Characterization of plant growth promoting bacteria isolated from water in mangrove ecosystem

S Widawati*, Suliasih1, A Sugiharto1, Suyadi1 and I M Sudiana1

Research Center for Biology, Research Organization for Life Sciences, National Research and Innovation Agency (BRIN), Jl. Jakarta-Bogor Km. 46, Cibinong Science Center, Cibinong, Bogor 16911, Indonesia

*Corresponding author e-mail: widadomon@yahoo.com

Abstract. Mangrove is a unique and dynamic coastal ecosystem that is characterized by high salinity. It creates an ecological niche with varied environmental conditions and microbial communities, including Nitrogen Fixing Bacteria (NFB) and Phosphate Solubilizing Bacteria (PSB), which have great potential as Plant Growth Promoting Bacteria (PGPB). The study aims to obtain isolates of indigenous bacteria in a varied saline environment of the mangrove ecosystem that has the characteristics to promote plant growth or potential as PGBP. The bacteria were isolated from the mangrove ecosystem, which is the water of brackish zone using specific media with total plate count (TPC) technique. Furthermore, the PGBP characterization was analyzed using nitrogenase, ACC-deaminase, cellulose, salinity, Indole-3-Acetic Acid (IAA), dissolved P, and phosphatase (PME-ase) indicators in triplicate. The results showed that water salinity and pH in the mangrove ecosystem ranged from 1.12 to 1.73 ppt and 6.56 to 7.44, respectively. Furthermore, the bacteria isolated from water in the mangrove ecosystem produced IAA (6 isolates), dissolved P, acid and alkaline PMEase (11 isolates), saline tolerance (33 isolates), cellulose (14 isolates), nitrogenase (18 isolates), and ACC-deaminase (2 isolates) activity. One of the isolates (AZT5.1) identified as *Bacillus cereus* has potential as PGBP with the value of IAA production (18.61 ppm), 1.1cm P halo zone dissolved, 569.45 ppm dissolved P, 11.2 μg/pnitrofenol g⁻¹h⁻¹ acid, and 12.85 μg/pnitrofenol g⁻¹h⁻¹ alkaline PME-ase production, 1.30 cm cellulose halo zone, saline tolerance, nitrogenase, and ACC-deaminase activity. Therefore, the PGBP from this bacterium was the highest compared to other isolates.

Keywords: Characterisation of PGBP, mangrove ecosystem.

1. Introduction

In Indonesia, Mangrove forests are mostly dominated by the genera Avicennia and Rhizophora [1] and are the most productive zones rich in organic material sources, which contribute to the coastal ecosystems [2]. However, the mangrove ecosystem still lacks nitrogen, phosphorus, and the carbon cycle often decreases [3] due to high and low tides, temperature, nutrients, and salinity fluctuations [4].

Salinity is considered the most detrimental environmental factor, especially in coastal marine ecosystems due to its ability to reduce the population of indigenous soil fertilizing microbes [5]. In addition, it reduces the productivity of microbial life that maintains the mangrove ecosystem [4], the function of microbes in the nutrient cycle process, availability of aquatic nutrients such as nitrogen, phosphate, and carbon [6], plant growth in the coastal environment [7], and changes the physical and chemical properties of the soil [8].
One of the ways of improving fertility and nutrition (N and P) in saline soil is to isolate indigenous bacteria from saline environments, such as mangrove ecosystems, and develop their activities through the screening of Indole-3-Acetic Acid (IAA), nitrogenase, phosphatase (PME-ase), dissolved P, cellulose, ACC-deaminase, and salinity test. When re-inoculated in saline soil, these indigenous bacteria are expected to adapt and associate with plants by playing an active role in providing nutrients.

The indigenous bacteria in mangrove ecosystems include Nitrogen Fixing Bacteria (NFB), Phosphate Solubilizing Bacteria (PSB), Sulfur Solubilizing Bacteria (SSB), Anoxygenic Photosynthetic Bacteria (APB), Methanogenic Bacteria (MB), and Enzyme Production Bacteria (EPB). These are a group of microorganisms that play an important role in the carbon cycle process, nutrient cycles such as N and P elements [9]. In addition, they have function as stabilizers of the nutrient cycle in the mangrove ecosystem, and as Plant Growth Promoting Bacteria (PGPB) for plants around the coast.

