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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 envi-

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ronment. 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 |

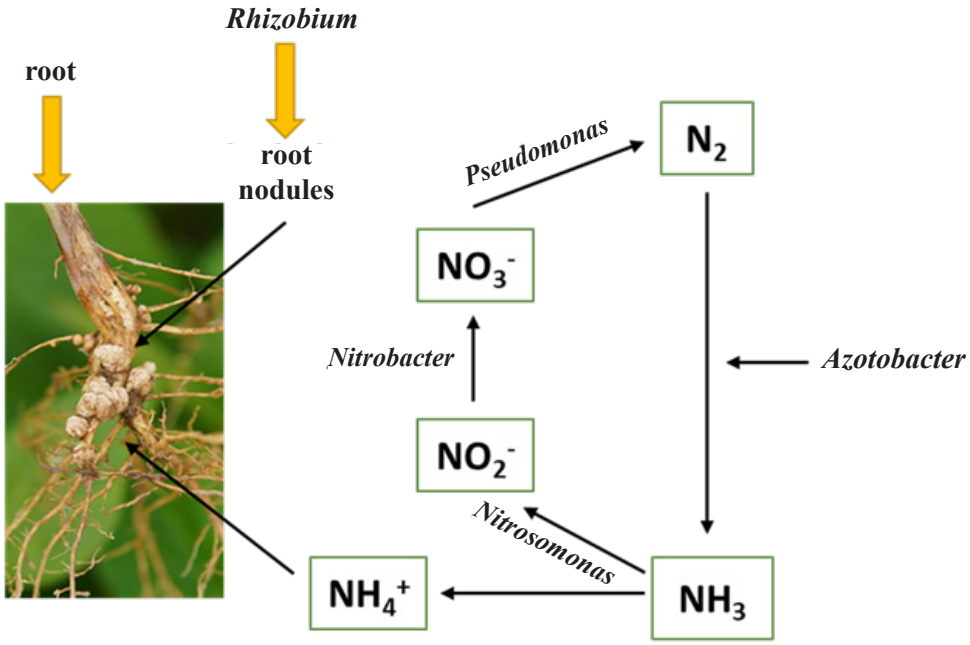



Figure 1. Contribution of microorganisms to the nitrogen cycle in nature (Gothandapani et al., 2017; modified).

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 °C. These bacteria do not tolerate high temperatures, although they can survive at 45–48 °C 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

## REFERENCES

**Aasfar A., Bargaz A., Yaakoubi K., Hilali A., Bennis I., Zeroual Y., Kadmiri I.M., 2021.** Nitrogen fixing *Azotobacter* species as potential soil biological enhancers for crop nutrition and yield stability. Frontiers in Microbiology, 12: 1-19, doi: 10.3389/fmicb.2021.628379.

**Adamczyk B., Godlewski M., 2010.** Various strategies of nitrogen by plants. Kosmos, 102: 211-222. (in Polish + summary in English)

**Ahmadi-Rad S., Gholamhoseini M., Ghalavand A., Asgharzadeh A., Dolatabadian A., 2016.** Foliar application of nitrogen fixing bacteria increases growth and yield of canola grown under different nitrogen regimes. Rhizosphere, 2: 34-37, doi: 10.1016/j.rhisph.2016.08.006.

**Akinrinlola R.J., Yuen G.Y., Drijber R.A., Adesemoye A.O., 2018.** Evaluation of *Bacillus* strains for plant growth promotion and predictability of efficacy by in vitro physiological traits. International Journal of Microbiology, 5686874, doi: 10.1155/2018/5686874.

**Akram M., Rizvi R., Sumbul A., Ansari R.A., Mahmood I., 2016.** Potential role of bio-inoculants and organic matter for the management of root-knot nematode infesting chickpea. Cogent Food and Agriculture, 2(1): 1183457, doi: 10.1080/23311932.2016.1183457.

**Aloo B.N., Tripathi V., Makumba B.A., Mbega E.R., 2022.** Plant growth-promoting rhizobacterial biofertilizers for crop production: The past, present, and future. Frontiers in Plant Science, 13, doi: 10.3389/fpls.2022.1002448.

**Andjelković S., Vasića T., Radovića J., Babića S., Markovića J., Zornića V., Djurić S., 2018.** Abundance of *Azotobacter* in the soil of natural and artificial grasslands. Soil Science Society of Serbia, pp. 172-175.

**Ansari M.F., Tipre D.R., Dave S.R., 2015.** Efficiency evaluation of commercial liquid biofertilizers for growth of *Cicer aeritinum* (chickpea) in pot and field study. Biocatalysis and Agricultural Biotechnology, 4: 17-24, doi:10.1016/j.bcab.2014.09.010.

