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Functional diversity of bacteria in various saline soil plant vegetations around Sialang Buah Coast, North Sumatra, Indonesia

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ABSTRACT

Environmental conditions profoundly influence microbial diversity and activity in soil. For optimal growth, soil microbes face limiting factors such as temperature, moisture, pH, and salinity levels. This study aims to find types of functional bacteria that are able to live in saline soils. The study was conducted in the Soil Biology Laboratory at Universitas Sumatera Utara (USU), Indonesia. Soil samples were collected around the Sialang Buah Coast, Serdang Bedagai Regency, North Sumatra, Indonesia. The method employed in this research was random composite sampling taken from three vegetation types: mangrove forests, grasslands, and oil palm plantations, with sample collection locations influenced by tidal fluctuations. Ten sampling points were taken at each location and then composited for each vegetation type. The results of the study showed that there were ten species of bacteria that were able to live in saline soil, namely *Pseudomonas aeruginosa*, *Burkholderia gladioli*, *Enterobacter cloacae*, *Brucella ciceri*, *Ochrobactrum oryzae*, *Achromobacter xylosoxidans*, *Priestia flexa*, *Enterobacter quasiroggenkampii*, *Bacillus cereus* and *Ochrobactrum oryzae*. All bacteria found were able to grow on Pikovskaya, Alexandrov, and Jensen media and only seven species of bacteria were able to form biofilms, namely *P. aeruginosa*, *B. gladioli*, *E. cloacae*, *B. ciceri, A. xylosoxidans, P. flexa,* and *E. quasiroggenkampii*.

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1. INTRODUCTION

The presence of microorganisms from one place to another is highly diverse. One of the factors contributing to this diversity is the unique characteristics of microorganisms and varying optimal environmental conditions that influence their reproductive capacity. Salinization is defined as an excess of soluble salts on the soil surface (Kang et al., 2014; Khan et al., 2019; Machado & Serralheiro, 2017; Shahbaz & Ashraf, 2013). The primary constraint under saline conditions is the elevated content of salts, particularly NaCl. Increased salt concentration in the soil affects turgor, photosynthesis, and/or specific enzyme activities (Acosta-Motos et al., 2017; Kaushal, 2020), reducing growth hormones like auxins and cytokinins while increasing ethylene and abscisic acid hormones. It is a well-established fact that soil microbial communities (bacteria or actinomycetes) alter their responses to excessive salt content (Barnawal et al., 2017; Misra et al., 2017; Singh et al., 2015). Additionally, salt stress limits microbial biomass production, microbial respiration, and enzymatic activity in the soil.

NaCl is the primary salt found in saline soils. In such lands, NaCl content ranges between 2-6%. Classification of electrical conductivity (EC) values, divided into five classes: non-saline (0-2 mmhos cm^{-1}), very low saline (2-4 mmhos cm^{-1}), slightly saline (4-8 mmhos cm^{-1}), saline (8-15 mmhos cm^{-1}), and highly saline (>15 mmhos cm^{-1}) (Abrol et al., 1988). Soils with EC values >4 mmhos cm^{-1} are categorized as saline soils (Shahbaz & Ashraf, 2013). High NaCl content in saline soils disrupts soil structure, resulting in poor aeration and low permeability. Absorption by soil particles causes swelling and closure of soil pores, worsening gas exchange, and colloidal material dispersion

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(Naher et al., 2013; Panhwar et al., 2014). Consequently, microbial populations in saline soils are minimal. This is due to the low aeration of saline soils, hindering soil microbes from respiration due to restricted gas exchange. Functional bacteria have the potential to serve as biofertilizers, playing a crucial role in improving soil quality biologically (Shrivastava & Kumar, 2015). They also play an important functional role in food chains, being an integral part of biogeochemical cycles such as carbon, sulfur, nitrogen, and phosphorus cycles. Certain bacteria can thrive in salt-affected alkaline lands, providing essential nutrients, especially nitrogen, and phosphate, while producing plant growth hormones to aid plant development (Ahemad & Kibret, 2014; Sembiring & Sabrina, 2022a; Sharma et al., 2013).

