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SHORT COMMUNICATION

Antagonistic Potential of Phosphate Solubilizing Bacteria (*Bacillus* spp.) from Peat Soil against *Erwinia* sp. (BP1) from the Leaves Zig-Zag Spots Symptoms Disease of the Pontianak Siamese Citrus Plants (*Citrus nobilis* var. *microcarpa*)

MUKARLINA*, SITI KHOTIMAH, RAHMAWATI & SHELLA MIDA JUNIARTI

Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak, West Kalimantan, Indonesia
*Corresponding author: mukarlina@fmipa.untan.ac.id
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ABSTRACT

The Pontianak Siamese orange (*Citrus nobilis* var. *microcarpa*) is one of the horticultural crops that is a leading commodity in the West Kalimantan area. However, in recent years, the production of Siamese oranges has decreased. One of the causes of the decline in the production of Siamese orange plants is the presence of diseases caused by bacteria. The *Erwinia* sp. bacteria, as the test pathogenic bacteria, were isolated from orange leaves with symptoms of zig-zag spots. Diseases in plants can be controlled using rhizosphere bacteria as biological agents. The phosphate-solubilising bacteria used as biological control agents in this research are rhizosphere bacteria originating from forest peat soil in Kubu Raya Regency, West Kalimantan. This research used a Completely Randomized Design (CRD) with five treatments. The *in vitro* antagonist test uses the dual culture method. The results showed that all phosphate-solubilising bacteria originating from peat soil *Bacillus* sp. (SGB1), *Bacillus* sp. (SGB2), and *Bacillus* sp. (SGB3) had antagonistic potential against the bacteria *Erwinia* sp. BP1.

Keywords: Antagonist test, *Erwinia sp.*, Phosphate-solubilizing bacteria, Siamese lime leaf

Pontianak Siamese oranges (*Citrus nobilis* var. *microcarpa*) are one of the local citrus varieties which are very popular because they have a sweet taste and contain high levels of vitamin C. Sambas Regency is the production center for Pontianak Siamese oranges in West Kalimantan. Sambas Regency produces 107,096 tons out of 142,917 tons, or around 75% of Pontianak Siamese orange production in West Kalimantan. The productivity of Pontianak Siamese orange plants from 2018 to 2020 decreased from 12.69 to 9.75, a decline of 30% (Kristiandi *et al*., 2021). One of the reasons for the decline in the productivity of Siamese orange plants is disease. Some symptoms of leaf disease on Siamese orange plants include Citrus Vein Phloem Degeneration (CVPD), scab, spots, powdery mildew, melanose, and leaf cancer, caused by fungi and bacteria (Setyaningsih *et al.,* 2021; Puspito *et al*., 2018). The pathogenic bacterium used in this study was *Erwinia* sp., which was isolated from Siamese orange leaves with zigzag spot symptoms. This bacterium does not cause symptoms on citrus leaves but causes symptoms on citrus fruit. Several species of *Erwinia i*nclude *E. carotovora*, *E. amylovora*, *E. tracheiphila*, and *E. rhapontici*, which cause disease in fruits, tubers, corn, carrots, and beans (Huang *et al*., 2003; Sasu, *et al*., 2010; Fatmi, *et al*., 2011).

Disease control in citrus plants generally still relies on the application of synthetic compounds such as imazalil, azoxystrobin, mefenoxam, thiabendazole, pyrimethanil, and fludioxonil (Panebianco *et al*., 2015; Torres, 2021). The continued use of these chemicals can have negative impacts on plants and the environment. As an alternative to controlling diseases in plants, rhizosphere bacteria can be used. Phosphate-solubilizing bacteria (PSB) are one type of rhizosphere bacteria that can act as biological control agents in plants. This group of bacteria is termed plant growth-promoting rhizobacteria, which includes many genera such as *Serratia*, *Rhizobium*, *Pseudomonas*, *Paenibacillus*, *Flavobacterium*, *Enterobacter*, *Burkholderia*, *Bacillus*, *Azospirillum*, *Arthrobacter*, *Acinetobacter*, and *Alcaligenes* (Marra *et al.*, 2012; Zeng *et al.*, 2011). These bacteria can indirectly inhibit pathogens through antibiotic compounds and serve as a form of biological control (Asril *et al.*, 2022; Qingwei *et al.,* 2023).