**Ansari R.A., Mahmood I., 2019.** Plant Health Under Biotic Stress: Volume 1: Organic Strategies: Volume 1: Springer Singapore, ISBN: 978-981-13-6042-8, doi:10.1007/978-981-13-6043-5.

**Archana D.S., Nandish M.S., Savalagi V.P., Alagawadi A.R., 2013.** Characterization of potassium solubilizing bacteria (KSB) from rhizosphere soil. Bioinfolet – A Quarterly Journal of Life Sciences, 10: 248-257.

**Arora M., Saxena P., Abdin M.Z., Varma A., 2018.** Interaction between *Piriformospora indica* and *Azotobacter chroococcum* governs better plant physiological and biochemical parameters in *Artemisia annua* L. plants grown under in vitro conditions. Symbiosis, 75: 103-112, doi: 10.1007/s13199-017-0519-y.

**Aseri G.K., Jain N., Panwar J., Rao A.V., Meghwal P.R., 2008.** Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) in India Thar Desert. Scientia Horticulturae, 117(2): 130-135, doi:10.1016/j.scienta.2008.03.014.

**Aquilanti L., Favilli F., Clementi F., 2004a.** Comparison of different strategies for isolation and preliminary identification of *Azotobacter* from soil samples. Soil Biology and Biochemistry, 36: 1475-1483, doi: 10.1016/j.soilbio.2004.04.024.

**Aquilanti L., Mannazzu I., Papa R., Cavalca L., Clementi F., 2004b.** Amplified ribosomal DNA restriction analysis for the characterization of *Azotobacteraceae*: a contribution to the study of these free-living nitrogen-fixing bacteria. Journal of Microbiological Methods, 57: 197-206, doi: 10.1016/j.mimet.2004.01.006.

**Aung A., Sev T.M., Mon A.A., San Yu S., 2020.** Detection of abiotic stress tolerant *Azotobacter* species for enhancing plant growth promoting activities. Journal of Scientific and Innovative Research, 9: 48-53, doi: 10.31254/jsir.2020.9203.

**Baars O., Zhang X., Morel F.M., Seyedsayamdost M.R., 2016.** The siderophores metabolome of *Azotobacter vinelandii*. Applied and Environmental Microbiology, 82(1): 27-39, doi: 10.1128/AEM.03160-15.

**Baars O., Zhang X., Gibson M.I., Stone A.T., Morel F.M., Seyedsayamdost M.R., 2018.** Crochelins: siderophores with an unprecedented iron-chelating moiety from the nitrogen-fixing bacterium *Azotobacter chroococcum*. Angewandte Chemie International Edition, 130: 545-550, doi: 10.1002/anie.201709720.

**Bag P.B., Panda P., Paramanik B., Mahato B., Choudhury A., 2017.** Atmospheric Nitrogen Fixing Capacity of *Azotobacter* Isolate from Cooch Behar and Jalpaiguri Districts Soil of West Bengal, India. International Journal of Current Microbiology and Applied Sciences, 6(3): 1775-1788, doi:10.20546/ijcmas.2017.603.204

**Bagyaraj D.J., Menge J.A., 1978.** Interaction between a VA mycorrhiza and *Azotobacter* and their effects on rhizosphere microflora and plant growth. New Phytologist, 80(3): 567-573.

**Baj J., Markiewicz Z., 2007.** Biologia molekularna bakterii. Wydawnictwo Naukowe PWN, Warszawa, pp. 141.

**Barakat M.A.S., Gabr S.M., 1998.** Effect of different biofertilizer types and nitrogen fertilizers levels on tomato plants. Alexandria Journal of Agricultural Research, 43(1): 149-160.

**Baral B.R., Adhikari P., 2013.** Effect of *Azotobacter* on growth and yield of maize. SAARC Journal of Agriculture, 11(2): 141-147, doi:10.3329/sja.v11i2.18409

**Behl R.K., Sharma H., Kumar V., Narula N., 2003.** Interactions amongst mycorrhiza, *Azotobacter chroococcum* and root characteristics of wheat varieties. Journal of Agronomy and Crop Science, 189(3): 151-155, doi:10.1046/j.1439-037X.2003.00026.x

**Bellenger J.P., Wichard t., Kustka A.B., Kraepiel A.M.L., 2008.** Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. Nature Geoscience, 1(4): 243-246, doi:10.1038/ngeo161

**Ben Mahmud M.T., Ferjani E.A., 2018.** Influence of soil pH on *Azotobacter* population with using microbiological characteristics as bio-measurement in arable lands of Tripoli N.W. Libya. Al-Mukhtar Journal of Sciences, 33(2): 146-154. doi:10.54172/mjsc.v33i2.180

**Bjelić D., Marinković J., Tintor B., Tančić Živanov S., Nastasic A., Mrkovacki N., 2015.** Screening of *Azotobacter* isolates for PGP properties and antifungal activity. Zbornic Matice Srpske Za Prirodne Nauke, 65–72, doi:10.2298/ZMSPN1529065B

**Bopaiah B.M., Abdul Khader K.B., 1989.** Effect of biofertilizers on growth of black pepper (*Piper nigrum*). Indian Journal of Agricultural Science, 59: 682-683.

