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Potential of indigenous bacteria from West Sumatra (Indonesia) as plant growth-promoting rhizobacteria and biological soil conditioners in vitro

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Abstract. *Yulensri, Warman B, Noveri, Maulina F. 2025. Potential of indigenous bacteria from West Sumatra (Indonesia) as plant growth-promoting rhizobacteria and biological soil conditioners in vitro. Biodiversitas 26: 3378-3386.* Farmers depend on chemical fertilizers to increase rice production in the lowlands. As a result, rice fields have become critical due to physical, chemical, and biological degradation caused by the continuous use of chemical fertilizers and environmental pollution from residues. The use of potential indigenous bacteria could solve the problem. Therefore, this study aimed to isolate the indigenous bacteria from the banana stump in West Sumatra and the rhizosphere of the maize plant. The isolated bacteria were identified for their potential as plant growth-promoting rhizobacteria and biological soil conditioners. The experiment was conducted from March to May 2024 at the Plant Protection Laboratory of Politeknik Pertanian Negeri Payakumbuh and the Biotechnology Laboratory of the Institut Pertanian Bogor, Indonesia. The variables observed were the potential of bacteria as N fixers, P solubilizing, secretion of protease, kinase, cellulase enzymes, and exopolysaccharide (EPS). The results of the molecular analysis showed that potential isolates were *Azotobacter beijerinckii* Lipman, 1904 and *Alcaligenes faecalis* C. Both bacteria showed potential as PGPR and biological soil conditioners, which positively fixed nitrogen, can solubilize phosphate, produce auxins, and secrete chitinase, cellulase, protease enzymes, and EPS.

Keywords: Exopolysaccharide, identify, isolate, N-fixing, P-solubilizing

Abbreviations: IMO: Indigenous Microorganism, PGPR: Plant Growth-Promoting Rhizobacteria, EPS: Exopolysaccharide

INTRODUCTION

The problem for farmers is low rice production due to declining land productivity and pests, including disease infestation. In Indonesia, the degradation of rice fields has caused a significant decline in production. In 2018, Indonesia had 9,453,729 hectares of critical land and 4,552,721 highly critical lands. 4% of rice fields in 8 rice-producing provinces remained intact, 8% were slightly degraded, and the rest had moderate and severe degradation (Statistics Indonesia 2020). Degraded rice fields are characterized by decreased soil productivity, organic C content, nutrients N and P, and low available phosphate nutrients caused by complex bonds with Al and Fe. However, PGPR can unbind P in soil by releasing organic acids (Hasan 2024). Soil biological degradation is also characterized by a decrease in the quality and quantity of soil organic matter and soil microbial activity (Reddy et al. 2024).

Using soil conditioners is essential to minimize the loss of soil productivity and restore quality. In this context, selecting the right conditioners capable of addressing issues such as soil degradation, drainage, aggregation, compaction, hardening, and water repellency is necessary. The main objective is to create a favorable plant growth and production environment, promote soil biota development, and increase erosion resistance. These conditioners should facilitate the optimal availability of nutrients, water, and air, allowing maximum function as an ecosystem and providing efficient support for plant growth (Novia et al. 2021).

In line with recent developments, the production of Plant Growth-Promoting Rhizobacteria (PGPR) that produce Exopolysaccharides (EPS) as biological soil conditioners has been proven effective in solving degradation problems. The urgency of PGPR products functions as a biological fertilizer (N-fixing and P-solubilizing) and pesticide (controls pests and diseases). It also produces growth hormones to stimulate plant development and EPS, improving soil structure. Therefore, the PGPR product is expected to solve the overall problem of reducing the use of chemical fertilizers and pesticides, serving as biological soil conditioners that can improve soil structure (Fatimah et al. 2023).

According to Cilas et al. (2016), environmental differences in the tropics strongly influence pests, diseases, and bacteria biocontrol agents. Due to Indonesia's tropical climate and high microbiota diversity, the country has significant potential for microorganism exploration (Ni'matuzahroh et al. 2019). It suggests that high activity in the search for indigenous microorganisms will increase the discovery of new isolates with high efficacy, thereby improving the availability of genetic resources. Based on this relationship, there is a tendency for effective isolates as biofertilizers, biopesticides, and biological soil conditioners to originate from the environment where pests and diseases are found.

Biological soil conditioners are formulated with microbial agents, particularly EPS-producing microbes. These agents are carbohydrate or polysaccharide polymer molecules secreted outside the surface of microbial cell walls, helpful in protecting cells from environmental stresses such as drought, low pH, and high pH. Some reported EPS-producing bacteria include *Caulobacter* Henrici & Johnson, 1935, *Acinetobacter* Brisou & Prévot, 1954, *Agrobacterium* Conn, 1942, *Alcaligenes* Castellani & Chalmers, 1919, *Cytophaga* sp., *Flavobacterium* Bergey et al., 1923, *Pseudomonas* Migula, 1894, and *Rhizobium* Frank, 1889 (Kucuk et al. 2020).

