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Review Article

The sustainable eco-friendly approach to improve crop production through *Azotobacter*

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Abstract

Usually, chemical fertilizer is used to increase soil nitrogen content. Intensive and high-dose utilization of N_2 fertilizers can cause ammonia volatilization and nitrate accumulation in the soil after prolonged application. An integrated nutrient management system, which integrates bacterial inoculants, plays a crucial role in soil health and productivity in sustainable agriculture. Biological nitrogen fertilizers containing *Azotobacter* can be used to increase soil fertility by increasing nutrient availability, providing some metabolites to plants during growth, and minimizing fertilizer doses by replacing or adding to fertilizers. The purpose of this literature review paper is to discuss the utility of *Azotobacter* in agriculture, and the perspective of *Azotobacter* to increase yield and substitute chemical fertilizer in food crop production. This review highlighted the potential of *Azotobacter* sp. as an efficient biofertilizer with testified efficacy to enhance plant nutrition and soil fertility. This bacteria's use in soil reclamation has proven beneficial, indicating that it may be a potential tool for turning barren land into rich soil. In order to effectively target agricultural challenges (such as the nutrient deficit, and biotic/ abiotic stresses), *Azotobacter* sp. still needs to be carefully exploited. This requires attention to a number of factors, including their multi-trophic interactions, synergies, abundance distribution, biogeography, and biological functions.

Keywords: PGPRs, Biofertilizers, Diazotrophs, Nitrogen Fixation, Consortia

1. Introduction

Plant growth-promoting rhizobacteria (PGPR) are increasingly utilized in crop production because they can effectively replace toxic additives like pesticides and chemical fertilizers. The term "biological nitrogen fixation" (BNF) is used to a microbial process that turns atmospheric nitrogen (N_2) into ammonium that roots can readily absorb. “Diazotrophs," are groups of N_2-fixing bacteria that can physiologically fix N_2 in conjunction with plant roots. Especially, the symbiotic rhizobacteria that transform bacterial cells into specialized structures called nodules and alter the morphological and physiological characteristics of plant roots. Other N_2-fixing bacteria are free-living, widely distributed fixers in farmland around the world. In agricultural and natural habitats lacking symbiotic N_2 fixation (SNF), they provide a significant natural supply of nitrogen (N_2). The *Azotobacter* sp. are usually aerobic and free-living, motile soil bacteria that produce slimy capsules (Tejera et al., 2005). This is an important step of the nitrogen cycle that relies on soil-dwelling diazotrophs, which contain an extensive range of metabolic abilities, including the capacity to repair atmospheric nitrogen using ammonia. *Azotobacter* sp. can be identified based on their chemical and biological characteristics, some strains were found to possess a higher nitrogen-fixing capacity in comparison to others, (Burgmann et al., 2003). *A. chroococcum* is the most common species which can exist in various soils, however, it is less adaptable in the rhizosphere of agricultural plants and on untamed land. It is widespread and economic practice employing *Azotobacter* as a biofertilizer for a variety of commercially significant plants, including rice, wheat, cotton oat, barley, sunflower, jute, coconuts, mustard, sesame, linseeds, castor, maize, sorghum, bajra, sugarcane, sugar beets, tobacco, tea, coffee, and rubber. In addition to fixing nitrogen, *Azotobacter* also synthesizes indole-3 acetic acid (IAA), gibberellins (GA) riboflavin, and thiamine. When administered to seeds, *Azotobacter* can significantly increase germination rates. Additionally, it shields young seedlings from fungal diseases due to its antifungal properties, so they don't succumb to the disease. Moreover, it produces vitamins and phytohormones that assist plants in fending off plant diseases; as a result, it plays a significant part in biotic stress resistance (Kader, 2002). Also, it was discovered that *Azotobacter* possesses glucose dehydrogenase enzymes to symbolize minerals. Some Azotobacter strains produce extracellular polysaccharides that shield the cell against drought and protozoan threats so they can be utilized as biofertilizers as well as for phytoremediation, (Looijesteijn et al., 2001). Some known species of *Azotobacter* are *A. paspali, A. insignis, A. vinelandii, A. armeniacus, A. beijerinckii, A. nigricans,* and *A. macrocytogenes.*