**Brown., M.E., Jackson R.M., Burlingham S.K., 1968.** Growth and effects of bacteria introduced into soil. The Ecology of Soil Bacteria, 531-551.

**Bunas A., Tkach E., Dvoretsky V., Dvoretska O., 2022.** Efficiency of using biosystem POWER , KS (BioSistem POWER, SC) preparation to accelerate the destruction of post-harvest residues. Agroecological Journal, 3: 119-125, doi: 10.33730/2077-4893.3.2022.266417**.**

**Calvo P., Nelson L., Kloepper J., 2014.** Agricultural uses of plant biostimulants. Plant and Soil, 383(1-2): 3-41, doi:10.1007/s11104-014-2131-8.

**Chetverikov S.P., Loginov O.N., 2008.** New metabolites of *Azotobacter vinelandii* exhibiting antifungal activity. Microbiology, 78(4): 428-432, doi:10.1134/S0026261709040055.

**Das A.C., Saha D., 2007.** Effect of diazotrophs on the mineralization of organic nitrogen in the rhizosphere soils of rice (*Oryza sativa*). Journal of Crop and Weed, 3: 47-51.

**Dash B., Soni R., 2018.** Evaluation of stress tolerance of *Azotobacter* isolates. Biologija, 64(1): 82-93, doi:10.6001/biologija.v64i1.3662.

**Dąbrowska G., Hrynkiewicz K., Janczak K., Żurańska M., 2014.** Modified soil bacteria and their potential application to improving fitoremediation of trace metal-contaminated environment. Ochrona Środowiska, 36(1): 21–26. (in Polish + summary in English)

**Dąbrowska G., Zdziechowska E., Hrynkiewicz K., 2016.** Evaluation of Potential Suitability of Rhizobacteria for Phytodesalination of Soils. Ochrona Środowiska, 38(3): 9-14. (in Polish + summary in English)

**Debojyoti R., Manibrata P., Sudip K.B., 2014.** A Review on The Effects of Biofertilizers and Biopesticides on Rice and Tea Cultivation and Productivity. International Journal of Science, Engineering and Technology, 2(8): 96-106.

**Demange P., Wendenbaum S., Bateman A., Dell A., Meyer J.M., Abdallah M.A., 1986.** Bacterial siderophores: structures of pyoverdins and related compounds. pp. 131-147. In:

Iron, Siderophores, and Plant Diseases; eds.: Swinburne T.R.; Plenum Press, New York.

**Diep C.N., Hieu N., 2013.** Phosphate and potassium solubilizing bacteria from weathered materials of denatured rock mountain, Ha Tien, Kién Giang province Vietnam. American Journal of Life Sciences, 1(3): 88-92, doi: 10.11648/j.ajls.20130103.12.

**El_Komy M.H., Hassouna M.G., Abou-Taleb E.M., Al-Sarar A.S., Abobakr Y., 2020.** A mixture of *Azotobacter*, *Azospirillum*, and *Klebsiella* strains improves root-rot disease complex management and promotes growth in sunflowers in calcareous soil. European Journal of Plant Pathology, 156: 713-726. doi: 10.1007/s10658-019-01921-w.

**El-sayed S., Hassan H., El-Mogy M., 2014.** Impact of Bio- and organic fertilizers on potato yield, quality and tuber weight loss after harvest. Potato Research, 58: 67-81, doi: 10.1007/s11540-014-9272-2.

**Esmailpour A., Hassanzadehdelouei M., Madani A., 2013.** Impact of livestock manure, nitrogen and biofertilizer (*Azotobacter*) on yield and yield components wheat (*Triticum Aestivum* L.). Cercetari Agronomice in Moldova, 46(2): 5-15, doi: 10.2478/v10298-012-0079-5.

**Geng Y., Cao G., Wang L., Wang S., 2019.** Effects of equal chemical fertilizer substitutions with organic manure on yield, dry matter, and nitrogen uptake of spring maize and soil nitrogen distribution. PLOS One, 14(7), e0219512, doi: 10.1371/journal.pone.0219512.

**Glick B.R., 2012.** Plant Growth-Promoting Bacteria: Mechanisms and Applications. Scientifica, 963401, doi:10.6064/2012/963401.

**Gothandapani S., Sekar S., Padaria J.C., 2017.** *Azotobacter chroococcum*: Utilization and potential use for agricultural crop production: An overview. International Journal of Advanced Research in Biological Sciences, 4(3): 35-42, doi:10.22192/IJARBS.2017.04.03.004.

