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BIOLOGICAL CONTROL OF POTATO SOFT ROT DISEASE (*Erwinia carotovora*) USING *Bacillus subtilis* AND *Pseudomonas fluorescens*

Istiqomah¹⁾, Cokorda Javandira²⁾, Elis Siti Toyibah³⁾

¹⁾Crop Protection Department, Jalan Veteran, Malang Jawa Timur 65145, Indonesia

²⁾Crop Protection Department, Jalan Veteran, Malang Jawa Timur 65145, Indonesia

³⁾Agrribusiness Department, Jalan Veteran, Malang Jawa Timur 65145, Indonesia

e-mail : Istiqomah.faqih@gmail.com

Abstract

Potato (*Solanum tuberosum* L.) is one of the commodities of potential as a source of carbohydrates and important on Indonesia's economy. One of the constraints of potato production is soft rot disease caused by *Erwinia carotovora* (syn. *Pectobacterium carotovorum*) which is needed to be controlled. One alternative control measures is the used of bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* for biological control. The purpose of this research is to know the potential of *B. subtilis* and *P. fluorescens* in single and in combination to inhibit potato soft rot diseases caused by *E. carotovora*. The results showed that biological control agents *B. subtilis* and *P. fluorescens* in single or in combination both of them (T3 to T17) have potential in inhibiting the growth of pathogenic *E. carotovora* by showing the clear zone. Biological agents provides nearly the same effect if compared with the bactericide Agrept (T2). Based on the results of the statistical analysis, the treatment 11th and treatment 14th showed a clear zone wider than the Agrept treatment (T2). The treatment 11th contain combination of biological agents *B. subtilis* (10⁹ cfu/ml) and *P. fluorescens* (10⁵ cfu/ml), while the treatment 14th contain combination of biological agents *B. subtilis* (10⁷ cfu/ml) and *P. fluorescens* (10⁵ cfu/ml).

Keywords : *Erwinia carotovora*, *Bacillus subtilis* and *Pseudomonas fluorescens*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the potential commodities as a source of carbohydrates and has significant importance to Indonesia's economy. One of obstacles in the production of potato is soft rot disease caused by the bacterium *Erwinia carotovora* (syn. *Pectobacterium carotovorum*) either while still in the field or in storage ¹. Pathogen attack caused physical changes, physiology and chemistry in potato tubers that affect on the quantity and quality of potato tuber productions. *E. carotovora* is a species of bacteria generally causes soft rot symptoms in some horticultural crops ⁵. This bacteria was soil borne pathogen which is difficult to control chemically and is spreading very fast. The condition above gave the idea to do a control which environmentally friendly and sustained by utilizing biological agents.

Rhizosphere bacteria as *B. subtilis* and *P. fluorescens* were known have a potential as biological control agents for some pathogenic plants ². Capability of *B. subtilis* and *P. fluorescens* as biological control agents spertaining to the ability of compete in obtaining food substances or because it produces those compounds of metabolite like siderofor, an antibiotic or enzyme extracellular. The purpose of this research was to determine the potential of biological agents *B. subtilis* and *P. fluorescens* singly and combined both to control pathogenic *E. carotovora*.

MATERIALS AND METHOD

The research was conducted in the laboratory of Bacteriology Agricultural Faculty Brawijaya University from May 2011 until February 2012. Materials that used in the research consisted of tobacco plants, medium for test the physiology and biochemistry of bacteria which cause soft rot in potato tubers, Granola varieties potato, sodium hypochlorite, chlorofoam, bactericidal Agrept 20 WP and isolate biological agents *B. subtilis* and *P. fluorescens* FP-UB (Faculty of Agriculture University of Brawijaya) collections. The instruments which used in this research consisted of a petri dish, analytical scales, spectrophotometers, sprayer and a micro pipette.

Characterization Causal Bacteria of Soft Rot Disease in PotatoTuber

Potato tubers which showed infected by soft rot symptoms were obtained from potato ware house in Batu in East Java province were isolated and identified to the species level includes hypersensitivity test, test gram reaction to 3% KOH and staining gram, gelatin hydrolysis, oxidative-fermentative test and catalase test, test soft rot, arginine hydrolysis, nitrate reduction test, acid production from various carbon sources, the ability NB grow that 5% NaCl and grow that 37°C and sensitivity of streptomycin ⁵.