Some of the indigenous bacteria that are PGPB from saline ecosystems include Pseudomonas, Enterobacteria, Bacillus, Herbaspirillum [10], Azospirillum lipoferum, Azotobacter croccum, Bacillus megaterium, B. thuringiensis, B. pantothenicus, B. weihenstephanensis, Rhizobium sp., Serratia sp. [11,12], and other 36 types of bacteria that fix nitrogen, dissolve phosphate, produce the hormone IAA [13]. Meanwhile, their use as biofertilizers and biological control agents increases mineral content, plant tolerance to environmental stresses, protects plants from pathogens, N elements, P elements, and the production of indole-3-acetic acid or IAA [14].

Therefore, this study aims to obtain isolates of indigenous bacteria from various saline environments in mangrove ecosystems that have the characteristic to promote plant growth or potential as PGPB through isolation techniques with a specific medium, bacterial population calculations, and analysis for characterizing PGPB (nitrogenase, ACC-deaminase, salinity, cellulose, IAA, dissolved P, and PME-ase).

2. Materials and Methods

2.1. Sampling

Samples were collected in October 2020 in mangrove ecosystem of Segara Anakan, Cilacap, West Java, Indonesia. Purposive random sampling was applied to represent variation of mangrove ecosystem. Samples of water, cloudy mixed with sediment were taken at 9 locations of mangrove forests in Segara Anakan, Cilacap. The sampling points were (1) Bener River (S: 108.53’04.3” E: 07.43’05.0”), (2) Motean (S: 108.52’06.3” E: 07.42’12.5”), (3) Lempong Pucung River (S: 108.52’41.5” E: 07.42’52.4”), (4) Lempong Pucung Gate (S: 108.52’51.6” E: 07.42’31.1” ), (5) Gatel River (S: 108.55’25.5” E: 07.42’29.4”), (6) Terobosan River (S: 108.56’02.0” E: 07.42’59.3”), (7) Sigitsela (S: 108.51’17.8” E: 07.41’28.0”), (8) Terobosan River (S: 108.56’04.6” E: 07.42’48.4”), and (9) Sapuregel River (S: 108.59’04.1” E: 07.43’40.4”). These locations have a salinity 1.38, 1.29, 0.70, 1.61, 1.52, 1.88, 1.37, 1.69, and 2.14 ppt, and pH 7, 7, 7, 7.44, 7.22, 7.00, 6.56, 6.89, and 7.22, respectively. Furthermore, the dominant mangrove species are Rhizophora mucronata (1, 2), Bruguiera gymnorrhiza (3), Nypa fruticans (4, 5, 6), B. cylindrical (7), Aigiceras corniculatum (8), and Ceriops tagal (9).

2.2. Isolation, bacterial population count, and salinity test

Bacteria were isolated using a multilevel dilution technique (10⁻¹–10⁻⁵) by pipetting 0.2 mL of suspension from 10⁻³ and 10⁻⁵ dilutions and grown on various agar media such as NA, YEMA, mannitol Ashby, Caceres, and Pikovskaya [15]. This was carried out to obtain more varied types of bacteria where all media were made with pH 7. After 7 days of incubation at 28-30 °C, the total population (single colony) was calculated using the plate count method from Vincent [16] and transferred to the same medium for purification. Furthermore, the pure bacterial isolates were re-grown on each specific medium containing salt (1% - 6% or NaCl 10g L⁻¹ - 60g L⁻¹) and the colonies that grew were counted. Meanwhile, some of the pure bacterial isolates were subcultured on the slant agar media for characterization analysis of PGPB properties and identification.
2.3. **PGPB trait characterization**

2.3.1. **Nitrogen fixation activity.**

The activity of nitrogenase (nitrogen fixation) by bacteria was qualitatively used as a semi-solid medium NFB (nitrogen-free medium). Furthermore, 1 öse of each pure bacterial isolate was grown into semi-solid NFB media and incubated for 7 days at 28-30°C [17].

2.3.2. **ACC-deaminase activity**

The activity of bacteria in producing ACC-deaminase was analyzed using the method by Jasim et al. [18]. The pure bacterial isolates were grown (cultured) in liquid TSB media and shaken for 24 hours at a speed of 120 rpm. A total of 5 µL of liquid culture was taken and put it into Eppendorf, then centrifuged at 1000 rpm for 5 minutes. The natan was taken and rinsed 2 times with distilled water. Meanwhile, approximately 5 µL of culture was taken and grown on Dworkin-Foster solid medium (DF) using the Dworkin and Foster method [19] and 3mM of ACC-deaminase precursor (1-aminocyclopropane 1-carboxylic acid) was added. Furthermore, the incubation was carried out for 7 days at a temperature of 28-30°C.

