Proceeding

"Enhancing Academic Collaboration Through ASEA-UNINET Scientific Meeting"

February 15, 2016
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FOREWORD

This proceeding is a collection of papers presented at the Scientific and Plenary Meeting ASEA-UNINET 2016 held in Bukit Jimbaran Campus, Udayana University, Bali, Indonesia from 15th to 18th of February 2016. The committee has accepted 67 papers from 7 countries (Austria 3 papers; England 1 paper; Indonesia 51 papers; the Philippines 2 papers; Poland 1 paper; Vietnam 7 papers; Thailand 1 paper; Laos 1 paper).

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POTENTIAL BACILLUS SP. AS BIOCONTROL AGENT OF BACTERIAL WILT RALSTONIA SOLANACEARUM IN VITRO

Diah Kharismawati Djereng
diah04djereng@gmail.com*

Retno Kawuri
mycrobiologylaboratory@yahoo.com

Yan Ramona
yan_ramona@yahoo.com

Biology Department, Faculty of Mathematics and Natural Sciences, Udayana University, Kampus Bukit Jimbaran, Bali, Indonesia

*Corresponding Author


Abstract

Pepper is one of horticultural commodities are cultivated by farmers in Indonesia. One cause reduced production of chili is the presence of diseases, common diseases that attack the bacterial wilt, caused by Ralstonia solanacearum. This study will be isolated antagonistic bacteria Bacillus sp. contained in CustomBio products, and tested the inhibition of Bacillus sp. against R. solanacearum in vitro in laboratorium menggunakan dual culture method with 3 replications, measured the diameter of inhibition zone is formed. It was found four isolates of Bacillus sp. isolated Bacillus sp. 1 (B1), Bacillus sp. 2 (B2), Bacillus sp. 3 (B3), and Bacillus sp. (B4) with a diameter of inhibition zone respectively 15 mm, 8 mm, 21 mm, 0.0 mm, 0.00 mm compared to the negative control, and positive control 25 mm.

Keywords: Bacillus sp., In vitro, Ralstonia solanacearum.

1 INTRODUCTION

Pepper is one of horticultural commodities are cultivated by farmers in Indonesia. Diseases in pepper is very complex, both in the rainy season and the dry season. It can even lead to substantial losses and declining quality of chili (Duriat, 2009). Common diseases that attack the pepper plant are bacterial wilt, caused by Ralstonia solanacearum (Fegan & Prior, 2005). In addition to pepper, these pathogens can also infect tomato plants (Aspiras & de la Cruz, 1985), potatoes (Gunawan, 1995), tobacco (Arwiyanto, 1998), ginger (Mulya et al., 2000), banana (Sumardiyono et al., 2001) and patchouli (Nasrun et al, 2007).

In other studies has also been reported that Bacillus spp Bc 26 effectively control the disease patchouli directly (antagonist) through the production of antibiotics and siderophores ranging from laboratory, greenhouse, till in the field (Chrisnawati et al., 2009). Bacillus bacteria is also reported as a plant resistance inducers and as Plant Growth

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Promoting Rhizobacteria (PGPR) that can enhance plant growth (Schipper et al., 1987). It is caused by the ability of PGPR bacteria dissolve phosphate (Premono, 1998) and produce a plant growth hormone include indole acetic acid (IAA) (Marwiyah, 2009).

Based on this background, this research will be isolated antagonistic bacteria contained in the product CustomBio, and tested the inhibition of Bacillus sp. against R. solanacearum in vitro in laboratorium menggunakan dual culture method with 3 replications, measured the diameter of inhibition zone is formed.

2 MATERIALS AND METHODS

Samples were collected from Buahan Kaja, Gianyar, Bali, Indonesia. Samples collected were part of a plant that is thought to be infected by R. solanacearum. A total of 10 g organ infected plants at the root of chili, put into 90 mL sterile water. Isolation of Bacillus sp. using the dilution method (Platting Method). Taken as many as 1 mL B5 is then inserted into the tube which already contains 9 mL of sterile water. Macroscopic observation includes observations form a colony, the colony surface shape, the shape of the edge of the colony and the colony color. Observations adapted to the macroscopic structure of bacterial colonies based on the book Bergey's Manual of Bacteriology Determination Test 9th Edition 1994. inhibition of Bacillus sp. against R. solanacearum using the dual culture on Petri dishes containing media SPA (Sucrose Peptone Agar). Suspension of R. solanacearum as much as 100 mL incorporated into the Petri dish, and the Petri dish is rotated to the right and to the left until homogeneous. Let the media NA becomes congested, after the inoculated suspension of Bacillus sp. taken using a pipette micron to 20 mL, and then placed in the hole diffusion with a diameter of 5 mm Petri. Then incubated at a temperature of 37°C for 1x24 hours. Then do the observation and measurement of the diameter of the inhibition zone formed from bacterial isolates of Bacillus sp. obtained on the growth of R. solanacearum. Inhibition zone can be seen through the formation of a clear zone around the area of Bacillus sp. Inhibition zone diameter of the most substantial and potentially.

