

## CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON *Capsicum annuum*

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Received : July 30, 2021

Accepted : October 08, 2021

DOI: 10.15575/biodjati.v6i2.13191

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**Abstract.** Plant Growth Promoting Bacteria Rhizobacteria (PGPR) is one of the potential bacteria to enhance of *Capsicum annuum* through inhabitation the growth of pathogenic fungi. This study aimed to characterize PGPR in chili plants (*Capsicum annuum*). PGPR was isolated from the soil habitat of the red chili plant in Cillegon, Indonesia. Screening was then carried out with the dual culture method on Petri dishes and tested through in vivo method on the red chili plant. The selected bacteria were characterized morphologically, biochemically, and physiologically. The results revealed that there were 14 single isolates of bacteria from the roots of the red chili plants. The five single bacterial isolates, namely *Azostobacter*, *Azospirillum*, *Pseudomonas*, *Serratia*, and *Beijerinckia* have good potential as PGPR based on multiple culture screening by producing clear zones and positively effect the growth of chili plants.

**Keywords:** *Capsicum annuum*, Characterization, Chilli Plants, Dual Culture, PGPR.

### Citation

Sari, I. J., Wahyuni, I., Khastini, R. O., Awaliyati, E., Susilowati, A., Utari, E. & Aryantha, I. N. P. (2021). Characterization of Plant Growth Promoting Rhizobacteria (PGPR) on *Capsicum annuum*. *Jurnal Biodjati*, 6(2), 255–263.

### INTRODUCTION

Indonesia is a country that is rich in agricultural products, including horticulture. The most cultivated horticulture plant is the red chili plant (*Capsicum annuum* L.). According to BPS (2016), centers of red chili production in Indonesia are located in West Java, Aceh, North Sumatra, Bali, Banten, South Sulawesi, and D. I. Yogyakarta. Based on these data, Banten Province is one of the center areas of red chili production in Indonesia.

Anthraxnose is a disease causes severe

damage to adult fruits in the field (Saxena et al., 2016), including chili plant causing declined in production. *Colletotrichum truncatum* (capsici), *Colletotrichum gloeosporioides*, *Colletotrichum acutatum* are species that caused anthracnose disease in red chili plants originated from the fruit (Than et al., 2008; Saxena et al., 2014). Anthracnosis causes extensive pre and post-harvest damage to chili, caused by anthracnose lesions. Many post-harvest fruit diseases do not show symptoms until the fruit is ripe. Typical symptoms of anthracnose disease are wet fruit

with a production of pink to orange conidial mass, circular or angular concave lesions that could occur in leaves, fruit, and stems (Than et al., 2008). Biological control techniques can be used to control the anthracnose (*Colletotrichum* sp.) and optimizing the chili production. Microorganisms that naturally associated with plant roots or better known as PGPR can improve growth and control plant diseases (Batool & Altaf, 2017).

Several studies have been done mainly investigate the role of PGPR in chili plants to inhibit anthracnose fungal. Thilagam & Hemalatha (2019) found that *Streptomyces violaceoruber* can be applied as a biological fertilizer and biocontrol agent to grow chilies against *C. capsici* fungal pathogen attack. Sutariati & Wahab (2010) also found *Bacillus* spp., *P. fluorescent*, and *Serratia* spp. can inhibit the growth of anthracnose pathogens. *B. subtilis* exhibited sufficient antibiosis capability due to its good inhibitory performance against *C. capsici* (Rajkumar et al., 2018). Application of bio fungicides using *Streptomyces* spp. exhibited the increase in chili plant growth parameters such as plant height, plant stem fresh/dry weight, root fresh/dry weight, root length, and fruit yield (Suwan, 2018).

In this study, we used a dual culture method for screening PGPR that can inhibit the growth of anthracnose pathogens on chili plants. Dual culture methods provide a way of preliminary screening to exclude environmental factors that may impact practical biocontrol. So, we can observe an antagonist and pathogen interaction using in vitro observation (Bunbury-Blanchette & Walker, 2019). Sari & Aryantha (2021) used dual culture as an initial screening to get the indigenous Mushroom Growth Promoting Bacteria (MGPB) increase the growth of *Volvarella volvacea*. Besides, Rupela et

al. (2003) used the dual culture method to get the potential microorganisms suppressing fungal plant pathogens as this method could visualize the characteristic zone of no growth in the petri dish grown beside each other on a culture plate.