2.3.3. **Cellulase activity**

The bacterial activity in degrading cellulose was carried out qualitatively using Carboxymethyl Cellulose or CMC media. Furthermore, approximately, 1 öse of pure bacteria were grown on CMC media and incubated for 7 days at 28-30°C. The growing bacterial colonies were dropped with Congo red solution and the halo zone was measured (halo zone diameter – colony diameter) [20].

2.3.4. **IAA production activity**

The IAA production by bacterial isolates was measured qualitatively and quantitatively according to the method of Gravel et al. [21] and Dawwam et al. [22]. In qualitative analysis, solid media of tryptic soy broth or TSB 50% sterile was used and 200 ppm of L-Tryptophan precursor was added to the media based on Mehboob et al [23]. Furthermore, 1 öse of each bacterial isolate was grown on Petridis, which contained the media and incubated for 3-5 days at 28-30°C. The growing bacterial colonies were dripped with Salkowski’s reagent and incubated in the dark for 3 hours at 28-30°C. Meanwhile, the analysis was carried out by growing bacteria into sterile liquid TSB (20 mL) medium with the addition of 200 µg mL⁻¹ L-Tryptophan, while non-bacterial media was used as a control. There was also 1 mL of the supernatant mixed with 4 mL of Salkowski’s reagent and incubated in a dark room for 20 minutes at a temperature of 28-30°C. The absorbance of the supernatant solution was later measured with a spectrophotometer at a wavelength of 530 nm and the IAA value was calculated using the IAA standard chart in ppm.

2.3.5. **Inorganic and organic phosphatase (PME-ase) dissolving activity**

Qualitative phosphate solubilization activity by bacteria was used in solid Pikovskaya media. Meanwhile, the liquid Pikovskaya media (without agar) with molybdate blue color was used from the method of Nagul et al. [24]. The amount of dissolved P was calculated using standard P (KH₂PO₄) and Pikovskaya medium without bacteria was used as control. Furthermore, the activity of organic phosphate (acid-base PME-ase) was measured using p-nitrophenyl phosphate disodium (pNPP 0.115 M) as a substrate. The p-nitrophenol (pNP) formed in the absorbance (yellow color) was measured by a spectrophotometer at a wavelength of 400 nm. Meanwhile, a solution of p-nitrophenol with a concentration of 1-10 ppm was used as a standard, while distilled water was used for blanks [25].

2.4. **Identification of bacteria**

Only bacterial isolates with 6 characterizations of the tested PGPB properties were identified. The PCR amplification method was used on 16 S rDNA with Primer 27 F: 5’ - AGA GTT TGA TCC TGG CTC AG - 3’ and Primer 1492 R: 5’ - GGT TAC CTT GTT ACG ACT T - 3’ [26]. Furthermore, the Yoon
method [27] was used for BioEdit and BLAST, while the Stover and Cavalcanti method was used for taxon similarity [28].

2.5. Statistical Analysis
Data were calculated statistically using SPSS software for windows version 23 with one-way analysis, and the variance test (ANOVA) was used for the comparison of several samples. To compare the significant differences between the treatments was used Duncan’s Multiple Range Test (DMRT) at P < 0.05 level.

3. Results and Discussion

3.1. Isolation and Population of Bacteria
The results of the isolation of water samples from 9 mangrove waters obtained 33 pure bacterial isolates with a population which ranged from 1.00 x 10^5–30.00 x 10^6 CFU mL^{-1} on the 5 media (Tables 1 and 2). Meanwhile, the highest population in YEMA media was dominated by isolates from locations 1, 2, 3, 4, 5, and 6, Caceres media by isolates from locations 7 and 9, Ashby mannitol media by isolates from locations 2, 3, and 8, and Pikovskaya media were dominated by isolates from locations 2 and 7. However, the lowest bacterial populations were from locations 8 (YEMA and Caceres), 1 and 7 (Ashby mannitol), and 2 (Pikovskaya). This result was higher than the bacterial population from the mangrove areas in Badung-Bali (1.3 x 10^4 CFU mL^{-1}) and the Amed coast - Bali (0.8 x 10^4 CFU mL^{-1}), but lower than the population from the Rambut Siwi beach - Bali (1.4 x 10^7 CFU mL^{-1}) [11]. In this study, the bacterial population in mangrove waters with the same vegetation (Rhizophora) was higher (33 isolates) than the results of Yahya et al. [29] which obtained 6 isolates from the coastal mangrove waters of Kraton-Pasuruan. This occurred by factors of pH, dissolved oxygen, salinity, availability of organic matter (chemical), temperature, current, water depth, and the type of mangrove vegetation (physical). According to Marwan et al. [30], the population of bacteria in the waters of mangrove forests is influenced by the type of mangrove plants that grow in the area.

