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Free-living bacteria of the genus *Azotobacter* – significance, mechanisms of action and practical use in crop production and sustainable agriculture

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Abstract. Crops grown today show high yield potential, and one of the conditions for realising this potential is to meet their increased nutrient requirements. Arable soils often lack adequate nitrogen, which results in reduced yields and reduced profitability of production. The low nitrogen content of the soil is usually supplemented by the application of mineral fertilisers, which can cause ammonia volatilisation and nitrate accumulation in the soil. Long-term and intensive use of nitrogen fertilisers also contributes to soil acidification, groundwater contamination and an imbalance in the biological ecosystem, for example by increasing the proportion of fungi in the soil microbial population. It is therefore crucial to develop integrated crop production strategies that sustainably increase crop productivity. No less important is the maintenance of soil quality and the reduction of soil degradation problems. Therefore, there is a growing interest in non-chemical methods of fertilisation and plant protection. Biologically active agents are being sought to protect plants and promote their growth. A number of biopreparations are available on the market that improve the humus-forming properties of soil, do not disturb the biological balance and increase plant yields. Their advantages include increasing the availability of elements needed by plants (nitrogen, phosphorus), as well as stimulating plant growth and development through the synthesis of phytohormones. The market for biopreparations is growing rapidly and forms the basis of plant protection in line with the European Green Deal and the ‚Field to Table' strategy, which promote the reduction of use of synthetic mineral fertilisers and pesticides in favour of biological solutions. One of the preparations available on the market are vaccines containing free-living atmospheric nitrogen-fixing bacteria belonging to the genus *Azotobacter*.

This article highlights the importance of bacteria belonging to the genus *Azotobacter* as a potential ingredient in biopreparations to improve the growth, development and yield quality of many crops. Furthermore, the mechanisms used by *Azotobacter* spp. to promote plant growth (fixation of atmospheric nitrogen, solubilisation of phosphate, potassium and zinc, synthesis of phytohormones, vitamins, siderophores and protection against pathogens) are discussed. Attention has been drawn to the ability of bacteria of the genus *Azotobacter* to form cysts, which enables them to survive under adverse environmental conditions (high temperature, acidic pH, salinity). Current information on the importance and practical use of *Azotobacter* spp. in crop production and sustainable agriculture is also reviewed. The use of biopreparations based on *Azotobacter* spp. strains in agriculture allows for increased crop production and can contribute to meeting the food needs of the world's ever-growing human population.

Keywords: *Azotobacter* spp., biopreparations, plant breeding, plant growth-promoting mechanisms

INTRODUCTION

Due to the strategic role of food in human life, ensuring food security is one of the most important challenges in the modern world (Kozłowska-Burdziak, 2019). In order to meet the increasing demand for agricultural products for food, synthetic fertilisers are used as a method to improve the yield of many crops (Yousaf et al., 2017; Geng et al., 2019). Unfortunately, over-application and intensive use of mineral fertilisers has led to degradation of the soil environment. It is important to remember that these fertilisers are also a source of greenhouse gas emissions and contribute significantly to climate change (Hindersah et al., 2020).

Awareness of the impact of food on human health has increased significantly in recent years, and there is a growing demand for organic agriculture food products worldwide (Rahman et al., 2024). Associated with agricultural intensification, the indiscriminate use of synthetic fertilisers and pesticides has resulted in pollution of the environment, soils, water and food, disruption of biodiversity and progressive climate change (Sivasakthi et al., 2017). Therefore, great importance is now attached to maintaining high soil quality and protecting the environment through, among other things, the popularisation of biopreparations, through which it is possible to supply nitrogen, phosphorus and phytohormones to plants. Protecting soils from degradation while achieving satisfactory yields, is one of the objectives of sustainable agriculture. This objective can be achieved, among others, by using plant-growth promoting rhizobacteria (PGPR), characterised by their ability to grow vigorously, metabolise many compounds and adapt to varying environmental conditions (Calvo et al., 2014). These bacteria stimulate plant growth either directly or indirectly. Direct plant growth stimulation is based on the production of phytohormones (auxins, cytokinins and gibberellins), the reduction of ethylene levels and the intensification of mineral uptake by increasing root surface area or inducing ion uptake systems. Indirect plant growth stimulation, in turn, involves biological control of pathogens and induction of systemic resistance (Dąbrowska et al., 2014, 2016). Some types of PGPR bacteria have found commercial use as biofertilizers (Glick, 2012). These include the genera: *Azotobacter*, *Azosprillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas* and *Serratia* (Table 1).

