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Consortia of Indigenous Rhizobacteria to Control Bacterial Wilt in Ginger

Naziha Diyanatur Rosiyah[1*], Ayu Wike Widiasari[1], Achmad Roekhan[1], and Restu Rizkyta Kusuma[2]
[1]Agroecotechnology, Faculty of Agriculture, University of Brawijaya, Indonesia
[2]Department of Plant Pest and Diseases, Faculty of Agriculture, University of Brawijaya, Indonesia

Email Address: naziha.diyana@student.ub.ac.id

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Abstract Ginger (Zingiber officinale Rosc.) is a type of rhizome plant that has high potential to be developed in Indonesia as a medicinal plant or spice. Inhibiting factors in ginger production is Ralstonia solanacearum which causes bacterial wilt disease. Rhizobacteria is a potential biological agent to control the disease. It is necessary to review from various study on rhizobacteria both the use of antagonistic bacteria singly or in a consortia. Aimed to analyze various sources of scientific literature regarding the potential of antagonistic bacteria singly or consortia compared to synthetic bactericides in controlling of R. solanacearum. The results showed that three recommended antagonist bacteria were found, namely Pseudomonas fluorescens, Bacillus subtilis, and Bacillus thuringiensis. which has the potential to control bacterial wilt disease. The rhizobacterial consortia are more effective as a sustainable, safe and environmentally friendly control on bacterial wilt disease with a percentage of disease incidence 10%. In addition, the rhizobacterial consortia are three and six times more effective than the single antagonistic bacteria and bactericides respectively.

Introduction

Ginger (*Zinger officinale* Rosc.) is a rhizome plant that has high potential to be developed in Indonesia as a medicinal plant or spice (BPPP, 2017). Several studies have reported that ginger essential oil contain of 0.82 to 1.68% contains active chemical compounds gingerol, zingerone, shogaol, and zingiberene which able to increase the activity of the immune system, treat various diseases, and as an antibacterial agents (Aryanta, 2019). Indonesian ginger has good competitiveness in the global market, Indonesia is ranked 4th in the world ginger exporting countries, under India, Vietnam, and China (Anggrasari & Mulyo, 2019). Ginger production decreased 4.24% in 2018, from 216,587 tons (BPS, 2018).

The main problem in ginger cultivation is bacterial wilt disease caused by pathogenic bacteria *Ralstonia solanacearum* (Hartati, 2012). This pathogen cause a decrease in the quality of the ginger rhizome and yields loss of up to 90% (Prameela & Bhai, 2019). Bacterial wilt disease in ginger plants has completely destroyed ginger cultivation in most of Asia, such as India, Thailand, the Philippines, and Indonesia (Mulya *et al*., 2000). These pathogens attack the stems and rhizomes of ginger and cause the plants to wither, yellow leaves and over time the plants will die. This pathogen is difficult to control because it attacks plants in the growth phase and causes land contamination so that it cannot be planted for a long time (Paret *et al.,* 2010).

In general, bacterial wilt disease can be controlled by several control methods such as resistant varieties and bactericides. Pathogen-free seedlings and resistant varieties are difficult to do because almost 85% of ginger planted areas are infected by pathogens and there are no ginger varieties that are resistant to these pathogens (Hartati, 2012). In other

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hand, the use of bactericides has a negative impact on the environment because it leaves toxic and residues (Tahat & Sijam, 2010). An environmentally friendly control is biological control, such as the use of antagonistic bacteria to control *R. solanacearum* as causal agent of bacterial wilt disease in single or in a consortia (Khan *et al.,* 2018). The use of consortia of antagonistic bacteria has the potential to inhibit the growth of pathogens by 25-70% (Bora *et al.,* 2016). Therefore, it is necessary to do review from various sources of scientific literature to examine the potential of consortia of antagonistic bacteria in controlling *R. solanacearum* as causal agent of bacterial wilt disease in ginger crops.