| Code | NA (General target) | Yema (Rhizobium target) | Caceres (Azospirillum target) | Ashby Mannitol (Azotobacter target) | Pikovskaya (BPF target) |
|------|---------------------|--------------------------|-------------------------------|-----------------------------------|-------------------------|
| 1    | 1.00± ± 0.00        | 36.00± ± 0.00            | 2.20± ± 0.00                 | 1.00± ± 0.00                      | 25.25± ± 0.00           |
| 2    | 10.00± ± 0.00       | 34.75± ± 0.00            | 1.85± ± 0.00                 | 20.00± ± 0.00                     | 37.75± ± 0.00           |
| 3    | 4.55± ± 0.00        | 30.50± ± 0.00            | 3.45± ± 0.00                 | 25.00± ± 0.00                     | 17.50± ± 0.00           |
| 4    | 1.18± ± 0.00        | 33.75± ± 0.00            | 0.05± ± 0.00                 | 7.5.00± ± 0.00                    | 29.75± ± 0.00           |
| 5    | 1.00± ± 0.00        | 33.05± ± 0.00            | 1.10± ± 0.00                 | 3.00± ± 0.00                      | 32.25± ± 0.00           |
| 6    | 10.00± ± 0.00       | 32.25± ± 0.00            | 0.33± ± 0.00                 | 6.00± ± 0.00                      | 36.5± ± 0.00            |
| 7    | 30.00± ± 0.00       | 22.50± ± 0.00            | 12.50± ± 0.00                | 1.00± ± 0.00                      | 32.25± ± 0.00           |
| 8    | 10.0± ± 0.00        | 21.00± ± 0.00            | 1.00± ± 0.00                 | 20.00± ± 0.00                     | 31.50± ± 0.00           |
| 9    | 10.00± ± 0.00       | 21.75± ± 0.00            | 15.00± ± 0.00                | 7.50± ± 0.00                      | 32.75± ± 0.00           |

Notes: Values followed by the same letter in the same column (1-9) are not significantly different from DMRT at the 5% level. (n=3).

3.2. PGPB Trait Characterization
The analysis of the characterization of PGPB using indicators such as nitrogenase, ACC-deaminase, salinity test, cellulase, Indole-3-Acetic Acid (IAA), dissolved P, and PME-ase acid-base are shown in Tables 2, 3, 4, 5, and Figure 1.

3.2.1. Nitrogen fixation activity.
Nitrogen fixation screening for characterization of PGPB properties obtained 22 bacterial isolates. The ability of 22 bacterial isolates to fix free N on MFB media was shown by the formation of a white ring
(pellicle) under the surface of the media without nitrogen (Figure 1A). Meanwhile, Jha et al. [31] stated that the indicator of nitrogenase activity by bacteria was shown by the formation of a white ring-shaped pellicle under the surface of the NFB medium or a change in the color of the medium from yellowish green to bluish [32] due to the nature of the indicator, which is bromthymol blue at alkaline pH [33]. The ability to fix nitrogen in 22 bacterial isolates was higher than the 3 bacterial isolates from the Peniti-Mempawah mangrove forest [34]. This is due to different abilities, characters, and species of bacteria.

Table 2. The qualitative results of PGPB trait characterization.