**Hafez M., Elbarbary T.A., Ibrahim I., Abdel-Fatah Y., 2016.** *Azotobacter vinelandii* evaluation and optimization of Abu Tartur Egyptian phosphate ore dissolution. Saudi Journal of Pathology and Microbiology, 1: 80-93, doi: 10.21276/sjpm.2016.1.3.2.

**Hakeem K.R., Sabir M., Ozturk M., Akhtar M.S., Ibrahim F.H., 2017.** Nitrate and nitrogen oxides: sources, health effects and their remediation. Reviews of Environmental Contamination and Toxicology, 242: 183-217. doi: 10.1007/398_2016_11.

**Hayat R., Ali S., Amara U., Khalid R., Ahmed I., 2010.** Soil beneficial bacteria and their role in plant growth promotion: a review. Annals of Microbiology, 60(4): 579-598.

**Herridge D.F., Peoples M.B., Boddey R.M., 2008.** Global imputs of biological nitrogen fixation in agricultural systems. Plant and Soil, 311(1):1-18, doi: 10.1007/s11104-008-9668-3.

**Hindersah R., Nuraniya Kamaluddin N., Samanta S., Banerjee S., Sarkar S., 2020.** Role and perspective of *Azotobacter* in crops production. SAINS TANAH – Journal of Soil Science and Agroclimatology, 17(2): 170-179, doi: 10.20961/stjssa.v17i2.45130.

**Huyer M., Page W.J., 1988.** $Zn^{2+}$ increases siderophores production in *Azotobacter vinelandii*. Applied and Environmental Microbiology, 54(11): 2625-2631.

**Iswaran V., Sen A., 1960.** Mahua (*Modhuca indica*) cake as a carrier of ammonia to soil. Journal of Scientific and Industrial Research, 19C: 127.

**Jaipaul S., Dixit A., Sharma A., 2011.** Growth and yield of capsicum (*Capsicum annum*) and garden pea (*Pisum sativum*) as influenced by organic manures and biofertilizers. Indian Journal of Agricultural Sciences, 81: 637-642.

**Jensen V., Petersen E.J., 1995.** Taxonomic studies on *Azotobacter chroococcum* Bejjerinck and *Azotobacter beijerinckii* Lipman. J. R. Vet. Agric. Coll., 84: 107-126.

**Jnawali A.D., Ojha R.B., Marahatta S., 2015.** Role of *Azotobacter* in soil fertility and sustainability – An review. Advances in Plants and Agriculture Research, 2(6): 1-5, doi: 10.15406/apar.2015.02.00069.

**Kandil A.A., El-Hindi M.H., Badawi M.A., ElMorarsy S.A., Kalboush F.A., 2011.** Response of wheat to rates of nitrogen, biofertilizers and land leveling. Crop and Environment, 2(1): 46-51, doi: 10.3923/ajcs.2013.200.208.

**Kennedy I.R., Tchan Y.T., 1992.** Biological nitrogen fixation in non-leguminous field crops: recent advances. Plant and Soil, 141(1-2): 93-118.

**Khan Z., Tiyagi S.A., Mahmood I., Rizvi R., 2012.** Effects of N fertilization, organic matter and biofertilizers on the growth and yield of chilli in relation to management of plant-parasitic nematodes. Turkish Journal of Botany, 36(1): 73-81, doi:10.3906/bot-1009-60.

**Kizilkaya R., 2009.** Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. Journal of Environmental Biology, 30(1): 73-82.

**Kozłowska-Burdziak M., 2019.** Conditions for the food security of Poland (with special consideration of the Podlasie Voivodeship). Optimum Economic Studies, 3(97): 33-48, doi: 10.15290/oes.2019.03.97.03. (in Polish + summary in English)

**Kraepiel A.M.L., Bellenger J.P., Wichard T., Morel F.M., 2009.** Multiple roles of siderophores in free-living nitrogen-fixing bacteria. BioMetals, 22(4): 573-581, doi: 10.1007/s10534-009-9222-7.

**Kumar A., Kumar K., Kumar P., Maurya R., Prasad S., Singh S.K., 2014.** Production of indole acetic acid by *Azotobacter* strains associated with mungbean. Plant Archives, 14(1): 41-42.

**Kumari S., Chourasia S.K., Singh U., Rajnikant, 2017.** *Azotobacter*: Its role in sustainable agriculture. New Agriculturist, 28(2): 485-492.

**Lenart A., Chmiel M.J., 2008.** Wpływ wybranych jonów metali ciężkich na bakterie glebowe z rodzaju *Azotobacter* asymilujące azot atmosferyczny. ss. 199-205. In: Przemiany środowiska naturalnego a rozwój zrównoważony; red.: Kotarba M.J.; Wydawnictwo TBPŚ GEOSFERA, Kraków.

