Versi online: http://ojs3.unpatti.ac.id//index.php/bdp
DOI: 10.30598/jbdp.2025.21.1.1

Jurnal Budidaya Pertanian
Vol. 21(1):1-7 Th. 2025 ISSN: 1858-4322 (Print) ISSN: 2620-892X (On line)
Terakreditasi Peringkat SINTA 4, SK.No. 10/C/C3/DT.05.00/2025

PROSPECTS OF AZOTOBACTER AS A BIOFERTILIZER IN SALINE SOILS

Reginawanti Hindersah[1*], Betty N. Fitriatin[1], Mieke R. Setiawati[1], Aliya Z. Adawiah[2]

[1]Faculty of Agriculture Universitas Padjadjaran, Universitas Padjadjaran, Jalan Ir. Soekarno Km. 21 Jatinangor, Sumedang 45363, Indonesia
[2]Graduated from Soil Science Master Program of Universitas Padjadjaran, Jalan Ir. Soekarno Km. 21 Jatinangor, Sumedang 45363, Indonesia
*Corresponding Author E-mail: reginawanti@unpad.ac.id

ABSTRACT

Climate change causes high waves on the seas that can accelerate the salinization of agricultural land located in coastal areas. Saline soil does not support plant growth because of the limitations of plant roots in absorbing water and nutrients, especially Nitrogen. Increasing salinization causes challenges in finding proper soil management methods, including nature-based solutions. A potential method to strengthen plant growth in saline areas is the introduction of biofertilizers containing nitrogen-fixing bacteria (NFB) that are resistant to salinity. This article aims to review the tolerance of nonsymbolic NFB Azotobacter to salinity and discuss the potential of this beneficial rhizobacterium for mitigating plant cultivation in saline land. The article was compiled through a literature study analyzing various relevant references from national and international journals in the last ten years. Analysis of the references shows that several soil Azotobacter strains are resistant to certain salt levels and have specific physiological characteristics when living in saline environments. Azotobacter induces plant tolerance to high salinity in the pot experiment. Therefore, Azotobacter inoculation becomes one of the alternatives to increase the growth of crops in saline soil.

***Keywords**: climate change, food crops, physiological change, salinity tolerance*

ABSTRAK

Perubahan iklim menyebabkan gelombang tinggi di lautan dan dapat mempercepat salinasi tanah pertanian di daerah pantai. Tanah salin tidak mendukung pertumbuhan tanaman karena keterbatasan akar tanaman menyerap air dan nutrisi terutama Nitrogen. Meningkatnya salinisasi menimbulkan tantangan dalam menemukan metode pengelolaan tanah yang tepat, termasuk solusi berbasis alam. Metode yang berpotensi untuk dikembangkan untuk memperkuat pertumbuhan tanaman di area dengan salinitas tinggi adalah introduksi pupuk hayati yang mengandung bakteri pemfiksasi nitrogen (BPN) yang tahan terhadap salinitas. Artikel ini bertujuan untuk mengulas toleransi BPN nonsimbiotik Azotobacter terhadap salinitas dan membahas potensi rizobakteri ini untuk mitigasi budidaya tanaman di lahan salin. Artikel disusun melalui studi pustaka dengan menganalisis berbagai referensi yang relevan sepuluh tahun terakhir dari jurnal nasional maupun internasional. Analisis terhadap referensi memperlihatkan bahwa sejumlah strain Azotobacter tahan ~~tanah~~ terhadap kadar garam tertentu dan memiliki karakteristik fisiologi spesifik ketika hidup di lingkungan salin. Inokulasi Azotobacter menginduksi toleransi tanaman terhadap salinitas tinggi. Oleh karena itu, inokulasi Azotobacter menjadi salah satu alternatif peningkatan pertumbuhan tanaman di lahan salin.

Kata kunci: perubahan fisiologis, perubahan iklim, tanaman pangan, toleransi salinitas

INTRODUCTION

Crop productivity might be limited by abiotic stress factors in the soil since the genetic potential of natural plant metabolism is inadequate to overcome these stresses. Abiotic stresses include soil moisture status and extreme temperatures, the toxicity of certain elements, and salinity. The area of saline land in the world, according to FAO (2021), is 424 M ha of topsoil (0-30 cm) and 833 M ha of subsoil (30-100 cm). In Indonesia, saline land is found in coastal areas, swamps affected by seawater intrusion, and areas distressed by natural or anthropogenic disasters. Global climate change increases air temperature, rainfall, and sea level but declines relative humidity. Enhancements in sea levels have been identified as climate change indicators that significantly accelerate soil salinization (Mukhopadhyay *et al*. 2021). Saline water enters agricultural land in coastal areas in Indonesia, and increasing salinity in the root zone threatens agricultural productivity. Therefore, adaptation strategies against salinity are essential to increase productivity.