## MATERIALS AND METHODS

### Isolation of PGPR Bacteria

As much as 1 g of soil attached to the root surface was put into an Erlenmeyer containing 100 ml of sterile distilled water (10<sup>-1</sup> dilution) and homogenized. The suspension obtained was diluted to 10<sup>-2</sup>-10<sup>-6</sup>. Homogenization was carried out repeatedly using vortex in each dilution stage. The pour plate method was then carried out to inoculate the sample (Cappuccino & Sherman, 2004). The bacterial culture obtained was incubated in a room at 27°C for 24 hours. Every colony that grows was then isolated to make pure culture.

### Isolation of Pathogenic Fungi

Chili plants that showed disease symptoms were collected from the field. The symptomatic plant fruits were sterilized with 5% sodium hypochlorite for 10 minutes, then cultured on a PDA medium. The cultures were incubated at room temperature (28°C) for 48 hours. Growing colonies were then transferred into new plates and subcultured several times to obtain pure isolates (Cappuccino & Sherman, 2004). Furthermore, Koch's postulate test was performed on the pathogenic fungus.

### Screening Using Dual Culture

Dual culture is an initial selection method to obtain the potential isolates as biological agents. A single isolate of bacteria that have been grown on NB media for 24 hours was grown on PDA media using pour

plate method. After the PDA was solid, pieces of pathogenic fungi with a diameter of 0.5 cm were inoculated in a petri dish containing PDA medium. The culture was incubated at a temperature of 26<sup>o</sup>-28<sup>o</sup>C for 48 hours. For each bacterial isolate, the test was repeated three times. The observation was carried out for 24-48 hours to see the inhibition zone or clear zone around the growth of pathogenic fungi.

### Selected Bacterial Testing on Chili Seeds (In Vivo)

The surface of chili seeds were sterilized using 70% ethanol solution for 5 minutes. The seeds were then put into 2% NaOCl solution for three minutes and washed using sterile aquadest 3 times. The chili seeds were then soaked in selective bacterial culture and water as a control. The chili seeds were placed in sterile filter paper placed in a petri dish for seven days. The selected rhizosphere bacterial culture with a density of 10<sup>8</sup> CFU

ml<sup>-1</sup> was used and dissolved. The bacteria were inoculated by watering the zeolite medium with a gap between seeds of 10 cm. The parameter observed was the height of the chili plant (Widiyanti et al., 2016).

### Characterization of PGPR

The parameter observed to characterize PGPR were the color, shape, edges, and elevation of colonies. Gram staining, motility test, and catalase testing were also conducted. Lastly, the samples were adjusted used Bergey's Manual of Determinative identification book (Holt et al., 1994).

## RESULTS AND DISCUSSION

There were 14 single isolates of bacteria obtained from the roots of the red chili plants. The result of observations on the characteristics of the bacterial colonies are presented in Table 1.

Table 1. The result of observations single isolates

Isolate Name	Colony of Colony	Colony Morfology	Surface	Gram	Cell Morfology
A	White (opaque)	Round shaped	Flat		Bacil
B	Pink	Irregular	Flat	-	Coccus
C	Red	Irregular	Flat	-	Coccus
D	Red	Irregular	Convex -		Coccus
E	White (opaque)	Round shaped	Convex -		Bacil
F	White	Irregular	Flat	-	Coccus
G	Yellow	Irregular	Flat	-	Bacil
H	White	Round shaped	Flat	-	Bacil
I	White	Round shaped	Convex -		Coccus
J	White (opaque)	Round shaped	Flat	-	Bacil
K	White (opaque)	Irregular	Flat	-	Bacil
L	White (opaque)	Irregular	Flat	+	Bacil
M	White (opaque)	Irregular	Flat	+	Bacil

Figure 1. is a red chili infected with a pathogenic fungus. Based on observation, the symptoms that appear in red chili are a color change around the area of infection, skin tissue

damage and wet. Based on these symptoms, red chili may be affected by *Fusarium wilt* by *Fusarium oxysporum* (Bai et al., 2018). Figure 2 is pathogenic fungi isolate from infected

chilies that have characteristic of white color mycelium that change to be orange after more than 48 hours. Moreover, Figure 3 shows a spore of *Fusarium oxysporum*. According to Joshi et al. (2012), the *Fusarium oxysporum*

has thin macroconidia and generally walled 3-5 septate, addition the chlamydo spores were both rough and smooth-walled, generally intercalary, and sometimes terminal.



