Bacterial Antagonist Isolates in Controlling Bacterial Wilt Disease of Potato (*Ralstonia solanacearum*) in Aeroponic Cultivation System

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Abstract: The growing of potato plants in aeroponics system is considered as safe and ecologically friendly for producing natural, healthy plants and crops. However, it often faces with several constraints. Infection of bacterial wilt caused by *Ralstonia solanacearum* in the aeroponics cultivation system can spread rapidly through the nutrition flow and lead to plant death and crop failure. The objective of this study was to evaluate bacterial antagonist isolates belonging to *Clostridium sp* in reducing bacterial wilt disease of potato caused by *Ralstonia solanacearum* in aeroponic system and its effect on increasing the weight and number of potato tubers. The results showed that of the seven treatments tested on potato aeroponic system, treatment with a combination of isolates NS01 + G06 + S06 tend to be more effective in suppression of bacterial wilt disease (85%), increase the amount of seed tubers (162.5 tubers/treatment) and increase the average of tuber weight as much as 311.25 gr/treatment. It can therefore be concluded that a combination of three isolates of *Clostridium* sp i.e: NS01 + G06 + S06 had some synergic activities in potato aeroponic cultivation system.

Keywords: Potato; bacterial antagonist; *Ralstonia solanacearum*; aeroponic system

1. Introduction

Most potato growers in developing countries do not use quality seed, because of high costs and lack of access. As a result, there is a high need for cost-effective methods to produce quality seed that can be accessed by small farmers at affordable cost. Aeroponics offers the potential to improve production and reduce costs compared to conventional methods or to the other soilless method of hydroponics (CIP, 2010). Due to the automation of most parts of the process, plants could be cloned and grown by the hundreds or even thousands. In short, cloning became easier because the aeroponic apparatus initiated faster and cleaner root development through a sterile, nutrient rich, highly oxygenated, and moist environment (Stoner, 1983). Compared with the total production in hydroponics, the tuber yield per plant in the aeroponic system was almost 70% higher and the tuber number is more than 2.5 fold higher. Ideally, the environment is kept free from pests and disease so that the

plants may grow healthier and more quickly than plants grown in a medium. However, since most aeroponic environments are not perfectly closed off to the outside, pests and disease may still cause a threat. In the earlier study, disease intensity were varies from 43 to 78% (Zulkarnain, 2007, in Ariyanti, 2010). The National Seed Agency of Indonesia requires that the production of G0 (seed sources) must be free from *R. solanacearum* wilt disease (Baharuddin *et al.,* 2012) since the bacterial wilt can also be transmitted through seeds.

Application of several control techniques including biological control by using rhizobacteria has been undertaken (Campbell, 1989). Microorganisms that are able to survive in aeroponics system has better interaction with plant rhizosphere, adaptic in higher aeroponic nutrient concentrations, more competitive and are more potentially used as biocontrol agents (Ariyanti, 2010). Four anaerobic bacteria isolates from the rhizosphere of potato cultivated in aeroponic system, which could inhibit the growth of *R. solanacearum in vitro,* were successfully isolated in this study. All four isolates, NS01, NG02, S06, and G06 were identified as *Clostridium* sp.

Effectivity of each bacterial antagonist has also been tested in other study. The results showed that each bacterial isolates has different effects on potato in aeroponic system. NS01 isolate was able to suppress the bacterial wilt infction by 35%, while the G06 isolate increase the amount of seed tubers as much as 10.15 tubers/plants, NG02 isolate increase the weight of tubers about 150 grams/plants, whereas isolate S06 gave no significant effect in reduction of bacterial infection as well as in increasing of weight and number of potato tuber (Baharuddin, 2012). Based on these facts, the objective of this paper is to evaluate four bacterial antagonist isolates i.e: NG02, NS01, G06, and S06 belonging to *Clostridium* in the suppression of bacterial wilt (*R. solanacearum*) and its effect on the number and weight of potato tubers in an aeroponic cultivation system.

2. Materials and Methods

2.1 Medium Preparation and Planting

Growth medium using a Styrofoam box with coconut husk at the bottom was used in this study. The plant nutrients were circulated by irrigation from reservoir tank that flow into the next pipe located on the plant and has been fitted with a nozzle. Electric pump was used to encourage the nutrients flow to all plants. Potato seedlings with 5-7 leaves and ± 15 cm height were planted on surface of styrofoam box that has been perforated in 15 cm x 15 cm distance.