2. *Azotobacter* as a potential candidate for biofertilizer

Azotobacter constitutes a major proportion of soil microbiota (Gradova et al., 2003) and plays a very crucial role in bioremediation. The *Azotobacter* genus has been shown to utilize a wide range of organic substrates as Corban and energy sources, including mannitol, benzoic acid, phenolic compounds, and other organic acids, and then convert them into biologically active compounds that promote rhizospheric microbial proliferation. (Onwurah and Nwuke, 2004). Oil hydrocarbons are assimilated by *Azotobacter* both in the presence of fixed nitrogen and during nitrogen fixation, so adding the bacteria to oil-contaminated soil accelerates the process. *Azotobacter chroococcum* stimulates the growth of hydrocarbon-oxidizing bacteria in microbial preparations such as Devoroil (Gradova et

al., 2003). An eco-friendly bioremediation system based on *Azotobacter vinelandii* was investigated to determine the physicochemical parameters of olive oil mill wastewater (OMWW) and the ability of the bacterium to degrade characteristic constituents (Piperidou et al., 2000). Results showed that *A. vinelandii* could grow in OMWW utilizing its own components, turning it into organic liquid fertilizer. The ability of Azotobacter sp. to use aromatic compounds has long been known. It has the ability to degrade aromatic chemical compounds such as 2, 4-D, 2, 4, 6-trichlorophenol, protocatechuic acid, benzoate, and p-hydroxy benzoate. *Azotobacter* sp. has also been reported to degrade a range of other chlorinated phenols like 2-Chlorophenol, 4-Chlorophenol, 2, 6-Dichlorophenol, and 2, 4-6-Trichlorophenol by *Azotobacter* sp. (Wu et al., 2004). The sole carbon source, 2, 4-dichlorophenoxyacetic acid (2, 4-D) was considerably metabolized by *A. chroococcum* (Kumar et al., 2016a). Comparing 2, 4-D modified medium with non-amended medium, the growth rate of *A. vinelandii* remained the same (Ferrer et al. 1986). At lower concentrations, such as 10 ppm, specific strains of *A. chroococcum* have demonstrated efficacy in lindane degradation. (Anupama and Paul, 2009). The ability of *A. chroococcum* to convert a common herbicide, pendimethalin, into non-toxic compounds was shown (Kole et al., 1994), proving that the bacterium is crucial for both healthy crop production and environmental harmony.

Microorganisms employ a variety of heavy metal resistance and detoxification mechanisms, contributing significantly to the biogeochemical cycling of dangerous heavy metals and the repair of metal-contaminated habitats. (Kannaujiya and Shikha, 2022). Abo-Amer et al. (2014) found that among 10 strains of the isolated *Azotobacter* found in wastewater-contaminated soil were highly resistant to heavy metals like Co_2^+, Ni_2^+, Zn_2^+, and Cu_2^+. The study thereby highlighted the possible utilization of such bacterial isolates for the bioremediation of metal-contaminated system. Wheat plants grown in heavy metal-polluted soils are significantly controlled in their uptake by the heavy metal-resistant *Azotobacter* strain because of its strong potential to bind with Cd and Cr both in vitro and in vivo (Joshi and Juwarkar, 2009).

3. Mechanism of action to enhance the growth of the plants

Fig. 1 shows, azotobacter mechanism of action to promote plant growth. It has been seen that *Azotobacter* inoculums increase the crop yield within a few days (Shende et al., 1987; Kızılkaya, 2008). Keeping in mind the ability of *Azotobacter* to fix N_2, which is one of the most important macronutrients for plant growth, it is stated that BNF clearly plays a crucial role in the process. Also, these bacteria are capable of solubilizing insoluble phosphate in the soil (Nosrati et al., 2014). Two of the potential mechanisms of action suggested by some studies are the production of phytohormones and other similar substances that alter the morphology, alter the nitrate reduction mechanism of bacteria, and slow down plant growth, which causes enhancement of nitrogen accumulation in plants injected with *Azotobacter* strains (Wani et al., 2016). German workers obtained higher yields and nitrogen uptake in sand cultures of peas after inoculating plants with *Azotobacter*. During sowing time, Kurguzov (1954) observed that *Azotobacter* inoculation enhanced the available NO_3, P_2O_5, and K_2O at the root area. Further, adding fresh plant residues improves *Azotobacter* growth since it cannot grow in isolation and needs organic matter and compost as other complements.