The Antagonism Test of Biological Agents *B. subtilis* and *P. fluorescens* against Pathogen *E. carotovora*

Testing the nature of biological agents bacterial antagonist *B. subtilis* and *P. fluorescens* against bacterial pathogen *E. carotovora* was done in spray method (fogging) ³. Biological agents bacteria *B. subtilis* and *P. fluorescens* which have been incubated for 48 hours was taken by ose needle which was made in 12 ml suspension of sterile distilled water and measured the OD 660 nm=1 with spectrophotometer (Spectronic 21, Milton Roy Company) and then adjusted to concentration which specified in Table 1. Furthermore, sterile filter paper with diameter of 5 mm was inserted into the suspension for ± 1 minute and drained for 2 hours. Then the filter paper which has dry planted in the middle of the NA media of petridish 9 cm in diameter and incubated for 2days. After incubation for 2 days, bacterial antagonists murdered by giving chloroform vapors by adding chloroform in cover of cultivated petri dish culture in an inverted for 1 hour. After that the cultivated was atomized by pathogenic bacteria *E. carotovora* suspension at a concentration of 109 cfu/ml. All treatments were incubated for 2 days. Index of bottleneck area (clear zone) which produced by isolat of biological agents bacteria was calculated by the formula ⁸:

$$I = \frac{\text{Diameter of Clear Zone}}{\text{Diameter of Biological Agents Colonies}} \quad (8)$$

Statistical Analysis

Data which obtained from clear zone observation analyzed more with analysis of range and Duncan multiple range test on the real level of 5%, then the presentation of the data conducted with descriptive analyzes (Microsoft Excel).

Table 1. The design of treatment antagonism trials biological agents *B. subtilis* and *P. fluorescens* against pathogen *E. carotovora*

Treatments	Control	Bacterial Biological Agent <i>B. subtilis</i>	Bacterial Biological Agent <i>P. fluorescens</i>	Bacterial Pathogen <i>E. carotovora</i>
P1	Sterile distilled water	0	0	10^9 cfu/ml
P2	Bactericidal Agrept 20 WP	0	0	10^9 cfu/ml
P3	0	10^9 cfu/ml	0	10^9 cfu/ml
P4	0	10^7 cfu/ml	0	10^9 cfu/ml
P5	0	10^5 cfu/ml	0	10^9 cfu/ml
P6	0	0	10^9 cfu/ml	10^9 cfu/ml
P7	0	0	10^7 cfu/ml	10^9 cfu/ml
P8	0	0	10^5 cfu/ml	10^9 cfu/ml
P9	0	10^9 cfu/ml	10^9 cfu/ml	10^9 cfu/ml
P10	0	10^9 cfu/ml	10^7 cfu/ml	10^9 cfu/ml
P11	0	10^9 cfu/ml	10^5 cfu/ml	10^9 cfu/ml
P12	0	10^7 cfu/ml	10^9 cfu/ml	10^9 cfu/ml
P13	0	10^7 cfu/ml	10^7 cfu/ml	10^9 cfu/ml
P14	0	10^7 cfu/ml	10^5 cfu/ml	10^9 cfu/ml
P15	0	10^5 cfu/ml	10^9 cfu/ml	10^9 cfu/ml
P16	0	10^5 cfu/ml	10^7 cfu/ml	10^9 cfu/ml
P17	0	10^5 cfu/ml	10^5 cfu/ml	10^9 cfu/ml

RESULTS AND DISCUSSION

Characterization Of Causal Soft Rot Bacteria In Potato Tuber

The results of physiological and biochemical characterization tested could be seen in table 2. Based on the isolates characterization that bacteria included in the genus *Erwinia*, then included in the species *Erwinia carotovora*.

Table 2. The results of physiological and biochemical characterization of bacterial isolates *E. carotovora*

