogen-fixing ability and plant growth-promoting properties. A characteristic feature of this species is the production of a brown to black, water-insoluble pigment that becomes increasingly evident in aging cultures. Owing to its broad ecological distribution and agronomic relevance, *A. chroococcum* is frequently used as a representative species in ecological, physiological, and biofertilizer studies (Kennedy et al., 2015; Garrity et al., 2005).

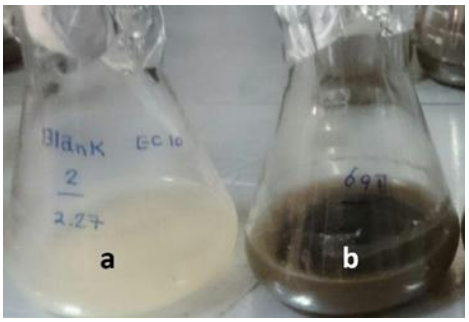

Figure 5. Production of a water-soluble brown pigment by *A. salinestris* in Vinogradsky medium
(Original photograph by the author).

5.2. *Azotobacter vinelandii*

Among all species of the genus, *A. vinelandii* is undoubtedly the most intensively studied. Originally isolated from soil in Vineland, New Jersey, it has become a model organism for research on BNF, respiratory protection, alginate biosynthesis, and polyhydroxybutyrate production. The species typically produces a water-soluble yellow-green pigment and exhibits broad metabolic versatility. Its well-developed genetic tools and extensive genomic characterization have established *A. vinelandii* as one of the most important experimental systems in diazotroph research (Kennedy et al., 2015).

5.3. *Azotobacter salinestris*

A. salinestris is distinguished from most other species by its adaptation to saline environments and partial dependence on sodium-containing growth conditions. The species commonly forms pairs or short chains and may produce a water-soluble brown pigment in liquid culture (Figure 5). Because of its tolerance to elevated salinity, A. salinestris has attracted interest as a potential microbial resource for saline and degraded soils. Subsequent taxonomic studies demonstrated that it represents a distinct species rather than a sodium-dependent variant of A. chroococcum (Khosravi and Dolatabad, 2020; Page and Shivprasad, 1991).

5.4. *Azotobacter armeniacus*

A. armeniacus, first isolated from Armenian soils, is characterized by the production of a distinctive red-to-purple, water-soluble pigment. While sharing many morphological characteristics with other species, it differs

Table 5. Some characteristics of different *Azotobacter* species

Characteristic	*A. chroococcum*	*A. armeniacus*	*A. beijerinckii*	*A. nigricans*	*A. paspali*	*A. salinestris*	*A. vinelandii*
Motility	+	+	−	−	+	+	+
Peritrichous flagella	+	+	−	−	+	+	+
Peroxidase activity	d	d	d	d	−	+	+
Urease activity	+	+	+	+	+	+	+
Oxidase activity	+	d	+	+	+	nd	+
Nitrate reduction ($NO_3^- \rightarrow NO_2^-$)	+	−	+	+	−	+	+
H_2S production from thiosulfate	d	−	d	d	+	nd	+
H_2S production from cysteine	−	−	−	−	d	nd	d
Dispersible polysaccharide production	+	d	d	+	−	d	d

+ = positive; − = negative; d= delayed or variable; nd= not determined

in several physiological aspects, particularly in its restricted utilization of certain inorganic nitrogen sources. These unique pigmentation and metabolic features have made *A. armeniacus* a subject of continuing taxonomic and physiological interest. (Thompson and Skerman, 1981).

5.5. *Azotobacter nigricans*

A. nigricans was initially described as a non-motile, non-flagellated species. It is distinguished by the formation of dark pigments in older cultures and by its relatively low tolerance to high temperatures. However, its taxonomic status has remained controversial in later systematic investigations, reflecting the broader complexities of species identification and classification within the genus (Kennedy et al., 2015).

5.6. *Azotobacter beijerinckii*

Named in honor of Beijerinck, *A. beijerinckii* shares many morphological features with *A. chroococcum* but differs in a number of physiological and biochemical properties. It is typically non-motile and displays distinctive carbon-substrate utilization profiles, including the capacity to metabolize compounds such as sorbitol and aconitate. These characteristics have long been employed as valuable taxonomic markers for species differentiation and identification within the genus.

5.7. *Azotobacter paspali*

A. paspali occupies a unique ecological position within the genus. Unlike most members, which occur primarily as free-living soil inhabitants, this species maintains a close association with the rhizosphere of *Paspalum notatum* and related grasses. It is characterized by marked cellular elongation during active growth and by the production of a yellow-green, water-soluble pigment resembling that of *A. vinelandii*. These distinctive ecological and physiological features have made *A. paspali* an important model organism for studying plant–microbe interactions and rhizosphere adaptation among free-living diazotrophs. (Page and Shivprasad, 2008; Kennedy et al., 2015)

6. Physiological and Biochemical Characteristics of *Azotobacter*

6.1. General physiological and biochemical characteristics

In addition to their distinctive morphology, *Azotobacter* spp. exhibit a wide range of physiological and biochemical characteristics that contribute to their ecological adaptation, taxonomic differentiation, and functional diversity. These characteristics include enzymatic activities, nutrient utilization patterns, respiratory metabolism, responses to environmental stress, and the production of extracellular metabolites. Comparative analyses have demonstrated considerable variation among species in traits such as motility, oxidase and urease activities, nitrate reduction, hydrogen sulfide production, pigment formation, and polysaccharide synthesis. Such differences provide valuable diagnostic criteria for species identification while also reflecting adaptation to diverse ecological niches (Kennedy et al., 2015; Garrity et al., 2005).

A comparative summary of major physiological and biochemical characteristics of recognized *Azotobacter* species is presented in Table 5 (Kennedy et al., 2015; Garrity et al., 2005).