| No. | Isolates ID* | IAA | Nitrogenase | Dissolved P | Celullase | ACC-deaminase | Salinity test |
|-----|--------------|-----|-------------|-------------|-----------|---------------|--------------|
| 1   | Y1.1         | -   | +           | -           | -         | -             | +            |
| 2   | Y2.1         | -   | +           | -           | +         | -             | -            |
| 3   | Y2.2         | -   | -           | -           | -         | -             | +            |
| 4   | Y3.1         | -   | +           | -           | +         | -             | +            |
| 5   | Y3.2         | -   | +           | -           | -         | +             | -            |
| 6   | Y4.1         | -   | +           | -           | -         | +             | -            |
| 7   | Y5.1         | -   | +           | -           | +         | -             | +            |
| 8   | Y6.1         | -   | -           | -           | +         | +             | -            |
| 9   | Y7.1         | -   | +           | -           | -         | -             | +            |
| 10  | Y8.1         | -   | -           | -           | +         | -             | +            |
| 11  | NA1.1        | -   | +           | -           | +         | -             | -            |
| 12  | NA2.1        | -   | +           | -           | -         | +             | -            |
| 13  | NA3.1        | +   | +           | -           | -         | -             | -            |
| 14  | NA4.1        | -   | -           | -           | +         | -             | +            |
| 15  | NA4.1        | +   | -           | -           | -         | -             | +            |
| 16  | NA5.1        | -   | +           | +           | -         | -             | -            |
| 17  | NA7.1        | -   | +           | +           | -         | -             | -            |
| 18  | NA8.1        | -   | +           | +           | -         | -             | +            |
| 19  | NA9.1        | -   | +           | +           | -         | -             | +            |
| 20  | AZT1.1       | -   | +           | -           | +         | -             | -            |
| 21  | AZT5.1       | +   | +           | +           | +         | +             | -            |
| 22  | AZT7.1       | +   | +           | +           | +         | -             | +            |
| 23  | AZT8.1       | -   | +           | +           | +         | -             | +            |
| 24  | AZP1.1       | -   | +           | -           | -         | -             | +            |
| 25  | AZP2.1       | -   | +           | -           | +         | -             | -            |
| 26  | AZP7.1       | -   | +           | +           | -         | -             | +            |
| 27  | AZP7.2       | -   | +           | +           | +         | -             | -            |
| 28  | AZP8.1       | +   | +           | -           | -         | -             | +            |
| 29  | AZP9.1       | -   | +           | -           | -         | -             | +            |
| 30  | PIK1.1       | -   | +           | -           | +         | -             | -            |
| 31  | PIK4.1       | +   | +           | +           | -         | -             | +            |
| 32  | PIK4.2       | -   | -           | -           | +         | +             | -            |
| 33  | PIK5.1       | +   | -           | -           | -         | -             | +            |

*Yema (Y), Nutrient Agar (NA), Mannitol Ashby (AZT), Caceres (AZP), and Pikovskaya (PIK) media. The number in the first and second digits are the sampling location code and the repetition.

3.2.2. ACC-deaminase activity.

The qualitative screening of ACC-deaminase obtained 2 bacterial isolates, namely AZT5.1 and PIK4.2 (Table 2). These bacteria used the ACC substrate which was added to the DF medium as a nitrogen source (Figure 1B). According to Shahzad et al. [35], bacteria that are capable of using ACC as a source of N grow well on ACC media. Furthermore, Jasim et al. [18] stated that bacteria are positive as
producers when they grow in DF+ACC media after 3 days of incubation at 28-30°C. Moreover, their activity in this study (2 bacterial isolates) was lower than the results of Husen and Salma [36] that obtained 292 ACC deaminase producing and saline tolerant putative isolates (minimum salt medium + ammonium sulfate) isolated from the rhizosphere of rice and grass wild. As stated by Jacobson et al. [37], the character of bacterial isolates in producing ACC deaminase was due to the ability to grow isolates in media enriched with ammonium sulfate and the ability to use ammonium sulfate in its activity.

Figure 1. Qualitative analysis of bacteria on Activity: A. Nitrogenase; B. ACC-deaminase; C. Cellulase; D. Salinity; E. IAA; F. Dissolved P

Table 3. The results of cellulase activity by bacteria.

| Isolats ID | 0 Halo zone (cm) |
|------------|-----------------|
| Y2.1       | 1.20 ± 0.0      |
| Y3.1       | 0.80 ± 0.0      |
| Y5.1       | 1.30 ± 0.0      |
| Y6.1       | 0.60 ± 0.0      |
| Y8.1       | 1.20 ± 0.0      |
| NA1.1      | 1.40f ± 0.0     |
| NA4.1      | 0.80b ± 0.0     |
| AZT1.1     | 1.00g ± 0.0     |
| AZT5.1     | 1.30f ± 0.0     |
| AZT7.1     | 1.00d ± 0.0     |
| AZT8.1     | 0.90c ± 0.0     |
| AZP2.1     | 0.80b ± 0.0     |
| AZP7.2     | 1.00d ± 0.0     |
| PIK1.1     | 1.20e ± 0.0     |

Notes: Values followed by the same letter in the same column (1-9) are not significantly different from DMRT at the 5% level. (n=3).