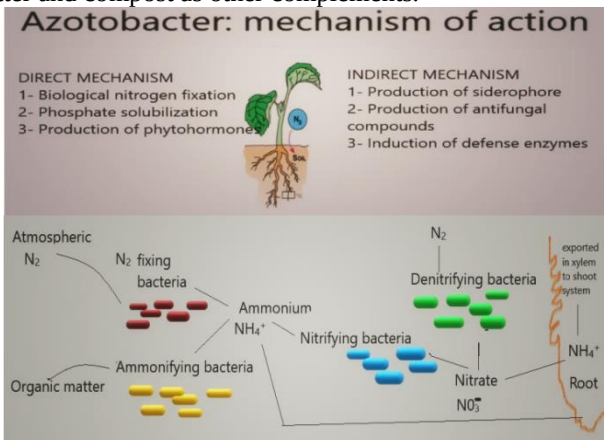

Fig.1 Azotobacter mechanism of action to promote plant growth

Azotobacter species are crucial in maintaining soil (N) status, they can fix up to 15 kg of nitrogen a year from the soil in free-living forms (Saha et al., 2017), while others reported about 60 kg a year (Bhattacharyya and Jha, 2012). Environmental variability, technical aspects, genotypic variability, and cropping practices related to estimation methods of BNF contribute to variations in the estimated non-symbiotic BNF rates (Ladha et al., 2016). Under aerobic conditions, this BNF process is the dominant trait of this species, which is extremely resistant to oxygen in the process of N_2 fixation. In addition to nitrogen, phosphorous (P) is also a significant nutrient that is crucial for the physiology of plants, microorganisms, and the biochemistry of plants. Insoluble forms of phosphate like ferrous phosphate (Fe_3PO_4), aluminum phosphate (Al_3PO_4), and tricalcium phosphate ($Ca_3PO_4)_2$ are all frequently found in soils. P is the slightly mobile and minimally accessible nutrient for plants compared to the other key nutrients, even when the overall soil P level is substantially above plant requirements (Nosrati et al., 2014). The strong reactivity of P ions with various soil components is the cause of the limited mobility of soil phosphate (Hinsinger, 2001). Thus only <1 mg of phosphate is usable for plant growth per year (Richardson et al., 2009). Insoluble P can, however, be convertible to soluble phosphate through soil phosphate-solubilizing microbes (PSMs) (Sharma et al., 2013; Kumawat et al., 2017). Soil microflora has also been found to solubilize insoluble phosphate components into soluble components which can be easily taken up through plants (Sashidhar and Podile, 2010). Phosphate-solving Azotobacter species are well known in the scientific community, for example, *A. vinelandii* strains are capable of solubilizing about 43% of the Egyptian Abu Tartur phosphate rock (El-Badry et al., 2016). In addition, Yi et al., (2008) reported the development of microbial, that were able to sufficiently solubilize tricalcium phosphate (TCP) was improved through variation caused by mutations, beginning with soil isolates, showing that exopolysaccharides (EPS) play a significant role in this process (Kumar et al., 2001). The microorganisms can thus be used to replace or to increase the effectiveness of chemical fertilizers (Narula et al., 2000; Nosrati et al., 2014). Soil physicochemical factors such as temperature, pH, organic component, salinity, moisture, and depth affected the populations of *Azotobacter* sp. in soil ecosystems (Kizilkaya, 2009). The NaCl concentrations have an impact on the PGPR properties of *Azotobacter*,