6.2. Biological nitrogen fixation (BNF)

The ability to fix atmospheric nitrogen is the defining physiological characteristic of the genus *Azotobacter*. Unlike symbiotic diazotrophs, this genus are capable of reducing atmospheric nitrogen independently of plant hosts, thereby contributing directly to nitrogen cycling in terrestrial ecosystems.

Nitrogen fixation is catalyzed by the nitrogenase

Table 6. Nitrogen fixation activity of different *Azotobacter* species at varying pH levels (Kennedy et al., 2015)

pH range	*A. armeniacus*	*A. chroococcum*	*A. vinelandii*	*A. nigricans*	*A. beijerinckii*	*A. salinestris*	*A. paspali*
5–5.5	−	−	−	D	d	−	−
6	−	+	+	D	+	d	d
6.5–9.5	+	+	+	+	+	+	+
10	d	+	+	+	+	−	+

+ all strains positive; − all strains negative; d variable response among strains.

enzyme complex, whose activity is influenced by multiple environmental and nutritional factors, including oxygen concentration, temperature, pH, carbon availability, phosphorus supply, and trace elements. Among micronutrients, molybdenum is particularly important because it forms an essential component of the conventional Mo-nitrogenase system. Under molybdenum-limited conditions, some species can express alternative vanadium-dependent or iron-only nitrogenases, although these systems generally operate with lower efficiency (Stacey et al., 1992).

One of the most remarkable physiological features of this genus is its ability to maintain nitrogen fixation under aerobic conditions. This capability is primarily attributed to exceptionally high respiratory activity, which reduces intracellular oxygen concentration and thereby protects the oxygen-sensitive nitrogenase enzyme system (Robson and Postgate, 1980; Kennedy and Smith, 1995). In addition to respiratory protection, *Azotobacter* spp. employ conformational protection mechanisms mediated by specific protective proteins such as the Shethna protein, which shields nitrogenase from oxygen damage under conditions of elevated oxygen tension (Robson and Postgate, 1980; Kennedy et al., 2015). Regulatory control of nitrogenase expression is also tightly linked to cellular energy status and environmental nitrogen availab(ility.

Reported nitrogen fixation rates vary widely among species, strains, and environmental conditions. Under controlled laboratory conditions, nitrogen fixation efficiencies of approximately 10–15 mg N per gram of glucose consumed have been reported, although substantially higher or lower values may occur depending on carbon source, oxygen level, and culture conditions (Wani and Lee, 2002; Subba Rao, 1986). In natural ecosystems, actual nitrogen inputs are further constrained by soil physicochemical properties, carbon availability, microbial competition, and environmental stresses (Kennedy and Smith, 1995; Page and Shivprasad, 2008). Nitrogen fertilization generally suppresses nitrogenase activity through feedback inhibition, whereas phosphorus fertilization often enhances bacterial growth and nitrogen fixation by improving energy metabolism and root exudation patterns (Wani and Lee, 2002). Consequently, the contribution of *Azotobacter* to soil nitrogen fertility depends on the complex interaction between microbial physiology and environmental conditions. Table 6 summarizes reported differences in nitrogen fixation activity among *Azotobacter* species under varying pH conditions.

6.3. Carbon utilization

Metabolic versatility is one of the most important ecological characteristics of *Azotobacter*. Members of the genus are capable of utilizing a broad spectrum of carbon substrates, including monosaccharides, disaccharides, organic acids, alcohols, aromatic compounds, and various plant-derived metabolites. This wide substrate range reflects the relatively large genome size and flexible central metabolism of *Azotobacter*, which allows adaptation to heterogeneous soil environments (Page and Shivprasad, 2008; Kennedy et al., 2015).

The range of utilizable substrates varies considerably among species and strains and has historically been used as a phenotypic criterion for species differentiation within the genus (Garrity et al., 2005). Such metabolic flexibility enables *Azotobacter* populations to persist in soils where carbon availability fluctuates spatially and temporally, particularly in rhizosphere environments enriched with root exudates (Kennedy and Smith, 1995; Wani and Lee, 2002). Species-specific substrate preferences further reflect ecological adaptation to distinct soil and plant-associated niches, contributing to their differential

An overview of carbon source utilization patterns among different *Azotobacter* species is presented in Table 7.

6.4. Pigment production

water-soluble and water-insoluble pigments have been reported, and the type, color, and intensity of pigmentation may vary among species as well as according to culture conditions, age of culture, and environmental factors (Page and Shivprasad, 2008).

Historically, pigment production served as a useful taxonomic marker in the phenotypic classification of *Azotobacter*. For instance, *A. chroococcum* is commonly associated with dark brown to black insoluble pigments, whereas *A. vinelandii* and *A. paspali* are known to produce yellow-green water-soluble pigments. *Azotobacter armeniacus* has been described as producing reddish to purple pigmentation under specific growth conditions (Garrity et al., 2005; Kennedy et al., 2015).