As one of the EPS-producing bacteria, *Azotobacter* Beijerinck, 1901 has the potential to absorb heavy metals, such as Cr and Zn. It also plays a role as a PGPR that works through the mechanism of nitrogen fixation and phytohormone production (Jeyanthi et al. 2018). These organisms can inhibit fungi growth (fungistatic), including certain highly pathogenic fungi such as *Alternaria* Nees ex Wallroth, 1816 and *Fusarium* Link, 1809 (Janatiningrum and Lestari 2022).

This study aimed to identify isolates of *Azotobacter* and *Alcaligenes* indigenous to West Sumatra and determine their potential as biofertilizers (N-fixing, P-solubilizing), pests and disease control (secret enzymes chitinase, cellulase, protease), and biological soil conditioners (producing EPS).

MATERIALS AND METHODS

Study area

This study was conducted from January to June 2024. *Azotobacter* bacteria are the Indigenous Microorganism (IMO) isolated from the banana weevil. *Alcaligenes* were isolated from the rhizosphere of *Zea mays* L. roots in the organic farming experimental field. Both isolates were collected in the experimental garden of Politeknik Pertanian Negeri Payakumbuh, West Sumatra, Indonesia, as shown in Figure 1.

Procedures

Isolation of Azotobacter *and* Alcaligenes

The banana weevil was collected in the morning before the sun shone and then cut into small pieces. Then, brown sugar was added in the ratio of 1:1. Subsequently, it was placed in a plastic box and incubated at a temperature of 25°C for 7 days, followed by isolation of *Azotobacter* bacteria under sterile conditions.

Alcaligenes were isolated from the roots of *Z. mays*. Soil samples were collected using the diagonal sampling method. Specifically, isolation was performed using the dilution method, and suspected colonies containing *Alcaligenes* were cultured on NA medium until pure isolates were obtained.

One gram of soil sample was mixed with 100 mL of 0.9% physiological NaCl and stirred until homogeneous to isolate bacteria. Then, 1 mL of the mixture was added to 9 mL of 0.9% physiological NaCl to prepare dilutions up to 10^{10}. The last 3 dilutions were collected, namely 10^{8}, 10^{9}, and 10^{10}, while 1 mL of each dilution was poured into a Petri dish and added with 20 mL of liquid nutrient agar. Subsequently, the sample was stored in an incubator for 1 day, followed by a purification process (Dewiyanti et al. 2022).

