Identification of endophytic and rhizosphere bacteria in rice (Oryza sativa L.) in the experimental field at Payakumbuh State Agriculture Polytechnic, West Sumatra, Indonesia

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Abstract. The decrease of soil fertility and fewer soil microorganisms will lower crop production, particularly rice, thus threatening the national food security program. This study is (a) to isolate and identify the bacteria in the endophytic and rhizosphere of rice plants (b) to study the bacteria from the endophytic and rhizosphere of rice plants which potentially stimulate plant growth. The experiment was carried out at the Laboratory of Food Crop Cultivation at Payakumbuh State Agriculture Polytechnic, Limapuluh Kota Regency, West Sumatra for four months. The sampling method was carried out by random sampling at rice planting in the Payakumbuh State Agriculture Polytechnic Experimental Field. Endophytic bacteria were taken from the root tissue of rice plants, and rhizosphere bacteria were taken from a layer of soil around rice roots. Isolation of bacteria was carried out by using the pour plate and scratchplate methods. Four bacteria were identified using the 16S rRNA sequencing method. The identification results showed that in the rice root tissue found the bacteria Chromobacterium rhizoryzae and Brevibacillus brevis. In the rice rhizosphere, Bacillus pseudomycoides and Bacillus thuringiensis are found. Bacteria are dominated by the Bacillus genera which can stimulate plant growth.

1. Introduction
Lowland rice production in Limapuluh Kota Regency from 2012 to 2016 decrease continuously, from 23,771.28 ha, 21,440.00 ha, 23,392.00 ha, 23,388.00 ha, and 22,585.00 ha. This decrease is caused by the transfer of land functions to non-agricultural functions [1]. Besides the decrease in land area, there was also a drastic decrease in paddy fields' fertility. One cause is the continuous use of inorganic fertilizers with excessive doses, resulting in reduced soil organic matter that makes soil microorganisms reduced or dead. Microorganisms play an important role in controlling soil and plant productivity. The existence of eco-friendly microorganisms, especially bacteria, guides sustainable agricultural management [2]. Bacteria closely related to plants and the soil environment are endophytic bacteria and rhizosphere bacteria around plant roots. Experiments on identifying and characterizing bacteria in plant tissue and rice rhizosphere are needed to determine the types of bacteria beneficial for plants and survive in soil fertility conditions.

The nature of the bacteria influences endophytic bacteria found in plant tissue, the host plant's conditions, and the environment [3, 4]. Pathogenic bacteria are not included in endophytic bacteria, because the group of bacteria found in plant tissue does not cause damage to plants. Endophytic bacteria are plant-beneficial bacteria that grow in plants and increase growth in normal and suboptimal conditions [4]. Endophytic bacterial colonization is more commonly found in plant roots than in plant stems. [5]
found that endophytic actinomycetes are more diverse in colonized roots than stems of rice plants. Endophytic bacteria that live in plant tissue originate from the root rhizosphere, which colonizes the roots and penetrates other hosts' inner tissues [6]. Endophytic bacteria colonize the host plant tissue through gaps or wounds formed when roots emerge in the lateral or root elongation zone and root differentiation and then spread to other plant parts such as stems and leaves.

Bacteria on plant roots have a positive effect through direct and indirect effect mechanisms. The direct mechanism is to contribute nutrients to plants and indirectly increase plant resistance by producing growth-promoting hormones such as auxins and gibberellins [7]. Plant roots are dominated by growth-promoting bacteria, which can support plant health by increasing nutrient availability and absorption [8]. Bacteria have a direct effect on plants through their role as plant growth stimulants, with nitrogen binding mechanisms, P and K dissolution, siderophore production, production of phytohormones in the form of IAA [9].

The following mechanism is that plants through their roots will release exudate in the form of organic acids. Bacteria around the roots will metabolize some organic acids as a source of carbon and nitrogen. Bacteria will produce several organic acids and minerals and be taken back by plants for growth and development [10]. The rice rhizosphere bacteria can produce organic acids such as succinic acid, propionic acid, oxalic acid, malic acid, and mineralize insoluble P to make it available for rice plants [2]. [11] stated that plant growth-promoting rhizobacteria (PGPR) in the soil can support plant growth and development by secreting different metabolites in the rhizosphere and available to plants.

Exploration, isolation, and characterization of bacteria will provide information on the potential of beneficial bacteria from endophytes and the rhizosphere of lowland rice plants as growth and abundance promoters. This study aims to identify, characterize, and calculate the abundance of bacteria from the endophytic and rhizosphere of rice plants in the Payakumbuh State Agriculture Polytechnic Experimental Field.