Table 7. Carbon sources used by different *Azotobacter* species (Kennedy et al., 2015)

Carbon substrate group	Carbon source	*A. chroococcum*	*A. armeniacus*	*A. beijerinckii*	*A. nigricans*	*A. paspali*	*A. salinestris*	*A. vinelandii*
Simple sugars	Glucose	+	+	+	+	+	+	+
	Fructose	+	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+	+
	Trehalose	+	+	d	+	−	+	−
	Maltose	+	+	d	d	−	+	+
	Melibiose	+	+	+	d	−	+	+
	Rhamnose	−	−	−	−	−	−	+
Organic acids	Acetate	+	+	+	+	+	+	+
	Pyruvate	+	+	+	+	+	+	+
	Fumarate	+	+	+	+	+	+	+
	Malate	+	+	+	+	+	+	+
	Succinate	+	+	+	+	+	+	+
	Lactate	+	+	+	+	+	+	+
	Oxoglutarate	+	+	+	+	+	+	+
	D,L-Glutarate	+	+	+	+	+	+	+
	Glutarate	−	d	−	−	−	−	+
	Malonate	+	−	−	d	−	+	+
	Glycolate	−	−	−	−	−	−	+
Alcohols & polyols	Glycerol	d	−	d	−	−	d	+
	Mannitol	+	+	d	d	−	+	+
	Sorbitol	+	+	d	d	−	+	+
	Propan-1-ol	−	−	d	d	−	−	+
	meso-Inositol	−	+	+	−	−	−	+
Aromatic & complex compounds	Benzoate	d	−	+	−	−	d	+
	Phenol	−	−	d	−	−	−	+
	β-Phenyl propionate	d	−	−	−	−	d	+
	Caprate	+	−	−	−	−	+	+
	Caprylate	−	+	−	−	−	−	+
	N-Butyrate	+	+	+	d	−	+	+
	Propionate	+	d	+	−	−	+	+
Special substrates	Acetylmethyl carbinol	+	+	+	+	+	+	+
	Hydrolyzed starch	+	+	d	d	−	+	−
	D-glucuronate	−	−	+	−	−	−	+
	D-galacturonate	−	+	+	−	−	−	−

Although molecular phylogenetic approaches have largely replaced pigmentation-based traits in formal taxonomy, pigment production remains a supportive phenotypic feature for species differentiation in classical microbiology. In addition, these pigments, often associated with melanin-like or phenazine-like compounds, have been suggested to contribute to protection against oxidative stress and may influence interactions with soil minerals and metals (Page and Shivprasad, 2008; Kennedy et al., 2015).

The distribution of pigment production among recognized species is summarized in Table 8.

Table 8. Comparative production of water-soluble pigments in different *Azotobacter* species (restructured from Kennedy et al., 2015; Garrity et al., 2005)

Pigment category	Pigment type	*A. chroococcum*	*A. armeniacus*	*A. beijerinckii*	*A. nigricans*	*A. paspali*	*A. salinestris*	*A. vinelandii*
Fluorescent pigments	Green-yellow fluorescent	−	−	−	−	+	−	+
Green pigments	Green	+	+	−	−	−	−	d
Brown/black pigments	Brown-black	−	−	−	d	−	+	−
	Black-brown to purple-red	−	+	−	+	−	−	−
Red-purple pigments	Red-purple	−	+	−	d	+	−	d

+= produced by all strains; − = not produced; d = variable or strain-dependent production

Table 9. The growth of various species within the *Azotobacter* at different temperatures

Temperature (C°)	Species						
	A. chroococcum	*A. armeniacus*	*A. beijerinckii*	*A. nigricans*	*A. paspali*	*A. salinestris*	*A. vinelandii*
9	-	-	d	d	-	-	-
14	d	-	+	d	+	-	+
18	+	d	+	+	+	-	+
32	+	+	+	+	+	d	+
37	d	d	-	-	+	+	+

+: all strains react; -: no strain reacts; d: Different strains react differently.

6.5. Temperature responses

Azotobacter. Although most species are classified as mesophilic, notable variation exists among species and strains in terms of temperature tolerance and optimal growth conditions (Page and Shivprasad, 2008; Kennedy et al., 2015).

In general, *Azotobacter* species grow over a relatively broad temperature range, with optimal growth commonly reported between approximately 28 and 35°C under laboratory conditions. Deviations from this optimum range can significantly reduce growth rate, alter respiratory activity, and decrease nitrogenase activity due to temperature sensitivity of enzyme systems involved in nitrogen fixation (Robson and Postgate, 1980; Wani and Lee, 2002). Species and strains adapted to specific ecological niches may exhibit broader or narrower thermal tolerance ranges, reflecting environmental adaptation and selection pressures in soil ecosystems (Kennedy and Smith, 1995; Page and Shivprasad, 2008).Comparative information on temperature-dependent growth responses of different *Azotobacter* species is presented in Table 9.

6.6. Plant growth-promoting mechanisms of *Azotobacter*

Azotobacter are widely recognized as important plant growth-promoting rhizobacteria (PGPR) because they enhance plant development through multiple direct and indirect mechanisms. Unlike microorganisms that rely on a single beneficial trait, *Azotobacter* simultaneously influences plant nutrition, hormone balance, soil structure, and stress tolerance, making it one of the most multifunctional bacterial genera used in sustainable agriculture.

6.7. Phytohormone production

Azotobacter spp. are multifunctional plant growth-promoting rhizobacteria (PGPR) capable of synthesizing several phytohormones, including indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins, and, in some strains, abscisic acid (ABA). Among these, IAA is the predominant phytohormone and is mainly synthesized through tryptophan-dependent pathways, particularly the indole-3-pyruvate (IPyA) and indole-3-acetamide (IAM) pathways, although the contribution of individual pathways varies among strains. Root-exuded tryptophan serves as a key precursor for bacterial auxin biosynthesis, while the production of gibberellins and cytokinins further enhances root elongation, cell division, nutrient acquisition, and stress adaptation. In addition to phytohormone biosynthesis, *Azotobacter* contributes to plant growth through biological nitrogen fixation, phosphate solubilization, and exopolysaccharide production, making it one of the most effective multifunctional PGPR (Orozco-Mosqueda et al., 2023).