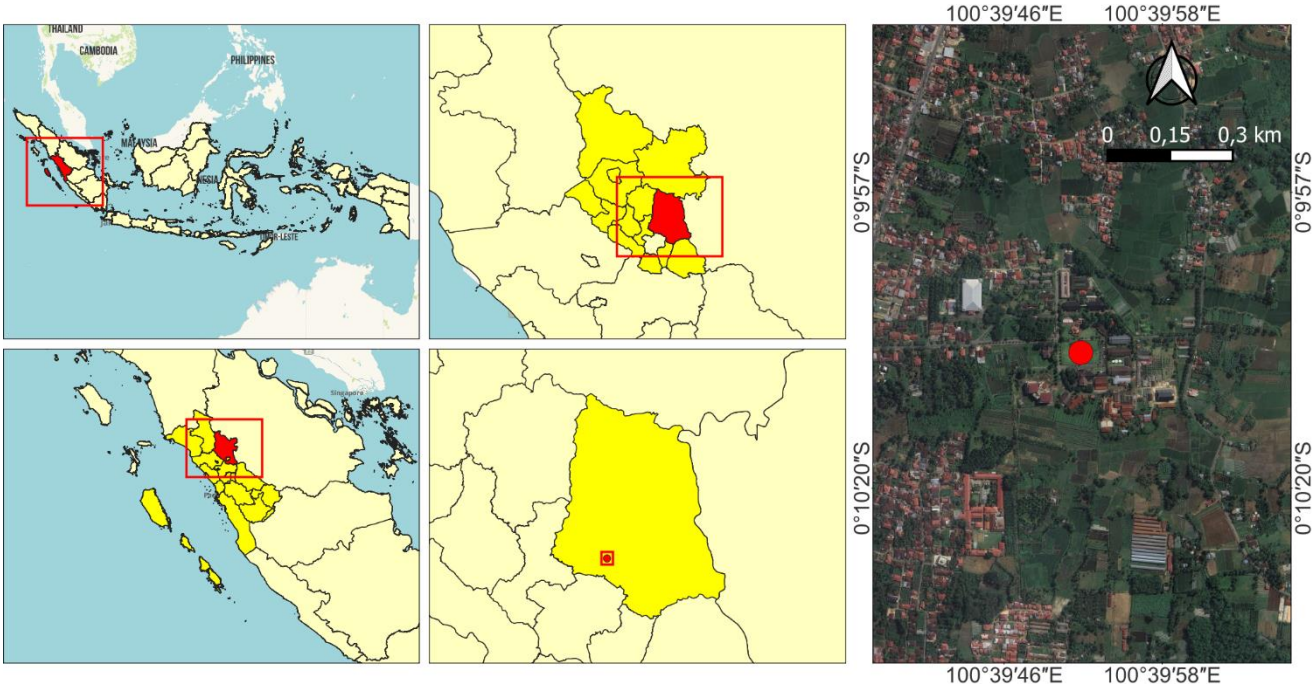


Figure 1. Map of sampling location

Identification of Azotobacter *and* Alcaligenes
Gion analysis

The 16S rRNA method identified selected isolates, where genomic DNA was extracted using Presto™ Mini gDNA Bacteria Kit (Genaid). Amplification for the 16S rRNA gene was achieved using two primers, namely E8F: 5-AGA GTT TGA TCC TGG CTC-3 for forward and 1541R: 5-AAG GAG GTG ATC CAG CCG CA-3 for reverse. Initial denaturation was at 95°C for 5 min, followed by 35 cycles of 95°C denaturation for 30 sec, 55°C annealing for 30 sec, and extension to 72°C for 1.5 min. A final extension step was set at 72°C for 10 min, while amplified products were verified by agarose gel electrophoresis and the Gel Doc system (BioRad, USA). Furthermore, verified DNA samples were sent to First Base (Malaysia) for DNA sequencing. The initial sequence analysis was undertaken using the Basic Local Alignment Search Tool (BlastN). Phylogenetic analyses were carried out with MEGA X using the Maximum Likelihood method. The tree used the Maximum Composite Likelihood (MCL) method for determining evolutionary distances (Sanjaya et al. 2021).

N-fixing potential of Azotobacter *and* Alcaligenes

The ability of Azotobacter and Alcaligenes to fix N from the air was tested by culturing the two bacteria on a free-N medium (Jensen's medium). The ability of bacteria to grow on a free-N medium indicated their ability to fix N from the air.

The potential of Azotobacter *and* Alcaligenes *as phosphate-solubilising bacteria*

The qualitative test of phosphate solubilisation ability was performed by inoculating isolates on Pikovskaya agar (PKV) and incubating at 30°C. Isolates were considered to dissolve phosphate when there was a translucent zone around the colony.

Potential Azotobacter *and* Alcaligenes *as a biological control of pests and diseases of the plant*
Chitinase enzyme excretion

The qualitative analysis of chitinase activity was carried out following the method of Masri et al. (2021). Colloidal chitin was used in the chitin agar medium. Chitin from crab (20 g) was dissolved with concentrated HCl (400 mL) and incubated for 24 hours at 24°C. Translucent zones around the hole containing bacteria suspension indicated the ability to excrete the chitinase enzyme.

Protease enzyme excretion

The qualitative analysis of protease activity was performed using a gelatin substrate. The test medium was prepared by adding gelatin (4 g) to 50 mL of sterile distilled water and sterilized using an autoclave. After incubation, the medium was soaked with saturated ammonium sulfate solution (5 mL) and observed for a translucent zone around the hole containing bacterial suspension (Meidong et al. 2017).

Cellulase enzyme excretion

The cellulase activity was qualitatively analyzed using a carboxymethyl cellulose (CMC) substrate. The substrate contained 4 solutions. A solution contained NaCl (0.25 g), K_2HPO_4 (1.5 g), CMC (2.5 g), and distilled water (400 mL). CMC was slowly added to distilled water and shaken using a shaker at a speed of 100 rpm for 24 hours at 50°C. B solution: $MgSO_4$ (1.0 M). C solution: Na2HPO4 (3.0 g), NH_4Cl (0.5 g), glycerol (2.5 mL), yeast extract (0.5 g), agar (6.5 g), and distilled water (100 mL), and D solution: 7.5% (w/v) $CaCl_2$. After incubation, the medium was stained with 10 mL of Congo Red (0.1%) and observed under a microscope. The test was carried out using a consortium of the two isolates. The ability of bacteria isolates to produce EPS was performed using a gravimetric test at the Laboratory of Land Resources and Soil Chemistry of Universitas Padjadjaran, Indonesia. The ability of isolates to excrete the growth regulator auxin was tested at the IPB Biotechnology Laboratory.