Azotobacter spp. are among the best-studied bacteria found in the root zone of plants and stimulate their growth and development. The presence of these bacteria has been reported in the rhizosphere of rice, maize, sugarcane, bajra and many other crops (Mazid, Khan, 2015). Due to their ability to fix atmospheric nitrogen and make it available to higher plants, produce substances that stimulate plant growth and development, and their ability to produce pathogen-inhibiting compounds, they are used in the production of biofertilisers, biostimulants and bioprotectants (Mohamed, Almaroai, 2016; Subedi et al., 2019).

Azotobacter-based biofertilisers are widely used in India, China and Indonesia, in many European countries, including Poland. The use of biofertilisers is becoming a modern biotechnological solution to support the development of agricultural practices that minimise environmental pollution and soil degradation (Hindersah et al., 2020).

The main aim of the article is to highlight the importance of free-living diazotrophs of the genus *Azotobacter* in sustainable agriculture, discussing the mechanisms used by these bacteria to promote plant growth and development, as well as their practical use as a biological agent in the production of biofertilisers, biostimulants and bioprotectants.

PLANT GROWTH-PROMOTING MECHANISMS

Atmospheric nitrogen fixation

Nitrogen is an essential plant nutrient, crucial for satisfactory, high-quality yields. This element is a component of many organic compounds, such as proteins, nucleic acids, nucleotides, plant hormones and energy carriers (ATP), which determine the normal development of living organisms. This element is therefore responsible for many plant processes, such as chlorophyll and protein synthesis, and therefore its deficiency contributes to a strong reduction in plant growth and development (Adamczyk, Godlewski, 2010; Ueda et al., 2017). Despite the presence of nitrogen in the geosphere, most, as much as 98%, is present in a form that is not available to living organisms, and only 2% of nitrogen can be utilised either directly or after conversion to readily available forms (Herridge et al., 2008). In soils, nitrogen occurs in organic and inorganic forms and is only taken up by plants in the form of NH_4^+ and NO_3^- ions (Paśmionka, 2017). Cultivated soils are often deficient in bioavailable nitrogen, resulting in reduced plant growth

Tabela 1. Plant growth promoting rhizobacteria commercial available as biofertilizers.

Bacteria	Biofertilizer	References
Azotobacter	Azotobakteryna, Bactim Nutri N+	Subedi et al., 2019
Azospirillum	NovobaktAzo+	Zeffa et al., 2019
Bacillus	Bi Azot, FitoProtect, BactoFos	Akinrinlola et al., 2018
Bradyrhizobium	Legume Fix, Turbosoy, MasterFix	Savala et al., 2022
Burkholderia	Ino Bact P Myc	Paungfoo-Lonhienne et al., 2016
Enterobacter	BioSistem POWER SC, Ecostern	Bunas et al., 2022
Pseudomonas	Proradix, SuperPower, Bacto Tarcza P	Qessaoui et al., 2019
Rhizobium	Nitragina, Rhizobium Bio-Gen, Nitraces	Aloo et al., 2022