Compared with other extensively studied PGPR such as Pseudomonas and Bacillus, *Azotobacter* exhibits a broad but strain-dependent phytohormone production profile. Pseudomonas species are generally recognized as highly efficient IAA producers and frequently possess complementary plant growth-promoting traits, including ACC deaminase activity, siderophore production, and induction of systemic resistance. Likewise, Bacillus species produce IAA, gibberellins, cytokinins, and diverse bioactive metabolites while benefiting from exceptional environmental persistence through endospore formation. Although the phytohormone yield of *Azotobacter* is often lower than that of elite Pseudomonas or Bacillus strains, its unique integration of nitrogen fixation, phytohormone biosynthesis, nutrient mobilization, and stress-mitigating activities provides a broader spectrum of plant growth-promoting functions, making it an attractive candidate for sustainable agriculture and microbial biofertilizer development (Abou Jaoudé et al., 2024).

6.8. Phosphate solubilization

Phosphorus in many soils is predominantly present in insoluble inorganic and organic forms that are not readily available for plant uptake. Several *Azotobacter* strains have been reported to enhance phosphorus availability through various phosphate-solubilization mechanisms, including the production of organic acids, proton extrusion, and the release of chelating compounds that facilitate the dissolution of mineral phosphates (Wani and Lee, 2002).

These biochemical processes can reduce pH in the immediate microenvironment and promote the mobilization of sparingly soluble phosphate compounds such as tricalcium phosphate, iron phosphate, and aluminum phosphate. However, the efficiency of phosphate solubilization varies considerably among strains and is strongly influenced by environmental factors, including carbon source availability, soil pH, nutrient status, and microbial competition (Kennedy et al., 2015; Sharma et al., 2013). Consequently, phosphate solubilization is regarded as a strain-dependent and context-specific trait rather than a universal characteristic of the genus.

Numerous studies have demonstrated significant phosphate-solubilizing activity among different isolates, highlighting their potential as biofertilizers for improving phosphorus nutrition in crops and reducing dependence on chemical phosphorus fertilizers (Nosrati et al., 2014). In addition to enhancing phosphorus availability, these activities may contribute to improved nutrient-use efficiency and greater sustainability of agricultural production systems.Potassium Mobilization

Recent research has highlighted the ability of certain *Azotobacter* strains to mobilize potassium from silicate minerals and clay-associated potassium reserves. Through the production of organic acids and other weathering-related metabolites, these bacteria facilitate the release of mineral-bound potassium into plant-available forms.

This characteristic extends the ecological role of *Azotobacter* beyond nitrogen fixation and emphasizes its contribution to integrated nutrient cycling within agricultural soils (Sarikhani, 2016; Shirinbayan et al., 2019).

6.9. Siderophore production

Iron availability is often limited in aerobic soils because ferric iron (Fe^{3+}) has very low solubility under neutral to alkaline pH conditions. Several strains have been reported to produce siderophores, specialized low-molecular-weight iron-chelating compounds that enhance iron solubility and facilitate its uptake by microorganisms and, indirectly, by plants (Sharma et al., 2003; Alexander and Zuberer, 1993).

In addition to improving iron acquisition, siderophore production may contribute to plant growth promotion by altering iron availability in the rhizosphere and increasing microbial competitiveness. Under certain conditions, this can indirectly suppress phytopathogens by limiting iron access to competing microorganisms; however, the magnitude of this effect is strongly strain- and environment-dependent and varies with soil physicochemical properties and microbial community structure (Ahmed and Holmström, 2014).

6.10. Extracellular polysaccharides and soil improvement

The production of extracellular polysaccharides (EPS) represents one of the most important ecological and functional traits of *Azotobacter*. These biopolymers play a critical role in improving soil aggregation, enhancing water retention capacity, facilitating biofilm formation, and strengthening root–soil interactions.

Beyond their contribution to bacterial survival under adverse environmental conditions, EPS improve soil physical properties and promote rhizosphere stability. By enhancing soil structure and microbial activity in the root zone, EPS-producing *Azotobacter* strains can positively influence both plant growth and soil health. Consequently, these bacteria contribute not only to plant productivity but also to the long-term sustainability and resilience of agricultural ecosystems (Shirinbayan et al., 2019; Ali et al., 2023).

6.11. Integrated plant growth promotion

The beneficial effects of *Azotobacter* on plant growth are generally attributed to the synergistic action of multiple plant growth–promoting mechanisms rather than to any single functional trait. These mechanisms include biological nitrogen fixation (BNF), production of phytohormones such as indole-3-acetic acid (IAA), enhancement of nutrient availability through phosphorus solubilization and iron mobilization, siderophore production, and improvement of soil structure via

extracellular polysaccharide (EPS) secretion (Wani and Lee, 2002; Vessey, 2003; Kennedy et al., 2015).

The integration of these diverse functions underlies the considerable interest in *Azotobacter* as a key component of biofertilizers and microbial inoculants for sustainable agricultural systems. By simultaneously improving nutrient acquisition, stimulating plant development, and enhancing soil quality, this genus can contribute to increased crop productivity and ecosystem sustainability. Nevertheless, the relative importance of each growth-promoting mechanism varies among strains and is strongly influenced by environmental conditions, plant genotype, and soil properties, emphasizing the context-dependent nature of its agronomic performance and effectiveness (Vessey, 2003; Backer et al., 2018).

7. Response of Plants to *Azotobacter* Inoculation

Inoculation with *Azotobacter* spp. has been widely reported to enhance plant growth and productivity across a broad range of agricultural and horticultural systems. However, the overall response is not uniform and is strongly influenced by soil conditions, crop genotype, microbial strain specificity, and nutrient management practices. The beneficial effects are primarily attributed to a combination of biological nitrogen fixation, phytohormone production (particularly auxins and gibberellins), improved nutrient solubilization, and enhanced rhizosphere interactions.

