Imperata cylindrica root as PGPR pressing the pathogen *phytophthora infestant* the cause of wilt disease in potato plant (*Solanum tuberosum* L.)

Dyah Roeswitawati [1*], *Sutarman* Sutarman [2], and *Dian* Indratmi [1]

[1]Department of Agrotechnology, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, 65144 East Java, Indonesia
[2] University of Muhammadiyah Sidoarjo, Jl. Raya Lebo No. 4 Sidoarjo 61261, East Java, Indonesia

Abstract. Potatoes (*Solanum tuberosum* L.) are a staple food worldwide, in addition to wheat, corn, rice, and wheat. In Indonesia, the potato commodity has received priority for development because it is a source of non-rice carbohydrates and has the potential for food diversification programs. Since 2016, Indonesian potato production has decreased due to attacks by the fungus *Phytophthora infestans*. Potato plants attacked by *Phytophthora infestans* show symptoms in small wet spots coloured pale green to dark green. So far, potato farmers have only relied on chemical pesticides to control these pathogens. The research aimed to test the potential of the *Imperata cylindrica* root to suppress the *Phytophthora infestans* pathogen that causes potato disease (*Solanum tuberosum* L.). The results show that the *Imperata cylindrica* root as PGPR can reduce the intensity of *Phytophthora infestans* attacks on potato. This is not significantly different compared to factory-made PGPR (Biopharma) but is significantly different compared to PGPR, which comes from the roots of bamboo plants. It is suspected that in the rhizosphere of the plant there are bacteria that release secondary metabolites so that they can suppress the *Phythtopthora infestant* pathogen through the process of antibiosis.

1. Introduction

Apart from increasing agricultural production, agro-industrial activities also produce waste. The use of agricultural production facilities, including chemical fertilizers and pesticides, has caused various side effects, namely environmental pollution in factories producing these fertilizers and pesticides and on agricultural lands that use them. If it enters the food chain, the toxic nature of these chemicals can cause various diseases such as cancer, mutations, birth defects, and CAIDS (Chemically Acquired Deficiency Syndrome) [1]. Potatoes (*Solanum tuberosum* L.) are a staple food worldwide besides wheat, corn, rice and wheat. European countries and the United States use potatoes as a staple food [2-3]. In Indonesia, the potato commodity has received priority for development because it is a source of non-rice

*Corresponding author: dyahwati@umm.ac.id

carbohydrates and has the potential for food diversification programs; however, since 2016, it has experienced a decline of 3.9% caused by attacks by the fungus *Phytophthora infestans* [4-5]. *Phytophthora infestans* attacks potato plants starting from the leaves, stems and tubers, causing crop failure [6].

Plant Growth Promoting Rhizobacteria (PGPR) is a group of soil microbes that live around the root surface [6]. These microbes are directly or indirectly involved in plant growth activities [7-8]. It was further stated that these bacteria actively colonize the root areas of plants, encouraging plant growth and development so that they play a role in the plant growth process. Research results show that PGPR can produce the hormones IAA, Cytokinin, Gibberellin, and Ethylene [7] and can act as a bioprotectant that protects plants from pathogens [9-12]. PGPR can be obtained from several plant roots such as bamboo roots, corn, beans, reeds, and elephant grass. Among these materials, PGPR from bamboo roots has the best influence on plant growth [13-18]. Furthermore, it was stated that the bacteria found in bamboo roots can dissolve minerals to break down organic materials into nutrients that plants need [19-21].

PGPR promotes plant growth through associative nitrogen fixation, phosphate solubilization, phytohormone production, and volatile organic compounds. PGPR also neutralizes plant stress caused by biotic and abiotic factors by increasing nutrient absorption, accumulation of osmolytes, and production of antioxidant enzymes and metabolites (20; 22).

In the rhizosphere, there are several soil bacteria called rhizobacteria, which play a role in promoting plant growth (PGPR). These bacteria colonize the root surface and stimulate plant growth and health through antagonistic and synergistic interactions [18; 23]. PGPR can be free-living, symbiotic, parasitic, or saprophytic and plays an important role in encouraging plant growth and productivity. Free-living as well as associations and symbiotic rhizobacteria species belonging to the genera *Bacillus*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, and *Serratia*, is reported as PGPR [23]. *Agrobacterium*, *Serratia*, *Azospirillum*, *Bacillus*, *Erwinia*, *Micrococcus*, and *Pseudomonas* are examples of ePGPR. IPGPR is an endophytic symbiotic bacterium found in root cells, generally in unique nodular structures, for example, *Mesorhizobium*, *Rhizobium*, and *Frankia* [23]. *Actinomycetes* such as *Micromonospora* sp., *Streptomyces* sp., *Streptosporangium* sp., and *Thermobifida* sp. which dominate the rhizosphere region are also reported to enhance plant growth and control root-associated fungal pathogens [24].