Number	Test of Physiology and Biochemistry	Bacterial Isolates	Literature
		Ec 2	Schaad <i>et al.</i> (2001)
1	Test of Soft Rot	+	+
2	Test of Pathogenicity	+	+
3	Test of Hypersensitivity	+	+
4	Medium Growth of CPG	+	+
5	Test of Gram Reaction		
	a. KOH 3%	-	-
	b. Painting Grams	-	-
6	Test of Catalase	+	+
7	The Growth of NB 5%NaCl	+	+
8	Test of Gelatinhydrolysis	+	+
9	Test of Oxidative-Fermentative	Fermentative	Fermentative
10	Test of Mortality	+	+
11	Test of Growth 37 ⁰ C	+	+
12	Sensitivity to Mankozeb	-	-
13	The Grwoth in Medium Kings'B	+	+
14	Pigmen Fluorescens in Medium Kings'B	-	-
15	Sensitivity to Streptomycin	+	+
16	The Production of Acid from various sources carbon:		
	a. Glycerol	+	+
	b. Maltose	+	+
	c. Laktose	-	+
	d. Arabinose	+	+
	e. Sucrose	+	+
	f. Galactose	-	-
	g. Sorbitol	+	-
	h. Manitol	+	+
17	Test of Reduction of Nitrate	+	+

Test of Biological Agents Antagonism *B. subtilis* and *P. fluorescens* against Pathogen *E. carotovora*

Based on the test media antagonism of Gelatine Nutrient (NA) in a petri dish (Figure 1) all treatments influenced in inhibiting the growth of pathogenic *E. carotovora*. On the control treatment (P1) was the NA media added pathogenic *E. carotovora*, it seen that there was no clear zone. The use of bactericidal Agrept 20 WP was made from active Streptomycin 20% sulfate influence to hamper the growth of pathogenic *E. carotovora* (P2). Biological agents *B. subtilis* and

P. fluorescens well as the combination of both agents (P3 to P17) indicated the potential for inhibiting the growth of pathogenic *E. carotovora* by produced the clear zone. Biological agents gave almost the same influence when compared with bactericidal Agrept. Based on the statistical analysis that on the 11th treatment and 14th treatment showed clear zone wider than bactericidal treatment Agrept (P2). The 11th treatment (Figure 2A) was the bacterial biological agents *B. subtilis* (10^9 cfu / ml) and bacterial biological agents *P. fluorescens* (10^5 cfu / ml), whereas 14th treatment (Figure 2B) was the bacterial biological agents *B. subtilis* (10^7 cfu / ml) and bacterial biological agents *P. fluorescens* (10^5 cfu / ml).

Figure 1. Test of biological agents antagonism *B. subtilis* and *P. fluorescens* against pathogenic *E. Carotovora*

Figure 2. A. The clear zone which resulted from the 11th treatment (*B. subtilis* bacterial biological agents 10^9 cfu/ml and *P. fluorescens* 10^5 cfu /ml); B. The clear zone which resulted from The 14th treatment (*B. subtilis* bacteria biological agents 10^7 cfu/ml and *P. fluorescens* 10^5 cfu /ml). Arrows indicate the diameter of the clear zone.

Biological agents *B.subtilis* and *P.fluorescens* on its activities discovered the various control mechanisms such as chemical compounds and enzymes bacteriolitic antibiotics ⁷. The Bacterial biological agents *B. subtilis* and *P. fluorescens* could control pathogenic bacteria *E. carotovora* with produce antibiotic compounds that can damage the protective function of the cell membrane of bacteria *E. carotovora*. Antibiotic compound was the result of secondary bacterial metabolism. Bacterial biological agents *B. subtilis* produces some antibiotic compounds such as bacitracin, basilin, basilomisin B, difisidin, oksidifisidin, lecithinase, and subtilisin ⁹. Bacterial biological agents *P. fluorescens* produces antibiotic compounds pyuloteorin, oomycin, Penazine1-carboxylic acid/ 2,4-diphloroglucinol ⁶. The damage to the membrane cell that led to the cell depreciation, so the bacterial cell *E. carotovora* will lost water and undergo plasmolisis ⁴. These obstacles could be done with the influence of the antibiotic compound to destroy the cell walls of pathogenic bacteria. So the metabolic activities of pathogenic bacteria become distracted. Thus the metabolism activities of pathogenic bacteria disturbed and causes cell pathogenic bacteria would be died. The influence of antibiotic compounds have a role in the synthesis of cell proteins. Cell protein synthesis could be inhibited when exposed to an antibiotic compound, so that cells would be damaged and could not synthesize the proteins.

CONCLUSION

Based on the isolation and identification to the species level, found that the symptomatic bacterial soft rot in potato tubers are *Erwinia carotovora*. In the test of antagonism on petri dishes that biological agents *Bacillus subtilis* and *Pseudomonas fluorescens* which were in oculated singly and the combination of both have the potential to control the soft rot disease in potato tuber (*Erwinia carotovora*).

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