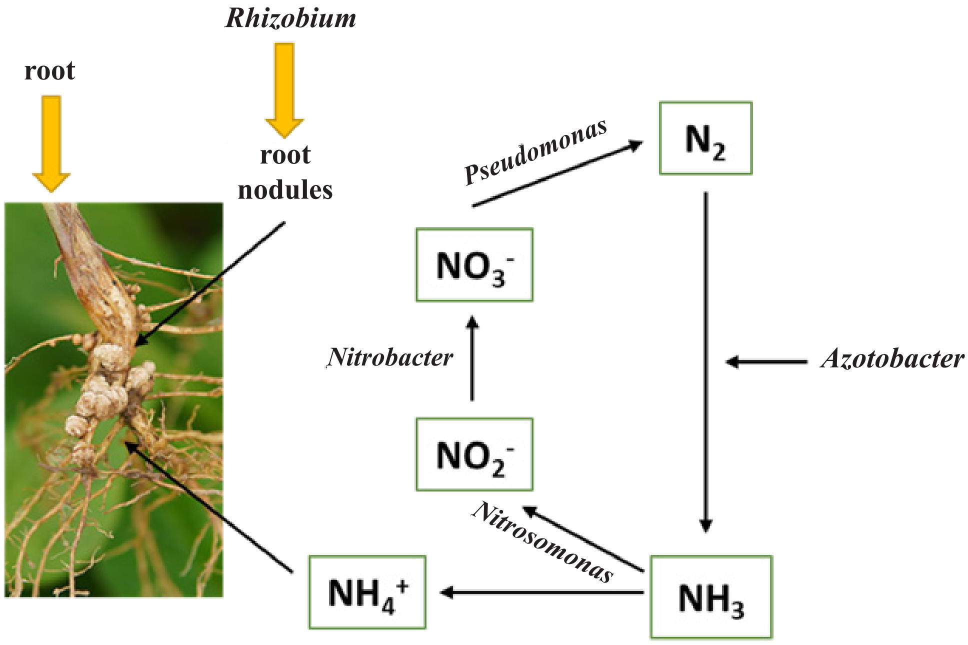

Figure 1. Contribution of microorganisms to the nitrogen cycle in nature (Gothandapani et al., 2017; modified).

and biomass production, even in environments with suitable climatic conditions and water availability (Martinez-Espinosa et al., 2011). The circulation of nitrogen in nature and its availability to plants are very much dependent on microorganisms, which are an integral part of the soil environment and play a key role in nutrient mineralisation (Figure 1).

The process of biological fixation of atmospheric nitrogen (BNF) contributes approximately 140–170 million tonnes of this element to the Earth's nitrogen cycle every year, which is of great importance from both an ecological and practical point of view. From a chemical point of view, the process of biological nitrogen fixation involves the conversion of molecular nitrogen, which is unassimilable to plants and animals, into a reduced form of this element, such as ammonia, which can be further utilised by living organisms. All microorganisms capable of carrying out this process have an enzyme complex, called nitrogenase, that enables them to fix nitrogen (Sivasakthi et al., 2017). Nitrogenase is an enzyme that is very sensitive to the presence of oxygen, which irreversibly inactivates it. Strictly aerobic microorganisms, for which molecular oxygen is essential for life, use mechanisms to protect nitrogenase from its deleterious effects. Bacteria of the genus *Azotobacter* have several such protective strategies. Firstly, the enzyme is protected by thick mucosal envelopes, which become a physical barrier that impedes the passage of oxygen from the external environment. Secondly, *Azotobacter* ssp. bacteria have a branched respiratory chain and when fixing atmospheric nitrogen they utilise those of its branches where only one phosphorylation site is present, and the reduction of O_2 to H_2O takes place with high efficiency. A final mechanism to protect nitrogenase from the toxic effects of oxygen is the formation of a complex of this enzyme with a special protein found in the cytoplasm under conditions of high oxygen concentration inside the cell. Once bound to the protein, nitrogenase becomes inactive and cannot be inactivated by oxygen (Baj, Markiewicz, 2007; Hakeem et al., 2017).