2.2 Cultivation and Application of Bacteria

All bacterial isolates were cultivated in Nutrient Agar (NA) before use. Application of bacterial antagonists was conducted seven days after planting in concentration of 10^8 cfu/ml, whereas inoculation of *R. solanacearum* was carried out 30 days after planting in same concentration. Completely Randomized Design (CRD) consisting of seven treatments with four replications was used in this study as follows:

NG02 + NS01 + S06 isolate + *R. solanacearum*
NG02 + NS01 + G06 isolate + *R. solanacearum*
NS01 + S06 + G06 isolate + *R. solanacearum*
NG02 + S06 + G06 isolate + *R. solanacearum*
NG02 + NS01 + S06 + G06 isolate + *R. solanacearum*

Control (-) without bacterial antagonist isolates
Control (+) with *R. solanacearum*

A total of 240 plants were used in this study. Observation of the disease incidence on plants began on 7th days post inoculation (dpi) with *R. solanacearum.* Percentage of disease incidence using the formula from Kelman (1954):

$$I = \frac{\sum (ni \times vi) \times 100\%}{Z \times N}$$

Description:
I = disease incidence (%)
ni = number of plants which revealed symptoms
vi = scale category in each observed plant
N = Total number of observed plants
Z = the highest of scale category

Category:
0: no symptom
1: one or two leaf were wilted/yellowing
2: 3 up to 10 leaf were wilted/yellowing
3: more than 10 leaf were wilted/yellowing
4: all the leaves were wilted or yellowing
5: plant dead

The tuber production data were calculated based on the number of tubers production in each treatment, while the weight of tubers were determined by weighing the number of tubers produced in each treatment. Differences in the disease incidence of *R. solanacearum* were assessed with one-way ANOVA at the end of each treatment. Duncan's multiple range test was applied when one-way ANOVA revealed significant differences ($P<0.05$).

3. Results and Discussion

3.1 The Disease Incidence of R. solanacearum

The mean disease incidence (DI) of *R.solanacearum* between the treatments with antagonist isolates in potato aeroponic cultivation chambers varied. The lowest DI was found on treatment NS01 + S06 + G06 isolates + *R. solanacearum.* on 14 until 42 days post inoculation (dpi). The percentage of DI in this treatment was only 15% (Table 1).

Table 1. Average of bacterial wilt disease incidence, 7 - 42 dpi (days post inoculation)

Treatments	Disease Incidence (%) day... post inoculation (dpi)					
	7	14	21	28	35	42
NG02+NS01+S06+*R.solanacearum*	0	2.50	5.00	9.50	14.5	24.0
NG02+NS01+G06+*R.solanacearum*	0	2.00	2.50	9.00	13.0	18.0
NS01+S06+G06+*R.solanacearum*	0	0.50	1.50	7.00	10.5	15.0
NG02+S06+G06+*R.solanacearum*	0	6.00	8.00	18.0	24.5	40.0
NG02+NS01+S06+G06+*R.solanacearum*	0	6.50	10.5	19.0	30.0	41.5
Control (-)	0	2.00	6.0	31.0	42.5	47.0
Control (+) *R.s*	0	79.0	100	100	100	100

Observations until 42 days after inoculation (dpi) showed that the mean disease incidence caused by *R. solanacearum* did not reach 50% in all treatment with different combinations of antagonist isolates and was lower than the controls. The low average of *R. solanacearum* infection suggested due to the presence of antagonist isolates. Microbial antagonism of plant pathogens occurs in several ways, the most common mechanisms being parasitism and predation, competition for nutrients or space, production of antimicrobial substances and induced resistance. These mechanisms has increased, it has become apparent that antagonism often involves the synergistic action of several mechanisms. Competition occurs when two or more organisms require the same resource for growth and survival. The use of this resource by one organism reduces the amount available to the other (Cook, 1983).

The occurrence of competition for space and nutrients lead many microorganisms to form dormant structures that are resistant to extreme environment. Competition space and nutrients can also affect the growth of other microbes. The rhizosphere is a region of intense microbial activity where there may also be competition for oxygen.

Combination of bacterial isolates in suppression of plant pathogen was demonstrated in the research of Taylor and Guy (1981), by using of *Clostridium* and *Bacillus* isolates in suppression of *Peniophora sacrata.* Spores of the bacteria present in sites of former anaerobic activity are stimulated to develop in the new anaerobic environment produced by the flooding, known to produce anaerobic conditions within 24 hr (Ponnamperuma, 1972). This would enable the anaerobic bacteria to colonize an entire soil. A similar kind of control may be responsible for reduction of *Ralstonia solanacearum* of potato in aeroponic system. Bacteria inoculation to nutrient solution seems promising in increasing seed production using aeroponics.