Increasing salinization raises significant concerns for the global food supply system. Nowadays, the concept of adaptation methods to soil salinity is Nature-based solutions (NBS), such as the use of saline soil rice varieties

(Hasrianda *et al*., 2024; Tarolli *et al*., 2024) and the utilization of natural barriers, such as mangrove forests and seagrass meadows (Temmerman *et al*., 2013).

A promising method for adaptive crop cultivation in saline soil is the utilization of N-fixer Azotobacter, the rhizobacteria widely used in biofertilizer formulation. The natural property of Azotobacter requires Na^+ salt for metabolism (Paul *et al*., 2014), which opens the way for using halotolerant bacteria in saline soil mitigation. Naturally, Azotobacter have the Nif genes cluster responsible for changing the inert N_2 to NH_3; the last molecule was reduced to NH_4^+, subject to nitrification to produce the NO_3^- that low in saline soil. The availability of fixed nitrogen limits agricultural crop production in the tropics, and the presence of Azotobacter in the soil and rhizosphere can increase N-NH_4^+ and N-NO_3^- availability.

Azotobacter's resistance to saline or alkaline soils has been demonstrated in in vitro cultures. Several studies have explained that salt-tolerant Azotobacter are multiresistant to other biotic or abiotic stresses. Four Azotobacter isolates proliferate in broth with 3% NaCl and support chickpeas in saline soil (Abdiev *et al*., 2019). The dual function of Azotobacter is to provide available N and adapt to high salinity, which benefits plant growth in saline soil with low availability of N. However, Azotobacter application may not overcome severe saline soil with a high salt level.

Despite the abundance of reviews of Azotobacter's potential as a biofertilizer, the review concerning the potency and prospect of Azotobacter utilization as a bioremediation agent to improve plant adaptability to saline soil is still limited. The review aimed to describe the properties of saline soil, Azotobacter tolerance to salinity, and mechanisms by which Azotobacter develop their resistance to salinity, and provide recent data on the salinity tolerance of plants inoculated with Azotobacter. The review focused on Azotobacter’s resistance that enables crops to develop resistance to salinity. Therefore, the review can address the limited growth of crops in saline soil.

METHODOLOGY

The article was compiled through a literature study, analyzing various references from national and international journals, mainly from the last ten years. The article search utilized the keywords Azotobacter, saline soil, bioremediation, crops’ tolerance to salinity, osmolytes, osmoprotectant, halotolerant, and mechanisms of bacteria for saline tolerance from the databases of SINTA, Google Scholar, Scopus, and Web of Science. Some older references were cited to obtain basic knowledge that has not changed. Since the research on saline soil in Indonesia is not yet as intensive as in other countries, the literature from Indonesia is limited. The literature study covered the results of general knowledge, basic, and applied research, especially concerning the saline soil, the resistance of Azotobacter to saline environments, and the saline resistance of plants grown with Azotobacter inoculation. The inclusion criteria were general concepts of salinity resistance mechanisms of bacteria, and Azotobacter-induced crops' resistance to salinity. The existing knowledge and data from the articles were analyzed and checked about the objective of the review articles. The methods used to accomplish this purpose were recording, observations, analyzing, and evaluating published research. The credibility of the research findings was carried out by cross-referencing with similar studies (Kakar *et al*., 2023).

RESULTS

Azotobacter genera are aerobic and heterotrophic bacteria (Figure 1) that form cysts to protect vegetative cells in dry environments (Sivapriya and Priya, 2017). The cell morphology of Azotobacter is pleomorphic, Gram-negative, capsule-forming, and motile (Ravikumar *et al*., 2004; Hindersah *et al*., 2024). The pigment of Azotobacter colonies is usually brownish black (Figure 1) and is related to protecting the nitrogenase system from oxygen during fixation (Banerjee *et al*., 2014).