3.2.3. Cellulase activity
The qualitative analysis resulted 14 isolates of cellulolytic bacteria (Table 2) which have potential to produce cellulase enzymes. Meanwhile, the enzyme was indicated by the formation of a halo zone around bacterial colonies on CMC media after being dripped with Congo red (Figure 1 C). The results of the measurement of halo zone diameter varied between 0.60 – 1.30 (Table 3). The halo zone of 1 cm was indicated by isolates Y5.1, Y8.1, NA1.1, AZT1.1, AZT5.1, AZT7.1, AZP7.2, and PIK1.1. Based
on the results, the highest halo zone diameter was shown by isolate AZT5.1 and the lowest by isolate Y6.1. The variation in the halo zone diameter was caused by differences in the ability of bacterial isolates (assisted by Congo red dye) to degrade carboxymethylcellulose on media in Petidis. Ponnambalam et al. [38] stated that the colonies that produced different halo zone diameters are used as a basis for comparison between bacterial colonies and the isolates that produced the largest diameters with the highest cellulolytic activity.

3.2.4. Salinity test.

The salinity test of 33 indigenous mangrove isolates on specific media with 1% - 6% NaCl (10g - 60g NaCl L\(^{-1}\)) was positive for saline tolerance (Table 2 and Figure 1D). This shows that bacterial isolates can be tested or used in the future in saline soils. Meanwhile, saline tolerant bacteria are also required for the continuous growth of mangrove plants in coastal ecosystems that always get stagnant seawater with a salinity of 2-22% (equivalent to brackish water) or 38% (equivalent to saltwater) [39]. These results increased the number of saline-tolerant bacterial isolates that had been obtained in previous studies such as Azotobacter chroococcum which tolerated salinity at a concentration level of about 35g NaCl L\(^{-1}\) [40]. In addition, Widawati et al. [41] have obtained and tested several salt-resistant bacteria as biofertilizers on rice plants in saline sandy soil.

3.2.5. IAA Production activity.

The main characterization of PGPB is the production of growth hormone or Indole 3 Acetic acid (IAA), which helps the growth and organize the root system to efficiently absorb nutrients [42].

| ID isolates | Incubation 0 hours | Incubation 24 hours | Incubation 48 hours | Incubation 72 hours |
|-------------|--------------------|---------------------|---------------------|---------------------|
| NA3.1       | 3.56\(^{ab}\) ± 1.13| 7.40\(^{b}\) ± 3.62  | 13.19\(^{ab}\) ± 1.70| 8.07\(^{a}\) ± 0.22 |
| NA4.1       | 0.60\(^{a}\) ± 0.05 | 9.53\(^{b}\) ± 2.68  | 16.29\(^{bc}\) ± 0.49| 14.19\(^{c}\) ± 0.67|
| AZT5.1      | 4.41\(^{b}\) ± 1.91 | 12.34\(^{b}\) ± 2.20  | 18.61\(^{c}\) ± 2.69 | 16.95\(^{e}\) ± 0.29|
| AZT7.1      | 1.41\(^{ab}\) ± 0.25 | 0.57\(^{a}\) ± 0.08  | 9.12\(^{a}\) ± 0.20  | 9.89\(^{b}\) ± 0.20 |
| PIK4.1      | 3.33\(^{ab}\) ± 1.31 | 7.84\(^{ab}\) ± 0.23  | 13.30\(^{ab}\) ± 1.81| 15.55\(^{d}\) ± 0.11|
| PIK5.1      | 1.18\(^{ab}\) ± 0.05 | 7.18\(^{b}\) ± 0.18  | 11.52\(^{ab}\) ± 0.34| 16.48\(^{de}\) ± 0.02|

Notes: Values followed by the same letter in the same column (1-9) are not significantly different from DMRT at the 5% level. (n=3).

Based on the qualitative test, 6 bacterial colonies produced a pink color after being dropped with Salkowski’s reagent (Table 2, Figure 1E). According to Kovacs [43], the red color formed was caused by the interaction of IAA with Fe which forms a complex compound [Fe\(_2\)(OH)\(_3\)]\(_2\)(IAA)\(_4\)] and the dark red color indicated the high production of IAA by bacteria. Meanwhile, 6 bacterial isolates were further tested quantitatively using 200 ppm of an essential amino acid (L-tryptophan) as a precursor used by bacteria to produce IAA (Table 4).

