Potency of Plant Growth Promoting Rhizobacteria (PGPR) in *Ipomea pes-caprae* roots: Initial microscopic and macroscopic identification on South Sulawesi’s coastal resources

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Abstract. The paper reports on an initial study aimed to isolate and identify plant growth promoting rhizobacteria in the roots of *Ipomea pes-caprae* (locally named as *Katang-katang*) abundantly found in the coastal area of South Sulawesi. Plant roots samples were obtained from the coastal area in Gantarangkeke Village, Bantaeng Regency, South Sulawesi Province, Indonesia. The isolation and identification were carried out at the Biofertilizer and Potential Microbial Laboratory, Faculty of Agriculture, Hasanuddin University from August to October 2019. The isolation was conducted by previously extracting the roots of the plant and then fermented for 14 days. One millilitre of the solution was used to inoculate the bacteria in a sterile petridish containing NA media using spread and streak methods and then left to an incubation period. Identification of the bacteria was conducted using three methods namely gram reaction test, catalase test, and microscopic colouring method. The bacteria samples were observed macroscopically and microscopically. The catalase test results reveal that bacteria in the *Katang-katang* roots was from the genus of *Bacillus* and *Pseudomonas*. Some of the implication of the results for agricultural purpose was discussed.

1. Introduction

*Ipomea pes-caprae* is a vegetation that dominates the formation of the pes-caprae which is in the highest tide area and on all open beaches in the tropics including in South Sulawesi Province of Indonesia. The plant has a relatively broad geographical distribution in tropical coastal regions and often found growing around the coastline, especially on the sand tongue (figure 1). The plant has an important role in coastal ecosystems, such as the natural protection of coastlines against erosion. This vegetation is able to live in harsh and unstable environmental conditions, such as sand tongue, because this species has a great tolerance to sea water which affects its initial growth [1].

*Ipomea pes-caprae*, locally known as *Katang-katang*, is an annual herbaceous plant with thick roots and grows on the stem segments. The stem, about 5-30 m long and spreads, is round, wet and brownish green. This vegetation has a single leaf, thick, smooth and shiny. The flowers are pink-purple and rather dark at the base of the flower. The fruit is in the form of a round capsule until somewhat flat with four black seeds and tight hair. Fruit size is 12-17 mm, while seeds are 6-11 mm. This species grows from sea level to 600 m, usually on sandy beaches, but also right on the coastline [1].
In a previous study conducted by Aiman et al. [2], it was found that from plants dominated the beach sand, 13 microbial isolates were identified with potential to be used as Plant Growth Promoting Rhizobacteria (PGPR). The PGPRs were proven for their ability to produce IAA and Phosphate by the presence of bright zones on Pikovkaya media. Of the 13 microbes that have been isolated, the bacteria C7, K2, K9 and K15 showed higher production of IAA compared to other isolates. IAA produced from the four bacteria were 0.63 ppm, 0.40 ppm, 0.60 ppm, and 0.59 ppm for the C7, K2, K9, and K15 isolates, respectively.

**Figure 1. Ipomea pes-caprae** (Local name: Katang-katang) plants on the coastal area of South Sulawesi, Indonesia.

PGPR is a group of bacteria living in the plant root area that can increase plant growth and crop yields through several mechanisms. Several mechanisms employed by PGPR in increasing plant growth include as biological fertilizer, produce phytohormone, produce siderophore, phosphate dissolving, as a biological control agent, and as a biological fungicide. Application of PGPR in plants can modulate root growth and development by producing phytohormones, secondary metabolites and enzymes. The most common is a reduction in primary root growth and an increase in the length and number of lateral roots and hair roots [3].

Biotechnology engineering between soil microbes and biomass will support the three aspects of soil fertility, namely chemical, physical and biological aspects of the soil. Viewed from the physical aspects of the soil including the structure and texture of the soil which makes the soil loose. Viewed from the biological aspects of the soil will increase the availability of sufficient immersion in the soil which will also increase the activity and propagation of soil microorganisms. Viewed from the aspect of soil chemistry, the activity and propagation of microorganisms will help to degrade old immersion molecules into elements that can increase soil fertility so that they are available to plants [4].

According to research conducted by Gholami et al. [5], the administration of PGPR can increase the synthesis of hormones such as Indole acetic acid (IAA) or giberalin (GA3) as a trigger for amylase enzyme activity that plays a role in germination. In the other hand, a study conducted by A’yun et al. [6] showed that the use of PGPR can reduce the incubation period, the intensity of Tobacco Mosaic
Virus (TMV) attacks and increase the height of cayenne pepper plants. Application of microorganisms on plant provide better crop yields, and phosphate solvents expand the range of plants’ ability to absorb water and nutrients.

