**Indole Acetic Acid (IAA) Producing Bacteria from Saline Paddy Soil in Kebumen**

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**Abstract.** Paddy cannot grow optimally on saline soil, one of which is due to the low production of plant hormones. One crucial plant growth hormone is auxin, the Indole Acetic Acid (IAA). The application of saline-resistant bacteria producing IAA can promote the paddy growth of saline fields. This study aimed to explore saline-resistant bacteria capable of producing IAA from paddy fields around Jetis beaches, Kebumen, Central Java, and to test them on hydroponic systems and to develop the bacteria into biofertilizer. The following steps were followed for this study, soil sampling, bacterial isolation, bacterial selection using saline medium, IAA production measurement, and screening in planta with hydroponic systems. The results showed that five isolates of saline-resistant bacteria produced IAA. Isolates JB 2 exhibited the highest IAA (26.34 ppm). Screening in planta showed that JB2 addition to the growing media had higher plant heights and weights.

1. **Introduction**

Paddy (*Oryza sativa* L.) is a crop that has become a staple food for almost all of Indonesia population. Rice consumption in 2017 has reached 150 kg capita-1 year -1, approximately 39.3 million tons (Indonesian Central Bureau of Statistics). An increase population of Indonesia increases the need for food, but the demand for rice is often higher than the growth of available rice production. It is necessary to add more rice fields not less than 20,000 ha year-1 to fulfill food needs [1]. This situation is challenging to achieve if only relying on rice production from irrigated and rainfed rice fields. One method to increase the rice production is by managing saline field for rice production.

Indonesia is a country whose coastline is the second-longest in the world, after Chile. There are extended areas that have not been optimized, especially for rice farming, along the coastline because of its salinity. Indonesian agricultural land, which is gripped by salinity, reaches 0.44 million hectares [2]. Saline land has a salt content above the standard limit, thus harsh environment for plant to grow due to osmotic pressure disturbance. Increasing of salinity per 1 unit of electrical conductivity (EC) value above 3.0 dS m-1 can reduce rice production by 12% [3]. A study has concluded that EC water values above 2.0 dS m-1 could cause a significant decrease in rice production [4]. Rice production can decrease to 1 t ha-1 due to an increase of one unit EC value of inundated water with EC > 2 dS m-1 [5]. One effect of salinity stress on paddy is decreased in plant growth hormones. One crucial growth hormone for plants is auxin including Indole Acetic Acid (IAA).
IAA is an active form of natural auxin hormones found in plants that play a role in crop quality and quantity improvement. The IAA increases cell development, stimulates new root formation, growth, flowering, and increases enzyme activity. Efforts to increase IAA quantity in a plant are necessary to meet plant auxin requirement in saline fields. Rhizosphere bacteria, known as Plant Growth Promoting Rhizobacteria (PGPR), can produce IAA beneficial for plants [3].

The PGPR is beneficial for soil to increase its fertility. The bacteria can activate soil microorganisms to decompose soil organic matter resulting in soil fertility and to increase IAA production. Thus, IAA production is as required for plant growth to stimulate rapid root growth and greater nutrient absorption resulting in faster plant growth [6]. PGPR can be used as a biofertilizer, which useful to enhanced soil fertility. The use of biofertilizer can improve the physical, chemical, and biological properties of the soil, so that the content of macro and micronutrients is sufficient [6]. The purpose of this study was to determine the diversity of IAA-producing bacteria and to observe the effects of bacteria on paddy growing in saline medium.

2. Methods

2.1 Soil Sampling
Soil samples were taken from marginal rice fields, which are less than 1 Km from the beach. Five locations were selected from rice farms near Jetis Beach, Kebumen, Central Java. Soil of the rhizosphere area was collected.

2.2 Isolation of Soil Bacteria
One gram of soil was taken from the sampling location and diluted multilevel with 9 mL sterile distilled water. Dilutions were carried out until 10-8, and the last two dilutions were poured into NA media and incubated for 2 x 24 hours. Isolates that grow well were selected and purified. The pure isolates were cultured on stock culture made in oblique media, stored, and used in the next step.

2.3 Bacterial Resistance Test at Saline medium
Stock bacteria were inoculated on Nutrient Broth (NB) medium without salinity, and NB with 5000 ppm NaCl. The bacteria were incubated for 2 x 24 hours then grown on the NA medium. The bacterial population was calculated using the Total Plate Count (TPC) method. Well grown bacteria in the saline medium were selected as potential isolates and used in the next step.