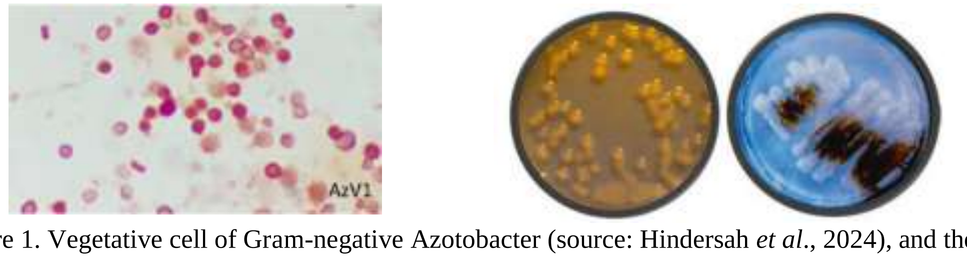

Figure 1. Vegetative cell of Gram-negative Azotobacter (source: Hindersah *et al*., 2024), and the colony morphology with brownish black pigment (Source: Dar *et al*., 202)

Nitrogen fixation is a specific property of Azotobacter living in the soil, rhizosphere, and phyllosphere (Kumar *et al*., 2018). *Azotobacter vinelandii* is a robust aerobic N-fixation model that protects the N-fixer apparatus from oxygen (Barron *et al*., 2024). Other essential functions of Azotobacter related to soil-quality increments are phytohormones, organic acid, and exopolysaccharides synthesis (Hindersah *et al*., 2020). Nonetheless, Azotobacter reduce the incidence of soil-born diseases (Hindersah *et al*., 2018). The Azotobacter resistence to heavy metal has been reported (Hindersah *et al*., 2017; Zulaika and Prasidya, 2017; Subardja *et al*., 2022).

Saline Soil

The chemical properties of saline soils are sodium (Na) content between 8-15%, pH < 8.5, electrical conductivity (EC) > 4 dS/m (equivalent to 4 mhos/cm), and sodium adsorption ratio (SAR) > 15% (Yan *et al*., 2015). Saline soil is one of the most damaging abiotic stresses that reduce crop growth, yield, and quality (Muhammad *et al*., 2024). The primary sources of salinity are the weathering of rocks and minerals that release dissolved salts, precipitation that washes salts downstream, wind-borne salts from the ocean, and seawater intrusion inland (Grundmann *et al*., 2016). Salinity can also be caused by climate change; decreasing precipitation and increasing temperature by 1.1^0C has markedly changed soil salinity in Kuwait (Bannari & Al-Ali, 2020). The Agronomic classification of saline soils based on electrical conductivity (EC) is non-saline (0-2 dS/m), slightly saline soil (2-4), saline soil (4-8), strongly saline soil (8-16) and extremely saline soil (>16) according to Hammam and Mohamed (2020).

In coastal ecosystems, most of the salinity in agricultural areas is caused by the use of salt-contaminated groundwater for irrigation (Duan, 2016). Excessive groundwater pumping and slow groundwater recharge reinforce the process of saltwater intrusion into the land (Grundmann *et al*., 2016) due to differences in water osmotic pressure. Soils above the salinized water table can be damaged due to the transport of saltwater under capillary channels and its evapotranspiration to the surface soil (Hui *et al*., 2022). Salt accumulation that increases the soil EC leads to the dispersion of clay particles and the limitation of water and nutrient uptake (Mindari *et al.*, 2015; Awedat *et al*., 2021).

Saline soils in Indonesian swamplands in the coastal plain reach 0.4 million ha with Na levels of 8-15% due to seawater intrusion (Haryono *et al*. 2012). For example, in Lohgung Village, Lamongan Regency, soil EC is 11.39 dS/m with a pH of 7.5 (Purwaningrahayu & Taufiq, 2018). The soil EC in Alue Raya Meulaboh, Aceh, was 2.5 dS/m at 0-20 cm depth and 5 dS/m at 20-40 cm after the 2004 tsunami. The topsoil of Cemara Village, Indramayu Regency, contains Na 35.17 mol/kg and a high SAR of 101.31 mmol/kg (Sutono, 2014).

Tolerance of Azotobacter to Salinity

Exposure to high osmolality in microorganisms triggers a rapid flow of cell water out of the cell along the osmotic gradient, resulting in decreased turgor and cytoplasmic dehydration. The process of plasmolysis disrupts cell characteristics, including cell volume (or relative volumes of cytoplasm and periplasm), turgor pressure, cell wall strain, cytoplasmic membrane tension, and concentration of solutes, salt ions, and uncharged biopolymers (Wood, 2015). Water uptake by roots is reduced in highly saline soils, as roots absorb many Na^+ and Cl^- ions that interfere with plant metabolic processes (Mäser *et al*., 2002). Many explorations of Azotobacter biodiversity in saline soils have been conducted, and their tolerance to various levels of NaCl has been verified (Table 2).