2. Methods
The research was conducted at Payakumbuh State Agricultural Polytechnic, Limapuluh Kota Regency, West Sumatra for four months. The source of bacteria comes from lowland rice cultivation at the Payakumbuh State Agricultural Polytechnic Experiment Field. Bacteria were isolated from the endophytic and rhizosphere of lowland rice plants. Isolation and identification of bacteria were carried out in the Food Crops laboratory of Payakumbuh State Agricultural Polytechnic and continued with identification using molecular analysis based on 16S rRNA fragments at LIPI Bogor.

Materials needed include Nutrient Agar, Nutrient Broth, and Pikovskaya's media, litmus paper, wrapping, alcohol, distilled water, and chemicals for molecular identification of bacteria. The tools used are beaker glass, Erlenmeyer, petri dish, test tube, one use hose needle, Bunsen lamp, analytical scales, oven, and laminar airflow

2.1 Isolation of Endophytic Bacteria
Endophytic samples were taken from rice plants' roots aged two months with criteria for healthy plants, green color, and normal growth. Root samples were washed with flowing water until clean and dried on filter paper. Sterilize the root surface by immersing in 70% ethanol solution for 2 minutes, 0.52% sodium hypochlorite solution for 5 minutes, rinse three times with sterile water, and dry on sterile filter paper. After dry, weigh 1 g and gently grind the roots in a sterile mortar, then put in the Erlenmeyer and add up to 10 ml of sterile distilled water. Stir by turning the Erlenmeyer. Take 1 ml of extract using a micropipette, put it in a tube, and add 9 ml of sterile distilled water. The same goes for a 10-7 dilution. Then take a 1 ml solution and grow it in a petri dish containing NA media. See the development of bacteria on the third day. The growing bacteria are separated and purified. The morphological characteristics of pure isolates of endophytic bacteria were based on shape, edge, elevation, surface, color, halo zone, and gram stain.

2.2 Isolation of Rhizosphere Bacteria
The soil was taken from a layer of soil around the same roots of rice plants. Dried the soil, take 1 g, then put it into Erlenmeyer and add sterile water to 10 ml. Then dilute it until 10-7. Next, take 1 ml of solution
and grown it on a Petri dish containing NA media. Look at the development of bacteria on the third day. Bacteria that grow are separated and purified. Pure isolates of endophytic bacteria are characterized by their morphology-based on the shape, edge, elevated surface, color, halo zone, and gram staining.

2.3 Identification of bacterial isolates
After that, the identification using molecular analysis based on 16S rRNA gene fragments. The extraction of bacterial genomic DNA using the GES method. Amplification of the 16S rDNA fragment was done using GoTaq (Promega) with a pair of general 27F primer (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5-GGTTACCTTGTTACGACTT-3') [11]. Then, sequencing data is processed with the Bioedit program. Isolates were identified using the EzTaxon server based on the 16S rRNA gene sequence data.

2.4 Calculation of Bacterial Populations
The bacterial population on NB media was calculated using a serial dilution method from dilution 10⁻¹, 10⁻², 10⁻³ until a dilution of 10⁻⁶. Each dilution was piped 0.1 ml and put into NB media, then incubated for 3 x 24 hours. To calculate the population used formula:

\[
\text{Bacterial density} = \text{Colony count} \times \frac{1}{\text{Dilution factor}} \times \left( \frac{\text{CFU}}{\text{ml}} \right)
\]

3. Results and Discussion
The results of bacterial isolation from root endophytes and rhizosphere of rice plants in Payakumbuh State Agriculture Polytechnic Experiment Field totalled 16 isolates. Still, four bacterial isolates were selected, which were dominant and potentially promote growth. The morphological characteristics of endophytic bacteria and rhizosphere of rice plants are shown in Table 1.

Table 1. The morphological characteristics of endophytic bacteria and rhizosphere of rice plants

| Source of bacteria | Isolate Code | Colony shape | Colony edge | Elevate | Surface | Color | Halo zone | Gram |
|--------------------|--------------|--------------|-------------|---------|---------|-------|-----------|------|
| Endophyte          | AP3A         | Irregular    | lobate      | raised  | smooth  | violet| 3 mm      | -    |
|                    | AP3B         | circular     | entire      | raised  | smooth  | cream | 4 mm      | +    |
| Rhizosphere        | TP1D         | rhizoid      | lobate      | flat    | smooth  | white | 3 mm      | +    |
|                    | TP3A         | circular     | wave        | raised  | smooth  | cream | 5 mm      | +    |

The four bacteria's morphological characteristics have various shapes, edges, elevations, colors, and halo zones. The colony's part, which is surrounded by clear areas (halo zone), indicates that the bacteria can dissolve P and suspected have properties that stimulate plant growth. These beneficial characteristics will open opportunities for bacteria to work together and can be used as biofertilizers. [12] state that close interactions between bacteria and plants, and between bacterias will form colonization as phytopathogens, so endophytic bacteria have great potential as biocontrol agents and biofertilizers.