Several types of soil organisms can provide essential nutrients such as Nitrogen (N), Phosphorus (P), and Potassium (K) to plants (Glick, 2014; Sembiring & Sabrina, 2022b). About 80% of microorganisms obtained from plant rhizosphere soils are capable of producing auxins like Indole-3-Acetic Acid (IAA) (Navarro-Torre et al., 2017; Olanrewaju et al., 2017; Sharma et al., 2016; Sorty et al., 2016). IAA is a pivotal hormone that enhances rhizobacterial-plant interactions (Ahemad & Kibret, 2014; Egamberdieva et al., 2017; Kang et al., 2014; Liu et al., 2013). Environmental factors play a critical role in the diversity of organisms across various agroecosystems and are instrumental in maintaining microbial interactions (Naher et al., 2016; Tan et al., 2020). Utilizing bacteria capable of solubilizing P and K (Sianturi et al., 2021), fixing atmospheric N (Zebua et al., 2020), producing biofilm, and generating IAA is anticipated to improve soil quality, and plant fertility, enhancing plant resilience against extreme environmental stresses, and protecting them against pathogen attacks (Naher et al., 2016; Panhwar et al., 2014; Paul, 2013).

In addition to producing growth-promoting compounds and controlling diseases, some rhizobacteria like Pseudomonas are also recognized as competitive bacteria that efficiently utilize nutrient sources in the rhizosphere environment. The benefits of these rhizobacteria include enhancing plant growth and synthesizing plant growth regulators (phytohormones). Soni et al. (2013) demonstrated that certain bacteria can thrive in NaCl concentrations up to 20% and can solubilize phosphorus from $Ca_3H_2(PO_4)$. However, salinity poses no issues for the existence of *Bacillus*, *Azotobacter*, and *Azospirillum* bacteria, particularly in terms of phosphorus solubilization. Some microbes can solubilize P and produce IAA hormones, but only *Bacillus megaterium, Stenotrophomonas maltophilia, Rhizobium sp, Azotobacter aerogenes, Azotobacter crococcum, Azospirillum lipoferum, Pseudomonas* and *Enterobacter* (Ali et al., 2014; Bharti et al., 2014) can produce ACC deaminase in saline regions. Research by Sembiring and Sabrina (2022a) showed that non-symbiotic nitrogen-fixing bacteria could increase total soil N content by 3.2%. Another study by Sembiring and Sabrina (2022b) demonstrated that *Dyella japonica, Bacillus subtilis, Pantoea dispersa, Enterobacter cloacae,* and *Ralstonia mannitolilytica* could produce IAA hormones and fix N from the air. *Bacillus amyloliquefaciens* enhanced exchangeable K by 67.64%, while *Talaromyces pinophilus* increased soil exchangeable K by up to 102.94% and P availability by up to 110.23%. Bacterial growth is strongly influenced by environmental conditions, high salt levels can inhibit bacterial growth so that the population and types are limited to saline soil. This study aims to find types of functional bacteria that are able to live in saline soils.

2. MATERIAL AND METHODS

This research was conducted at the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Sumatera Utara (USU). Soil samples were collected from around Sialang Buah Coast, Serdang Bedagai Regency, North Sumatra Province, Indonesia. Fresh soil samples were taken from three vegetation types: mangrove forests (Lat 3.59086° N, Long 99.093313° E), grasslands (Lat 3.59051° N, Long 99.093143° E), and oil palm plantations (Lat 3.553585° N, Long 99.053469° E). Soil sampling employed the random composite sampling method, with 10 sampling points taken from each vegetation type and then composited. Soil analysis conducted was soil pH soil, Organic C (Spectrophotometry), EC (Potentiometry) and bacteria population (Total plate count). The specific medium used was the Dworkin-Foster (DF) minimal salt selective medium (Dworkin & Foster, 1958) enriched with Ammonium sulfate following the procedure by Glick (2014). The composition of the DF medium was as follows: KH_2PO_4 4 g, Na_2HPO_4 6 g, $MgSO_4.7H_2O$ 0.2 g, $FeSO_4.7H_2O$ 0.001 g, H_3BO_3 0.00001 g, $MnSO_4$ 0.00001 g, $ZnSO_4$ 0.00007 g, $CuSO_4$ 0.00005 g, MoO_3 0.00001 g, Glucose 2 g, Gluconate Acid 2 g, Citric Acid 2 g, Agar 12 g (for solid media), and Distilled Water 1000 ml), Ammonium sulfate 2 g. Pikovskaya, Alexandrov, and Jensen media were used to test the microbial potential to enhance N, P, and K availability.