**Lenart A., 2012.** Occurrence, characteristics, and genetic diversity of *Azotobacter chroococcum* in various soils of southern Poland. Polish Journal of Environmental Studies, 21(2): 415-424.

**Limmer C., Drake H.L., 1996.** Non-symbiotic $N_2$-fixation in acidic and pH-neutral forest soils: aerobic and anaerobic differentials. Soil Biology and Biochemistry, 28(2): 177-183.

**Mahato S., Kafle A., 2018.** Comparative study of *Azotobacter* with or without other fertilizers on growth and yield of wheat in Western hills of Nepal. Annals of Agrarian Science, 16: 250-256, doi: 10.1016/j.aasci.2018.04.004.

**Martinez-Espinosa R. M., Cole J. A., Richardson D. J., Wartmough N. J., 2011.** Enzymology and ecology of the nitrogen cycle. Biochemical Society Transactions, 39(1): 175-178, doi: 10.1042/BST0390175.

**Martyniuk S., 2008.** The importance of biological fixation of atmospheric nitrogen in ecological agriculture. Journal of Research and Applications in Agricultural Engineering, 53(4): 9-14. (in Polish + summary in English)

**Martyniuk S., 2010.** Production of microbial preparations: symbiotic bacteria of legumes as an example. Journal of Research and Applications in Agricultural Engineering, 55(4): 20-23. (in Polish + summary in English)

**Mazinani Z., Asgharzadeh A., 2014.** Genetic diversity of *Azotobacter* strains isolated from soils by amplified ribosomal DNA restriction analysis. Cytology and Genetics, 48(5): 26-35, doi: 10.3103/S0095452714050041.

**Mazid M., Khan T.A., 2015.** Future of bio-fertilizers in Indian agriculture: An Overview. International Journal of Agricultural and Food Research, 3(3): 10-23, doi: 10.24102/ijafr.v3i3.132.

**Milošević N., Tintor B., Protić R., Cvijanović G., Dimitrijević T., 2012.** Effect of inoculation with *Azotobacter chroococcum* on wheat yield and seed quality. Romanian Biotechnologicals Letters, 17(3): 7352–7357.

**Mirzakhani M., Ardakani M.R., Rejali F., Rad A.H.S., Miransari M., 2014.** Safflower (*Carthamus tinctorius* L.) oil content and yield components as affected by co-inoculation with *Azotobacter chroococcum* and *Glomus intraradices* at various N and P levels in a dry climate. pp. 153-164. In: Use of Microbes for the Alleviation of Soil Stresses: Volume 2: Alleviation of Soil Stress by PGPR and Mycorrhizal Fungi; eds.: Miransari M.; Springer, New York, doi: 10.1007/978-1-4939-0721-2_9.

**Mohamed H., Almaroai Y., 2016.** Effect of Inoculated *Azotobacter chroococcum* and Soil Yeasts on Growth, N-uptake and Yield of Wheat (*Triticum aestivum*) under Different Levels of Nitrogen Fertilization. International Journal of Soil Science, 11: 102-107, doi: 10.3923/ijss.2016.102.107.

**Mrkovacki N., Milic V., 2001.** Use of *Azotobacter chroococcum* as potentially useful in agricultural application. Annals of Microbiology, 51(2): 145-158.

**Natywa M., Selwet M., Ambroży K., Pociejowska M., 2013.** The effect of nitrogen fertilization and irrigation on the number of *Azotobacter* in the soil under maize at different stages of plant development. Polish Journal of Agronomy, 13: 53-58. (in Polish + summary in English)

**Nieto K.F., Frankenberger W., 1989.** Biosynthesis of cytokinins by *Azotobacter chroococcum*. Soil Biology and Biochemistry, 21(7): 967-972.

**Nongthombam J., Kumar A., Sharma S., Ahmed S., 2021.** *Azotobacter*: A complete Review. Bulletin of Environment Pharmacology and Life Sciences, 10(6): 72-79.

**Okon Y., Itzigsohn R., 1995.** The development of *Azospirillum* as commercial inoculant for improving crop yields. Biotechnology Advances, 13: 415-424.

**Omer A., Emara H., Zaghloul R., Abdel M., Dawwam G., 2016.** Potential of *Azotobacter salinestris* as plant growth promoting rhizobacteria under saline stress conditions. Research Journal of Pharmaceutical Biological and Chemical Sciences, 7(6): 2572-2583.

**Palanché t., Blanc S., Hennard C., Abdallah M.A., Albrecht-Gary A.M., 2004.** Bacterial iron transport: coordination properties of azotobactin, the highly fluorescent siderophores of *Azotobacter vinelandii*. Inorganic Chemistry Journal, 43(3): 1137-1152, doi: 10.1021/ic034862n.