The results of the quantitative test showed that the AZT5.1 isolate had the highest IAA production values of 4.41 ppm (0 hours), 12.34 ppm (24 hours), 18.61 ppm (48 hours), and 16.95 ppm (72 hours) compared to the other isolates. Meanwhile, the optimum production of AZT5.1 isolate was shown at an incubation time of 48 hours. This is similar to other isolates, except PK4.1 and PK5.1 which still showed an increase in IAA production value at 72 hours incubation time due to the stationary phase that occurred from 24 hours to 48 hours, and 48 hours to 72 hours. A study by Patil et al. [44] showed that the optimum production of IAA by bacteria occurs at the beginning of the stationary phase. Moreover, IAA is a secondary metabolite of bacteria produced in the stationary phase of growth [45]. Furthermore, the production of IAA isolates AZT5.1, PIK4.1, and PIK5.1 isolated from Cilacap mangrove waters was higher than MI11 isolates (174 mg L\(^{-1}\)=1.74 ppm) isolated from Beautiful Beach Estuary, Deli Serdang, North Sumatra [46]. Also still higher than Avicennia marina rhizobacteria isolate, which was 5.3 ppm [47]. This occurred because the effect of bacterial activity to produce IAA depends on the presence of...
L-tryptophan content in the medium. Saidah [48] stated that rhizobacteria in producing IAA were influenced by the availability of tryptophan, cell activity, environmental conditions, and different growth phases of bacteria, which led to varied results. Therefore, the production of IAA by bacteria increases when the addition of L-tryptophan in the culture medium is increased.

3.2.6. Inorganic and organic phosphate dissolving activity.

Based on the qualitative analysis, the selection of 11 bacterial isolates produced a halo zone around the colony. Halo zone is an indicator of bacterial activity in dissolving P from Ca\(_3\)(PO\(_4\))\(_2\) bonds in solid Pikovskaya media (Tables 2, Figure 1F). The largest and smallest halo zone diameters are shown by AZT5.1 (1.20 cm) and Y1.1 (0.40 cm), respectively. The number of phosphate solubilizing bacteria (11 isolates) with the highest halo zone diameter of 1.20 cm (12 mm) obtained in this study, was more numerous and larger in diameter than the isolates isolated from Beach Estuary, Deli Serdang, North Sumatra (9 isolates and 7.5 mm) [46]. The dissolution results of P (halo zone) varied due to differences in the species ability to produce PME-ase enzymes that play a role in dissolving P. However, the extent of the clear area produced by the solubilizing bacteria (PSB) activity is not used as a standard for the amount of dissolved P. This is because there are differences in dissolved P values in solid and liquid Pikovskaya media (Table 5). This is in line with Lynn et al. [49] which stated that the halo zone area produced by bacteria is not a quantitative indicator of phosphate dissolution value. Therefore, halo zone size around the bacterial colony is not an indicator for the ability of bacteria to dissolve P but is a signal to know the ability of the bacteria.

### Table 5. The results of dissolving inorganic and organic phosphates activity by bacteria

| Isolates ID | 0 Halo zone (cm) | P available (ppm) | PME-ase acid (μg/pnitrofenol g\(^{-1}\)h\(^{-1}\)) | PME-ase base (μg/pnitrofenol g\(^{-1}\)h\(^{-1}\)) |
|-------------|-----------------|--------------------|-----------------------------------------------|-----------------------------------------------|
| Y1.1        | 0.40 ± 0.00     | 221.45 ± 7.85      | 4.50 ± 0.17                                   | 5.64 ± 0.15                                   |
| NA5.1       | 1.10 ± 0.00     | 541.26 ± 5.73      | 11.98 ± 0.01                                 | 5.42 ± 0.00                                   |
| NA7.1       | 0.80 ± 0.00     | 522.16% ± 5.59     | 7.27 ± 0.20                                   | 5.61 ± 0.19                                   |
| NA8.1       | 0.90 ± 0.00     | 501.58% ± 4.19     | 6.58 ± 0.19                                   | 5.36 ± 0.17                                   |
| NA9.1       | 0.60 ± 0.00     | 467.45% ± 8.28     | 5.15 ± 0.16                                   | 7.32 ± 0.17                                   |
| AZT5.1      | 1.20% ± 0.00    | 569.45% ± 9.13     | 11.12% ± 0.16                                | 12.85% ± 0.10                                |
| AZT7.1      | 0.80% ± 0.00    | 541.05% ± 9.49     | 4.75% ± 0.12                                 | 5.95% ± 0.11                                 |
| AZT8.1      | 1.00% ± 0.00    | 522.37% ± 3.89     | 5.63% ± 0.08                                 | 5.56% ± 0.08                                 |
| AZP7.1      | 1.20% ± 0.00    | 98.37% ± 0.24      | 4.54% ± 0.03                                 | 5.27% ± 0.00                                 |
| AZP7.2      | 1.00% ± 0.00    | 130.32% ± 1.76     | 5.16% ± 0.02                                 | 4.47% ± 0.00                                 |
| PIK4.1      | 0.60% ± 0.00    | 180.71% ± 3.45     | 11.56% ± 1.45                                | 4.35% ± 1.40                                 |