Biological fixation of atmospheric nitrogen is one of the most important biological processes occurring on the Earth's surface after photosynthesis (Vance, Graham, 1995), and the ability of microorganisms to fix atmospheric nitrogen is one of their most important activities (Vojinoviv, 1961). This process plays an important role in nitrogen cycling in the biosphere (Wani et al., 2016), as well as in maintaining soil fertility and improving crop productivity (Vance, Graham, 1995). Atmospheric nitrogen fixation is a direct mechanism of action of *Azotobacter* spp. as biostimulators inducing plant growth and development. Free-living N_2 assimilators of the genus *Azotobacter* are model microorganisms in studies of the biochemistry and energetics of N_2 fixation, as well as in studies of the spatial structure and function of nitrogenase and the genetic regulation of biological atmospheric nitrogen fixation (Paul, Clark, 2000). Bacteria belonging to the genus *Azotobacter* can fix at least 20 mg N per 1 g of glucose consumed (Mazinani, Asgharzadeh, 2014; Jnawali et al., 2015; Bag et al., 2017). In the environment, the efficiency of atmospheric nitrogen fixation by these bacteria and other non-symbiotic diazotrophs is not high. This is due to the fact that free-living nitrogen assimilators only carry out this process during growth, using energy for metabolic processes related to cellular life activity. The low efficiency of N_2 fixation by the microorganisms in question is also related to the low availability of nutrients, especially readily available nutrients (Kennedy, Tchan, 1992; Martyniuk, 2008). Literature data suggest that the presence of iron and molybdenum in the soil increases the efficiency of atmospheric nitrogen fixation by *Azotobacter* spp. due to the fact that both these micronutrients are part of the nitrogenase active centres (Trncik et al., 2022). Also, calcium present in the soil environment promotes the proliferation of *Azotobacter* spp. and enhances their ability to fix atmospheric nitrogen (Iswaran, Sen, 1960). This indicates the importance of liming acidic soils to ensure their proper functioning and increase fertility by stimulating microbial activity, including the growth of *Azotobacter* spp. (Soleimanzadeh, Gooshchi, 2013). In contrast, elevated nitrogen levels have an adverse effect on the activity of *Azotobacter* spp. Research conducted by Natywa et al. (2013) shows that the application of nitrogen rates exceeding 80 kg/ha results in a reduction in the abundance of bacteria of the genus *Azotobacter*, which is associated with the accumulation of toxic substances, such as ammonia, a reduction in soil pH and a reduction in the growth of some microbial groups. Excessive doses of nitrogen fertilisers modify the qualitative composition of the biocenoses – *Arthrobacter*, *Azotobacter* and *Streptomyces* bacteria are reduced and fungi take over the dominance in the microbiocenoses.

According to Kennedy and Tchan (1992), bacteria of the genus *Azotobacter* provide only small amounts of plant-available nitrogen to the soil, but according to Martyniuk (2010), it is these small amounts of assimilated nitrogen that have a beneficial effect on soil metabolism and fertility. According to the literature, properly selected and characterised strains of these bacteria are successfully used in crop production as an alternative method to reduce the use of mineral nitrogen fertilisers in agriculture (Kizilkaya, 2009; Esmailpour et al., 2013). Various reports are available confirming the reduced nitrogen fertiliser requirements of crop plants inoculated with *Azotobacter* spp. Romero-Perdomo et al. (2017) reported that the use of *Azotobacter*-based formulations allows a reduction in the use of mineral nitrogen fertilisers by up to 50% in cotton cultivation under greenhouse conditions.

Production of phytohormones and siderophores

Phytohormones (plant hormones) are biologically active substances produced by both microorganisms and plants. They regulate different physiological and biochemical processes in plants, including developmental and growth processes (Ansari, Mahmood, 2019; Gothandapani et al., 2017). Bacteria of the genus *Azotobacter* synthesise and secrete significant amounts of biologically active substances that stimulate plant growth and development, i.e.: auxins, gibberellins, cytokinins and B vitamins (nicotinic acid, pantothenic acid) (Aquilanti et al., 2004a; Patil, 2011; Vikhe, 2014; Arora et al., 2018; Aasfar et al., 2021). By secreting phytohormones into the substrate, they increase the amount of phytohormones in the environment, and this has a stimulating effect on the yield of many crops (Taller, Wong, 1988; Zahir et al., 2005; Kumari et al., 2017). The ability of *Azotobacter* spp. to produce phytohormones is a well-known phenomenon, the importance of which has been repeatedly confirmed by numerous experiments. Based on laboratory work has demonstrated the presence of three phytohormones, i.e. indolyl-3-acetic acid (IAA), cytokine and gibberellin, in liquid cultures of *Azotobacter* spp. (Rubio et al., 2013; Vikhe, 2014). Indolyl-3-acetic acid (IAA) is produced after approximately five days of culture of *Azotobacter* spp. on medium supplemented with 5 mg tryptophan/ml, a precursor of auxins (Patil, 2011). Increasing the tryptophan concentration in the medium results in a more intense synthesis of indolyl-3-acetic acid (Kumar et al., 2014; Zulaika et al., 2017). Brown et al. (1968) found the presence of three gibberellin-like compounds in 14-day cultures of *Azotobacter chroococcum*, with concentrations ranging from 0.01 to 0.1 µg/ml. In contrast, Nieto and Frankenberger (1989) identified five cytokinins in the filtrate of *Azotobacter chroococcum* cultures. The effect of phytohormones produced by *Azotobacter chroococcum* was further confirmed in field experiments conducted on different crops. Positive effects of *Azotobacter* spp. have been found on the yield of crops, such as wheat, barley, maize, oats, chickpea, cucumber and tomato (Barakat, Gabr,1998; Mrkovacki, Milic, 2001; Baral Adhikari, 2013; Debojyoti et al., 2014; Akram et al., 2016; Mahato, Kafle, 2018).