Data analysis

Data were analyzed qualitatively and observed for translucent zones around the hole containing bacterial suspension. The positive result in 1 replicate is denoted as (+), while 2 and 3 are represented by (++) and (+++), respectively.

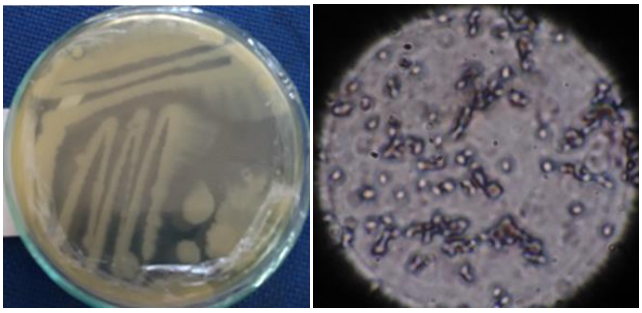
RESULTS AND DISCUSSION

Isolation of *Azotobacter* and *Alcaligenes*

Isolation of *Azotobacter* from the IMO weevil produced 3 isolates coded with BP1, BP2, and BP3. Among the 3 isolates grown on Free N medium, only 1 grew and was coded as Azo (Figure 2). The results of the multi-step dilution of soil from *Z. mays* roots produced 1 isolate suspected to be *Cytophaga* sp. and coded as CYT (Figure 3). Gram staining was performed using iodine/lugol, safranin, and crystal violet.

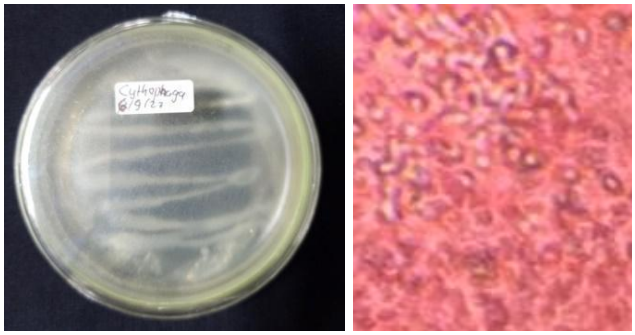
Azotobacter colony morphology is rod-shaped, Gram-negative, obligately aerobic, and larger than other prokaryotes, with a cell diameter of 2-4 µm or wider. *Alcaligenes* sp. bacteria are Gram-negative and rod-shaped (Figures 3.A and 3.B). The result of molecular identification shows that the *Azotobacter* indigenous to the IMO weevil in West Sumatra is 100% similar to the *Azotobacter beijerinckii* Lipman, 1904 in 165 rRNA, partial sequence (Table 1). Although the sample is suspected of *Cytophaga*, it is 100% identical to the *Alcaligenes faecalis* C strain BFE13 gene for 165 ribosomal RNA, partial sequence (Table 2). The phylogenetic tree of *A. beijerinckii* and *A. faecalis* is shown in Figure 4.

This study performed an evolutionary analysis using the maximum likelihood method and the Kimura 2-parameter model (Kimura 1980). Based on the results, the tree with the highest log-likelihood value was -6225.43. Furthermore, the percentage of trees clustered with associated taxa was shown next to the branches. The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the MCL method. It was followed by selecting the topology with the superior log-likelihood value. This analysis included 8 nucleotide sequences, with codon positions consisting of 1st+2nd+3rd+ noncoding, leading to 1569 positions in the final data set. Evolutionary analyses were performed in MEGA II (Tamura et al. 2021).



A B

Figure 2. A. Morphology of *Azotobacter* colony, B. *Azotobacter* cells



A B

Figure 3. Colony morphology and cell shape of *Alcaligenes*. A. *Alcaligenes* colonies, B. *Alcaligenes* cells

Table 1. Identification of *Azotobacter* and *Alcaligenes*

Description	Query cover (%)	Similarity (%)	E value	Acc. number
Azotobacter beijerinckii gene for 16S rRNA, partial sequence	100	100	0.0	AB429527.1
Azotobacter chroococcum strain P208 16S ribosomal RNA gene, partial sequence	100	98.7	0.0	MK841645.1
Azotobacter chroococcum strain SP1 16S ribosomal RNA gene, partial sequence	100	98.7	0.0	MK780064.1
Azotobacter chroococcum strain P207 16S ribosomal RNA gene, partial sequence	100	98.7	0.0	MK567897.1
Uncultured bacteria gene for 16S rRNA, partial sequence, gene: L2CLN32	100	98.7	0.0	AB696361.1