3.2 Number and Weight of Tubers

Figure 1 showed that the highest mean number of tubers (162.5) and weight of the potato tuber (311.25 g) found with treatment containing NS01 + S06 + G06 + *R. solanacearu*. In contrast, the lowest mean number of 59.5 tubers and 105 g weight of tubers was found in treatments NG02 + NS01 + S06 + G06 + *R. solanacearum*. There was a high disease incidence which reached up to 41.5% thereby reducing the plant productivity. However, statistical analysis indicated that the average number and weight of tubers produced in the treatment of NS01 + S06 + G06 + *R. solanacearum* was not significantly different with other treatment except the treatment with NG02 +NS01 + S06 + G06 + *R. solanacearum*, control (-) and control (+).

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line defense against root attack by pathogens. Lwin and Ranamukhaarachchi (2006) showed that the application of bio-control agents also may affect the antagonist's action prior to pathogen attack. Pathogens encounter antagonism from rhizophere microorganisms before and during primary and secondary root infection. According to Weller (1988), root-associated

bacteria are an important group of beneficial microorganisms for controlling soil-borne pathogens and promoting plant growth promotion.

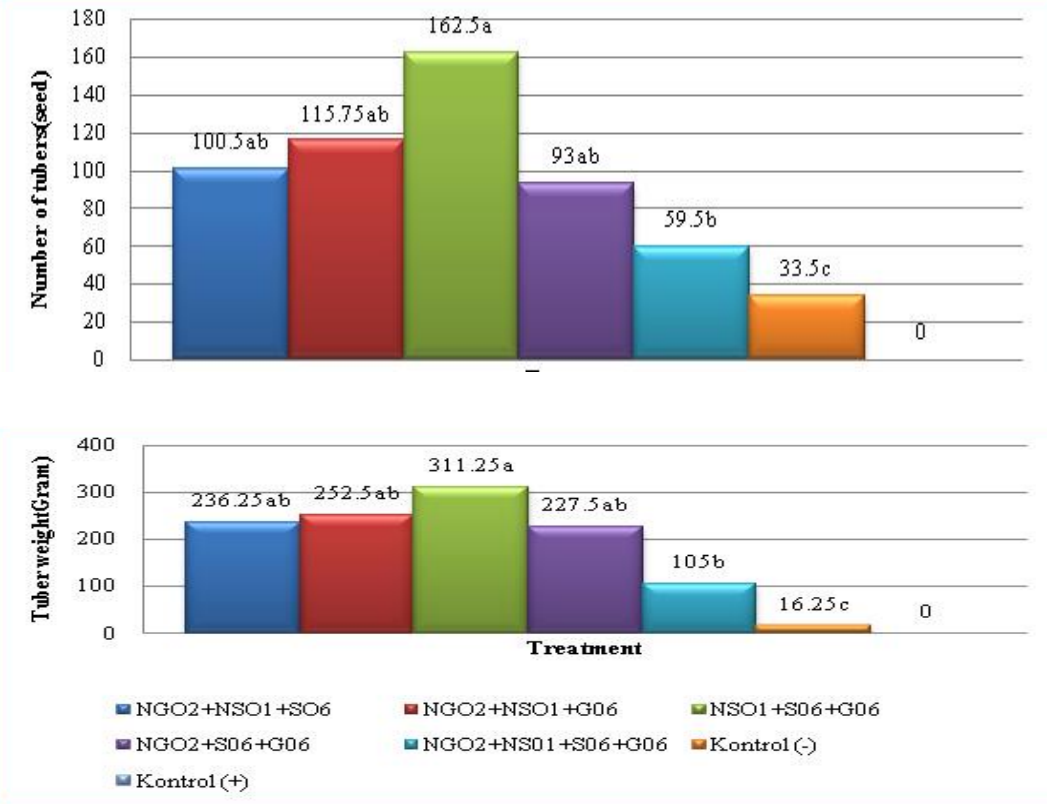

Figure 1. Average number (top) and weight of potato tubers (bottom) in all treatments

4. Conclusion

A combination of three isolates of *Clostridium* sp i.e: NS01 + G06 + S06 had the highest synergistic activities in potato aeroponic cultivation system. They were more effective in suppressing the bacterial wilt disease (85%) where the amount of seed tubers (162.5 tubers/treatment) and the average weight of tubers (311.25 g/ treatment) were both increased.

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