Iron acquisition is an important process affecting microbial growth and development. For bacteria belonging to the genus *Azotobacter*, iron is an essential micronutrient that is part of the nitrogenase active centres (Baars et al., 2016). *Azotobacter* spp. have the ability to bind iron from the rhizosphere through the production of siderophores (Wichard et al., 2009). These bacteria form Fe-siderophore complexes, which are absorbed by cell membrane-bound receptors (Palanché et al., 2004). The presence of siderophores in the vicinity of plant roots may protect them from many pathogens by binding to chelates all available forms of iron and making it unavailable to pathogenic organisms (Hayat et al., 2010). *Azotobacter vinelandii* under iron-deficient conditions in the environment secretes a yellow-green fluorescent siderophore called nitrogenobactin, a member of the pyoverdin family (Demange et al., 1986). Strains of *A. vinelandii* also produce catechol siderophores (Tindale et al., 2000). The siderophores produced by *A. vinelandii* have been shown to have the ability to bind molybdenum and vanadium (Bellenger et al., 2008), as well as heavy metals such as tungsten and zinc (Huyer, Page, 1988; Kraepiel et al., 2009). In addition, siderophores of

A. vinelandii stimulate the growth of some freshwater algae in mixed culture by providing them with atmospheric nitrogen. This is due to fact that algae are able to absorb bacterial siderophores and assimilate the metals they contain. Algae such as *Neochloris oleoabundans* and *Scenedesmus* spp. grow on media with purified *Azotobacter vinelandii* siderophores as the sole nitrogen source or in mixed cultures with *Azotobacter vinelandii* (Villa et al., 2014).

Plant protection against pathogens and environmental stresses

In addition to their ability to synthesise phytohormones, bacteria of the genus *Azotobacter* produce compounds that inhibit pathogens, particularly fungi (Lenart, Chmiel, 2008). *Azotobacter vinelandii* synthesises sucrose polythiophosphatetramine showing fungicidal activity against some phytopathogenic species, such as: *Helminthosporium* sp., *Macrophomina* sp. and *Fusarium* sp. (Chetverikov, Loginov, 2008; Bjelić et al., 2015). Based on studies, this metabolite produced by *Azotobacter chroococcum* was found to inhibit the growth of fungi such as *Bipolaris sorokiniana, Botrytis cinerea, Pythium debaryanum, Verticillium dahliae* and *Fusarium* spp. (Ponmurugan et al., 2012; Bjelić et al., 2015). On the other hand, El_Komy et al. (2020) showed that the application of a mixture of bacteria from the genera *Azotobacter*, *Azospirillum* and *Klebsiella* significantly inhibits the mycelial growth of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani*. The ability of *Azotobacter* spp. to solubilise phosphate (Hafez et al., 2016), potassium (Archana et al., 2013) and zinc (Baars et al., 2018; Aung et al., 2020) is also an important feature for promoting plant growth. The enhancement of Zn bioavailability in soil by *Azotobacter chroococcum* was confirmed by Wu et al. (2006). The primary mechanism for the release of soil zinc by this bacterial species is the lowering of soil pH through the production of organic acids (Aung et al., 2020). Another mechanism of zinc solubilisation by *A. chroococcum* is related to the production of siderophores, i.e. vibrioferrin, amphiphactin and crochelin. These siderophores enable the bacteria to extract both iron and zinc (Baars et al., 2018). The ability of *Azotobacter* spp. to solubilise potassium has been confirmed by numerous studies (Singh et al, 2010; Sangeeth et al, 2012; Archana et al, 2013; Diep, Hieu, 2013). Bacteria of the genus *Azotobacter* can not only dissolve potassium, but also play an important role in potassium assimilation by plants (Wu 2005; Singh et al., 2010). Free-living nitrogen assimilators belonging to the genus *Azotobacter* are also characterised by their ability to synthesise the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACC), which hydrolyses ACC (a direct precursor of the plant hormone ethylene) to NH_3 and α-ketobutyrate, consequently reducing the inhibitory effect of ethylene on plant growth (Omer et al., 2016). Melanins synthesised by *Azotobacter chroococcum* promote the growth of some plants and, due to their affinity for metals, can be used in the bioremediation of heavy metal-contaminated soils and waters (Aasfar et al., 2021). Shivprasad and Page (1989) assessed the effect of *Azotobacter* spp. on overall soil microbial activity by determining soil dehydrogenase activity, which is an indicator of metabolic intensity of microorganisms. They found that dehydrogenase activity increased in all combinations inoculated with *Azotobacter* spp.