2.1. Sample Collection

Samples were collected from mangrove forests, grasslands, and oil palm plantations near Sialang Buah Coast, Teluk Merkudu District, Serdang Bedagai Regency, North Sumatra Province, Indonesia. The soil type is Alluvial, sampling locations were close to the coast, thus influenced by tidal fluctuations. Soil samples were taken at depths of 0-40 cm below the soil surface using a soil auger.

2.2.Isolation of Saline Soil Microbes

Ten grams of fresh soil were put in a 250 mL Erlenmeyer flask containing 50 mL sterile physiological saline (dilution 10^{-1}). The mixture was shaken for 30 minutes. Serial dilutions were made, with 1 mL taken from the 10^{-1} dilution and placed into a test tube containing 9 mL physiological saline (10^{-2} dilution), then shaken over a vortex until homogeneous. From the 10^{-2} dilution, 1 mL was pipetted and put into a test tube containing 9 mL of physiological solution (10^{-3} dilution). The procedure was repeated, resulting in dilutions up to 10^{-7}. The diluted solutions were spread onto petri dishes containing DF + Ammonium Sulfate 2 g medium and allowed to solidify. The petri dishes were then incubated at 28 °C for 3-7 days. After incubation, bacterial colonies were observed and counted Glick (2014)..

2.3. Purification

Purification aimed to obtain pure cultures without contaminants from other microorganisms. Microbial colonies

were selected based on morphological differences in color and surface texture, ensuring the isolation of pure strains.

2.4. Molecular Identification (PCR)

To identify bacteria at the molecular level, universal primers of 63f (5'CAG GCC TAA CAC ATG CAA GTC 3'), Primer 1387r (5' GGG CGG WGT GTA CAA GGC 3') were used to amplify the gene sequence of 16S bacteria rRNA through PCR. DNA amplification was conducted in 29 cycles with denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 1 minute 30 seconds, primer annealing at 55 °C for 45 seconds, and extension at 72 °C for 1 minute. Amplified DNA from bacterial colonies was subjected to sequencing. Similarity searches for the resulting sequences were performed using the BLAST program from the National Center for Biotechnology Information (NCBI) (Marchesi Julian et al., 1998).

2.5. Microbial Potential Test for Nutrient Availability Enhancement

The microbial potential was evaluated by their ability to release clear zones on Pikovskaya media for P solubilization (Pikovskaya, 1948), Jansen media for atmospheric N fixation (Jensen, 1951) and Alexandrov media for K solubilization. The observation lasted for 7 days, focusing on colony growth and clear zones formed on each medium (Prajapati & Modi, 2012).

2.6. Biofilm Production Capability Test

Cultural characterization of biofilm formation using the microtiter plate biofilm assay (Merritt et al., 2006). Each TSB culture (48-hour-old, at 28 °C) of the HM resistant isolate was diluted to 1:100 µL with sterile H_2O water. The bacterial suspensions (6×10^9 CFU mL^{-1}) were transferred into duplicate wells of a disinfected and dried flat bottom microtiter plate. The plate was covered and incubated at 28 °C for four days. The biofilm adhering to the bottom and wall of the washed wells was stained with 125 µL of 0.1% crystal violet for 10 min at 28 °C. The wells were washed with H_2O and excess liquid was removed. The qualitative observation was done by visual comparison with positive control (biofilm-producing *Pseudomonas aeruginosa*) and uninoculated TSB in wells.