Table 2. Sources of bacteria, bacterial species, strains, homology, and TPC of bacteria.

| Source of Bacteria | Bacterial Species            | Strain          | Homology | TPC CFU/ml |
|--------------------|-----------------------------|-----------------|----------|------------|
| **Endophyte**      | *Chromobacterium rhizoryzae*| LAM1188         | 99%      | 5.96 x 10⁷ |
|                    | *Brevibacillus brevis*      | NBRC 15304(T)   | 99.86%   | 6.78 x 10⁸ |
| **Rhizosphere**    | *Bacillus pseudomyoides*    | FJAT-40028      | 99%      | 4.6 x 10⁶  |
|                    | *Bacillus thuringiensis*    | BT              | 99%      | 7.96 x 10⁸ |
Bacteria originating from the endophytic and rhizosphere of rice plants were dominated by the genera Bacillus from the *Bacillus cereus* group with 95% genetic relationship (Figure 1). This group of bacteria can dissolve phosphates called Phosphate Solubilizing Bacteria (PSB). According to [2], PSB strains isolated from endophytes can dissolve P, produce organic acids, enzymes, IAA, siderophore and are competent for antagonistic effects against pathogens. P availability will increase in waterlogged soils such as lowland rice. Likewise, soils with acidity (pH) close to normal will increase the availability of P [13].

The abundance of bacteria in the endophytic and rhizosphere is quite high, and this is due to rice plants that grow in water which can accelerate the spread of bacteria. The factors influencing population density are plant age, type of bacteria, biotic, and non-biotic [14]. The types and abundance of microbes in rice plants' rhizosphere are mostly influenced by root secretions or exudates such as organic acids, amino acids, and sugars [15].

The composition of the nucleotide bases tested by molecular analysis based on the 16S rRNA gene fragment resulted in a similarity of bases (homology) between 99% - 99.86%. Phylogenetic tree based on the 16S rRNA gene for endophytic bacteria and rhizosphere processed using Mega 06 software (Figure 1)

![Figure 1. Phylogenetic joining between endophytic bacteria and rice rhizosphere based on the 16S rRNA gene sequence](image)

![Figure 2. Bacterial isolates with codes AP3A, AP3B, TP1D, and TP3A](image)

In this study, rice plant endophytic bacteria the *Chromobacterium rhizoryzae* strain of LAM 1188T is the dominant bacteria found in rice root endophytes. Seeing the name of the species, it is suspected that bacterium originated from the rhizosphere which succeeded in colonizing plant roots and living in the endosphere of rice plants. [16] Explain bacterial colonization's interaction on the root surface through the phases of bacterial movement, attachment, recognition, and penetration of bacteria in the root epidermis. Characteristics of the genera Chromobacterium is the production of violins and pigments, whose synthesis is regulated by quorum sensing and acts as a biocontrol against certain diseases [17]. Most of the genera Chromobacterium are found to live freely in the environment of water, soil, and the rhizosphere. Some of these bacterial species are found in plant roots and can suppress plant diseases [17]. Previous research by [18] that these bacteria have been found in rice plants' roots at The Experimental Demonstration Base at Huazhong Agricultural University, Wuhan City, China.
Some plant exudates that out through the roots can act as repellents against microorganisms, while others act as attractants to accommodate microbes. This exudate composition depends on the physiological status and species of plants and microorganisms [10]. [19] reported that four isolates from the endophytic origin and rhizosphere of rice plants, including Chromobacterium sp. MWU328 which potentially can produce bioactive compounds. Natural bioactive compounds are found in antimicrobial, anti-fungal, or antibiotic forms [20] stated that fifty percent of all endophytic bacteria isolated were antagonistic to rice pathogens.

*Brevibacillus brevis NBRC 15304(T)* in the endophyte of rice plants have the highest abundance because the bacteria *B. brevis* is multifunctional. According to [8] *B. brevis* is a PGP bacteria capable of producing growth stimulants on cotton plants. *B. brevis*, which is co-inoculated to the seeds, will reduce the disease caused by Fusarium oxysporum and Fusarium lycopersici in tomato plants, increase plant and root height growth [21]. *B. brevis* bacterial isolate is very effective in reducing nickel toxicity, increasing shoots, and plant root biomass on nickel-isolated soil [22]. Plant the IAA phytohormone influences growth and development. *B. brevis* bacteria can produce IAA closely related to improving root growth and nodule production [23]. The antimicrobial activity of ethylparaben from *B. brevis* can inhibit harmful bacteria's growth and fungi [24], so that plant health is maintained and plant growth is getting better. Some of the benefits of endophytic bacteria to their host plants are increasing plant growth-promoting activities, modulation of plant metabolism, and phytohormones signalling [14] ; [25].

