Inhibition test of Rhamnolipids *P. aeruginosa* IFO 3924 on the growth of
destroyer microbe of potato crop from Lembang and Pengalengan

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Abstract

Rhamnolipids biosurfactants compound which is very popular because it can be applied to some industries, including the agricultural industry. The aim of this study was to test the inhibition of rhamnolipids on the growth of destroyer microbe of potato plants. Rhamnolipids was produced using *Pseudomonas aeruginosa* IFO 3924 in modified Basal salt medium with carbon source palm oil curd 7g/L, 100-liter fermenter capacity with mixing 16 rpm, temperature 30°C, with aeration flow 2.5 L/min are used in this experiments. Fermented broth is separated by centrifugation at 3000×g for 30 min, 30°C temperature. This supernatant is used as rhamnolipids, it has 100% concentration. For inhibition tests performed with the following stages: (1) Preparation of substrate: each of these isolates (from Lembang and Pengalengan) were cultured in medium V8 and incubated at 18°C for 15 days to obtain concentration of 10^6 spores/ml. On the surface, mycelium is cut into pieces and added as much as 20ml of sterile distilled water, the spore suspension on the surface water, the determination of the amount of spore are hemacytometer; suspension containing 10^6 spores/ml was used as destroyer microbe of potato plants; (2) Preparation of dilution series rhamnolipids of 10%, 5%, 2.5%, 1.25%, 0.625%, 0.3125%, 0.15625% and 0%; (3) circle leaves potato plants, age of 1.5 months using cork Bohrer was prepared. (3) each circle potato leaves dipped in the rhamnolipids last for 15 seconds and placed upside down in the petri dish then sprayed with suspension prepared in stage number 1, (4) The growth of destroyer microbe was observed on the surface of the leaves for 6 days. The results showed that the higher concentration rhamnolipids used the higher inhibitory capability. The spraying of rhamnolipids on Lembang’s Potato is more effective to inhibit destroyer microbe compared with that of the Pengalengan’s potatoes. Rhamnolipids *P. aeruginosa* IFO 3924 has potential as an anti destroyer microbe of potato plants.

Keywords: Rhamnolipids, *P. aeruginosa* IFO 3924, destroyer microbe of potato plants

Introduction

Potato crop damage is generally caused by a variety of microorganisms such as small worms or nematodes, some types of fungi and also other types of microorganisms. Eradication of pests has been done in several ways such as by use of a synthetic pesticide. The residue of synthetic pesticides actually produce harmful substance to society. According to study reported by Rochman [8] the decline in production due to pest of potato crops such as Globodera rostochiensis pest of microorganisms group of worms that live in the roots of potato plants and to reduce the production of between 32-71%. The potato crop damage caused by microorganisms is very disadvantage. While the damage caused by fungi also big enough impact on potato production. Therefore the use of biopesticide is recommended.

Consumption and production of potatoes in Indonesia is dominated in West Java and Central Java. Table 1 below illustrates the production and consumption of potatoes in Indonesia.

<table>
<thead>
<tr>
<th>Year</th>
<th>Production (tons)*</th>
<th>Consumption (kg/kap/yr)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>1,176.304</td>
<td>1,721</td>
</tr>
<tr>
<td>2010</td>
<td>1,060.805</td>
<td>1,825</td>
</tr>
<tr>
<td>2011</td>
<td>955.488</td>
<td>1,564</td>
</tr>
<tr>
<td>2012</td>
<td>1,094.240</td>
<td>1,460</td>
</tr>
<tr>
<td>2013</td>
<td>1,124.282</td>
<td>1,480</td>
</tr>
</tbody>
</table>

*Badan Pusat Statistik (2013), **Buletin Koernare edisi (2013)

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Potato production and consumption is fluctuative, the consumption per capita per year trend to decrease due to increasing of variety of staple food in Indonesia. Purwanti [7] reported in her study that diseases caused lobiod by virulent pathogenic fungus Phytophthora infestans can lower the production of potatoes up to 90% of the total potato production in very short time.

In this study, the experiment was conducted to test the rhommolipids produced by Pseudomonas aeruginosa 1PO 3924 using a batch fermentation process with the media Modified Basal Salt Medium (MBSM). Rhommolipids generated is used to inhibit the growth of mold destroye potato plants such as Phytophthora infestans in laboratory scale using a potato leaf in the cup petri dish.

Material
Isolate Phytophthora infestans from potato centre plant district Baiduong in the area Pematang and District. Baiduong district area Lembang. Media V8 consist of 100 ml V8, CaCO3 1.2 g, and agar 20%, Media Water Agar, free fungicide Potato leaf, Petri-dish, Rhommolipids pasteurized on 10°C for 5 Minutes, and rhommolipids without pasteurized. V8 is mix vegetable consisting of eight type of vegetable.