Across cereal crops, especially wheat and maize, *Azotobacter* inoculation has been consistently associated with improvements in root system architecture, biomass accumulation, and grain yield. These responses are largely linked to stimulated root proliferation and enhanced nutrient uptake efficiency, particularly nitrogen and phosphorus. In several studies, yield increases ranging from moderate (8–11%) to substantial (up to ~20%) have been reported, particularly under field conditions with integrated nutrient management. Synergistic effects have frequently been observed when *Azotobacter* is co-inoculated with Azospirillum or arbuscular mycorrhizal fungi, indicating strong functional complementarity within the rhizosphere microbial community. Nevertheless, some multi-location field trials have reported weak or inconsistent responses, particularly in maize and wheat, emphasizing that environmental variability and native soil microbial communities can significantly modulate inoculation efficiency (Khosravi et al., 2024; Rai and Gaur 1988; Jarak et al., 2006; Khosravi et al., 2013; Bahrani et al., 2010).

In vegetable and greenhouse systems, *Azotobacter* generally exhibits more consistent positive effects on both growth and yield quality. Improvements in leaf area development, chlorophyll content, root biomass, and reproductive traits have been documented in crops such as tomato, cucumber, onion, lettuce, radish, cabbage, and squash. A notable feature in these systems is the partial replacement of chemical nitrogen fertilizers without yield penalties, indicating improved nitrogen use efficiency. In several cases, inoculation combined with reduced fertilizer inputs (typically 25–50% reduction) maintained or even enhanced productivity compared with full fertilization. These findings suggest that *Azotobacter* is particularly effective in intensively managed systems where nutrient availability and microbial responsiveness are higher (Balemi et al., 2007; Mishra and Tripathi 2011; Chamangasht et al., 2012; Shirvan et al., 2017; Subedi et al., 2019).

In industrial crops such as safflower, canola, tobacco, sunflower, and soybean-based rotation systems, *Azotobacter* inoculation has been linked to improvements in yield components, chlorophyll content, and nutrient uptake efficiency. Importantly, many studies indicate that partial substitution of synthetic nitrogen fertilizers (often 50–75%) combined with inoculation can sustain or enhance yield levels, highlighting its potential role in reducing chemical fertilizer dependency. In legume-based systems, co-inoculation with symbiotic nitrogen fixers further enhances nodulation and system productivity, suggesting additive or synergistic microbial interactions (Balemi et al., 2007; Mishra and Tripathi 2011; Chamangasht et al., 2012; Bhushan et al 2013; Shirvan et al., 2017; Mirzakhani et al., 2009; Amirhandeh et al., 2012; Gosal et al., 2012; Naderifar and Daneshian 2012; Rawat et al., 2013; Subhashini et al., 2016).

In fruit tree species, including apple, mango, citrus, pear, peach, pomegranate, and pineapple, *Azotobacter* has been shown to improve seed germination, seedling vigor, root development, nutrient uptake, and fruit yield and quality. These effects are often more pronounced at early growth stages and in nursery or young orchard conditions. The underlying mechanisms are generally attributed to phytohormone production, improved nutrient mobilization, and enhanced microbial activity in the rhizosphere. Co-inoculation with mycorrhizal fungi and other plant growth-promoting bacteria often produces stronger and more stable responses than single inoculation treatments (Damiano and Monticelli 1998; Sharma and Bhutani 1998; Sudhakar et al., 2000; Aseri et al., 2008; Khosravi et al., 2009; Mohamed and Massoud 2017).

Representative studies reporting the effects of Azotobacter inoculation on growth and yield of agricultural and horticultural crops under field and greenhouse conditions (Table 10).

Despite generally positive trends reported under controlled and field conditions, the effectiveness of *Azotobacter* inoculation is not universally consistent. Variability in field performance is a well-documented issue and is mainly attributed to strain–environment mismatches, soil physicochemical constraints (particularly pH, organic matter content, and nutrient status), competition with native microbial communities, and limited or inconsistent root colonization under field conditions (Bashan et al., 2014; Vessey, 2003).

Table 10. Representative studies reporting the effects of *Azotobacter* inoculation on growth and yield of agricultural and horticultural crops under field and greenhouse conditions.

Crop	*Azotobacter* species/strain	Experimental condition	Measured response	Increase (%)	Reference
Rainfed wheat (*Triticum aestivum*)	*A. chroococcum*	Field	Grain and shoot yield	36.2 / 37.8	Khosravi & Mahmoudi, 2013
Wheat (*Triticum aestivum*)	*A. chroococcum*	Field	Grain yield	10–30	Wani et al., 2016
Rice (*Oryza sativa*)	*A. chroococcum*	Field	Grain yield	8–20	Kannaiyan, 2002
Maize (*Zea mays*)	*A. chroococcum* (Azc10)	Field (multi-location)	Grain yield	up to 20	Khosravi, 2019
Tomato (Solanum lycopersicum)	*A. chroococcum* 76A	Greenhouse	Fruit fresh weight	39	Ruzzi & Aroca, 2015
Tomato (*Solanum lycopersicum*)	*A. chroococcum* 76A	Greenhouse	Fruit number	49	Ruzzi & Aroca, 2015
Tomato (*Solanum lycopersicum*)	*A. chroococcum* 76A	Greenhouse	Shoot dry weight	45	Ruzzi & Aroca, 2015
Cucumber (*Cucumis sativus*)	*Azotobacter* sp.	Greenhouse	Plant biomass	20–35	Baset Mia et al., 2010

Consequently, the agronomic efficiency of *Azotobacter* should be considered highly context-dependent rather than stable across diverse agroecosystems. This context dependency reflects the complex interactions between microbial inoculants, plant hosts, and soil environments, which often limit the translation of laboratory performance to field conditions (Kennedy et al., 2015).