Note: Sample code: 12-23-0001/AZO (*Azotobacter*), *Azotobacter beijerinckii*, shows the sequences selected as reference

Table 2. Identification of *Alcaligenes*

Description	Query cover (%)	Similarity (%)	E value	Acc. Number
Alcaligenes aquatilis strain RC43 16S ribosomal RNA gene, partial sequence	100	100	0.0	MT572474.1
Alcaligenes faecalis strain IMJ8 16S strain P208 16S ribosomal RNA gene, partial sequence	100	100	0.0	MT516335.1
Alcaligenes faecalis strain BFE13 16S ribosomal RNA gene, partial sequence	100	100	0.0	MT415328.1
Alcaligenes faecalis strain BJD1 16S ribosomal RNA gene, partial sequence	100	100	0.0	MT378145.1
Alcaligenes faecalis strain P2 16S ribosomal RNA gene, partial sequence	100	100	0.0	MT277037.1

Note: Sample code: 12-23-0002/CYT (*Cytophaga*), number 3 shows the sequences selected as reference

The potential of *Azotobacter beijerinckii* and *Alcaligenes faecalis* as biological fertilizers, biological control of plant pests and diseases, auxin-producing hormones, and biosoil neutralizers

Table 3 shows that *A. beijerinckii* could survive on Free N medium in 3 replicates, while *A. faecalis* survived only in 1 replicate. It indicated that *A. beijerinckii* can take atmospheric nitrogen better for their life.

The potential of *Azotobacter beijerinckii* and *Alcaligenes faecalis* as phosphate-solubilizing bacteria

The ability of isolates to grow on soluble phosphate media is shown by a translucent zone around the colony (Table 4). The presence or absence of translucent zones around the colony indicates the ability of isolates to dissolve phosphate. The results on the ability of *A. beijerinckii* to dissolve phosphate showed that only 1 produced a translucent zone from 3 replicates in the suspension well, as shown in Table 4. This finding indicates the presence of dissolved phosphate in the Pikovskaya medium. Therefore, *A. beijerinckii* had the potential to dissolve phosphate despite its limited potential. In the *A. faecalis* bacteria, 2 samples produced translucent zones in the suspension well, showing the presence of dissolved phosphate.

The potential of *Azotobacter beijerinckii* and *Alcaligenes faecalis* as biological control agents

Azotobacter beijerinckii bacteria excrete the chitinase enzyme, as shown by the formation of a halo around bacteria well on a colloidal chitin medium. It is due to the presence of dissolved chitin, which shows the activity of the chitinase enzyme. *Alcaligenes faecalis* bacteria can also grow in the chitin medium, producing a halo around the bacteria well (Table 5).

Protease enzyme production

Azotobacter beijerinckii and *A. faecalis* grown on a gelatin medium showed protease enzyme activity characterized by a translucent zone around the colony wells. The translucent zone for *A. faecalis* was wider than for *A. beijerinckii* (Table 6).

Table 7 shows that *A. beijerinckii* and *A. faecalis*-can excrete the cellulase enzyme. It is indicated by the presence of a translucent zone around bacteria well on the CMC

medium due to the presence of dissolved chitin, indicating the activity of the chitinase enzyme. *Alcaligenes faecalis* bacteria can grow in the chitin medium and produce translucent zones.

Congo red was used to identify the clear zone, which shows the presence of soluble cellulase. The interaction of Congo red with the ÿ-(1,4)-D-glucan linkages in CMC produced a red color on CMC agar media (Table 7) (Zulaika et al. 2022).

Growth regulator activity of *Azotobacter beijerinckii* and *Alcaligenes faecalis*

Table 8 shows that both bacteria produce the growth hormone auxin. *Azotobacter beijerinckii* produced more auxin than *A. faecalis.*

Biosoil neutralizer activity of *Azotobacter beijerinckii* and *Alcaligenes faecalis*

Table 9 shows that *A. beijerinckii* and *A. faecalis* can produce EPS in liquid and flour formulations. The consortium of both bacteria on flour media produced EPS of 44 g/mL based on the test performed by the gravimetric method.