Rice plant rhizosphere bacteria the *Bacillus pseudomycoïdes* are found in the rhizosphere of lowland rice plants in the Payakumbuh State Agricultural Polytechnic Experimental Field. Based on the 16S rRNA gene sequence analysis and its relationship, *B. pseudomycoïdes* is one of the 12 species of *B. cereus* members [26]. These bacteria are rod-shaped, facultatively anaerobic, gram-positive stain. Several types of *B. pseudomycoïdes* from plant rhizosphere that have been reported can produce cytotoxic compounds. [27] found that *B. pseudomycoïdes* species with Pseudomycoïcidin production as a new antibiotic with antimicrobial activity produced heterologous in E. coli. These bacteria are heterotrophic bacteria that can inhibit the growth of pathogenic bacteria with the antimicrobials they produce.

*Bacillus thuringiensis* is well known in the biological insecticide industry (bioinsecticide). The main products of these bacteria are compounds with antimicrobial activity in the form of several bacteriocins. The by-product of *B. thuringiensis* can synthesize Cry, Vip, and Cyt proteins and active against insects and parasites [28]. The results of previous research [29] found *B. thuringiensis* in the rhizosphere of cotton plants. Bacterial isolates are inoculated into plants and can dissolve phosphate, IAA production, and siderophore so that these bacteria have the potential as biological fertilizers. Bacterial inoculation experiments on several plants show maximum plant growth [29]. *B. thuringiensis* bacteria belong to the *B. cereus* group [26]. *B. thuringiensis* is one of the bacteria used as an insecticide because this bacterial gene can form spores, has toxic parasporal crystal inclusions for insect larvae. As an entomopathogenic bacterium, *B. thuringiensis* also has the potential to produce growth stimulants [8]. Previous researchers reported that some Bacillus species in the rhizosphere could mineralize insoluble P to become available to plants.

The mechanism for bacteria's presence starts from the roots that release metabolic secretions in the form of exudates that become food for microbes around the plant's rhizosphere. It was reported by [30] that root exudates provide an abundant source of energy and nutrients to microbes. Root and microbial exudates are important components of the rhizosphere's ecology and so important in changing the bioavailability of nutrients. Many factors determine bacteria's ability to live in the rhizosphere (Rhizocompetence), including bacterial strains, bacterial population, and soil fertility [31].

The Bacillus genera dominate bacterial species found in the endophytic and rhizosphere of rice in the Payakumbuh State Agricultural Polytechnic experimental garden. Likewise, the endophytic and rhizosphere bacteria of maize plant found by [32] at the same location (Kandang Lamo, Limapuluh Kota Region) were more dominated by the genera Bacillus. Testing bacteria on seeds by [33] that bacteria from the genera Bacillus and Pseudomonas inoculated on corn seeds will affect maize seeds' germination metabolism. The mechanism is bacteria with the help of carbohydrates in the endosperm to multiply, dissolve phosphate in the endosperm and produce IAA as a growth-promoting substance. The availability of phosphate will stimulate embryo growth in forming root and leaf growth of corn.
Previous studies indicated that differences in soil chemical composition could affect the endophytic microbial community of rice plants. The production of siderophore by endophytic bacteria will improve plant growth by binding to available iron [8]. Plant growth promoter bacteria can increase plant growth and protect plants from disease through various mechanisms [9]. Exudate organic acid compounds will regulate the soil microbial community in the root zone [11]. Utilization of endophytic bacteria from plants to obtain phytohormones or growth stimulating substances can be inoculated into agricultural products. [34] stated that endophytes affect plant growth through N fixation, phytohormone production, nutrient acquisition, and tolerance for abiotic and biotic stresses. All bacteria have the potential to dissolve phosphate, produce IAA and siderophores, all of which can stimulate plant growth.

4. Conclusion
The identification results showed that in the rice root tissue found the bacteria Chromobacterium rhizoryzae 5.96 × 10^5 CFU/ml and Brevibacillus brevis 6.78 × 10^6 CFU/ml. Bacillus pseudomycoides 4.6 × 10^6 CFU/ml and Bacillus thuringiensis 7.96 × 10^8 CFU/ml are found in the rice rhizosphere. Bacteria are dominated by the Bacillus genera which can stimulate plant growth.

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