Method
Every isolate was cultured in media V8 and incubated for 10-15 days on the temperature of 18°C. It was prepared inoculum from each isolate at a concentration of 106 spores/ml on the surface of mycelium was cut in the small pieces and add 20 ml sterile distilled water, and left over night, so conspore swam on the surface. This suspension was injected into test tube and then put in haemocytomiser to calculate the conspore. It was prepared the potato leaves age 1.5 months, which is free of rhommolipids, and then washed off all the dirt and rinsed with sterile distilled water, and continued with draining. It was created a circle of a potato leaves with diameter 1 inch using cock holder. Rhommolipids dilution series was prepared to be tested, starting from 10% as much as 7 root treatment and control, which is 10%, 8%, 6%, 5%, 4%, 3%, 2%, and 1% by volume. Each rhommolipids dilution series was dipped into series rhommolipids solution previously prepared for 15 seconds. A total of 9 pieces of circle potato leaves for each treatment in petri dish, then drained. Circle potato leaves that have been dipped with rhommolipids was then sprayed with inoculum containing Phytophthora infestans from each area of origin of the isolates. Circle potato leaves which were dipped in rhommolipids tested and which was sprayed with inoculum was placed into the petri dish containing water agar medium. 9 circle potato leaves for one treatment, it was prepared another petri dish filled agar water medium and was placed 9 circle potato leaves free from rhommolipids and free from P. infestans. All it was incubated at temperature of 18°C in an incubator with 12 hours of light and 12 hours dark for 5 days. At the end, it was observed every day the development of the growth of P. infestans isolates.

Results and discussion
In general, the results of the experiments showed that the response of P. infestans differently to the two types of rhommolipids treatment. Actually the use of rhommolipids to kill consporeis, e.g. Phytophthora infestans has been done and it was effective [10]. In our experiment the treatment without heating, gave clearer effect on the inhibition of growth of P. infestans compares to that rhommolipids with heating. It might be explained, that the heating of rhommolipids caused the decrease of activity of rhommolipids. During heating possibility rhommolipids molecular changes, as a result, its effectiveness may be reduced to destroy cell walls of fungi. The degrading activity of rhommolipids due to heating is not always, depend on the type of the molecule of rhommolipids. In Table 1 showed that the infection intensity of P. infestans on potato leaves sprays with non pasteurized rhommolipids on the day after infection was very effective.

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_P. aeruginosa_ is a pathogenic microorganism. Either in table 1 and table 2 the higher concentration of rhizomoloids used the damage of potato leaves more slowly, and vice versa. Without the use of rhizomoloids potato leaves which infected by _P. infestans_ within two days, all leaves experiment either from Lembang or Pengalengan, wilt attacked by _P. infestans_ (table 1 and 2, treatment H 1 or picture 1 and 2 treatment H). The control (Table 1 and 2 treatment I 1, or picture 1 and 2 treatment I) potato leaves without infection from _P. infestans_ and without dipping in rhizomoloids the potato leaves were fresh until 6 days observation.

**Picture 1**: Potato leaves from Lembang and Pengalengan sprayed with non-pasteurised rhizomoloids and infected with _Pythium aphanidermatum_.

The growth of _P. infestans_ was clearly inhibited by unpasteurised rhizomoloids, it was demonstrated by the fresh leaves of potato experiment (figure 1), whereas the growth of _P. infestans_ was less inhibited by pasteurised rhizomoloids, it was demonstrated by the withering and drying leaves of potato experiment (figure 2). The principle action of rhizomoloids biodistroyed zoospore produced by _P. infestans_, and then reacted with plasma membrane of zoospore which not protected by cell wall [9]. Stanghellini, et al., 1997 and 1998 in Duesse et al., [1] reported that mono rhizomoloids and dibharmoloids have zoosporicidal activity against phytopathogenic microorganism. Moreover, the rhizomoloids could destroy plasma membrane of zoospore of _Pythium aphanidermatum_, _Plasmopara lachne-radic_, and _Pythophthora capsici_. In our experiment the plasma membrane of zoospore of _P. infestans_ was also ruptured by rhizomoloid, it was demonstrated by still fresh the potato leaves experiment. The rupturing of plasma membrane of zoospore will lead to its invasive metabolism process in zoospore of fungus _P. infestans_, thus the ability to destroyed potato leaves in our experiment was reduced. This condition inhibits the proliferation of zoospores so, the leaves of potato plant tested was still fresh.
Conclusion

Rhamnolipids produced by Pseudomonas aeruginosa IPO 3924 and pasteurized has ability to inhibit the growth of mild destroy (P. infestans) of potato plants. The higher the concentration the more effective rhamnolipids used to kill the fungus destroy. Both potatoes from Lentosha and Pengengan can be sprayed with pasteurized Rhamnolipids every three days to prevent the growth of mild destroy.

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References