Table 2. Salinity-tolerance level of specific Azotobacter

	NaCl level	Reference
A. chroococcum	0.8 mM Na^+	Page, 1987
A. salinestris sp. nov.	1.5%	Page & Shivprasad, 1991
Azotobacter spp.	6-10%	Akhter *et al*., 2012
Azotobacter isolates	7%	Bjelić *et al*., 2015
Azotobacter salinestris	1 %	Omer *et al*. 2016
Azotobacter spp.	3.5 %	Hindersah *et al*., 2019
A. chroococcum	8 mM	Yaghoubian, *et al*., 2021

Decades ago, *A. salinestri*s sp. nov., which required Na+ ions for growth, was isolated from slightly saline soils (Page and Shivprasad, 1991). This finding is supported by Wang *et al*. (1993), who isolated the sodium-dependent Azotobacter species in New South Wales, Australia, examining their distribution and association with saline and alkaline soils. Nowadays, different species of saline-resistant Azotobacter have been isolated. Recent finding are biofilm- and exopolysaccharide-production *A. chroococcum* SC8, *A. beijerinckii* SC10, and *A. tropicalis* SC4 that proliferate in 150 and 300 mM NaCl (Çam *et al*., 2022).

Mechanism of Azotobacter Resistance to Salinity

Among soil microbes, Azotobacter has natural halotolerant characteristics, making it important for nutrient management in saline farms. *Azotobacter salinestris* tolerate 8% salt and becomes a typical Azotobacter in approaching saline conditions (Chennappa *et al*., 2016). Halotolerant Azotobacter have been successfully isolated using Ashby's mannitol agar contaminated with 1%, 2% and 3% NaCl (Bhavna *et al*., 2019). Moreover, *Azotobacter* isolated from saline paddy soil can proliferate in N-free medium supplemented with 0.85%, 1.7%, and 3.4% NaCl (Hindersah *et al*., 2019).

The accumulation of intracellular osmoprotectant in the cells of the N_2-fixer Azotobacter is the main mechanism by which the bacteria withstand the saline soil. Azotobacter salinestris produced osmoprotectant substances such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme, Salicylic acid (SA), proline, and exopolysaccharide (EPS) in saline conditions (Omer *et al*., 2016). Azotobacter chroococcum 67B and 76A synthesized osmolyte superoxide dismutase, catalase, proline, and ACC deaminase in response to elevated salt concentration in a laboratory experiment (Viscardi *et al*., 2016).

The EPS, which is a complex polysaccharide synthesized on the outer surface of the cell wall of Azotobacter, is well known to bind the alkali metal sodium (Na^+) and hence limit its uptake by the bacterial cell and reduce Na^+ availability for plant uptake (Geddie and Sutherland, 1993). The capacity of soil bacteria to produce EPS can lead to metal ions undergoing biomineralization (Bhagat *et al*., 2021). In the case of saline soil with a high concentration of Na, EPS is an extracellular barrier for preventing excessive Na^+ enter the cell via sodium-substrate cotransport (Igiri *et al*., 2018; Wilson and Ding, 2001).

The salt accumulation in cytoplasm is characteristic of members of the Halobacteriaceae bacteria, such as *Halobacterium salinarum*, an extreme halophilic archaeobacteria that requires more than 5 M NaCl for optimal growth (Vauclare *et al.*, 2020). Proline is an essential amino acid with diverse biological functions that accumulates in the cells of many bacteria and plants as an osmoprotectant agent and in response to osmotic stress (Ingrisano *et al*., 2023).

Azotobacter-Mediated Plant Tolerance to Salinity

Azotobacter inoculation triggers the formation of osmotolerant compounds under salt stress (Table 2). Plants produce various types of osmoprotectants, generally proline. The accumulation of osmoprotectants has the potential to select saline soil productivity and reduce the effect of salt stress on growth parameters (Table 3).