**Parmar N., Dadarwal K.R., 1999.** Stimulation of nitrogen fixation and induction of flavonoid-like comounds by rhizobacteria. Journal of Applied Microbiology, 86(1): 35-44.

**Paśmionka I., 2017.** Mikrobiologiczne przemiany azotu glebowego. Kosmos, 66: 185-192.

**Patil V., 2011.** Production of indole acetic acid by *Azotobacter* sp. Recent Research in Science and Technology, 3(12): 14-16.

**Paul E.A., Clark F.E., 2000.** Mikrobiologia i biochemia gleb. Red. Jędrych M.; Wydawnictwo Uniwersytetu Marii Curie-Skłodowskiej, Lublin, 400 pp.

**Paungfoo-Lonhienne C., Lonhienne T.G.A., Yeoh Y.K., Donose B.C., Webb R.I., Parsons J., Liao W., Sagulenko E., Lakshmanan P., Hugenholtz P., Schmidt S., Ragan M.A., 2016.** Crosstalk between sugarcane and a plant-growth promoting *Burkholderia* species. Scientific Reports, 6(1): 37389, doi: 10.1038/srep37389.

**Ponmurugan K., Sankaranarayanan A., Al-Dharbi N.A., 2012.** Biological activities of plant growth promoting *Azotobacter* sp. isolated from vegetable crops rhizosphere soils. Journal of Pure and Applied Microbiology, 6(4): 1689-1698.

**Qessaoui R., Bouharroud R., Furze J.N., El Aalaoui M., Akroud H., Amarraque A., Van Vaerenbergh J., Tahzima R., Mayad E.H., Chebli B., 2019.** Applications of New Rhizobacteria *Pseudomonas* Isolates in Agroecology via Fundamental Processes Complementing Plant Growth. Scientific Reports, 9(1): 12832, doi: 10.1038/s41598-019-49216-8.

**Rahman A., Baharlouei P., Yan Koh E.H., Pirvu D.G., Rehmani R., Arcos M., Puri S., 2024.** A Comprehensive analysis of organic food: Evaluating nutritional value and impact on human health. Foods, 13, 208, doi: 10.3390/foods13020208.

**Ramakrishnan K., Selvakumar G., 2012.** Effect of biofertilizers of growth and yield on tomato (*Lycopersicum esculentum* Mill.). International Journal of Research in Botany, 2(4): 20-23.

**Ritika B., Utpal D., 2014.** Biofertilizer, a way towards organic agriculture: A review. African Journal of Microbiology Research, 8(24): 2332-2343, doi: 10.5897/AJMR2013.6374.

**Romero-Perdomo F., Abril J., Camelo M., Moreno-Galvan A., Pastrana I., Rojas-Tapias D., Bonilla R., 2017.** *Azotobacter chroococcum* as a potentially useful bacterial biofertilizer for cotton (*Gossypium hirsutum*): Effect in reducing N fertilization. Revista Argentina de Microbiologia, 49(4): 377-383, doi: 10.1016/j.ram.2017.04.006.

**Rubio E.J., Montecchia M.S., Tosi M., Cassán F.D.,Perticari A., Correa O.S., 2013.** Genotypic Characterization of Azotobacteria Isolated from Argentinean Soils and Plant-Growth—Promoting Traits of Selected Strains with Prospects for Biofertilizer Production. The Scientific World Journal, 519603, doi: 10.1155/2013/519603.

**Saeed K., Ahmed S. A., Hassan I. A., Ahmed P. H., 2015.** Effect of bio-fertilizer and chemical fertilizer on growth and yield in cucumber (*Cucumis sativus* L.) in green house condition. American-Eurasian Journal of Agricultural and Environmental Sciences, 15: 353-358.

**Sangeeth K.P., Bhai R.S., Srinivasan V., 2012.** *Paenibacillus glucanolyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* I.) rhizosphere. Journal of Spices and Aromatic Crops, 21: 118-124.

**Saribay G.F., 2003.** Growth and Nitrogen Fixationdynamics of *Azotobacter chroococcum* in Nitrogen- Free and OMW Containing Medium. Master's thesis, Middle East Technical University, Ankara.

**Sarkar A., Mandal A. R., Prasad P. H., Maity T. K., Chandra B., Viswavidyalaya K., 2010.** Influence of nitrogen and biofertilizer on growth and yield of cabbage. Journal of Crop and Weed, 6(2): 72-73.

**Sarma I., Phookan D. B., Boruah S., 2015.** Influence of manures and biofertilizers on carrot (*Daucus carota* L.) cv. Early Nantes growth, yield and quality. Journal of Ecofriendly Agriculture, 10(1): 25-27.