Overall, accumulated evidence indicates that *Azotobacter* functions as a multifunctional plant growth–promoting bacterium, exerting both direct effects (BNF and phytohormone production) and indirect effects (nutrient mobilization, improved nutrient cycling, and modulation of microbial interactions) on plant performance. Its most consistent benefits are generally observed under integrated nutrient management systems and when applied in combination with other beneficial microorganisms, supporting its role as a component of sustainable agricultural strategies rather than a complete substitute for mineral fertilizers (Vessey, 2003; Bashan et al., 2014).

8. The Role of *Azotobacter* in Natural Ecosystems and Non-Agricultural Soils

Azotobacter spp. are free-living diazotrophic bacteria that contribute to nitrogen cycling in a wide range of natural ecosystems independent of direct plant association. Unlike symbiotic nitrogen fixers, they survive in soil organic matter and rhizosphere environments where they utilize available carbon sources while fixing atmospheric nitrogen under favorable ecological conditions. Their persistence in diverse habitats indicates ecological adaptability across soils with varying organic matter content, moisture regimes, and vegetation cover (Aasfar et al., 2025).

In natural ecosystems, BNF is not uniform but strongly regulated by environmental constraints such as carbon availability, oxygen diffusion, soil moisture, and temperature. While it has been suggested that nitrogen fixation by free-living diazotrophs may be relatively more pronounced in certain non-agricultural systems, particularly in low-input or undisturbed soils, direct comparisons with symbiotic systems should be interpreted cautiously due to methodological differences in estimation approaches.

Altitude-related variations in nitrogen fixation are primarily associated with changes in temperature, soil organic carbon inputs, and vegetation structure rather than oxygen pressure alone. Therefore, increased nitrogen fixation rates in some high-altitude ecosystems are more accurately explained by shifts in microbial activity and plant-derived carbon inputs rather than a direct physiological advantage of reduced oxygen tension for free-living diazotrophs. (Ladha et al., 2022).

Across ecosystem types, reported nitrogen fixation rates indicate that both free-living and symbiotic systems contribute substantially to the global nitrogen cycle, with symbiotic associations generally dominating in highly productive ecosystems such as tropical forests and managed grasslands, while free-living diazotrophs contribute relatively more in systems where symbiotic hosts are limited or absent.

Estimated ranges of nitrogen fixation across different biomes are summarized in Table 11, based on synthesized literature data (Reed et al., 2011). These values highlight the substantial variability in both free-living and symbiotic nitrogen fixation across ecosystems, reflecting strong environmental control over microbial activity.

Table 11. Estimated rates of BNF in free-living and symbiotic systems across ecosystems

Ecosystem type	Free-living BNF (kg N ha^{-1} yr^{-1})	Symbiotic BNF (kg N ha^{-1} yr^{-1})
Boreal forests	0.3–3.8	0.3–6.6
Temperate forests	0.01–12	1–160
Temperate grasslands	0.1–21	0.1–10
Tropical plains	3–30	3–90
Evergreen forests	0.1–6	5.5–16
Tropical floodplains	4.1–12	14–28.5
Tropical deciduous forests	3.3	7.5–30
Mediterranean shrublands	1	1.1–10
Desert ecosystems	0.01–13	0.7–29.5

Source: adapted from Reed et al., 2011

9. The Role of *Azotobacter* in Bioremediation of Inorganic and Organic Contaminants

Azotobacter spp. have been increasingly recognized as environmentally relevant microorganisms with potential involvement in the transformation and attenuation of both inorganic and organic contaminants in soil and aquatic systems. Their contribution to bioremediation is primarily indirect and is mediated through metabolic activity, extracellular polymeric substances (EPS) production, and redox-related biochemical processes rather than specialized obligate degradation pathways.

In the case of inorganic contaminants, particularly heavy metals, *Azotobacter* can influence metal mobility and bioavailability through multiple mechanisms, including adsorption onto cell surface functional groups, EPS-mediated complexation, and changes in local microenvironmental conditions such as pH and redox potential. These processes may result in either immobilization or altered speciation of metals, thereby reducing their immediate bioavailability and toxicity. Experimental evidence has shown that *A. vinelandii* can contribute to the removal of cadmium and lead from aqueous systems, likely through EPS-associated binding mechanisms (Pasetti et al., 1996).

Some strains of *Azotobacter* also exhibit tolerance to elevated concentrations of toxic metals such as Pb^{2+}, Hg^{2+}, Zn^{2+}, and Cr^{6+}, suggesting adaptive resistance mechanisms rather than direct detoxification pathways (Aleem et al., 2003). In mercury-contaminated environments, enzymatic systems such as mercury reductase and organomercurial lyase have been reported in certain bacterial communities, enabling the transformation of highly toxic Hg species into less reactive forms, although the ecological relevance of these pathways in *Azotobacter* populations may vary depending on strain specificity and environmental conditions (Ghosh et al., 1996).

Regarding organic contaminants, particularly petroleum hydrocarbons, *Azotobacter* may contribute to biodegradation processes indirectly through co-metabolic activity and biosurfactant production, which enhances the bioavailability of hydrophobic compounds. Reported studies indicate that combined inoculation with *Azotobacter* and hydrocarbon-degrading fungi can accelerate degradation of crude oil residues, suggesting synergistic microbial interactions in contaminated environments (Fauzi and Suryatmana, 2016). Additionally, biosurfactant-mediated emulsification may facilitate the dispersion and subsequent microbial breakdown of hydrocarbon fractions (Sianipar et al., 2016).