In this study, three test strains of phosphate-solubilizing bacteria (PSB) from different *Bacillus* genera were used, namely, *Bacillus* sp. SGB 1, *Bacillus* sp. SGB 2, and *Bacillus* sp. SGB 3, which were isolated from the rhizosphere of forest peat soil in West Kalimantan. The aim of the research was to determine the ability of the three bacterial strains to inhibit the growth of Erwinia sp. BP 1 bacteria.

In this study, bacterial strains *(Erwinia* sp. BP1), (*Bacillus* sp. SGB1), (*Bacillus* sp. SGB2), and (*Bacillus* sp. SGB3) were selected. The bacterial strains were obtained from the Laboratory of the Biology Department, Faculty of Mathematics and Natural Sciences, Tanjungpura University, West Kalimantan. The treatments used in this research are: a negative control that uses distilled water, a positive control that uses amoxicillin, and the antagonist bacteria used are *Bacillus* sp. (SGB1), *Bacillus* sp. (SGB2), and *Bacillus* sp. (SGB3). Three isolates of phosphate-solubilizing bacteria (PSB) were re-cultured by taking 0.1 ml of each PSB isolate from the stock culture and adding it to 10 ml of liquid NB medium (composition: beef extract and peptone) in each test tube, followed by shaking for 24 hours at a speed of 120 rpm. The bacterial suspension was then spread onto solid Pikovskaya agar medium and incubated at room temperature at pH 7 for 24 hours. An isolate of *Erwinia* sp. was recultured by taking two doses from the stock culture, placing them in a test tube containing 10 ml of sterile distilled water, and shaking it for 24 hours at a speed of 120 rpm. The bacterial suspension was taken from the test tube and spread evenly onto a petri dish containing solid NA medium, then incubated at 37°C and pH 7 for 24 hours.

The antagonism test uses a double-test method or dual-culture method, which is carried out by making a suspension of *Erwinia* sp. isolates and a suspension of antagonistic bacterial isolates, namely taking 1–2 loop of each bacterial isolate, placing them in separate test tubes that contain 10 ml of NB media, then shaking for 24 hours. A 1 ml suspension of pathogenic bacteria and antagonistic bacteria were pipetted into each test tube containing 9 ml of sterile distilled water, and the turbidity was equalised with a 1 McFarland's solution. The NA medium was poured into a petri dish (approximately 20 ml) and cooled. Then, the suspension of pathogenic bacteria, which had been equalized with a 1 McFarland's solution, was taken using a cotton swab and spread evenly over the surface of the NA medium (once solidified) until evenly distributed. Next, pieces of sterile filter paper (diameter ~0.5 cm) were immersed in each of the antagonistic bacterial suspensions, antibiotic suspensions, and sterile distilled water for approximately 30 minutes. The filter paper pieces were placed on the edge of the petri dish containing pathogenic bacteria and incubated for 24 hours at room temperature (Bonev *et al.*, 2008)

The observation method is carried out by measuring the horizontal and vertical diameters, as well as the diameter of the filter paper. The average diameter of the inhibition zone for antagonistic bacteria, based on the theory put forward by Jawetz *et al.* (2008), can be calculated using the following formula, Eq. (1):

$$\text{Zone of Resistance, } R = \frac{(Dv - Dc) + (Dh - Dc)}{2} \qquad \text{Eq.(1)}$$

Where:

R = Average zone of resistance that appears (mm)

Dv = Vertical diameter

Dh = Horizontal diameter

Dc = Diameter of filter paper

From the data, the mean values for the zones of growth inhibition of the plant extracts were calculated using statistical analysis of variance (ANOVA), and the result was expressed with a 95% level of confidence ($p<0.05$) (Kumar *et al*., 2006).