**Savala C.E.N., Wiredu A.N., Chikoye D., Kyei-Boahen S., 2022.** Prospects and Potential of *Bradyrhizobium diazoefficiens* Based Bio-Inoculants on Soybean Production in Different Agro-Ecologies of Mozambique. Frontiers in Sustainable Food Systems, 6: 908231, doi: 10.3389/fsufs.2022.908231.

**Shivprasad S., Page W.J., 1989.** Catechol formation and melanization by Na –Dependent *Azotobacter chroococcum*: a protective mechanism for Aeroadaptation? Applied and Environmental Microbiology, 55: 1811-1817.

**Siddiqui A., Shivle R., Magodiya N., Tiwari K., 2014.** Mixed effect of *Rhizobium* and *Azotobacter* as biofertilizer on nodulation and production of chick pea, *Cicer arietinum*. Bioscience Biotechnology Research Communications, 7(1): 46-49.

**Singh G., Biswas D.R., Marwaha.S., 2010.** Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (*Zea mays*) and wheat (*Triticum aestivum*): a hydroponics study under phytotron growth chamber. Journal of Plant Nutrition 33: 1236-1251, doi: 10.1080/01904161003765760.

**Singh A., Maji S., Kumar S., 2014.** Effect of biofertilizers on yield and biomolecules of anti-cancerous vegetable broccoli. International Journal of Bio-resource and Stress Management, 5(2): 262, doi: 10.5958/0976-4038.2014.00565.X.

**Singh S.K., Sharma H. R., Shukla A., Singh U., Thakur A., 2015.** Effect of biofertilizers and mulch on growth, yield and quality of tomato in mid-hills of Himachal Pradesh. International Journal of Farm Sciences, 5: 98-110.

**Sivasakthi S., Saranraj P., Sivasakthivelan P., 2017.** Biological Nitrogen Fixation by *Azotobacter* sp. – A review. Indo- Asian Journal of Multidisciplinary Research, 3(5): 1274-1284, doi: 10.22192/iajmr.2017.3.5.6.

**Subedi R., Khanal A., Aryal K., Chhetri L., Kandel B., 2019.** Response of *Azotobacter* in caulifower (*Brassica oleracea* L. var. *botrytis*) production at Lamjung, Nepal. Acta Scientifica Malaysia, 3: 17-20, doi: 10.26480/asm.01.2019.17.20 .

**Sumbul A., Ansari R.A., Rizvi R., Mahmood I., 2020.** *Azotobacter*: A potential bio-fertilizer for soil and plant health management. Saudi Journal of Biological Sciences, 27(12): 3634-3640, doi: 10.1016/j.sjbs.2020.08.004.

**Taller B.J., Wong T., 1988.** Cytokinins in *Azotobacter vinelandii* culture medium. Applied and Environmental Microbiology, 55(1): 266-267.

**Tejera N., Lluch C., Martinez-Toledo M.V., Gonzalez-Lopez J., 2005.** Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. Plant and Soil., 270(1): 223-232, doi: 10.1007/s11104-004-1522-7.

**Tilak K.V.B.R., 1995.** Vesicular-arbuscular myccorhizae and *Azospirillum brasilense* rhizocoenosis in pearl miller in semiarid tropics. pp. 177-179. In: Proceedings of Third National Conference on Mycorrhiza; eds.: Adholeya A., Singh S.

**Tilak K., Sharma K.C., 2007.** Does *Azotobacter* help in increasing the yield. Indian Farmers Digest, 9: 25-28.

**Tindale A.E., Mehrotra M., Ottem D., Page W.J., 2000.** Dual regulation of catecholate siderophore biosynthesis in *Azotobacter vinelandii* by iron and oxidative stress. Microbiology, 146(7), 1617–1626, doi: 10.1099/00221287-146-7-1617.

**Trncik Ch., Müller T., Franke P., Einsle O., 2022.** Structural analysis of the reductase component *anfH* of iron-only nitrogenase from *Azotobacter vinelandii*. Journal of Inorganic Biochemistry, 227: 111690, doi: 10.1016/j.jinorgbio.2021.111690.

**Ueda Y., Konishi M., Yanagisawa S., 2017.** Molecular basis of the nitrogen response in plants. Soil Science and Plant Nutrition, 63(4): 1-13, doi: 10.1080/00380768.2017.1360128.

**Vance C.P., Graham P.H., 1995.** Nitrogen fixation in agriculture: application and perspectives, pp. 77-86. In: Nitrogen fixation: Fundamentals and applications. Current plant science and biotechnology in agriculture; eds.: Tikhonovich I.A., Provorov N.A., Newton W.E.; Springer, Dordrecht.