Overall, the role of *Azotobacter* in bioremediation should be interpreted as complementary rather than primary, as these bacteria generally function within complex microbial consortia where contaminant transformation results from synergistic interactions among multiple microbial groups. The efficiency of these processes is strongly dependent on strain characteris

10. The Role of *Azotobacter* in Mitigating Drought Stress in Plants

Azotobacter spp. have been reported to contribute to plant tolerance against drought stress through a combination of direct and indirect mechanisms that primarily act at the rhizosphere level. A key functional trait is the production of extracellular polymeric substances (EPS), which enhance soil aggregation, improve soil water-holding capacity, and facilitate the formation of stable soil microenvironments around the root zone. These structural modifications can improve water retention and regulate the diffusion of water and nutrients toward plant roots, thereby supporting plant performance under limited moisture conditions (Khosravi and Mahmoudi, 2013; Shirinbayan et al., 2019).

In addition to physical soil modification, certain *Azotobacter* strains may alleviate drought-induced physiological stress in plants through the production of phytohormones and the enzyme ACC deaminase, which can reduce stress ethylene levels. This modulation of

ethylene signaling is considered an important mechanism for maintaining root growth and delaying stress-induced senescence under water deficit conditions (Gamalero et al., 2023).

Experimental studies have also indicated that *Azotobacter* inoculation, particularly in combination with other beneficial microorganisms such as rhizobia, can improve plant water status, including relative water content and nutrient uptake under drought conditions. For instance, co-inoculation approaches have been associated with improved physiological performance in legumes under water-limited environments, suggesting synergistic microbial effects on plant stress adaptation (Dashadi et al., 2011).

Beyond drought tolerance, some studies have reported antagonistic effects of *Azotobacter* against soil-borne phytopathogens under controlled conditions. These effects are often attributed to competition for nutrients such as iron via siderophore production, as well as possible production of antimicrobial metabolites. For example, reduced disease incidence caused by fungal pathogens such as Sclerotinia, Fusarium, Rhizoctonia, and Pythium has been observed in laboratory or controlled experiments, although the consistency of such effects under field drought conditions remains less well established.

In maize under drought stress, inoculation with *Azotobacter* has been associated with improved growth parameters and nutrient uptake, indicating that its beneficial effects are not limited to a single physiological pathway but rather involve combined improvements in soil structure, microbial interactions, and plant physiological regulation (Shirinbayan et al., 2019).

Overall, the role of *Azotobacter* in drought stress mitigation should be interpreted as multifactorial and context-dependent, involving both soil-structuring effects (via EPS), plant physiological modulation (via hormone and ACC deaminase activity), and indirect biotic interactions within the rhizosphere. However, the magnitude of these effects is strongly influenced by strain specificity, soil texture, and environmental stress severity, and therefore cannot be generalized across all field conditions.

11. Effect of *Azotobacter* on Plant Disease Control

Azotobacter spp. have been reported as plant growth-promoting rhizobacteria with potential roles in the suppression of several soil-borne plant pathogens, although they are not classical biocontrol agents. Their contribution to disease reduction is mainly indirect and arises from rhizosphere competition, production of bioactive metabolites, and modulation of plant physiological status (Sumbul et al., 2020; Vessey, 2003).

One of the key mechanisms involved in pathogen suppression is competition for iron through siderophore production, which reduces iron availability to pathogenic fungi and limits their growth. This mechanism has been implicated in the suppression of pathogens such as Fusarium oxysporum and Rhizoctonia solani in different crop systems (Takemura et al., 2025). In addition, certain *Azotobacter* strains produce extracellular metabolites that can inhibit fungal growth and interfere with spore germination under in vitro and greenhouse conditions (Aasfar et al., 2025).

Experimental evidence also indicates that inoculation with *Azotobacter* can reduce the severity of soil-borne diseases in several host–pathogen systems. For instance, suppression of Fusarium, Rhizoctonia, and Sclerotinia species has been reported in laboratory and controlled experiments following application of *A. chroococcum* (Takemura et al., 2025; Hassouna et al., 1998). Similarly, reductions in fungal biomass and growth inhibition of Fusarium verticillioides have been observed in maize-associated systems, suggesting potential roles in both pathogen suppression and mycotoxin-related processes (Hamden et al., 2026).

In some systems, *Azotobacter* has also been associated with indirect suppression of plant-parasitic nematodes. For example, reduced incidence of Meloidogyne incognita has been reported in chickpea following inoculation with *A. chroococcum*, likely due to induced resistance and improved plant vigor (Sumbul et al., 2020).

However, it is important to note that the biocontrol efficiency of *Azotobacter* is generally inconsistent under field conditions. This is because its primary ecological function is nitrogen fixation rather than specialized pathogen antagonism, and its suppressive effects are strongly dependent on strain specificity, environmental conditions, and microbial community interactions (Vessey, 2003; Sumbul et al., 2020).

Overall, the role of *Azotobacter* in plant disease control should be considered complementary and indirect, contributing mainly through rhizosphere competition and enhancement of plant health rather than strong direct antibiosis or targeted pathogen elimination (Aasfar et al., 2025). Effects of Azotobacter spp. on plant disease suppression (biocontrol activity) presented in Table 12

12. Limitations of *Azotobacter* as a Biofertilizer

However, several biological and ecological constraints limit *Azotobacter* consistent agronomic performance. Compared with symbiotic diazotrophs such as Rhizobium, the nitrogen fixation efficiency of *Azotobacter* is generally lower and often insufficient to fully satisfy crop nitrogen demand under field conditions. Furthermore, as heterotrophic bacteria, their survival and activity are strongly dependent on the availability of organic carbon sources, which may restrict their performance in soils with low organic matter content (Ladha et al., 2022).