The results of the ANOVA test showed that the positive control treatment, negative control treatment, and the three tested strains of *Bacillus* sp. (SGB1), *Bacillus* sp. (SGB2), and *Bacillus* sp. (SGB3) applied against *Erwinia* sp. (BP1) at a 24-hour incubation period had a significant effect ($F4.10 = 42.697$, $P = 0.000$), as did the 48-hour incubation period ($F4.10 = 120.237$, $P = 0.000$), both affecting the diameter of the inhibition zone formed. Duncan's further test results at incubation times of 24 hours and 48 hours showed that the positive control treatment was significantly different from the negative control treatment and the phosphate-solubilizing bacteria *Bacillus* sp. (SGB1), *Bacillus* sp. (SGB2), and *Bacillus* sp. (SGB3). The testing of the inhibitory power of phosphate-solubilizing bacteria against *Erwinia* sp. (BP1) from the leaves of Siamese orange plants at incubation times of 24 hours and 48 hours showed the highest inhibition zone diameter in the *Bacillus* sp. (SGB2) treatment namely, 0.80 mm and 0.85 mm (Table 1). This condition indicates that bacteria belonging to the species *Bacillus* spp., with isolate codes SGB1, SGB2, and SGB3, have the ability to act as antagonistic agents against pathogenic bacteria from the leaves of Siamese orange plants through different inhibitory mechanisms and are thought to produce compounds that inhibit growth, varying in type and amount.

Several studies have reported the effects of the genus *Bacillus* isolated from the rhizosphere as having antimicrobial properties. The mechanism of action of antagonistic substances produced by *B. subtilis* against *Erwinia* spp. involved damaging the K^+ ion transport of sensitive phytopathogenic bacteria through their cell walls (Amin *et al*., 2015; Asril *et al*., 2022; Qingwei *et al.,* 2023).

The inhibitory activity of *Bacillus* is also due to the presence of synthesised siderophore compounds (Eman *et al*., 2018). The research results of Khotimah (2021) showed that isolates of phosphate-solubilizing bacteria of the genus *Bacillus* spp. that were isolated from the rhizosphere of forest peat soil in Kubu Raya, West Kalimantan, were able to produce exopolysaccharides, which are siderophores.

The resulting inhibition zone is classified as weak (Table 1). It is suspected that the antagonist bacteria used in this study have weak antagonistic activity against Gram-negative bacteria. *Erwinia* spp. are Gram-negative bacteria (Huang *et al*., 2003; Fatmi *et al.*, 2011). Oscariz *et al.* (1999) reported that *B. cereus* strains isolated from soil were active against most Gram-positive, but not Gram-negative bacteria. Aslim *et al.* (2002) demonstrated that *Bacillus* strains had greater effects on Gram-positive bacteria than on Gram-negative bacteria. As conclusion, phosphate-solubilizing bacteria *Bacillus* spp. with isolate codes SGB-1, SGB-2, and SGB-3, originating from peat soil in this study showed antagonistic potential against the pathogenic bacteria *Erwinia* sp. (BP1).

Table 1. Mean Diameter of the Inhibition Zone for the Antagonist Test of All Treatments Against *Erwinia* sp. BP1 from Siamese Orange Plant Leaves with Zig-Zag Spot Symptoms of the Disease.

Treatment	Diameter of Inhibition zone (mm)		Diameter Inhibition Zone Category (Surjowardojo *et al*., 2015)
	24 hours	48 hours	
BP1 vs K^+	$5,73 \pm 1,35^b$	$7,2 \pm 0,95^b$	Medium
BP1 vs K^-	0.00 ± 0.00^a	0.0 ± 0.00^a	Weak
BP1 vs SGB1	$0,63 \pm 0,03^a$	$0,73 \pm 0,15^a$	Weak
BP1 vs SGB2	$0,80 \pm 0,25^a$	$0,85 \pm 0,30^a$	Weak
BP1 vs SGB3	$0,63 \pm 0,24^a$	$0,68 \pm 0,31^a$	Weak

Numbers followed by the same letter indicate results that are not significantly different with a confidence level of 95%.

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