**Vikhe P.S., 2014.** *Azotobacter* species as a natural plant hormone synthesizer. Research Journal of Recent Sciences 3 (IVC): 59-63, ISSN 2277-2502.

**Villa J.A., Ray E.E., Barney B.M., 2014.** *Azotobacter vinelandii* siderophores can provide nitrogen to support the culture of the green algae *Neochloris oleoabundans* and *Scenedesmus* sp. BA032. FEMS Microbiology Letters, 351(1): 70-77, doi: 10.1111/1574-6968.12347**.**

**Vojinoviv Z., 1961.** Microbiological properties of main types soil in Serbia for nitrogen cycling. Journal for Scientific Agricultural Research, 43: 3-25.

**Wani S.A., Chand S., Wani M.A., Ramzan M., Hakeem K.R., 2016.** *Azotobacter chroococcum* – a potential biofertilizer in agriculture: an overview. pp. 333-348. In: Soil Science: Agricultural and Environmental Prospectives; eds.: Hakeem K.R., Akhtar J., Sabir M.; Springer, doi: 10.1007/978-3-319-34451-5.

**Wani S.P., Gopalakrishnan S., 2019.** Plant growth-promoting microbes for sustainable agriculture. pp. 19-45. In: Plant Growth Promoting Rhizobacteria (PGPR): Prospects for Sustainable Agriculture, Springer, Singapore, doi: 10.1007/978-981-13-6790-8_2.

**Wichard T., Bellenger J.P., Morel F.M., Kraepiel A.M., 2009.** Role of siderophores azotobactin in the bacterial acquisition of nitrogenase matal cofactors. Environmental Science and Technology, 43(19): 7218-7224, doi: 10.1021/es8037214.

**Wu S.C., Cao Z.H., Li Z.G., Cheung K.C., Wong M.H., 2005.** Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma, 125: 155-166, doi: 10.1016/j.geoderma.2004.07.003.

**Wu S.C., Luo Y.M., Cheung K.C., Wong M.H., 2006.** Influence of bacteria on Pb and Zn speciation, mobility and bioavailability in soil: a laboratory study. Environmental Pollution, 144: 765-773, doi: 10.1016/j.envpol.2006.02022.

**Yadav A.S., Vashishat R.K., 1991.** Associative effect of *Bradyrhizobium* and *Azotobacter* inoculation on nodulation, nitrogen fixation and yield of mungbean (*Vigna radiate* (L.) Wilczek). Indian Journal of Microbiology, 31(3): 297-299.

**Yasari E., Azadgoleh M.E., Mozafari S., Alashti M.R., 2009.** Enhancement of growth and nutrient uptake of rapeseed (*Brassica napus* L.) by applying mineral nutrients and biofertilizers. Pakistan Journal of Biological Sciences, 12(2): 127-133. doi: 10.3923/pjbs.2009.127.133.

**Yousaf M., Li J., Lu J., Ren T., Cong R., Fahad S., Li X., 2017.** Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. Scientific Reports, 7(1): 1-9, doi: 10.1038/s41598-017-01412-0.

**Yousefi S., Kartoolinejad D., Bahmani M., Naghdi R., 2017.** Effect of *Azospirillum lipoferum* and *Azotobacter chroococcum* on germination and early growth of hopbush shrub (*Dodonaea viscosa* L.) under salinity stress. Journal of Sustainable Forest, 36(2): 107-120, doi: 10.1080/10549811.2016.1256220.

**Zahir Z.A, Asghar H.N., Akhtar M.J., Arshad M., 2005.** Precursor (L-tryptophan) – inoculum (*Azotobacter*) interaction for improving yields and nitrogen uptake of maize. Journal of Plant Nutrition, 28: 805-817, doi: 10.1081/PLN-200055543.

**Zayadan B.K., Matorin D. N., Baimakhanova G. B., Bolathan K., Oraz G. D., Sadanov A. K., 2014.** Promising microbial consortia for producing biofertilizers for rice fields. Microbiology, 83(4): 391-397, doi: 10.1134/s0026261714040171.

**Zayed M.S., 2012.** Improvement of growth and nutritional quality of *Moringa oleifera* using different biofertilizers. Annals of Agricultural Science, 57(1): 53-62, doi: 10.1016/j.aoas.2012.03.004.

**Zeffa D.M., Perini L.J., Silva M.B., de Sousa N.V., Scapim C.A., Oliveira A.L.M., de Azeredo Goncalves L.S., 2019.** *Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes. PLOS One, 14(4), e0215332, doi:10.1371/journal.pone.0215332.

**Zulaika E., Solikhah F., Alami N.H., Kuswytasari N.D., Shovitri M., 2017.** Viability of *Azotobacter* consortium in auxin production. In AIP Conference Proceedings (Vol. 1854, hal. 20041). AIP Publishing LLC, doi: 10.1063/1.4985432.

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