3 RESULTS AND DISCUSSION

Fourth isolates have different characteristics macroscopically and microscopically. The following is a characteristic of the four isolates of Bacillus sp. which has been isolated. Isolates of Bacillus sp. 1 (B1) Irregularly shaped colonies and white milk, at the age of 24-48 hours, the edges of the colony uneven or bumpy, and catalase positive. At the top of the flat colonies. Microscopic bacill or rod-shaped chain with a diameter of 0.40 to 0.71 μm (Figure 1).

Figure 1. Macroscopic and microscopic characters B1. (A) Colonies B1 was 24 hours on nutrient agar medium (NA); (B) The character of microscopic shaped bacill (stem chain) and Gram positive. 1000x magnification with a light microscope (Yazumi).
Isolates of *Bacillus* sp. 2 (B2), colonies are round and white translucent, at the age of 24-48 hours, the edges of flat colony and catalase positive. At the top of the convex colonies. Microscopically shaped 0.31- cocobacill with a diameter of 0.45 μm (Figure 2).

*Figure 2. Macroscopic and microscopic characters B2. (A) Colonies B2 only 24 hours on the medium NA; (B) The character of microscopic forms cocobasill and Gram positive. 1000x magnification with a light microscope (Yazumi).*

Isolate *Bacillus* sp. 3 (B3), colonies are round and creamy translucent, at the age of 24-48 hours, the edges of flat colony and catalase positive. At the top of the flat colonies. *Bacill* microscopically shaped with diameter of 0.33 to 0.46 μm (Figure 3).

*Figure 4.3. Macroscopic and microscopic characters B3. (A) Colonies B3 was 24 hours in media NA; (B) The character of microscopic forms basill and Gram positive. 1000x magnification with a light microscope (Yazumi).*

Isolates of *Bacillus* sp. (B4), colonies are round and white milk, at the age of 24-48 hours, the edges of the flat colonies like billowy clouds and catalase positive. At the top of the flat colonies. *Bacill* microscopically shaped with a diameter from 0.28 to 0.39 μm (Figure 4).
Figure 4. Macroscopic and microscopic characters B4. (A) Colonies B4 24 hours old at NA media; (B) The character of microscopic forms and gram-positive bacilli. 1000x magnification with a light microscope (Yazumi).

Of the four isolates were found to have inhibitory zone variation different. Bacillus isolates sp.3 (B3) has a zone of inhibition of the largest and most potential, compared to isolates of Bacillus sp.1 (B1), Bacillus sp.2 (B2), Bacillus sp.3 (B3). Bacillus sp. has the ability to control the development of bacterial wilt disease caused by R. solanacearum. Bacillus produce antimicrobial substances such as bacteriocins. Bacteriocins are antimicrobial polypeptides or proteins produced by microorganisms that are bactericidal. Bacteriocins kill target cells to penetrate between the membrane and the resulting function of the target cell membrane becomes unstable, causing cell lysis (Compant et al., 2005).

Table 1. Inhibition of isolates of Bacillus sp. against R. solanacearum in vitro.

<table>
<thead>
<tr>
<th>Number</th>
<th>Isolates Bacillus</th>
<th>Inhibition Zone Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacillus sp. 1 (B1)</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus sp. 2 (B2)</td>
<td>8</td>
</tr>
<tr>
<td>3.</td>
<td>Bacillus sp. 3 (B3)</td>
<td>21</td>
</tr>
<tr>
<td>4.</td>
<td>Bacillus sp. 4 (B4)</td>
<td>0,0</td>
</tr>
<tr>
<td>5.</td>
<td>Control negative (–)</td>
<td>0,0</td>
</tr>
<tr>
<td>6.</td>
<td>Control positive (+)</td>
<td>25</td>
</tr>
</tbody>
</table>

Description: The values in the table ± standard deviation is the average of three replications. Different letters in the same column shows the results that were significantly different (P <0.05), according to Duncan test after ANOVA analysis beforehand.

It was found four isolates of Bacillus sp, which isolates B1, B2 isolates, isolates B3, and B4 isolates. Tested the ability of these isolates to inhibit the pathogen Ralstonia solanacearum in vitro. B3 isolates have inhibitory potential compared to the largest and most other isolates or control inhibition zone with a diameter of 21 mm.
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