Adaptation of *Azotobacter* to adverse environmental conditions

Azotobacter spp. inhabit many environments such as soil, water, sewage sludge, root and leaf surfaces. These bacteria are found in different climatic zones, with many species appearing in tropical and polar regions (Jensen, Petersen, 1995; Aquilanti et al., 2004b). For example, the species *Azotobacter chroococcum* and *Azotobacter vinelandii* occur more frequently and in greater numbers in tropical soils (Aasfar et al., 2021). Bacteria belonging to the genus *Azotobacter* prefer neutral to slightly alkaline soils. In acidic soils (pH < 6), these bacteria are rare, which is related to the lower availability of assimilable nutrients, unfavourable air and water conditions, and the presence of toxic aluminium ions (Al^{3+}) in the soil solution (Martyniuk, 2008; Mazinani, Asgharzadeh, 2014; Andjelković et al., 2018). Of the known species, *Azotobacter beijerinckii* is most commonly detected in acidic soils (Aasfar et al., 2021). Also, the environment of alkaline soils is much less conducive to the growth and multiplication of *Azotobacter* spp. than the environment of neutral soils. The unfavourable conditions for the growth of this group of bacteria are caused, among other things, by the limited availability of bioavailable forms of P and Mg. Numerous studies support the claim that *Azotobacter* spp. are most abundant in neutral soils (Limmer, Drake, 1996; Aquilanti et al., 2004a; Lenart, 2012; Mazinani, Asgharzadeh, 2014; Ben Mahmud, Ferjani, 2018). The sensitivity of bacteria of the genus *Azotobacter* to the pH of the soil environment is a species characteristic and so, for example, *Azotobacter chroococcum* is able to survive at pH 9 and its growth is not inhibited even at higher values. *Azotobacter salinestris*, on the other hand, is sensitive to an alkaline soil reaction and does not grow at pH values > 9 (Aasfar et al., 2021). Also, NaCl concentrations have a significant effect on the occurrence, abundance and metabolic activity of *Azotobacter*. As reported in the literature, some species of these bacteria, i.e. *A. chroococcum*, *A. vinelandii* and *A. beijerinkii*, tolerate up to 10% NaCl concentration (Dash, Soni, 2018; Aasfar et al., 2021). *Azotobacter* spp. are typical mesophilic bacteria that thrive best at temperatures between 25 and 30 ^oC. These bacteria do not tolerate high temperatures, although they can survive at 45–48 ^oC by transforming into a cyst form (Saribay, 2003). *A. salinestris* strains maintain an op-

Table 2. Effect of selected *Azotobacter-based* biofertilisers on crop yields.