3. RESULTS

The highest soil pH was recorded in the mangrove vegetation, ranging from 7.15 to 7.44, while the lowest pH was found in palm oil plantations (6.68 to 6.83). The higher soil pH in mangrove areas is attributed to various factors, such as the high salt content. Mangrove vegetation grows in areas subject to inundation by saline or seawater. Saline water contains elevated salt concentrations, and when tidal water infiltrates the soil, these salts can accumulate within it. EC levels and bacterial populations were highest in mangrove land and the lowest in oil palm land. The highest EC was 16.2 and the lowest was 5.6. The bacterial population in mangrove vegetation ranged from 31 – 56 × 10^4 CFU (Colony forming unit) while in palm oil vegetation it ranged from 24 – 34 × 10^4CFU. The high bacterial population at the research site can be attributed to the soil pH, which supports bacterial growth due to its proximity to neutral or alkaline conditions. The suitable pH range for organisms is 6.5 to 8.5, and even under acidic or alkaline conditions, some organisms remain tolerant. In grass vegetation, 6 isolate codes were found, namely A, D, E, H, K and L and in mangrove and oil palm vegetation there were four isolate codes each.

A sequence of 16S rRNA reveals that the bacteria obtained from saline soil include *Pseudomonas aeruginosa, Burkholderia gladioli, Enterobacter cloacae, Brucella ciceri, Ochrobactrum oryzae, Achromobacter xylosoxidans, Priestia flexa, Enterobacter quasiroggenkampii, Bacillus cereus, Brucella cicero* and *Ochrobactrum oryzae* (Figure 1). Each bacterial species possesses distinct characteristics that differentiate them from other species, encompassing morphological traits. Generally, the observed bacterial cell shape is cocci at 100x magnification (Figure 2).