2. Methodology

The material of the Katang-katang roots were sampled from Gantarangkeke Village, Gantarangkeke District, Bantaeng Regency, South Sulawesi Province. Isolation and identification of the Katang-katang root extract was carried out at the Biofertilizer and Potential Microbial Laboratory, Faculty of Agriculture, Hasanuddin University. The research was conducted from August to October 2019.

2.1. Preparation of PGPR

The PGPR solution was prepared by modifying method conducted by Nico et al. [7]. About 500 grams of Katang-katang roots were mixed with 1.5 kg Molasses, 1.5 kg of bran, 2.5 grams of shrimp paste, 2.5 Litres of rice water into a 30 L bucket and stirred until evenly distributed, and then water were added until the bucket is full. The PGPR solution was then fermented for 14 days. After the fermentation process is completed, PGPR was filtered with a sieve that is covered with a cloth into the jerry can.

2.2. Preparation of Nutrient Agar (NA) media

The preparation of NA media was carried out by weighing 8 grams of Nutrient Broth and 20 grams agar and then placed into a 1000 mL Erlenmeyer. About 500 mL of distilled water were added then stirred until the material is dissolved. Erlenmeyer is heated while stirring and adding distilled water little by little until the volume of the solution reached 1000 mL. When the solution is homogeneous, Erlenmeyer was covered with aluminium foil and then put into an autoclave to be sterilized for 15 minutes [8].

2.3. Media sterilization

Before starting the pouring of media, laminar air flow (LAF) sterilization is conducted by turning on the lights and spraying 70% alcohol to all parts of the LAF then wiping it using tissue. Then the LAF was covered with black plastic and the UV lamp is turned on for 30 minutes. After finishing UV sterilization, the LAF blower was turned on and all tools and materials used in the pouring media were inserted. NA media is poured on a petri dish around 15-20 mL, then covered and wrapped with plastic wrap and then labelled. The media were stored in a storage box and allowed to harden.

2.4. Bacteria inoculation

Bacteria inoculation was carried out in LAF, using two methods, namely the spreading and streaking methods with five petri dishes each. The Katang-katang root PGPR solution and NA media were placed into the LAF that have been previously sprayed with 70% alcohol. The spread method was carried out by taking a 1 mL PGPR solution using a micro pipette and then spreading it over NA media and levelled using a spatula. Whereas the streaking method was conducted by taking an ose needle then sterilized with Bunsen then dipped in a PGPR solution then zigzagging on the surface of NA media in a petri dish. All plates are again stored in storage boxes and carried out bacterial growth checks every day [9].

2.5. Identification of bacteria

Identification of bacteria was carried out using three methods [9], namely:

2.5.1. Gram Reaction Test. This bacterial identification method was conducted by placing 1 drop of 3% KOH on a glass preparation then the bacteria that have grown from the spreading and streaking method were taken with an ose needle and rubbed in a 3% KOH solution and observed. The category
of gram-negative bacteria obtained when mucus produced shows a positive reaction and the category of gram-positive bacteria if the mucus produced shows no negative reaction.

2.5.2. Catalase Test. This bacterial identification method was done by placing 1 drop of H$_2$O$_2$ solution on a glass preparation then the bacteria that have grown previously were taken with an ose needle and rubbed on the H$_2$O$_2$ solution and observed. The category of gram-positive bacteria is obtained if it produces bubbles (positive reaction) and the category of gram-negative bacteria if it does not produce bubbles (negative reaction).

2.5.3. Microscopic observations by the colouring method. The colouring method was carried out using methylene blue. Dropped 1-2 drops of methylene blue on the previously sterilized glass preparation and let dried, then dropped 1-2 drops of lugol solution on the preparation glass and leave for 1 minute then rinse with 70% alcohol for 30 seconds then dry. After drying, the preparation glass was covered with the deglass and then observed under a microscope. If there is a change in the colour of the bacteria to pink / purple, the bacteria are a group of Pseudomonas and if there is no change in colour, the bacteria are a Bacillus group.

3. Results

3.1. Gram reaction test

The gram reaction test results (table 1) show that the PGPR of the Katang-katang roots inoculated with spreading and streaking method produced mucus when testing with the gram reaction test using 3% KOH.

| Method    | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|-----------|----------|----------|----------|----------|----------|
| Spreading | -        | +        | +        | +        | +        |
| Streaking | +        | -        | +        | +        | +        |

(+): produce mucus; (-): did not produce mucus.