particularly BNF in the soil. It has been reported that *Azotobacter* can tolerate saline conditions as high as 10% sodium chloride For example, *A. salinestris* can resist 8% salt. As a typical mesophilic bacteria, *Azotobacter* flourishes at temperatures between 25 and 30 °C for optimal growth and activities. Apparently, *Azotobacter* grows best at temperatures less than 0^0C. The cells of *Azotobacter* cannot withstand high temperatures, but they can form cysts that germinate under favorable conditions at 45–48°C (Saribay, 2003). For example, *A. salinestris* can survive at 45^0C and gives optimum growth at 35^0C, the growth is reduced with increasing temperatures above 35^0C. It has been found that the pH of soil ecosystems regulates the prevalence of *Azotobacter* communities, lower pH (≤6.0) typically reduces the population of *Azotobacter* and, in sometimes entirely prevents their proliferation. Due to adverse elements including inadequate and physiologically active nutrients and ineffective air-water regimes, *Azotobacter* is exceedingly uncommon or even nonexistent in acidic soils. (Andjelković et al., 2018). For *Azotobacter*'s physiological processes, a pH range of 7 to 7.5 is ideal. *A. salinestris* is intolerant to pH levels more than 9, and above this range, growth cannot be seen, however *A. chroococcum* is able to thrive at pH 9 and higher pH levels do not affect its growth.

3.1 Cysts of the bacterium *Azotobacter* confer special survival and toleration properties

Cyst formation is one of the distinctive traits of *Azotobacter* sp. (Sadoff, 1975). Adverse and critical conditions like salinity, higher and lower temperatures, freezing, and drought conditions induce cysts formation naturally. The addition of some organic compounds in the medium like n-butane-1-ol, b-hydroxybutyrate, and ethanol, or altering the nutrient concentration in the medium also can induce cyst formation (Núñez ez et al., 1999). These physical alterations go along with metabolic changes, including modifications to catabolic processes, respiration, and macromolecule production. *Azotobacter's* cysts are spherical and made up of a "two-layered shell," a "central body" that is a scaled-down version of vegetative cells, and multiple vacuoles. While the exterior contains a hexagonal crystalline formation called exine, the interior of the shell contains a fibrous structure called intine. Alginate, a crucial element of the capsule, and alkylresorcinols, alkylpyrones and a phenolic lipid, are elements of the exine and are produced following encystment induction and take the place of the phospholipids in the cyst membranes. The central body always contains a large number of polyhydroxybutyrate granules (García et al., 2014). Studies have revealed that LEA (Late embryogenesis abundant) proteins and short RNAs are crucial for the growth of *Azotobacter* cysts as well as for their ability to withstand desiccation and other abiotic stresses (Rodriguez-Salazar et al., 2017). Cysts are able to survive under drought conditions for over 10 years while vegetative cells could not survive even 2 years, preserved under the same conditions (Vela, 1974). They have a twofold increase in UV resistance, in particular, also resistant to ultrasound, gamma radiation as well as drying, but not resistant to heat (Wyss et al., 1961). As discussed above, cyst formation in the laboratory can be achieved by inducing *Azotobacter* with some reagents such as n-butane-1-ol, ethanol or b-hydroxybutyrate. This procedure may be of significant importance for the bioformulation of *Azotobacter*, in particular. In the production of biofertilizer products, it was demonstrated that large-scale cyst formation employing *Azospirillum lipoferum, Sinorhizobium meliloti*, and *Azospirillum brasilense*, increased the product shelf-life while preserving its efficiency. Since this trait makes *Azotobacter* sp. more resistant to the conditions of the soil and environment as well as predators, more research is required to examine the nature of cysts in a natural soil environment.