The research aims to test several PGPR sources capable of suppressing wilt disease caused by the pathogenic fungus *Phytophthora infenstan* on potato plants so that they are competent to be developed as environmentally friendly biopesticides.

2. Materials and Methods

The research included a survey of potato planting locations showing symptoms of disease caused by the pathogenic fungus *Phytophthora infenstan*. The field survey was carried out in the potato farming fields in Pujon village, the Pujon sub-district, Malang Regency. Next, a portion of the diseased potato plant and the soil will be observed in the Agrotechnology laboratory, Faculty of Agriculture, Animal Husbandry, University of Muhammadiyah Malang.

2.1.Isolation and identification of pathogenic fungi *Phytophthora infenstan*

Isolation and identification of the pathogenic fungus *Phytophthora infenstan* using a combination method [6]. Pathogenic fungi are isolated from the soil of potato crop areas that show symptoms of leaf rot or potato tubers infected with pathogens. Isolation using the *Manalagi* apple variety as bait because this pathogen is classified as obligate. Make selective corn meal agar media with a composition of 12.5 g yellow corn flour, 10 ml Tween 80 and 3.8 g agar 300 ml distilled water [19], plus antibiotics rifampicine and pyramycine 30 ml, amphicycline and PCNB 30 µ. Manalagi apples variety are cleaned with 70% alcohol and hollowed out using a drill bit, then filled with soil and covered with tape, then incubated at 27^{o} C. After the apples show signs of rot, the skin surface is cleaned with 70% alcohol and rinsed with sterile distilled water. The skin of the fruit is peeled, and the border between rotten tissue and healthy tissue is cut into 1 cm x 1 cm cubes, then planted in a petri dish containing 10 ml of corn meal agar media and incubated for 3 x 24 hours until the fungal mycelium appears. After the white mycelium grows, it is transferred to a new petri dish, given PDA media identification and purification. The results of fungal isolation were identified macroscopically and microscopically; compared with the literature, *P. infestans* fungal spores are oval-shaped with blunt ends like kidneys [25].

2.2.Isolasi Plant Growth Promoting Rhizobacteria (PGPR)

PGPR is made from the roots of the *alang alang* plant (*Imperata cylindrica*) using a combination method [19; 26], namely: 200 g of *alang-alang* plant roots soaked in 1000 cc of water for seven days, then filtered and the filtrate stored as a bacterial starter. Make a bacterial growth medium, namely: 100 g of shrimp paste as a source of protein [7; 19], 1 kg of rice bran as a provider of fiber and carbohydrates, 500 g of sugar as a provider of glucose and fructose [18, 20], 5 g of lime as a pH neutralizer and 10 l clean water. All media ingredients are mixed and cooked until boiling, then cooled to room temperature. Next, 4 l of the media was mixed with 500 ml of bacterial starter and fermented for seven days until it smelled sour and had foam on the surface [27-28]. During the fermentation process, stirring is carried out every day.

2.3.Isolation and identification of rhizosphere bacteria

Rhizosphere bacteria were isolated from PGPR resulting from the extraction of *alang-alang* roots and elephant grassroots. Each PGPR was grown on agar extract soil media and identified based on observations of colony morphology, physiology and biochemical tests. The gram test includes gram staining and KOH solubility. Before isolating, all tools and materials used are sterilized first. Next, isolation was performed by making a dilution series (10^{-1}–10^{-5}), each repeated three times. The isolation process was carried out by pipetting 0.1 ml of the sample solution for each dilution series into a petri dish containing nutrient agar (NA) media, then incubating at 25 °C and observing bacterial growth daily. Microorganism growth media, making microorganism growth media using nutrient agar (NA) media. The composition of NA media is 8 g nutrient broth and 20 g agar, and then wet sterilization is carried out using an autoclave. Purification of bacteria is carried out using the scratch method, all colonies that have different shapes are purified on NA media to obtain pure isolates. The isolate obtained was then streaked and repeated until a single isolate was obtained. Next, incubation was carried out at 25 °C and growth was observed for two days.