Materials and Methods

Literature Review

Primary data searches were sourced from several national and international scientific literature databases, such as *Science Direct, Springerlink, Elsevier*, and SINTA accredited national journals. The search was carried out using a combination of specific keywords, viz bacterial consortium × *Ralstonia solanacearum* in different plants: “consortium rhizobacteria × control × *Ralstonia solanacearum*”, and others. Searches were limited to articles published in English and Indonesian from January 2000 to August 2020. The initial data consisted of 65 relevant literature references then screened and 20 literature references were determined as the primary data source. Reference sources were examined specifically to discuss the utilization of the bacterial consortium against *R. solanacearum* compared to other alternative controls (singly and bactericides). Specific discussions are classified based on the type of control, the method used and the inhibition results in vitro and in vivo.

Expert evaluation based on control alternatives

The literature references were compiled and tabulated based on alternatives to control bacterial wilt disease, and then consulted to the expert for further improvement and updating. Reference data criteria were made based on the “feasibility” of each management alternative using the effective criteria as pathogen control for *R. solanacearum*. The data were collected presented descriptively in the tabular form of each control alternative and compared based on its feasibility.

Result and Discussion

Characteristics of pathogens that cause bacterial wilt

Ralstonia solanacearum is a soil borne pathogen that causes the bacterial wilt disease in several crops. These bacteria are belonged to the Prokaryote, Division of Gracilicutes, Class of Proteobacteria, Family of Pseudomonadaceae, Species of *Ralstonia solanacearum* (Agrios, 2005). *Ralstonia solanacearum* is in irregular shape and form white mucus colonies. *Ralstonia solanacearum* is a rod shape, gram-negative, aerobic, non-spore, move with one flagellum at the poles, 0.5−0.7 × 1.5−2.0 µm, in soils with a pH of 4−8.5 and with suitable temperature ca. 13−37˚C (Istiqomah & Kusumawati, 2018). The characteristics of virulent *R. solanacearum* in TZC (*Triphenyltetrazolium Chloride*) media are indicated by irregular colony shape, cloudy white color, and pink color in the middle with flat edges. Meanwhile, avirulent bacteria are irregular in shape, smaller in size with a white border, and an overall red color in the middle (Ayin *et al*., 2019) (Figure 1).

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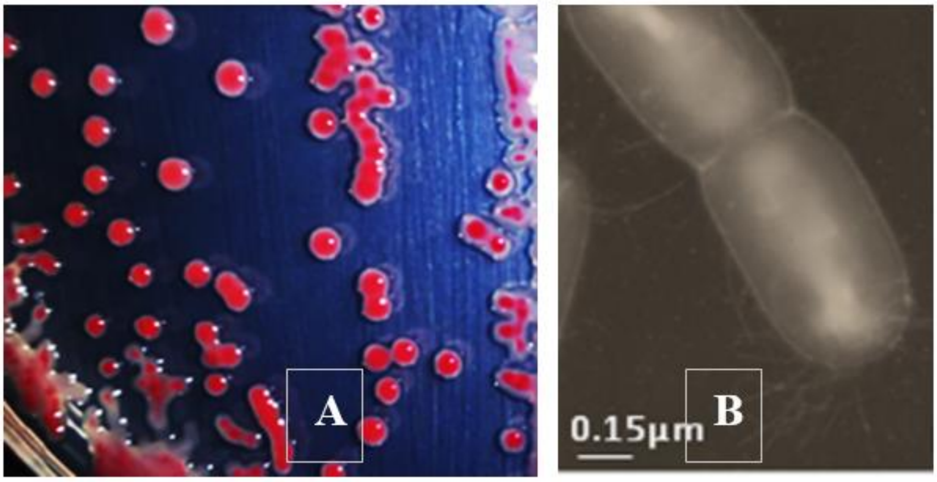

Figure 1. (A) Growth of *R. solanacearum* on TZC (Ayin et al., 2019). (B) Microscopic Appearance of *R. solanacearum* (Istiqomah & Kusumawati, 2018)

Ralstonia solanacearum Control with bactericide

Several bactericides with various active ingredients have been reported to be effective in inhibiting the development of *R. solanacearum*. Bactericides such as algicides (3- [3-indolyl] butanoic acid), fumigants (metam sodium, 1,3-dichloropropene, and chloropicrin) have been widely used to control bacterial wilt disease (Kurabachew & Wydra, 2014). Several bactericidal active ingredients can be used to control bacterial wilt disease caused by the pathogen *R. solanacearum* (Table 1).