3.2 Traits and compounds of *Azotobacter* to promote plant growth

Azotobacter's positive effects on plant growth can be attributed to its ability to enhance root development, improve mineral absorption by roots, and counter fungus and pathogen growth. Bioactive compounds such as biotin, heteroxins, B vitamins, gibberellin, nicotinic acid, and pantothenic acid, as well as other chemicals, are synthesized and secreted in large quantities by *Azotobacter*, promoting plant root growth, (Patil et al., 2020). Another characteristic used to identify growth-promoting *Azotobacter* strains and identify effective free-living N_2-fixing bacteria is the solubilization of inorganic and organic P. Auxin (IAA) synthesis is another characteristic of *Azotobacter* sp. which promotes plant growth. Auxin is a fundamental phytohormone reported by Halliday et al.,(2009), that instigates the formation of lateral roots, root elongation, and density root hairs, which helps in nutrient adsorption, (Datta and Basu, 2000). Auxin is essential for cell elongation, cell division, initiation of roots, leaves, flowers, fruits, and for senescence. Several studies suggested that *Azotobacter* species could promote plant development by synthesizing this phytohormone instead of fixing nitrogen. (Behl et al., 2012; Ahmed and Holmström, 2014). A few strains of *Azotobacter*, including *A. vinelandii, Atropicalis tropicalis* and *Azorhizophilus paspali* have also been noted for their ability to produce antifungal compounds that prevent the growth of phytopathogenic species, (Bjeli´c et al., 2015). El_Komy et al. (2020) found that the mycelial growth of some pathogenic fungi, including *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Fusarium solani*, was significantly inhibited by the mixture of *Azospirillum, Azotobacter, and Klebsiella.* Additionally, *A. Vinelandii* isolates have been shown to be capable of producing alginate-like polysaccharides, at rates ranging from 4.88 to 5.26 g/L. Production of Hydrogen cyanide (HCN) and siderophores are characteristic features of *Azotobacter* sp. (Baars et al., 2016). One of the major possibilities for *Azotobacter* to encourage plant growth is the solubilization of zinc (Zn) and potassium (K). Wu et al., (2006) found the capacity to boost Zn bioavailability in the soil system by the soil bacteria *A. chroococcum*. He also described acidification as a mechanism of this process. These microorganisms create organic acids in the soil that bind Zn cations and lower the pH of the surrounding soil (Alexander, 1997; Aung et al., 2020). Creating new siderophores by *A. chroococcum*, including vibrioferrin, crochelins, and amphibactins, which hold the iron in a hexadentate manner with the aid of iron-chelating amino acids, is another process that could contribute to Zn solubilization.

These siderophores not only assist bacteria in gaining access to iron resources but also aid in the management of soil-borne plant diseases, (Saravanan et al., 2011). Numerous studies have demonstrated that certain *Azotobacter* species can solubilize K, (Diep and Hieu, 2013). Other studies revealed that *Azotobacter* sp. could play a significant role in enhancing plant K uptake in addition to solubilizing K. The production of enzyme 1-aminocyclopropane1-carboxylate (ACC) deaminase is another

crucial characteristic of *Azotobacter* sp. (Omer et al., 2016). Ethylene gets lowered by ACC deaminase, excessive levels of which can harm or even kill plants, (Glick et al., 2007). ACC deaminase also lowers plant ACC levels, which is a plant ethylene precursor, by breaking down the ACC into ammonia and α-ketobutyrate. Moreover, some *Azotobacter* strains synthesize pigments that are important for other bacteria's metabolism. For instance, *A. chroococcum* produces the dark-brown pigment melanin which is water-soluble and occurs during BNF at high levels of metabolism. The nitrogenase system has been observed to be shielded from oxygen by this process. Shivprasad and Page (1989) used soil dehydrogenase activity, a measure of the level of microbial metabolism, to quantify the impact of *Azotobacter* on soil's overall microbial activity. Table 1 shows Azotobacter strains identified possessing growth-promoting traits.

3.3 *Azotobacter* mediated biotic and abiotic stress resistance to the plants

The main environmental factors limiting crop growth, quality, and yield include salinity and drought. Some salt-resistant *Azotobacter* strains have been found to successfully colonize the rhizosphere and stimulate plant development in challenging environments. In addition to their recognized function of nitrogen fixation, *Azotobacter* may have additional features that further add to their ability to reduce plant stress in a variety of ways. These all properties are found to enhance the resistance to biotic and abiotic stresses in injected plants (Ruzzi and Aroca, 2015). It has been found that *Azotobacter* enhances the growth of wheat plants when inoculated under salinity (Chaudhary et al., 2013).