Another major limitation of *Azotobacter* is the variability in establishment and competitiveness of introduced strains under field conditions. Native soil microbial communities can significantly influence

Table 12. Effects of *Azotobacter* spp. on plant disease suppression (biocontrol activity)

Pathogen / disease	Crop / system	*Azotobacter* strain	Mechanism of suppression	Observed effect	Reference
Fusarium oxysporum	Tomato / maize rhizosphere	*Azotobacter* spp.	Siderophore production, competition, antimicrobial metabolites	Reduced disease severity and fungal growth	Chauhan et al., 2012
Rhizoctonia solani	Cotton, guar, tomato	*A. chroococcum* isolates	Antibiosis + extracellular metabolites	Significant inhibition in greenhouse and pot studies	Chauhan et al., 2012
Fusarium verticillioides	Maize	*Azotobacter* spp.	Antifungal metabolites + fumonisin degradation	Growth inhibition and toxin reduction in vitro	Deepa et al., 2022
Meloidogyne incognita (nematode)	Chickpea	*A. chroococcum*	Induced resistance + rhizosphere effects	Reduced disease incidence	Akram et al., 2016 (cited in Sumbul et al., 2020)
Fusarium oxysporum, *Rhizoctonia solani*	Cotton/guar/vegetables	*Azotobacter* spp.	Siderophore + antimicrobial compounds	Suppression of fungal growth in vitro	Chauhan et al., 2012
Multiple soil-borne pathogens	Broad crops	*Azotobacter spp.*	Competition, IAA production, siderophores	Reduced infection pressure, improved plant health	Sumbul et al., 2020
Fusarium, *Curvularia*, *Rhizoctonia*	Rhizosphere systems	*Azotobacter spp.*	Siderophores + antimicrobial compounds	Inhibition of fungal growth (strain-dependent)	Aasfar et al., 2021
Soil-borne fungi (general)	Various crops	*A. chroococcum*	Antifungal metabolites + competition	Partial disease suppression in field/greenhouse	Chauhan et al., 2012

colonization success, leading to inconsistent plant responses across environments. In addition, not all strains simultaneously express multiple plant growth-promoting traits, making strain selection and formulation a critical challenge in inoculant development (Sumbul et al., 2020).

13. Future Prospects of *Azotobacter* as a Biofertilizer

The growing demand for sustainable agricultural production and reduced dependence on synthetic fertilizers has renewed interest in multifunctional plant growth-promoting microorganisms, including *Azotobacter*. Although *Azotobacter*-based inoculants have demonstrated beneficial effects on plant growth, nutrient acquisition, and stress tolerance, their widespread adoption remains constrained by inconsistent field performance and environmental variability. Consequently, future research should focus on improving the reliability, efficiency, and adaptability of inoculant formulations under diverse agroecosystem conditions.

One important research priority is the identification and selection of elite strains possessing multiple beneficial traits, including efficient nitrogen fixation, phytohormone production, phosphate solubilization, potassium mobilization, stress tolerance, and rhizosphere competence. Advances in genomics, transcriptomics, proteomics, and metabolomics are expected to accelerate the discovery of genetic and physiological determinants associated with superior plant growth-promoting performance.

Another promising direction involves the development of next-generation microbial formulations. Encapsulation technologies, improved carrier materials, and controlled-release formulations may enhance bacterial survival during storage and after field application. In addition, the use of microbial consortia combining *Azotobacter* with complementary microorganisms such as phosphate-solubilizing bacteria, potassium-solubilizing bacteria, arbuscular mycorrhizal fungi, and other plant growth-promoting rhizobacteria may provide more stable and reproducible agronomic responses than single-strain inoculants.

Future studies should also place greater emphasis on understanding rhizosphere ecology and the interactions among *Azotobacter*, host plants, native microbial communities, and environmental factors. Such knowledge is essential for predicting inoculant establishment, persistence, and functionality under field conditions. The integration of systems biology approaches with ecological studies may provide new insights into the mechanisms governing successful plant–microbe interactions.

In the context of climate change, the potential role of

Azotobacter in enhancing crop resilience to abiotic stresses such as drought, salinity, heat, and nutrient limitation warrants further investigation. Understanding the molecular basis of stress mitigation may facilitate the development of inoculants specifically adapted to challenging environments.

Finally, future commercialization efforts should prioritize large-scale multi-location field trials, standardized evaluation protocols, and long-term assessments of agronomic, economic, and environmental outcomes. Such studies will be essential for translating laboratory and greenhouse findings into practical agricultural applications.

Overall, the future of *Azotobacter* as a biofertilizer is likely to depend not on its use as a direct replacement for chemical fertilizers, but on its integration into holistic nutrient management strategies that combine microbial technologies, improved soil health management, and sustainable agricultural practices.

14. Conclusion

Azotobacter spp. are widely distributed free-living diazotrophic bacteria that play a multifunctional role in soil ecosystems through BNF, phytohormone production, and enhancement of nutrient cycling processes. A substantial body of evidence indicates that their application can improve plant growth and yield across a wide range of agricultural and horticultural crops, particularly under integrated nutrient management systems.

The effectiveness of *Azotobacter* inoculation is, however, highly context-dependent and is influenced by soil properties, environmental conditions, crop type, and strain specificity. Field performance is often variable, reflecting limitations in colonization efficiency and dependency on available organic carbon sources.

Beyond growth promotion, *Azotobacter* contributes to improved plant performance under abiotic stresses such as drought and nutrient limitation, mainly through indirect mechanisms including EPS production, improved soil structure, and modulation of plant physiological responses. In addition, certain strains may contribute to the suppression of soil-borne pathogens through competitive and biochemical interactions within the rhizosphere.

Overall, *Azotobacter* should be considered a complementary component of sustainable agriculture rather than a standalone substitute for chemical fertilizers. Future research should focus on improving strain selection, formulation stability, and microbial consortia approaches to enhance field reliability and agronomic efficiency.

Although the possibility of developing more intimate plant–*Azotobacter* associations has been discussed in the literature, current evidence does not support the existence of stable symbiotic systems comparable to the legume–Rhizobium interaction.

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