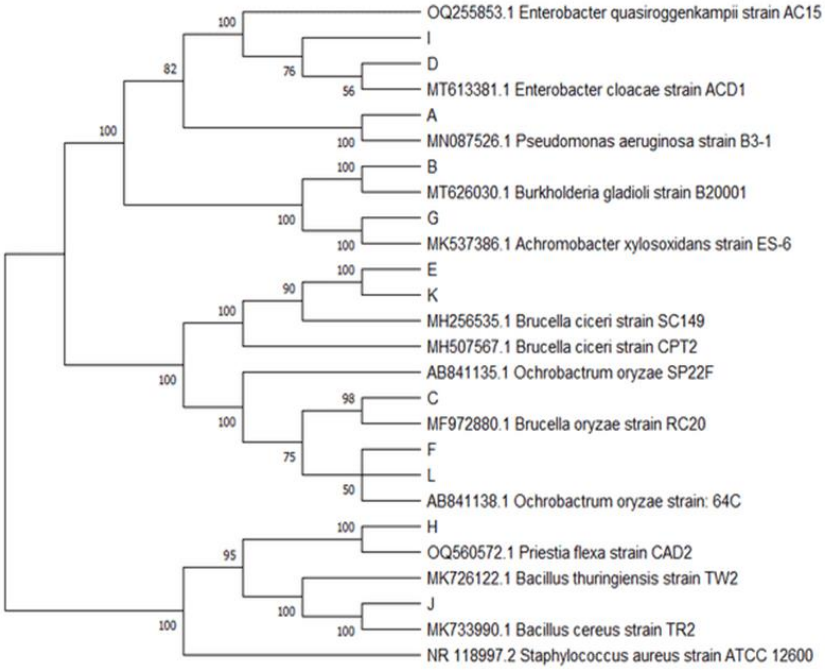

Figure 1. Phylogenetic Tree of Bacteria Found in Saline Soil

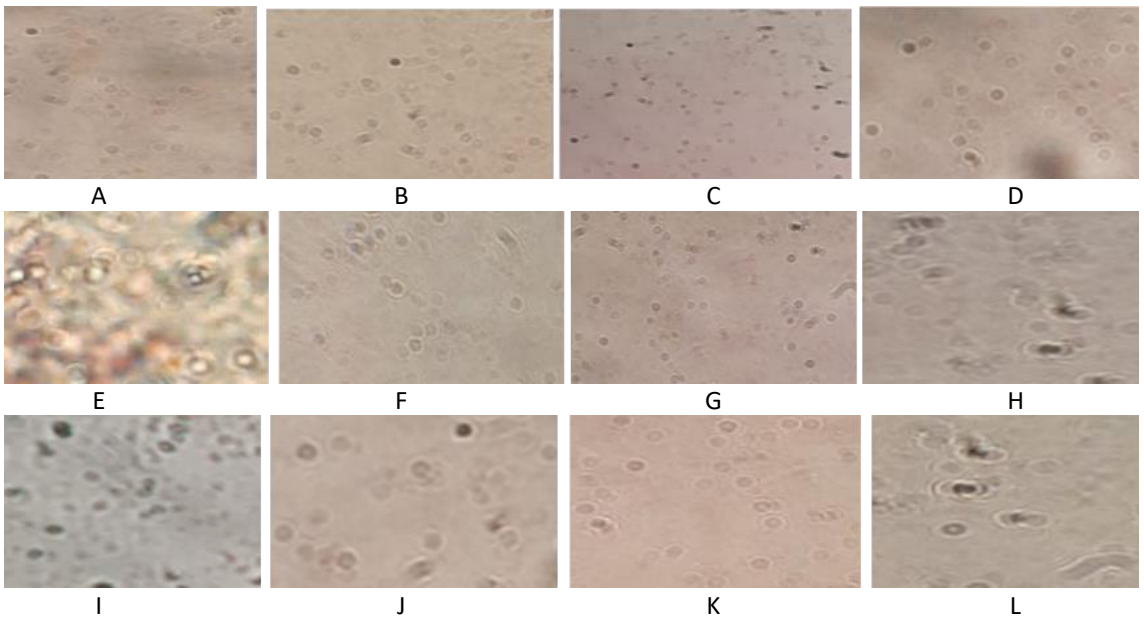

Figure 2. Colony Morphology of Bacteria Found in Saline Soil at 100x magnification

From the observation results of microbial biofilm production, there are seven microbial species capable of producing biofilm, namely *P. aeruginosa, B. gladioli, E. cloacae, B. ciceri, A. xylosoxidans, P. flexa* and *E. quasiroggenkampii*. Meanwhile, three species of bacteri are unable to produce biofilm, namely *B. oryzae, O. oryzae, and B. cereus*. The observation of microbial growth ability on the various media used indicates that all the microbes found are capable of growing on Pikovskaya, Alexandrov, and Jensen media, which suggests that these microbes may enhance the availability of nitrogen, phosphorus, and potassium (Figure 3).

4. DISCUSSION

The carbon content in saline soils is greatly influenced by vegetation. Palm oil vegetation exhibited the highest organic carbon (C-organic) content, ranging from 0.58% to 0.76%, whereas mangrove vegetation had the lowest organic carbon content (Table 1). This disparity is likely due to the limited plant diversity in mangrove areas, mainly consisting of mangrove trees. Any organic material present may be carried away during tidal fluctuations, causing its depletion. Two primary factors govern organic carbon content in salt-affected soil: (i) increased osmotic potential, hindering plant growth and consequently reducing organic carbon input (Wong et al., 2010; Zhao et al., 2018), and (ii) decreased microbial activity by microorganisms like bacteria and fungi which negatively impact decomposition (Setia et al., 2013; Setia et al., 2011).

The salinity values (EC) in mangrove vegetation ranged from 14.8 to 16.2 (very saline), whereas, in palm oil vegetation, the range was 5.6 to 8.6 (moderately saline/saline). This variation suggests that soil salinity is influenced by the proximity to the saline source (seawater). pH is one of the essential factors influencing bacterial growth. Bacteria require an optimal pH (6.5 - 7.5) for optimal growth, and the minimum and maximum pH values for the growth of most bacterial species are 4 to 9.