Figure 1. A red chili infected with a pathogenic fungus



Figure 2. Pathogenic fungi isolate from infected chilies

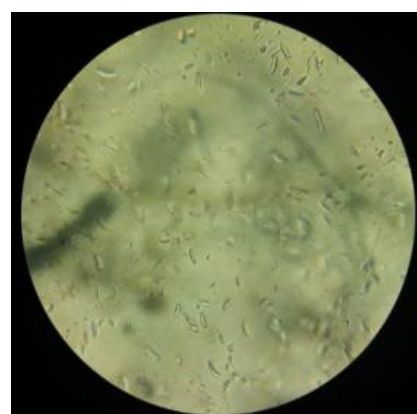


Figure 3. Spore of *Fusarium oxysporum* (Magnification 100x)

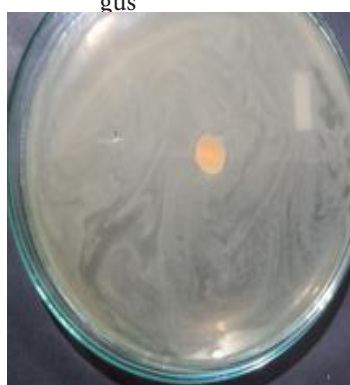


Figure 4. The absence of a clear zone



Figure 5a. isolate have a large clear zone

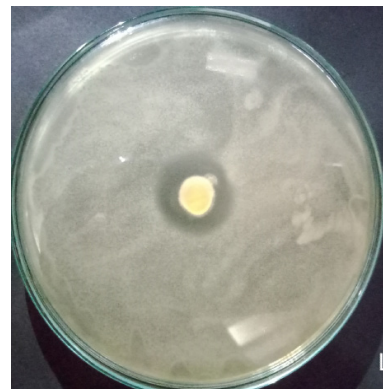


Figure 5b. isolate have a small clear zone

The dual culture results between pathogenic fungi and bacterial isolates to obtain PGPR bacteria are characterized by the presence of a clear zone around the pathogenic fungal mycelium on PDA (Figure 4). The absence of a clear zone on the presence of bacteria means that the bacteria are unlikely to have an impact on the growth of the pathogenic fungal mycelium (Figure 4). While the emergence of clear zones may indicate the presence of bacteria inhibiting the growth of

pathogenic fungal mycelium (Figure 5a and 5b). According to Zivkovic et al. (2010) and Mmbaga et al. (2018), the emergence of a clear zone is one of the bacterial mechanisms responsible for suppressing fungal pathogens, including competition, antibiotics, and metabolite production. The dual culture in Figure 5a has a large clear zone than that in Figure 5b. This is due to the different ability of PGPR to inhibit the pathogenic fungal mycelium. Shobha & Kumudini (2012) found



that the various mechanism of PGPR inhibits pathogenic fungi, such as the active enzymes, toxic metabolites from PGPR that can damage the fungal cellular walls.

The results of the growth of chili seeds treated with selective pathogenic fungi and bacteria are presented in Figure 6. Based on observations, bacterial isolates that gave the effect to growth of chilli seeds are bacterial isolates with code of A, B, E, G, H, I, K, and

N. The application of bacterial isolates results in higher plant height compared to control. Schillaci et al. (2019) stated that some nutrient-supplying bacteria at the root of the rhizosphere (rhizobacteria) are PGPR. This group of bacteria has many roles, including tethering nitrogen (N), producing IAA, dissolving phosphate, and inhibiting plant disease.