3.2. Catalase test

The catalase test results (table 2) show that the PGPR of Katang-katang roots sample showed reaction (bubbles).

| Method    | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|-----------|----------|----------|----------|----------|----------|
| Spreading | ++       | ++       | ++       | +        | ++       |
| Streaking | ++       | +++      | ++       | +++      | ++       |

Number of (+) marks based on the amount of bubbles produced at the test.
3.3. Bacteria genus determination results
The results of the laboratory identification of bacterial genus based on morphology, biochemistry as well as identification of family and genus of bacteria are presented in table 3.

| Parameter     | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---------------|----------|----------|----------|----------|----------|
| **Morphology**|          |          |          |          |          |
| Cell shapes   | Round    | Rod      | Round    | Round    | Rod      |
| Gram          | Negative | Positive | Negative | Negative | Positive |
| **Biochemistry**|          |          |          |          |          |
| Catalase      | Positive | Positive | Positive | Positive | Positive |
| Colour        | Blue     | Pink     | Blue     | Blue     | Pink     |
| **Family**    | Bacilliaceae | Pseudomanadaceae | Bacilliaceae | Bacilliaceae | Pseudomanadaceae |
| **Genus**     | Bacillus | Pseudomonas | Bacillus | Bacillus | Pseudomonas |

3.4. Microscopic observation of bacteria
Microscopic observations (figure 2) show that PGPR of dwarf roots in staining with methylene blue produces a blue color for the genus Bacillus and a pink / purple color for the genus Pseudomonas.

![Figure 2. Microscopic observations of bacteria under a microscope after the method of staining with methylene blue](image)

4. Discussion
Based on the positive response of Katang-katang root PGPR when tested using catalase test results, bacteria contained in the Katang-katang root can be categorized from the genus Bacillus. According to Hatmanti [10], two main characteristics that distinguish Bacillus from other endosporic-forming bacteria are the ability of Bacillus to live aerobically (although some are anaerobic facultative) and the majority of species produce catalase (positive response in catalase test). In addition, Bacillus is one of six endosporic-producing bacteria. The endospores of Bacillus are round, oval, elliptical or cylindrical, which form in vegetative cells [10]. Based on the microscopic observations of the bacteria in the Katang-katang root extract, there are round and oval-shaped bacteria produced, hence indicates that the bacterium is from the genus *Bacillus* spp.

According to Aiman et al. [2], among several types of rhizosphere of coastal plants, rhizosphere from the roots of *Katang-katang* contain the most probiotic bacteria and observed to have higher production of auxin hormone (IAA). The ability to decompose phosphate from microbes derived from the *Katang-katang* is relatively broader than the others. The IAA produced from the plant isolates ranged from 0.3 to 1.34. IAA is a key hormone for various aspects of plant growth and development so as to increase growth.

Plants in marginal land will be able to grow well if there is microbial participation, especially microbes that form colonies in the roots which are often referred to as that live around plant roots [11]. The microbial life colonizes the roots of plants and functions to stimulate plant growth, improve root
physiology, reduce disease and at the same time provide nutrients such as P, Fe, S and Cu so that they are available to plants [12]. Microbes in the PGPR of the Katang-katang roots is able to produce auxin hormone that will help to increase the plant growth and development. A’yun et al. [6] states that the mechanism directly carried out by the PGPR is by synthesizing metabolites such as compounds that stimulate the formation of phytohormones such as indole acetic acid (IAA), or by increasing the uptake of plant nutrients. IAA is one of the most important plant growth hormones. The addition of the Katang-katang root PGPR can also play a role in increasing the chloroplast content that will increase the photosynthetic capacity per leaf area. This is supported by Phabiola et al. [13] which states that PGPR treatment can increase leaf chlorophyll from 23.81% to 28.22% in 15 days after application of the PGPR formula. This increase is due to the ACC-Deaminase activity in PGPR that slow down the chlorophyll degradation process or increasing photosynthesis per leaf area compared to control plants. As a biofertilizer for plants, PGPR is a biological fertilizer capable of providing nutrients for plant growth, so that plants can grow optimally and have resistance to pests, diseases and stresses that come from the environment.

Some study reported that rhizobacteria from the Bacillus spp. and Pseudomonas spp. are able to dissolve phosphate isolates. These bacteria were also reported to be able to synthesize IAA, gibberellins and cytokinin growth hormones [14,16]. The use of non-pathogenic bacteria which is explored from plant roots (rhizobacteria) which belongs to the PGPR group is a contribution of biotechnology in an effort to increase crop productivity.

5. Conclusions
The identification of bacteria contained in the Katang-katang roots PGPR reveals that bacteria are from the genus of Bacillus and Pseudomonas.

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