Table 2. Plant tolerance mechanisms in saline soil after Azotobacter inoculation

Saline-resistance Azotobacter	Crops and Plant responses	Reference
A. salinestris NBRC 102611	Sorghum. Produce salicylic acid, proline, and exopolysaccharides	Omer *et al*., 2016
A.chroococcum 67B and 76A	Tomato. Accumulate Al-siderophore complexes on root	Viscardi *et al*., 2016
Azotobacter sp.	Tall fescue (*Festuca arundinaceae* schreb.). Induce proline synthesis	Massahi *et al*., 2018
A.chroococcum 67B and 76A	Tomato. Increase of relative water content and Ca^{2+}	Van Oosten *et al*., 2018
A. chroococcum and/or Alcaligenes faecalis	Canola. Boost soluble sugar, protein, proline & antioxidant enzymes, reduced hydrogen peroxide.	Latef *et al*., 2021
Azotobacter and/or Rhizobium	Chikcpeas. Enhance growth and grain.	Abdiev *et al*., 2019
Azotobacter sp.isolates S2 and K4	Tomato. Better seedling growth in soil with 3.4% NaCl	Hindersah *et al*., 2019
A. chroococcum and vermicompost	Maize. Increase Nitrogen retention and utilization.	Li *et al*., 2024

Generally, plants respond to Azotobacter inoculation by producing various osmoprotectants that increase their tolerance to salinity. Azotobacter is reported by McRose *et al*. (2017) to produce siderophores under low concentrations of iron (Fe), molybdenum (Mo), and vanadium (V). In response to saline-resistant Azotobacter, tomato roots accumulate siderophores that complex with aluminum (Al) in the root instead of the Fe-, Mo-, and V-siderophore complex (Table 2). Aluminum ions facilitate the remediation of deficiencies in other elements and activate genes that enhance tolerance to various stresses, although higher concentrations of Al are toxic to plants

(Ofoe *et al*., 2023). Nevertheless, Azotobacter-treated plants contained higher Relative Water Content (Table 2), suggesting Azotobacter activity might trigger the production and/or uptake of osmolytes, that maintain the favorable water uptake (Van Oosten *et al*., 2018). Increase of Ca^{2+} following Azotobacter inoculation is a mechanisms to protect the plant from osmotic and ionic stress (Van Oosten *et al*., 2018).

In saline soils, NH_4^+ content is lower than in non-saline soils due to the increased loss rate (Zhu *et al*., 2016), so that N availability to plants relies more on nitrate. Limitation of nitrogen (N) uptake has been shown in saline soils to result in the slowing of early vegetative growth and stunting (Assouline *et al*., 2015). Reduced crop yield in saline paddy fields affects seed germination, growth and yield of paddy (Reddy *et al*., 2017).

Prospect of Azotobacter to Alleviate Saline Soil

The N_2-fixer Azotobacter is a promising bioagent to address the harmful effects of saline soil on various coastal crops. Indonesia's coastline spans nearly 55,000 kilometers, making it the second longest in the world, only after Canada and Norway. Mangroves in coastal areas are crucial for protecting agricultural regions from saltwater intrusion; however, both man-made and natural disasters have diminished mangrove density in other parts of Indonesia.

Different species and strains of Azotobacter have been isolated from both saline and nonsaline soils, assessed for their salinity resistance, and evaluated for their capacity to enhance plant growth in saline conditions. This type of rhizobacteria serves multiple functions, acting as both a defensive agent for plants and a promoter of plant growth. Consequently, introducing Azotobacter can be an effective strategy to increase agricultural productivity in saline soils.

Nowadays, various Azotobacter species are used in biofertilizer formulation. Developing biofertilizer from a saline-resistant Azotobacter strain is a way to initiate the use of Azotobacter in the bioremediation of saline soil. The challenge of utilizing Azotobacter to improve plant growth and productivity in saline areas includes the effectiveness of strains to induce plants to synthesize osmotolerant compounds, reduced costs of bioagent formulation, farmers' acceptance and adaptation to new technology, and the price and affordability of Azotobacter inoculants in remote coastal areas.

CONCLUSION

The adaptation of N_2-fixing Azotobacter to osmotic stress is essential because of the limited availability of nitrogen in saline soil. Azotobacter is confirmed to synthesize intracellular osmolytes to cope with increased salinity in the hyperosmotic environment. Plants grown in saline soil with Azotobacter inoculation develop the adaptability mechanisms for maintaining growth by boosting osmolyte synthesis. Introducing N_2-fixing Azotobacter has the potential to contribute to sustainable agriculture in saline-coastal areas. Exploring saline-tolerance Azotobacter is required to develop specific Azotobacter-based biofertilizers for saline soil.

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