Injection of *Azotobacter* to maize plants increases potassium intake and sodium exclusion, which has been demonstrated to improve growth under salinity (Abdel Latef et al., 2020). Also, several plants found to be protected from the biotic stress of plant diseases through *Azotobacter*. Their ability to colonize the soil and rhizosphere, as well as their ability to compete with local microbial and fungal strains, determine their capability to withstand stresses. Plant pathogens, including *Curvularia, Rhizoctonia Fusarium*, and *Aspergillus* species, are known to be inhibited by siderophores and HCN production (Ponmurugan et al. 2012) as well as antimicrobial substances like 2,3-hydroxybenzoic acid, aminochelin, azotobactin, protochelin, and azotochelin, (Bhosale et al., 2013). The effectiveness of employing *Azotobacter* as a remedy for drought stress tolerance has been shown in numerous research, (Shirinbayan et al., 2019). Sunflowers treated with EPS produced by *Azotobacter* showed increased resilience to water stress, (Vurukonda et al., 2016). This is likely because of their capacity to promote rhizosphere soil physiology. Under drought conditions, *Azotobacter's* EPS is crucial for maintaining cellular hydration and biofilm formation.

4. Current research trends to use *Azotobacter* as a potential biofertilizer

As a non-symbiotic organism, *Azotobacter's* ability to increase growth can be fully utilized by co-injection along with other biofertilizers as opposed to its sole application. *Azotobacter*, when used in conjunction with other biofertilizers, accelerates the beneficial effects of these fertilizers, in conjunction with benefiting plants directly by improving mineral adsorption. Moreover, other reports suggest that microorganisms enhance the plant growth-promoting activity of *Azotobacter* and there are currently a number of examples of *Azotobacter* being used in combination with other bacteria, which is highly preferred by both researchers and farmers.

4.1 *Azotobacter's* consortium with other biocontrol agents

Phosphate-solubilizing mycorrhizal fungus have been found to work well in conjunction with *Azotobacter* to improve the characteristics of plant growth among the fungal biofertilizers (Behl et al., 2003). Several researchers have discovered synergistic interactions between *Azotobacter* and the AM fungus, (Akram et al., 2016). *Glomus fasciculatum* along with *Azotobacter chroococcum* inoculations, either separately or together, can affect the rhizospheric bacteria population in tomato plants, (Bagyaraj and Menge, 1978). They discovered that tomato plants that had been injected with both *G. fasciculatum* and *A. chroococcum* had many populations of bacteria (with *actinomycetes*) in their rhizosphere than plants that had either received *G. fasciculatum* or *A. chroococcum*. The rhizospheric *A.chroococcum* population was increased after tomato plants were injected with *G.fasciculatum* and sustained at a high density for a larger period of time. Further, *G. fasciculatum* infection and spore production increased when tomato roots were inoculated with *A. chroococcum*. Furthermore, tomato plants' dry matter was remarkably increased when injected with both *G. fasciculatum* and *A. chroococcum* in comparison to uninjected plants. Similar effects had been observed in wheat injected with *Azotobacter* along with AM fungus. Aseri et al. (2008) discovered that the application of a mixture of A. chroococcum and Glomus mosseae improved the ability of pomegranate (Punica granatum) plants to thrive in stressful environmental circumstances. According to Arora et al, (2018), the mutualistic symbiosis between the AM fungus *Piriformospora indica* and *A. chroococcum* improved the plant's physiological and biochemical characteristics, which increased the level of the active ingredient artemisinin. Table 2 shows consortium of Azotobacter with other microorganisms.