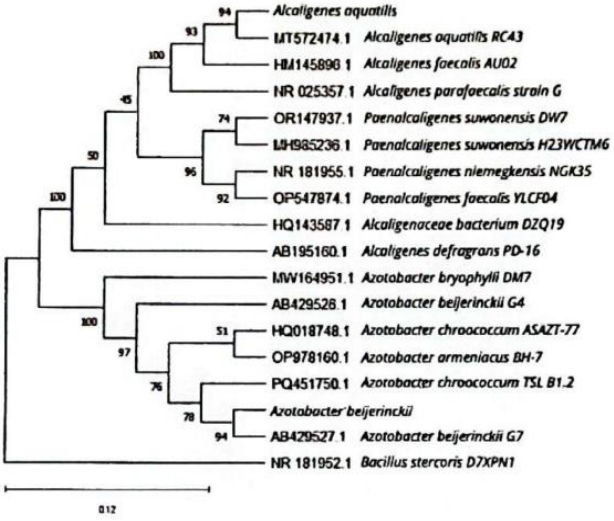


Figure 4. Phylogenetic tree of *Azotobacter beijerinckii* and *Alcaligenes faecalis* based on 16s rRNA. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances are in units of the number of base substitutions per site

Table 3. Rate of survival of *Azotobacter beijerinckii* and *Alcaligenes faecalis* on free N-medium

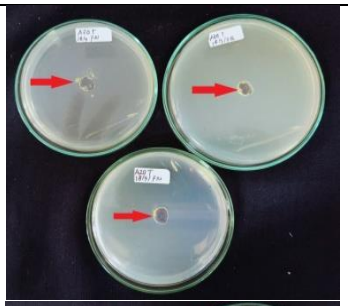
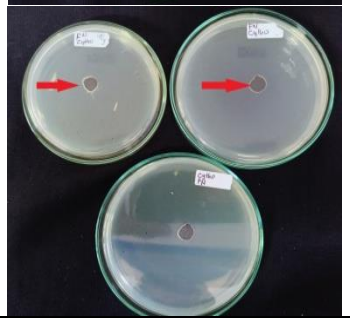
Treatment	Results	Documentation
Azotobacter beijerinckii	+++	
Alcaligenes faecalis	++	

Table 4. The formation of translucent zones on the Pikovskaya medium of *Azotobacter beijerinckii* and *Alcaligenes faecalis*

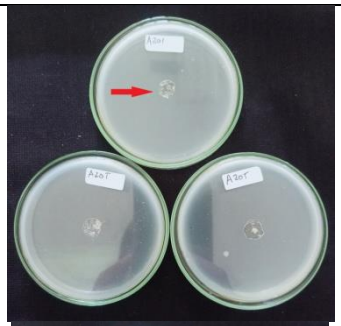
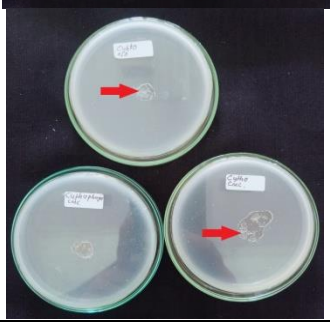
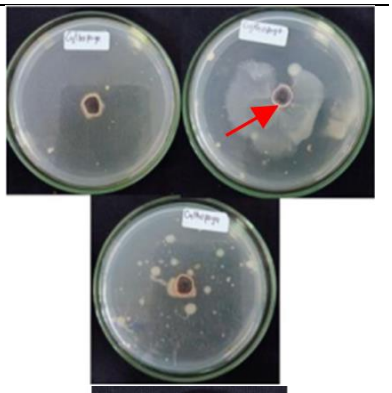
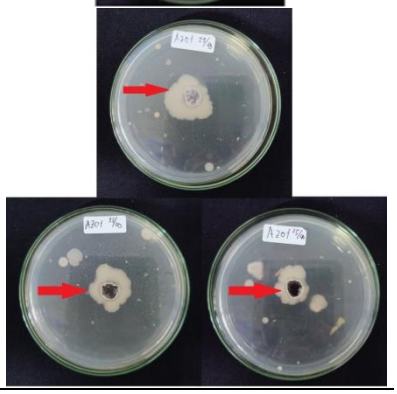
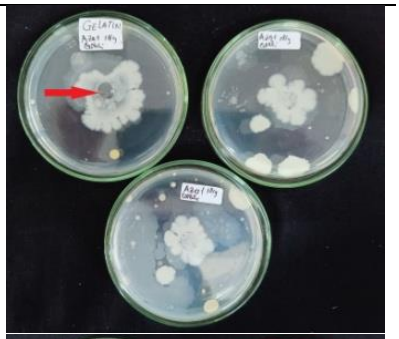
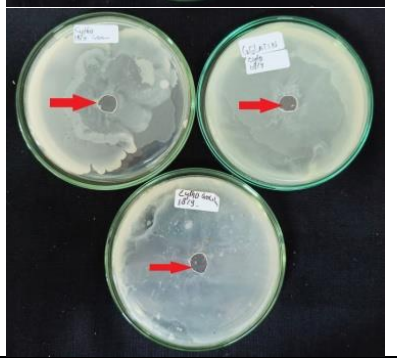
Treatment	Results	Documentation
Azotobacter beijerinckii	+	
Alcaligenes faecalis	++	