Biofertilizer components	Plant	Type of experiment	Increase in plant yield [%]	Source
Azotobacter azospirillum PSB	potatoes	field	62.32	El-sayed et al., 2014
Azotobacter PSB	paprika	field	30.01	Jaipaul et al., 2011
Azotobacter	cucumber	greenhouse	21.7	Saeed et al., 2015
Azotobacter	cabbage	field	12.9	Sarkar et al., 2010
Azotobacter PSB	broccoli	potted	17.27	Singh et al., 2014
Azotobacter PSB	tomato	field	23.8	Singh et al., 2015
Azotobacter PSB	carrot	field	19.6	Sarma et al., 2015
Azotobacter, Chlorella, Nostoc	rice	in situ analysis	26.92	Zayadan et al., 2014
Azotobacter	cotton	greenhouse	13.6	Romero-Perdomo et al., 2017
Azotobacter azospirillum	rapeseed	field	1.52	Ahmadi-Rad et al., 2016
Azotobacter	wheat	field	14.32	Milošević et al., 2012
Azotobacter glomus intraradices	safflower	field	2.63	Mirzakhani et al., 2014
Azotobacter PSB	peas	potted and field	35.5	Ansari et al., 2015

timal growth rate at 35 °C, which decreases with increasing temperature. Besides, the occurrence and population size of this group of bacteria is influenced by many other environmental factors, i.e. soil properties (organic matter content, moisture content, fertility, C/N ratio), or climatic conditions (Tejera et al., 2005).

EFFECT OF BIOFERTILIZERS BASED ON *AZOTOBACTER* SPP. ON PLANT GROWTH AND YIELD

The interest in bacteria of the genus *Azotobacter* is largely related to their properties, allowing these microorganisms to be used in agriculture, horticulture, forestry as biofertilisers, biostimulants and bioprotectants (Hindersah et al., 2020). Due to their ability to fix atmospheric nitrogen, produce substances that stimulate plant growth and development, their ability to produce pathogen-inhibiting compounds, and stimulate rhizosphere microbes, they are used for the production of bacterial soil preparations (Jnawali et al., 2015; Aasfar et al., 2021; Nongthombam et al., 2021). Bacteria belonging to the genus *Azotobacter* have important effects on seed germination, root development, root and shoot biomass, and leaf number and surface area (Wani et al., 2016). Numerous studies confirm that the application of *Azotobacter* spp. improves the growth, development and yield quality of many crops, including wheat, canola, rice, cotton, potato, pepper, cucumber, cabbage, tomato, carrot and pea (Table 2).

Research indicates that bacteria of the genus *Azotobacter* can be an alternative to conventional crop protection products. Ritika and Utpal (2014), in a field experiment, showed that the application of *Azotobacter* spp. as a component of a biofertiliser increased cauliflower yield by 40% and maize yield by 15–20% compared to the yield obtained with conventional fertilisers.

THE AGRICULTURAL IMPORTANCE OF BACTERIAL CONSORTIA INVOLVING *AZOTOBACTER* SPP.

In agricultural practice, bacteria of the genus *Azotobacter* are used to form bacterial consortia with specific functions towards crop plants. Consortia of beneficial microorganisms are one of the latest solutions to increase the quality, safety and efficiency of crop production (Sumbul et al., 2020). On the world market, there are both bio-fertilisers containing only experimentally selected bacterial strains of the genus *Azotobacter* and innovative and equally effective microbial preparations containing bacterial consortia. According to the literature, the use of *Azotobacter* spp. together with other microorganisms is highly effective both experimentally and in practice (Akram et al., 2016; Yousefi et al., 2017; Arora et al., 2018).