4.2 Molecular biology to enhance the bio-fertilizer property of *Azotobacter*

It has been suggested to use *Azotobacter* sp. as biofertilizers to plenish the nitrogen amount into the soil (Gauri et al., 2012). It is important to take into account the cost-effective techniques while enhancing the nutritional qualities of *Azotobacter* that can give the agriculture industry a cheaper source of biofertilizer. In order to maximize *Azotobacter's* development during fermentation and to maximize its biofertilizer properties, it is required to standardize cultural and nutritional conditions when contemplating its large-scale production. Because of their beneficial characteristics and potential to create new chemicals that can be used as a more potent tonic for managing soil and plant health, bacterial inoculants and the polymers they produce have gained increased biotechnological and industrial interest. Exopolysaccharides (EPS), siderophores, and poly—hydroxybutyrate (PHB), which are key biological compounds produced by *A. vinelandii*, are crucial for biotechnological applications (Diaz-Barrera and Soto, 2010). Genome editing can significantly improve the capacity of *A. vinelandii* to fix nitrogen by either adding or removing certain genes (s). Targeted gene modification is done in a way that causes common metabolites to produce terminal products from urea (Barney et al., 2015).

Table 1 Azotobacter strains identified possessing growth-promoting traits

Microorganism	Strain	Growth condition	Results	Reference
Azotobacter sp.	Ale-3		Field trials showed enhanced growth and yield of wheat	Hooda and Dahia (1992)
A. chroococcum	B12	Nitrogen-Free Burk's medium containing 1%(w/v) glucose as a carbon source	Higher N_2 fixation at neutral pH with low conc. Of KNO_3 And $(NH_4)_2SO_4$, also showed tolerance to NaCl.	Jana and Mishra (1994)
A. chroococcum	A-41, B-8005, BKMB-1030, MKU 201	Jensen's agar medium	Showed better growth and nitrogen fixation at a varied range of temp.	Rajkumar and Lakshmanan (1995)
A. chroococcum	MAC-27, WH-147, E-12, RH-30	Jensen's medium contains sodium glutamate as a nitrogen source and EDA-HCl		Kumar and Narula (1999)
A. chroococcum	Mala-11 and HT54	Jensens's N-free medium at 300C	Produce phytoharmones like IAA, Gibberellin, cKinetin	Verma et al. (2001)
A. chroococcum	BG-13 and BG-33	Jensens's N-free medium with sucrose	Degrade 2,4-D to chlorocatechol in the presence of sucrose as a carbon source	Gahlot and Narula (2004)
A.vinelandii		Burk's medium plus 2% sucrose or glucose and ammonium acetate as nitrogen source at 300C	Developed sucrose catabolic regulation	Johnson et al. (2006)
A.vinelandii	ATCC 9046	Burk's medium at room temp.	Enhanced growth of the test plant	Pena et al. (2008)
Azotobacter sp.		Cultured on Ashby's agar after serial dilution till 105	The root length, shoot length, protein, carbohydrate, fresh weight, and chlorophyll content of Trigonella plantlets were increased	Nagananda et al. (2010)
A.vinelandii		Nitrogen-deficient medium containing combined carbon sources at 320C in a rotary shaker for 5 days	Improved alginate production	Lozano et al. (2011)
A. chroococcum	Azt		Suitable probiotic in aquaculture, shown better growth parameter in Oreochromis niloticus aquaculture	Sayeda et al. (2011)
Azotobacter sp.	ST3, ST6, ST9, ST17 and ST24	Cultured in Jansen's broth at room temp.	Under salt stress, wheat grain production, total nitrogen, and biomass are increased.	Chaudhary et al. (2013)
Azotobacter vinelandii	O2, O4 and O6		phosphate solubilizing ability and enhanced growth rate	Nosrati et al. (2014)
A. vinelandii		Cultured in modified Burk's medium limiting Fe	Synthesis of a vibrioferrin-like compound, an α-hydroxy carboxylate siderophore characteristic of marine bacteria such as Vibrio parahaemolyticus	Baars et al. (2016)
Azotobacter sp.	Az63, Az69 and Az70		Provide drought resistance through phosphate and potassium solubilization, siderophore production and maximum growth in PEG 6000	Shirinbaan et al. (2019)
A. chroococcum			Improved growth characteristics, pigments, K+/Na+ ratio, and CAT, POD, and APX activity under salt stress in maize.	Abdel et al. (2020)