Table 5. Halo formation of *Azotobacter beijerinckii* and *Alcaligenes faecalis* on colloidal chitin medium

Treatment	Results	Documentation
Azotobacter beijerinckii	+	
Alcaligenes faecalis	+++	

Note: + = positive result for 1 plate

Table 6. Translucent zone formation of *Azotobacter beijerinckii* and *Alcaligenes faecalis* on a gelatin medium

Treatment	Results	Documentation
Azotobacter beijerinckii	+	
Alcaligenes faecalis	+++	

Note: + = positive result for 1 plate

Table 7. Translucent zone formation of *Azotobacter beijerinckii* and *Alcaligenes faecalis* in the CMC medium

Treatment	Results	Documentation
Azotobacter beijerinckii	++	
Alcaligenes faecalis	+++	

Note: + = positive result for 1 plate

Table 8. Auxin production of *Azotobacter beijerinckii* and *Alcaligenes faecalis* on Luria-Bertani medium

Treatment	Concentration IAA (PPM)	Documentation
Azotobacter beijerincki	31,9	
Alcaligenes faekalis	5,94	

Table 9. The ESP production of *Azotobacter beijerinckii* and *Alcaligenes faecalis*

Treatment	Produced Exopolysaccharide (EPS) (mg/L)
Azotobacter consortium of *Azotobacter beijerinckii* and *Alcaligenes faecalis* in a liquid formula	0,12
Azotobacter consortium of *Azotobacter beijerinckii* and *Alcaligenes faecalis* in the flour formula	44

Discussion

Isolation of *Azotobacter* was carried out from the weevil. The morphological character of the *Azotobacter* colony is rod-shaped, Gram-negative, and obligate aerobic, with a cell diameter of 2-4 µm or more (Figure 2.A and 2.B). Fatimah et al. (2023) and Hala et al. (2019) reported that the *Azotobacter* colony has the following characteristics: convex, flat edges, clear, average diameter of 1 mm, and slightly slimy. In addition, the morphological characters of *Alcaligenes* bacteria include Gram-negative and rod-shaped cells (Figure 3.A and 3.B). Ethica et al. (2018) reported that the morphology of *Alcaligenes* colonies was irregular, with entire edges, flat elevations, white color, coccobacillary cell morphology, Gram-negative, rod cell shape: rounded or spherical, 0.5-1.0 × 0.5-2.6 µm in size, and does not form endospores, aerobic and motile. The identification results show that *Azotobacter* indigenous from West Sumatra is 100% similar to the *A. beijerinckii* gene in 165 rRNA, partial sequence. This 16S rDNA gene amplification is the best tool to identify bacterial isolates due to sensitivity, reliability, and resistance (Foysal and Lisa 2018; Al-Amery and Alyousif 2022). However, the sample suspected to be *Cytophaga* has 100% similarity to *A. faecalis* strain BFE13 165 ribosomal RNA gene, partial sequence.

Azotobacter beijerinckii is a nitrogen-fixing microorganism that converts nitrogen (N_2) to ammonium (NH3) in sufficient quantities. It can fix 10-20 mg nitrogen/g sugar on a suitable medium; therefore, it can fertilize the soil and increase the biomass of crops (Aasfar et al. 2021). Several experiments conducted in temperate areas show that nitrogen fixation in soil inoculated with *Azotobacter* is not more than 10 to 15 kg N/ha/year, depending on the availability of carbon sources (Ladha et al. 2022). Although *Azotobacter* is an obligate aerobic bacterium, it catalyzes the binding of N_2 as a nitrogenase enzyme sensitive to O_2. A thick mucus capsule of *Azotobacter* helps to protect the nitrogenase from carbon. *Alcaligenes faecalis* can also live on a free nitrogen medium. However, *A. faecalis* cannot use the atmospheric N_2 for survival.

Azotobacter beijerinckii dissolves phosphate moderately and has lower activity than *A. faecalis.* Sembiring and Sabrina (2022) stated that phosphate-solubilizing microbes also produce various organic acids. *Azotobacter* can metabolize carbohydrates to produce organic acids, which facilitate phosphate solubilization. *Azotobacter chroococcum* Beijerinck, 1901 isolated from the rhizosphere can effectively solubilize phosphate in liquid media at 30 and 37°C (Nagaraja et al. 2022).