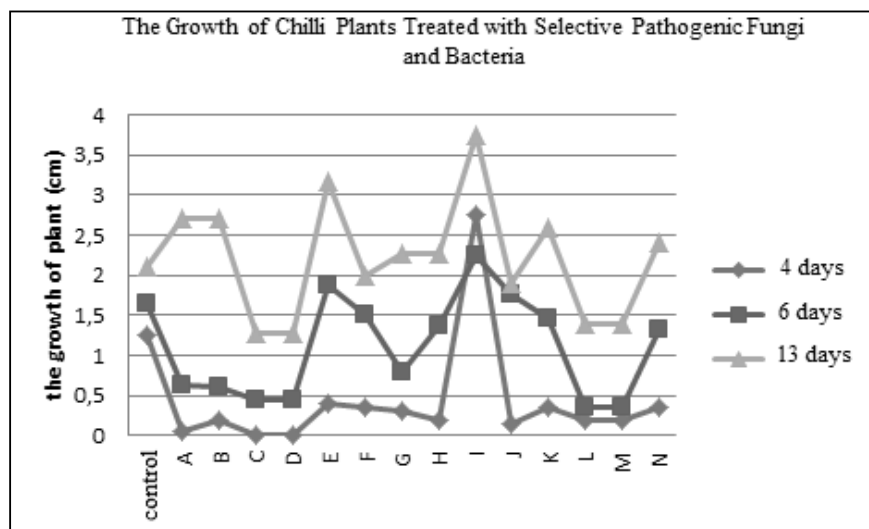


Figure 6 The chart of the growth of chilli seeds treated with selective pathogenic

Among fourteen bacterial isolates that had passed the dual culture test and produced a clear zone that also positively affected the growth of chili plants from the seed phase, are the bacterial isolates with codes A, B, E, I, and N (Figure 7). We then identified those bacterial isolates based on morphological observations in Table 1 and Figure 7. Isolate A has a small rounded colony, flat elevation, shiny colony surface, flat edge of the colony with white opaque color, and a bacillus (stem) cell shape and was gram-negative bacteria (Figure 7a). Meanwhile, the results on biochemical activity test showed that isolate A was positive in the catalase test, negative in the indole test, motile, and was aerobic. The results obtained indicated that isolate

might belong to the genus *Azotobacter*. The result is in accordance with Bergey's Manual of Determinative Bacteriology 9th edition, which explains that the genus *Azotobacter* has the characteristics of stem-shaped cells, gram-negative, motile, aerobic, can break down nitrogen, positive in catalase tests, and are generally classified as non-symbiotic (Holt et al., 1994). *Azotobacter* has the ability as N fixation by producing phytohormones, so *Azotobacter* could be used to enhance the growth of plants as PGPR (Kanchana et al., 2013).

Based on the results of morphological observations (Figure 7b), isolate B showed the characteristics of a rounded-shape colony, flat elevation shiny colony surface.

The edge of the colony is flat with pink, has a coccus shape, and is gram-negative. Meanwhile, biochemical activity test showed characteristics of the positive catalase test, the positive indole test, motile, and aerotolerant. The results obtained indicated that isolate B might belong to the genus *Azospirillum*. This is in accordance with Bergey's Manual of Determinative Bacteriology 9th edition, which explains that the genus *Azospirillum* has the characteristics of fibroid-shaped cells, pink, gram-negative, motile, and can break down nitrogen (Holt et al., 1994). *Azospirillum* has defense mechanisms during abiotic stresses through inducing systemic tolerance, mediated by antioxidants, osmotic adjustment, production of phytohormones, and defense strategies such as the expression of pathogenesis-related genes (Fukami et al., 2018).

The isolate E has the shape of a rounded colony, convex elevation, the shiny surface colony, the edge of the colony have milky white color, had the shape of bacilli (stem) cells and gram-negative (Figure 7c). Meanwhile, observations of the biochemical activity test showed isolate E has characteristics of positive catalase testing, negative indole testing, motile, and aerotolerant interactions. The results obtained indicated that isolate E was included in the genus *Pseudomonas*. This is in accordance with Bergey's Manual of Determinative Bacteriology 9th edition which explains that the genus *Pseudomonas* has the characteristics of stem-shaped cells, gram-negative, mobile, aerobic, and positive catalase tests (Holt et al., 1994). According to (Munir et al., 2019), *Pseudomonas* can be facilitators for siderophore, I-3-AA, ammonia production, phosphate solubilization, and tomatoes' growth in greenhouse conditions.