Numerous studies confirm that the use of free-living atmospheric nitrogen-fixing bacteria of the genus *Azotobacter* and mycorrhizal fungi leads to improved growth and yield quality in many crops (Behl et al., 2003). Synergism between *Azotobacter* spp. and AM (*arbuscular mycorrhizal*) fungi belonging to the genus *Glomus* has been confirmed by many researchers. Bagyaraj and Menge (1978) studied the effect of inoculation with *Glomus fasciculatum* and *Azotobacter chroococcum* on the growth and population of rhizosphere bacteria in tomato crop. The use of inoculation with both strains simultaneously significantly increased the population of bacteria (including radicle) in the rhizosphere of tomato plants compared to the effects observed when inoculating with *G. fasciculatum* or *A. chroococcum* alone. Inoculation of tomato plants with *G. fasciculatum* alone led to an increase in *A. chroococcum* abundance in the rhizosphere, whereas the use of *A. chroococcum* inoculation of tomato roots increased spore production by *G. fasciculatum*. Furthermore, inoculation with both strains had a significant effect on increasing the dry weight of tomato plants compared to non-inoculated combinations. Positive effects of dual inoculation with the arbuscular mycorrhizal fungus AM and the *Azotobacter* spp. strain on growth and grain yield of wheat were observed by Behl et al. (2003). Aseri et al. (2008) found that co-inoculation of *A. chroococcum* and *Glomus mosseae* mitigates the negative effects of environmental stress on the growth of pomegranate (*Punica granatum*). A study by Arora et al. (2018) indicated that application of *Piriformospora indica* and *A. chroococcum* improved the physiological and biochemical properties of *Artemisia annua* L., as evidenced by increased artemisinin content.

Free-living atmospheric nitrogen-fixing bacteria of the genus *Azotobacter* can interact with symbiotic bacteria of the genus *Rhizobium*, and the stimulating effect of inoculation with these bacteria on crop growth and yield has been demonstrated in both laboratory, pot and field experiments (Wani, Gopalakrishnan, 2019). A positive effect of inoculation of *A. chroococcum* and *Bradyrhizobium* on mung bean (*Vigna radiata*) was observed by Yadav and Vashishat (1991). Similar results were obtained by Siddiqui et al. (2014) inoculating chickpeas with the same bacterial strains. Numerous studies have shown that the use of inoculation with *Azotobacter* spp. and *Azospirillum* spp. improves growth, yield and quality of many crops i.e.: chickpea (Parmar, Dadarwal, 1999), mustard (Tilak, Sharma 2007), rapeseed (Yasari et al., 2009), chili pepper (Khan et al., 2012), wheat (Kandil et al., 2011), pearl millet (Tilak, 1995), black pepper (Bopaiah, Khader, 1989) and tomato (Ramakrishnan, Selvakumar, 2012). Das and Saha (2007) observed an increase in rice grain and straw yield by 4.5 and 8.5 kg/ha, respectively, using a combination of *Azotobacter* and *Azospirillum* bacteria. Co-inoculation with *Azotobacter* and *Azospirillum* bacteria also mitigates the deleterious effects of salinity on plant growth. Yousefi et al. (2017) observed that hopbush (*Dodonaea viscosa* L.) seeds inoculated *with Azospirillum* spp. and *Azotobacter* spp. and exposed to salinity stress showed a higher percentage of germination. The effect of inoculation with bacterial strains belonging to the genera *Azotobacter* and *Azospirillum* on the growth and yield of crop plants depends mainly on the ability of these bacteria to increase the weight and number of lateral roots, biological fixation of atmospheric nitrogen, antagonistic effects on plant pathogens such as fungi, bacteria and nematodes, and to a lesser extent on the alleviation of abiotic stress in plants (Okon, Itzigsohn, 1995). Research by Zayed (2012) also showed that the application of the bacteria *Azotobacter chroococcum*, *Azospirillum brazilense*, *Bacillus megatherium*, *Bacillus circulans*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* significantly improved the growth and nutritional value of horseradish tree (*Moringa oleifera*).

SUMMARY

Free-living nitrogen assimilators of the genus *Azotobacter* are components of biopreparations used in sustainable crop production to increase crop yields. Interest in these bacteria is related to their beneficial effects on plant growth and development through enrichment of the soil environment with nitrogen compounds, production of phytohormones, solubilisation of phosphates and ability to produce pathogen-inhibiting compounds. In addition, these bacteria support plants under stress conditions, increase their resistance to disease and improve soil fertility. Thanks to these characteristics, they are used in agriculture, horticulture and forestry as biofertilisers, biostimulants and bioprotectants. Microbial preparations comprising non-symbiotic atmospheric nitrogen-fixing bacteria are known and used worldwide. In order to obtain maximum benefit from the use of these biopreparations, it seems necessary to match the *Azotobacter* spp. strains to the specific plant genotype. Research to date indicates that the use of bacteria of the genus *Azotobacter* can be an alternative to the use of synthetic fertilisers, pesticides and artificial growth regulators

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