Azotobacter is a rhizosphere bacterium with nitrogen-fixing ability and a potential phosphate-solubilizing strain. *Azotobacter* can produce organic acids at a higher rate than others due to the presence of glucose dehydrogenase (GDH) enzyme. Based on previous reports, *Azotobacter* consortium increased dissolved phosphate by 18.1% compared to a single isolate (Thoyib et al. 2016). The existence of microorganisms that could dissolve insoluble phosphate into a soluble form allows plants to absorb it through dissolution and mineralization (Atekan et al. 2014). During phosphate dissolution, *A. faecalis* secretes various organic acids, such as oxalic, citric, malic, and other protein bands. The maximum alkaline phosphatase activity occurred at pH 9.0 (96.53 U/mL).

Azotobacter beijerinckii and *A. faecalis* bacteria secrete the chitinase, protease, and cellulase enzymes. *Alcaligenes*, a short rod-shaped cellulolytic species, can degrade macromolecules such as polysaccharides, including cellulose, chitin, starch, and pectin. According to Sayed et al. (2024) cell membranes of *Alcaligenes* contain endoglucanase and periplasmic endoglucanase that degrade cellulose. Although capable of digesting cellulose, *Alcaligenes* cannot produce cellulase; this cellulose is only attached to the cell envelope and binds to the mucus released during movement. Nikkhoy and Motamed (2019) reported that enzymes secreted by microbes were essential for the bioeconomy, with proteases accounting for approximately 60%. The protease enzyme produced by bacteria increases plant resistance to pathogens (Prihatiningsih et al. 2021). Additionally, the activity of proteolytic bacteria is often influenced by the nutrient source in the media (Fachrial et al. 2021).

Bacteria belonging to the Azotobacteraceae family are mainly free-living, nitrogen-fixing bacteria. *Azotobacter* isolated from soil or seed effectively increases the yield of cultivated plants on fertilized soil with sufficient organic matter content. In pure culture, *Azotobacter* can synthesize biologically active substances such as B vitamins, indoleacetic acid, and gibberellins. It can inhibit the growth of fungi (fungistatic), particularly certain highly pathogenic fungi such as *Alternaria* and *Fusarium*. These properties showed the beneficial effects of *Azotobacter* in increasing seed germination rates, plant growth, plant stand, and vegetative growth (Khandelwal et al. 2024).

Azotobacter beijerinckii and *A. faecalis* potentially excrete various EPS compounds and fatty acids (Karaca 2023). EPS functions as biosurfactants that can enhance the biodegradation of petroleum wastes (Whitfield et al. 2015; Wang et al. 2023). Karaca (2023) stated that fatty acids act as biosurfactants due to their amphiphilic nature, containing lyophobic and lyophilic groups. These bacteria also have the potential to excrete various EPS compounds and fatty acids. Several factors influence exopolysaccharide (EPS) production, including medium composition (primarily carbon and nitrogen sources), temperature, pH, fermentation time, and oxygen availability. The production of a specific type and amount of EPS can also be affected by the microbial strain and its growth phase (Petry 2000).

Based on the study's findings, *Azotobacter* isolated from the IMO weevil and *Alcaligenes* from the rhizosphere of corn roots are indigenous isolates of West Sumatra, which were identified as *A. beijerinckii* and *A. faecalis,* that can serve as biofertilizers with nitrogen-fixing and phosphate-solubilizing abilities. Potential as biocontrol agents is also attributed to chitinase, protease, and cellulase secretion. The production of auxins further supports their role as plant growth regulators, while EPS secretion functions as a biological soil conditioner. Applying the Intensification Rice System can increase seed viability, growth, and production, significantly reducing brown spot disease attacks by approximately 90% on leaves and 37.5% on grains. Furthermore, the physiological and pathological quality of seeds is improved. The consortium of *Bacillus cereus* Frankland & Frankland, 1887 strain ATCC 14579, *Bacillus subtilis* G subsp. *subtilis* strain 168, *Bacillus siamensis* Sumpavapol et al., 2010 strain KCTC13613, and *Pseudomonas fluorescens* Migula, 1895 are potential biopesticides due to the secretion of extracellular enzymes (chitinase, cellulose, protease) and suppression of blast disease attacks by *Pyricularia oryzae* Cavara with an inhibition percentage of ≥75% (Yulensri et al. 2021).

The molecular identification shows that *Azotobacter* from West Sumatra was identified as *A. beijerinckii* 165 rRNA gene, while *Alcaligenes* is similar to *A. faecalis* strain BFE13 for the 165 rRNA gene. *Azotobacter beijerinckii* and *A. faecalis* have the potential as biofertilizers, nitrogen fixation, dissolve phosphate, and biological agents for pests and diseases, secreting chitinase, cellulase, and protease enzymes. In addition, these bacteria also produce auxins and EPS, which act as growth regulators and biological soil conditioners.

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