Based on morphological observations (Figure 7d), isolate I showed characteristics

of rounded colony shape, convex elevation, shiny colony surface, flat edge colony with clear color, a bacillus (stem) cell shape and was gram negative. Meanwhile, biochemical activity test revealed that Isolate I was positive in catalase test, the negative indole test, motile and were anaerobic in nature. The results obtained indicated that isolate I might belong to the genus *Serratia*. This is in accordance with Bergey's Manual of Determinative Bacteriology 9th edition, which explains that the genus *Serratia* has the characteristics of rod-shaped cells, gram-negative, usually motile with peritric flagella, anaerobic facultative, positive in catalase tests and negative indole tests (Holt et al., 1994). Martinez et al. (2018) stated that *Serratia* strains have the ability as biofertilizers and could increase the root collar diameter, height, relative chlorophyll content, biomass, and nitrogen content of rauli plantlets.

The isolate N showed characteristics of rounded colony shape, convex elevation, shiny colony surface, flat edge of the colony with milky white color, bacillus (stem) cell shape and was gram negative (Figure 7e). Meanwhile, the results on biochemical activity test showed this isolate was the negative catalase test, the negative indole test, motile, and aerobic. Based on the results obtained indicates that isolate N might belong to the genus *Beijerinckia*. This is in accordance with Bergey's Manual of Determinative Bacteriology 9th edition, which explains that the genus *Beijerinckia* has the characteristics of stem-shaped cells, gram-negative, motile or non-motile, can fix nitrogen under aerobic and also microaerobic conditions, and positive catalase tests (Holt et al., 1994). *Beijerinckia* is one of the PGPR that has benefits for nitrogen fixation (Vejan et al., 2016).

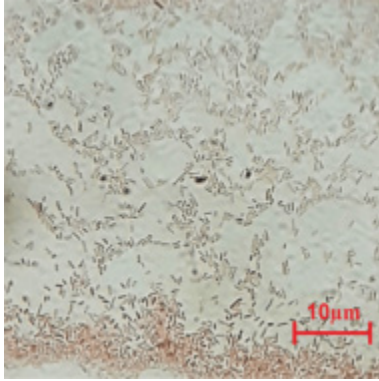


Figure 7a. *Azotobacter* sp.

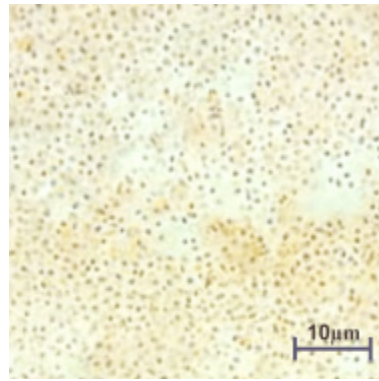


Figure 7b. *Azospirillum* sp

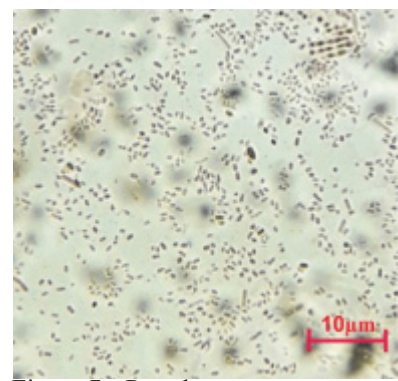


Figure 7c. *Pseudomonas* sp.

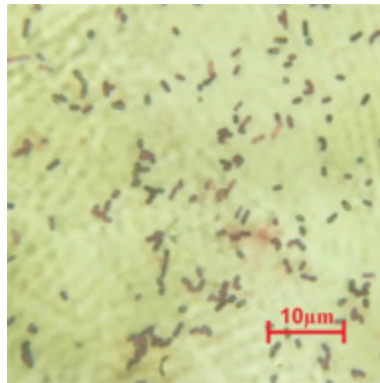


Figure 7d. *Serratia* sp.

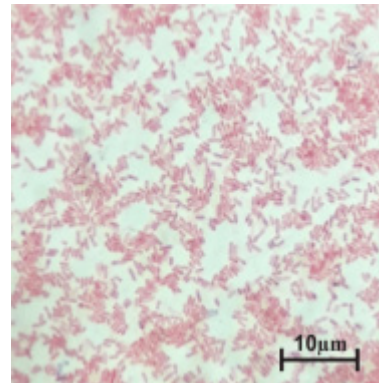


Figure 7e. *Beijerinckia* sp.

## ACKNOWLEDGEMENTS

Authors thank LPPM Universitas Sultan Ageng Tirtayasa who has funded this research through Penelitian Kerjasama antar Perguruan Tinggi (Pekerti).

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