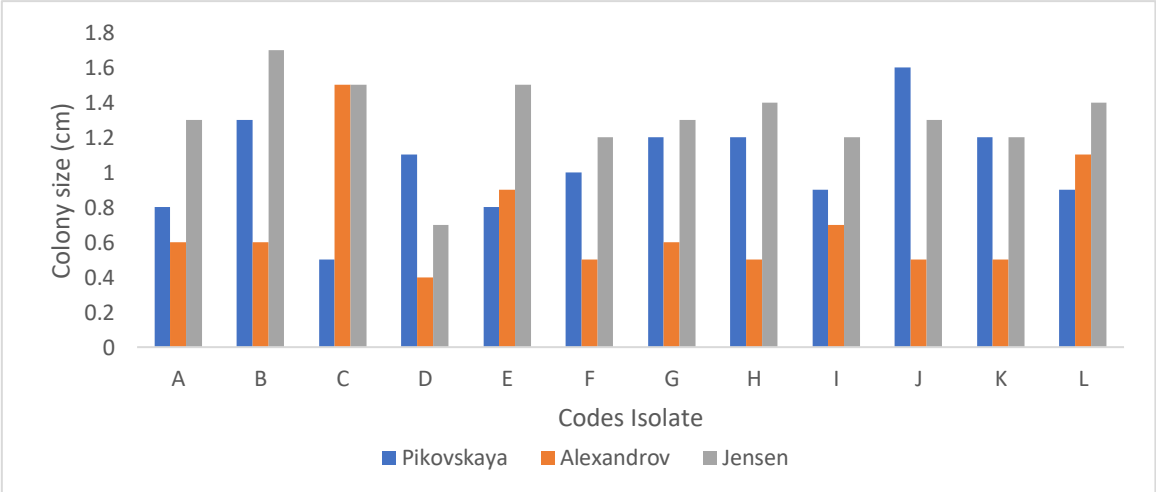

Figure 3. Microbial Growth Capability on Pikovskaya, Alexandrov, and Jensen Media

Table 1. Saline Soil Analysis in Various Vegetations

Vegetation Type	Parameters Observed				
Depth 0 – 40 cm	Soil pH	organic C (%)	EC (dS m^{-1})	Bacterial Population (10^4CFU)	Isolate Code
Mangrove	7.15 - 7.44	0.02 - 0.03	14.8 – 16.2	31 - 56	B, C, G, L
Grass	7.09 - 7.1 1	0.55 - 0.59	8.8 – 11.8	32 - 52	A, D, E, H, K, L
Palm Oil	6.68 - 6.83	0.58 - 0.76	5.6 – 8.5	24 - 43	I, J, F, L

The diversity of bacteria found in grass vegetation was more significant greater compared to other vegetation types. In grass vegetation, six isolates (A, D, E, H, K, L) were obtained, while in mangrove vegetation four isolates (B, C, G, L) and in palm oil vegetation four isolates (I, J, F, L) were identified. Among the numerous isolates found, the isolate with code L was able to thrive under varying salt conditions and different plant vegetation. Soil salinity has been proven to be a significant factor shaping the global distribution of soil microorganisms. Salinity is a crucial factor that impacts the diversity and composition of soil bacteria in numerous natural habitats (Rath et al., 2019; Zhao et al., 2020), and soil salinization regulates fungal diversity, community, and ecological functions (Kim et al., 2019; Mohamed & Martiny, 2011; Yang & Sun, 2020). According to Rodríguez-Blanco et al. (2015), *Pseudomonas* and *Enterobacter* bacteria (Ehis-Eriakha et al., 2022; Jiao et al., 2023), as well as *Priestia flexa*, are capable of atmospheric nitrogen fixation. The research findings by Sembiring and Sabrina (2022a, 2022b) indicate that several *Bacillus* genera act as phosphate and potassium solubilizers.

In the phylogenetic tree (Fig. 1), it can be observed that the bacteria obtained from saline soil include *P. aeruginosa, B. gladioli, E. cloacae, B. ciceri, O. oryzae, A. xylosoxidans, P. flexa, E. quasiroggenkampii, B. cereus* and *O. oryzae.* In Figure 1, it is evident that among the 12 obtained isolate codes, there are 10 bacterial species originating from various plant vegetation and different salinity levels, and two bacteria share the same isolate code as others, indicating that all these microbes are capable of thriving in diverse salt levels and plant vegetation. The research findings by Sembiring and Sabrina (2022a, 2022b) indicate that several *Bacillus* genera act as phosphate and potassium solubilizers in soil.