2.4. Test the inhibitory power of rhizosphere bacteria against pathogens

The ability of rhizosphere bacteria to suppress pathogenic fungi was tested using the Dual Culture Method, namely by growing each bacteria side by side with pathogenic fungi on potato dextrose agar (PDA) media with the composition: 200 g of extracted potatoes; 20 g dextrose; 15 g agar.
Pathogenic fungal colonies (P) measuring 7 mm were placed 2 cm from one side of the petri dish, likewise 7 mm colonies of rhizosphere bacteria (R) were placed 2 cm from the edge of the petri dish on the other side. In another petri dish, a colony of pathogenic fungi without rhizosphere bacteria was grown as a control (K). Next, all treatments were incubated at room temperature 28^0 C. Antagonistic activity can be observed for approximately four days after incubation by observing and measuring the diameter of the pathogenic fungal colony P, which leads to the rhizosphere bacterial colony (R) and the diameter of the control culture colony (K). Calculation of the percentage of inhibition uses the formula [29] as follows:

$$IP = \frac{R1-R2}{R1} \text{ x } 100\%$$

Notes :
IP : Percentage of Inhibitory Power (%)
R1 : the radius of the pathogenic fungal colony away from the antagonistic fungal colony.
R2 : the radius of the pathogenic fungal colony approaches the antagonistic fungal colony.

3. Result and Discussion

3.1.Result

The results of the isolation of the Phytophthora infestant pathogen using baiting of the Manalagi apple variety are presented in Figure 1A, and then identification and purification were carried out. The pathogen is identified based on the kidney-shaped morphology of the spores. Rhizosphere bacteria that act as PGPR isolated from the roots of *alang-alang* (*Imperata cylindrica*) were purified and presented in Figure 1B. Next, a test was carried out on the ability of rhizosphere bacteria to suppress the growth of pathogens, presented in Figure 1C.

A B C

Fig. 1: A, results of pathogen isolation *Phytophthora infenstant*
B, Rhizosphere bacteria (PGPR)
C, Rhizosphere bacterial inhibition test suppresses pathogens

3.1.1. Inhibitory Power

Measurement of the percentage of inhibitory power (IP) of rhizosphere bacteria against the pathogenic fungus *Phythtopthora infestant* is determined based on measurements of R1 (the radius of the pathogen away from the antagonistic bacterial colony) and R2 (the radius of the pathogenic fungus that approaches the antagonistic fungal colony). The average results of R1 and R2 measurements seven days after inoculation of pathogenic fungi are presented in Table 1. In Table 1, it can be seen that R1 has an average radius value that is greater than the radius of R2. This shows that rhizosphere bacteria (PGPR) can inhibit the growth of colonies of the pathogenic fungus *Phythtopthora infestant* that grow close to rhizosphere bacteria (PGPR).

Table 1. Average pathogenic radius of *Phythtopthora infestant* grown next to PGPR (Imperata cylindrica bamboo roots and biopharma)

PGPR Treatment	Day 7 after pathogen inoculation *P. infestant* (cm)		
	R1	R2	R1 vs R2
Imperata cylindrica root	2,76	1,9	2,76 > 1,9
Bamboo root	2,86	1,93	2,86 > 1,93
Biopharma	2,86	2,76	2,86 > 2,76

Based on the R1 and R2 radius values, it appears that the percentage of PGPR's inhibitory power to suppress the pathogenic fungus Phythtopthora infestant on the seventh day was 29.19% for *Imperata cylindrica* roots and 32.13% of bamboo roots. Rhizobacteria are indicated to produce secondary metabolite compounds that can inhibit the growth of colonies of the pathogenic fungus Phythtopthora infestant so that these secondary metabolite compounds can control pathogens.

The percentage of inhibitory power data was analyzed using SPSS 16.0 software through an ANOVA test, which aims to determine whether or not treatment affects the inhibiting power of Phytophthora infestant pathogenic fungal colony growth. The ANOVA results showed that there was a significant/significantly different effect of the treatment on the inhibition of the pathogenic fungus Phythtopthora infestant because the significance value was below 0.05 (0.03 < 0.05), and continued with the Duncan test, the results of which are shown in Table 2.

Table 2. Average percentage of PGPR inhibitory power against the pathogenic fungus *Phythtopthora infestant*

PGPR Treatment	Percentage of Inhibition Power (%) 7th day
Imperata cylindrica root	29,19 a
Bamboo root	32,13 b
Biopharma	28,09 a

3.1.2. Colony growth rate

The observation results showed that the colony growth rate and radial direction of the two microbes were different during the seven-day observation. Table 3 presents the average colony growth rate and radial direction at the age of seven days, the pathogenic fungus *Phythtopthora infestant*.