2.4 Indole Acetic Acid (IAA) Production Test in Bacteria
IAA production test began with making the IAA standard curve by a spectrophotometric method with a wavelength of 530 nm in Salkowski reagents with concentrations of 0, 20, 40, 60, and 80 ppm. The spectrophotometry results created by curves and regression analysis was performed to obtain equations. Bacterial isolates on 10 mL of NB medium were incubated for 1 x 24 hours then centrifuged (10,000 rpm, 10 minutes). A total of 0.5 mL of supernatant was taken, and 1.5 mL of Salkowski reagent was added, then incubated in a dark room for 30 minutes. They were measured for absorbance at a wavelength of 530 nm and measured by IAA levels using the IAA standard curve previously made.

2.5 Screening in Planta in Hydrophonic System
The method used was a modification of [7]. IAA producing bacteria were inoculated on the floating hydroponic system planted with rice with AB mix nutrients, which had salinity stress of 500 ppm NaCl or 0.75 μmos/cm. The control was a hydroponic system that was not inoculated with bacteria. The treatment was repeated three times to have 18 research units. The study was conducted for 30 days. Parameters in this research were plant height, leaf number, and plant dry weight.
3. Results

3.1. Soil Sampling

The soil was sampled at five points in rice fields located around the River Bodo. Soil pH at those locations were classified at the normal pH range, whereas for groundwater salinity, they showed a high level of salinity, except one location that was moderate (Table 1).

| Location | pH | EC (µS/cm) |
|----------|----|------------|
| 1        | 6,8| 448        |
| 2        | 6,4| 78         |
| 3        | 6,1| 201        |
| 4        | 6,2| 490        |
| 5        | 6,6| 524        |

3.2. Bacterial isolates resistant in Salinity

A total of 14 isolates were obtained from 5 different sampling locations. The method of selecting salinity-resistant bacteria was carried out by growing 14 bacterial isolates on NB + NaCl 5 g/L or equivalent to 5000 ppm and NB without salinity as a control. The results of bacterial population (CFU/mL) calculation showed that all bacterial isolates could grow on NB medium without NaCl. In contrast, in NB medium with 5000 ppm NaCl, four isolates could not live well, and ten other isolates could grow.

![Figure 1. Bacteria Population in NB and NB + NaCl](image)

3.3. IAA Production of Bacteria

A total of 10 selected saline isolates were tested for IAA production. The test of bacterial IAA production from isolation was carried out quantitatively by spectrophotometric method. Bacteria that have the potential to produce IAA turns to red or pink when combined with Salkowsi reagent and incubated in a dark room for 30 minutes. IAA standard curves were made and obtained by the IAA calculation formula to calculate the quantitative of IAA produced. There were 5 isolates capable of producing the IAA hormone after 24 hours. The highest concentration was produced by JB 2 isolate indicating 26.34 ppm IAA was produced (Table 2).
3.4. Screening in Planta in Hydroponic System

A total of 5 isolates that have the potential to produce IAA were screened in planta on rice plants using a hydroponic system with nutrients added with salinity stress. The results of screening in planta showed that plants given bacterial isolates with different IAA concentrations showed different results on plant height, plant weight, and leaf number (Table 3).

**Table 3. Height, weight and number of plant leaves in screening in planta**

| Isolate Code | Height (cm) | Weight (g) | Plant leaves |
|--------------|-------------|------------|--------------|
| JA 2         | 4.833       | 0.019      | 2            |
| JB 1         | 12.133      | 0.017      | 3            |
| JB 2         | 16.533      | 0.024      | 3            |
| JD 1         | 15.033      | 0.024      | 3            |
| JE 1         | 10.033      | 0.022      | 2            |
| Kontrol      | 14.267      | 0.015      | 3            |

Plants inoculated with JB 2 isolate reached the highest IAA production (26.34 ppm), better plant height and weight than control treatments (without bacterial addition). The weight of plants inoculated with JB 2 and JD 1 isolates had the best weight which was 0.024 g (Figure 2 and 3).
4. Discussion

The analysis demonstrated that the selected bacteria have the potential to be applied to saline soil since they are able to cope and live in a salt stress environment. Likewise, IAA is the primary member of auxin groups that control many critical physiological processes, including cell enlargement and division, tissue differentiation, and response to light [8]. Auxin produced by the selected bacteria can be absorbed by plants and improved the process of plant elongation in response to light. Plants inoculated by JB2 isolates were 2.27 cm higher than control manifested in 15.88% taller plants than the control. It is very likely that given more time, the JB2 plants might grow even taller. The weight of the JB2 plant increased to 60% and produced the highest IAA. Plant weight can be correlated with plant height. Higher plant weight can increase chlorophyll content, thus better photosynthesis, have more nutrition, and gain better weight [7].

5. Conclusion

There are five isolates of potential IAA-producing bacteria from paddy fields near the Jetis beach with the highest IAA production of 26.34 ppm. Inoculation of JB2 isolates that have the highest IAA production can increase the paddy plant weight and height at a screening in the hydroponic plants system with salinity stress.

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