Notes: Values followed by the same letter in the same column (1-9) are not significantly different from DMRT at the 5% level. (n=3).

The dissolution of P in a liquid medium is quantitatively indicated by a blue color. Meanwhile, the highest and lowest values of dissolved P concentration (available P) are produced by AZT5.1 (569.45 ppm) and AZP7.1 (98.37 ppm), respectively. This value is higher than the value of dissolved P concentration (215 ppm - 357 ppm) produced by rhizobacteria, isolated from the mangrove forest of Qeshm Island, Iran [50]. The difference in the value of dissolved P concentration (available) was caused by the ability of bacteria, incubation time, temperature, humidity, pH, food supply, enzyme production, organic acids, and environmental conditions during growth in the liquid Pikovskaya media. This is in line with Jumadi et al., [51] found that the difference in the ability of rhizobacteria in quantitatively dissolving phosphate was due to variations in the production of types and amounts of organic acids such as acetic, lactic, oxalic, tartrate, succinate, citrate, gluconate, ketogluconate, gluconate, and the phosphate solvent. According to Lestari et al. [52], the activity of rhizobacteria in liquid Pikovskaya media is influenced by the length of incubation time. Therefore, the process of dissolving phosphate in Ca\(_3\)(PO\(_4\))\(_2\) by bacterial activity depends on the high and low production of organic acids and enzymes such as acid-alkaline PME-ase.
The highest values of acid and alkaline PME-ase were produced by AZT5.1, namely 11.12 and 12.85 g/pnitrophenol g\(^{-1}\)h\(^{-1}\), the lowest acid PME-ase by Y1.1 (4.54 g/pnitrophenol g\(^{-1}\)h\(^{-1}\)) and Azp7.1 (4.54 g/pnitrophenol g\(^{-1}\)h\(^{-1}\)), and the lowest alkaline PMEase by PIK4.1 (4.35 g/pnitrophenol g\(^{-1}\)h\(^{-1}\)). These results were higher than Widawati [53], which obtained acid and alkaline PMEase (values of 0.82 and 0.92 g/pnitrophenol g\(^{-1}\)h\(^{-1}\)) from the activity of Azotobacter sp. in the same media type (Pikovskaya). This occurs due to the activity of phosphate solubilizing bacteria in dissolving bound P and producing phosphatase enzymes in the same medium. This showed that the high yield of soluble P was followed by the high production of PME-ase through the AZT5.1 isolate. This is in line with a study by Savin et al. [54] which showed that the higher the dissolved P, the higher the PME-ase enzyme produced. Therefore, the process of dissolving phosphate at Ca\(_{10}\)(PO\(_4\))\(_2\) by bacterial activity depends on the level of acid and alkaline PME-ase production.

3.3. Identification Result.

PGPB characterization test on 33 isolates from mangrove-Cilacap waters obtained 1 isolate (AZT5.1) which had 6 PGPB properties. The identification result of AZT 5.1 was Bacillus cereus. This is similar to the results (Bacillus sp.) which were obtained by Yahya et al., [29] from the mangrove waters of the Pasuruan Kraton Coast. According to Shome et al. [55], 50% of the mangrove ecosystem is dominated by Bacillus spp.

4. Conclusion

This study obtained bacterial isolates from the aquatic zone of the mangrove ecosystem that produced IAA (6 isolates), dissolved P, acid and alkaline PME-ase (11 isolates), cellulose (14 isolates), nitrogenase (18 isolates), and ACC-deaminase (2 isolates) activities. Meanwhile, 1 isolate (AZT5.1) from Gatel River with a pH of 7.22 and a salinity test of 1.52 ppm and was identified as Bacillus cereus with 6 characteristics of PGPB, namely IAA production (18 isolates), cellulose (14 isolates), acid and alkaline PMEase (values of 0.82 and 12.85 units) activities, and salinity test.

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