The ability of microbes to produce biofilm varies, which is related to a cluster of cells embedded in a matrix composed of exopolysaccharides (EPS), proteins, and sometimes DNA. Matrix production results in the formation of a characteristic complex biofilm architecture (Ahmed et al., 2020; Beauregard et al., 2013; Goodwine et al., 2019; Muhammad et al., 2020; Zea et al., 2020). The function of the EPS matrix is to physically prevent the access of specific antimicrobial agents into the biofilm by acting as an ion exchanger, thus limiting the diffusion of compounds, such as hydrophilic and positively charged antibiotics, from the surrounding environment into the biofilm. EPS can absorb or accumulate metals, cations, and toxins. EPS also protects against various environmental pressures, such as UV radiation, pH changes, osmotic shocks, and drought. Biofilm provides a single species or mixed bacterial community, especially non-sporulating formers like rhizobia, protection from fluctuating and often harsh rhizosphere conditions, such as drought, extreme pH levels, temperature, salt, and nutrient availability (Barraud et al., 2015; Rumbaugh & Sauer, 2020; Srinivasan et al., 2021). Biofilm is essential for bacterial colonization on plant roots (Srinivasan et al., 2021; Zea et al., 2020). Isolates A, B, D, E, G, H, and I are capable of forming biofilm, while isolates C, F, and J cannot form biofilm (Table 2). In general, several environmental factors affect biofilm production in bacteria, such as temperature, osmolarity, concentration of ferrous iron ions, nutrient availability, surface of the biofilm attachment material, and environmental acidity (Amankwah et al., 2021; Ponomareva et al., 2018; Toyofuku et al., 2016). Besides environmental factors, the genotype factor of bacteria is also suspected to play an important role in biofilm formation (Lade et al., 2019; Yin et al., 2019). The nutrient factor becomes a significant factor in biofilm formation in bacteria. This factor is the easiest to modify in vitro to enhance biofilm formation. Supplementation of several types of carbon sources has been reported to increase biofilm production on nutrient agar media. These carbon sources include glucose, sucrose, maltose, and lactose (Zou & Liu, 2020). In addition to carbon sources, NaCl also influences biofilm formation through osmolarity changes.

The ability of microbes to enhance nutrient availability in the soil is influenced by several factors, including their ability to produce organic acids, enzymes, and supportive environmental conditions. The ability of bacteria to grow on Jensen, Pikovskaya, and Alexandrov media is different for each microbe. This depends on the environmental and biological factors of the bacteria.

Table 2. Microbial Potential Test for Biofilm Formation

Isolate Code	Bacterial Names	Biofilm Production Capability
A	*Pseudomonas aeruginosa*	+
B	*Burkholderia gladioli*	+
C	*Brucella oryzae*	-
D	*Enterobacter cloacae*	+
E	*Brucella ciceri*	+
F	*Ochrobactrum oryzae*	-
G	*Achromobacter xylosoxidans*	+
H	*Priestia flexa*	+
I	*Enterobacter quasiroggenkampii*	+
J	*Bacillus cereus*	-
K	*Brucella ciceri*	+
L	*Ochrobactrum oryzae*	-

Notes: + capable of biofilm production, - incapable of biofilm production

The ability of bacteria to increase the solubility of P and K in media and soil depends on their ability to produce organic acids. The organic acids produced by phosphate-solubilizing bacteria can enhance P availability in the soil (Jiao et al., 2023; Khan et al., 2019; Sembiring & Sabrina, 2022a; Sharma et al., 2013; Zhao et al., 2018). Some organic acids resulting from microbial metabolism include citric acid, glutamate, succinate, lactate, oxalate, glyoxylate, malate, fuma tartrate, and α-ketobutyrate (Seshachala & Tallapragada, 2012; Zhao et al., 2014).

5. CONCLUSION

There are ten species of bacteria that can survive in saline soil, namely *P. aeruginosa*, *B. gladioli*, *E. cloacae*, *B. ciceri*, *O. oryzae*, *A. xylosoxidans*, *P. flexa*, *E. quasiroggenkampii*, *B. cereus* and *O. oryzae*. Among the ten bacteria found, seven species were able to form biofilms and all bacteria were able to grow on Pikovskaya, Alexandrov, and Jansen media, indicating that these bacteria are thought to be able to increase the availability of N, P, K.

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Declaration of Competing Interest

The authors declare that no competing financial or personal interests that may appear and influence the work reported in this paper.

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