Table 2 Consortium of Azotobacter with other microorganisms

Consortium	Test plant	Effect	Reference
Azotobacter chroococum+ arbuscular mycorrhiza fungi (AMF)	Wheat	Enhanced root length and increased root biomass	Behl et al. (2003)
G. fasciculatum and A. chroococcum	Tomato	The dry weight of tomato plants increased significantly	Bagyaraj and Menge (1978)
ZOB-1 *(Azotobacter sp. Chlorella vulgaris, and Anabaena variabilis)*	Rice	Enhanced germination and improved growth of rice plants	Zayadan et.al. (2014)
Piriformospora indica & Azotobacter chroococcum strain W5	Rice	Enhanced plant growth and improved nutrient uptake	Bandyopadhyay et.al. (2022)
Azotobacter and AMF	Jatropha	Increased shoot length, shoot diameter, LRWC, carbohydrates, proteins, and pigments that are used in photosynthesis	Kumar et al. (2016b)
Azotobacter, Enterobacter cloacae, Enterobacter hormaechei and Pantoea agglomerans strain	Wheat	Higher grain, straw, biological yield and harvest index	Jain et al. (2021)
A. chroococcum 76A *Trichoderma afroharzianum* T22, *and 6-pentyl-α-pyrone*	Basil	Enhanced total fresh weight of basil	Comite et al. (2021)
Piriformospora indica (Pi) and Azotobacter chroococcum (Az)	Annual mugwort	Enhanced activities of antioxidant enzyme and non-enzymatic antioxidants to combat salt stress	Arora et. al . (2020)
Azotobacter and Azospirillum	Eucalyptus	Enhanced growth, rooting and leaf number.	González-Díaz etal. (2019)
Azotobacter sp. + Burkholderia sp. +Sphingobacterium sp.	Onion	Improved plant growth parameters, yield characteristics, bulb quality characteristics, and decreased physiological weight loss after 15–120 days of room temperature storage.	Tinna et al. (2020)

The nifL gene of the nifLA operon system can be disrupted in a number of clever ways to increase the amount of ammonium secreted by *A. vinelandii*, (Ortiz-Marquez et al., 2012). Additionally, the soil ecology of various places varies greatly, which disables the same strains of *Azotobacter* to be functional in different regions and can't be effective as a biofertilizer universally. Given the significance of chemicals and EPS found in the bacterial ecosystem in agricultural soil, it is important to consider *Azotobacter* strains that have the highest capacity to fix nitrogen and create these compounds at higher rates. Furthermore, future agricultural practices may take into account to improve our understanding of manipulating these qualities to meet human requirements (Gauri et al., 2012).

5. Future prospective and conclusion

The ability of *Azotobacter* to fix nitrogen, produce growth hormones, solubilize phosphates, manage plant diseases, and improve soil health makes it an excellent choice for use as a biofertilizer for environmentally friendly and sustainable crop production. Understanding and utilizing all of *Azotobacter's* advantageous traits may prove to be of utmost importance for crop improvement projects in the future. However, further research is urgently needed to improve screening methods, characterize chemicals that promote plant growth and antimicrobials from bacterial isolates, and understand the molecular underpinnings of the mechanisms at play. Further research is also necessary to explore the potential of *Azotobacter* to improve soil fertility by using modern technology such as soil genomics. Scientists have a great possibility in identifying compatible partners, such as a specific strain of *Azotobacter* that will work well with a particular plant genotype to increase bio-fertilizer efficiency. Agrochemicals, which have several negative side effects on the environment, will eventually be replaced by these naturally occurring nitrogen-fixing bacteria.

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Author contribution-

Shivangi Awasthi: Written-original draft, formal analysis, conceptualization, methodology; Professor Shikha: Supervision, reviewing, Dr. Rajeeva Gaur: Supervision and reviewing.

Conflict of interest

There are no conflicts of interest declared by the authors.

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