Table 3. Average colony growth rate and radial direction of the pathogenic fungus *Phythtopthora infestant*

PGPR Treatment	Colony growth rate and radial direction (cm)
Imperata cylindrica root	5,29 a
Bamboo root	5,13 a
Biopharma	4,09 a

The results of analysis of variance showed that the colony growth rate and radial direction of Phythtopthora infestant were not significant. Observations of antagonistic interactions were carried out on the seventh day, namely when the growth rate of the pathogenic fungal colony Phythtopthora infestant and the rhizosphere bacterial colony came into contact with each other.

Table 4. Antagonist test mechanism for each PGPR treatment

PGPR Treatment	Antibiosis
Imperata cylindrica root vs *Phythtopthora infestant*	+
Bamboo root vs *Phythtopthora infestant*	+

Notes: +, antagonism mechanism occurs / there is an obstacle zone

Inhibition of *Phythtopthora infestant* pathogenic fungi by rhizobacteria of *alang2* roots and bamboo roots is indicated as an antibiosis mechanism. This mechanism occurs due to secondary metabolite compounds produced by rhizosphere bacteria, which is indicated by forming a clear zone between the two microbes [30]. Furthermore, it was stated that the antibiosis mechanism, apart from indicating the presence of a clear zone (barrier) between the growth of the two microbes tested, there was also a colour change in the media due to antibiotic compounds produced by the test microbes.

3.2. Discussion

The research results show that PGPR from *Imperata cylindrica* roots can suppress the growth of pathogenic fungi that cause potato wilt disease. The inhibition test results showed no difference between the PGPR of Imperata cylindrica roots compared to bamboo roots and factory-produced PGPR (biopharma).

Utilization (PGPR) is an alternative technology that increases agricultural production and is safer because soil bacteria live in the root area of plants and can stimulate plant growth [6;9]. These bacteria colonize/multiply aggressively in the plant rhizosphere due to the presence of plant exudates [14-16]. The existence of these microorganisms is beneficial for physiological processes and plant growth [17]. These microbes mobilize or facilitate the absorption of various nutrients in the soil, synthesize and change the concentration of growth-promoting phytohormones [13;18-22].

PGPR bacteria can fix free nitrogen; this nitrogen is converted into ammonia and then distributed to plants [23]. These bacteria are also able to provide several minerals that plants need, including: iron, phosphorus and sulfur [19;24-27]. The research results of Roeswitawati *et al*. [28] showed that the highest yield of potato tubers per hectare was obtained in the bamboo root PGPR treatment with a concentration of 20 ppm, although it was not significantly different from a concentration of 30 ppm. This is due to the beneficial role of PGPR for plant growth and production. As stated by [4-5;10-12;18-19], PGPR can prevent and control disease and stimulate plant growth [25].

Furthermore, it was also stated that PGPR can be used as a biological pesticide because of the presence of several antagonistic microbes that live around the roots. [7-8; 14-16]. Another opinion states that PGPR can increase growth due to the role of several phytohormones contained in it [7]

PGPR helps suppress pathogen activity by producing antibiotic compounds and stimulating plant growth, namely stimulating the formation of hormones or growth regulators, auxins, cytokinins and gibberellins so plants are more fertile. PGPR can be used as an additive to speed up the composting process [21;25-26]. Biological control agents that have been widely studied are the genera *Bacillus*, *Streptomyces*, *Pseudomonas* (*Pseudomonas fluorescens*), *Burkholderia* and *Agrobacterium* [29-30]. These microbes suppress disease growth through the following mechanisms: induction of systemic resistance in plants; production of siderophores that chelate (bind) iron so that iron is not available to the pathogen (*Pseudomonas fluorescens*); synthesis of antifungal metabolites such as antibiotics, enzymes that degrade fungal cell walls, or hydrogen cyanide that suppresses the growth of pathogenic fungi; able to compete with pathogens for nutrients or growth space in the rhizosphere [14; 20-22].

4 Conclusion

It was concluded that PGPR from the roots of the *Imperata cylindrica* plant was able to suppress the growth and development of the *Phythtopthora infestant* pathogen that causes potato wilt disease. This is not significantly different compared to factory-made PGPR (Biopharma), but is significantly different compared to PGPR which comes from the roots of bamboo plants. It is suspected that in the rhizosphere of the plant there are bacteria that release secondary metabolites so that they are able to suppress the *Phythtopthora infestant* pathogen through the process of antibiosis.

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