Table 1. Inhibition of *R. solanacearum* with various bactericidal active ingredients

Bactericidal active ingredients	Host Plant	Inhibition Results		Reference
		Inhibition *In Vitro*	Disease Incidence *In Vivo*	
Bismerthiazol 100 μg mL^{-1}	Tobacco	99%	60%	Su *et al.* (2017)
Dazomet polyethylene film	Ginger	95.8%	56.3%	Mao *et al.* (2017)
Magnesium Oxide 250 mg mL^{-1}	Ginger	67.78%	93.20%	Cai *et al.* (2018)
Copper hydroxide 2g L^{-1}	Tomato	61.54%	52.78%	James & Mathew (2015)
Thiodiazole-copper 100 μg mL^{-1}	Tobacco	35%	55.6%	Su *et al.* (2017)
Streptomycin 100 mg L^{-1}	Tobacco	2.24%	97.76%	Li *et al.* (2014)

Bismerthiazol 100 μg mL-1 has the highest percentage of pathogen inhibition ca. 99% with 60% disease incidence. While the inhibition of Copper hydroxide was 61.54%, with 52.78% disease incidence (Table 1). So that, the use of bactericides has not been said to be effective because there is still a high disease intensity (more than 50%). This is because of not all bactericidal active ingredients are effectively used to control plant pathogens (Cai *et al.,* 2018). According to research conducted by Li *et al*. (2014) in general farmers used streptomycin, dazomet, copper hydroxide, and bismerthiazol to control of bacterial wilt disease. Based on mechanism, the bismerthiazol inhibit the formation of pathogenic bacterial cell walls (Su *et al.,* 2017). Bactericides have the ability to inhibit

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the growth of plant pathogenic bacteria by producing antibiotic compounds i.e. bacteriostatic (by inhibit the growth of pathogenic bacteria), and bactericidal (by killing the pathogenic bacteria) *in vitro* (Li *et al.,* 2014).

Antagonistic Bacteria as Biocontrol of Ralstonia solanacearum

Several studies reported that the antagonist bacteria *Pseudomonas fluorescens* and *Bacillus subtilis* were effective and efficient in suppressing the growth of *R. solanacearum* bacteria in vitro (Singh & Jagtap, 2017). Some of the antagonistic bacteria were used to control bacterial wilt disease that caused by the pathogen *R. solanacearum* (Table 2).

Table 2. Antagonistic Bacteria as Biocontrol Agents of *Ralstonia solanacearum*

Antagonistic Bacteria	Host Plant	Inhibition Result		Reference
		Zone Inhibition *In vitro*	Disease Incidence *In vivo*	
Bacillus lincheniformis	Ginger		33%.	Prameela & Bhai (2019)
Pseudomonas fluorescens	Tomato	21.38 mm	40%	Istiqomah & Kusumawati (2018)
Bacillus subtilis		23.12 mm	50%	
Arthrobacter sp.	Ginger		55%	Zhang *et al.* (2018)
Pantoea dispersa			61%	
Burkholderia sp.			63%	
Pseudomonas fluorescence	Ginger	24.33 mm		Singh & Jagtap (2017)
Bacillus subtilis	Ginger	19.33 mm		
Pseudomonas diminuta	Ginger	3.6 mm		Sanjaya *et al.* (2016).
Pseudomonas fluorescens		1.8 mm		
Pseudomonas putida		1.3 mm		

The results of inhibition of antagonistic bacteria *in vitro* showed the ability to suppress the growth of pathogens with an *in vivo* incidence rate of 33-63%. (Table 2). Mechanism of *P. fluorescens, Pantoea dispersa, Burkholderia* sp., and *Arthrobacter* sp. are by producing a siderophore metabolite compound (Yuliar *et al.,* 2015). In detail, the mechanism of siderophore in inhibiting the growth of pathogens is binding to iron (Fe). Binding ion Fe (iron) is siderophore mechanism to inhibit pathogen growth. By iron (Fe) binding, pathogen cannot infect plant tissues (Prihatiningsih *et al.,* 2017). *Pseudomonas diminuta* breaks chitin polymers in pathogenic microorganisms by chitinase enzymes, and play a role in increasing plant resistance to pathogenic attacks (Sanjaya *et al.,* 2016). Meanwhile, *Bacillus subtilis* has the ability to produce 68 types of antibiotics and is effective as a biocontrol agent (Prameela & Bhai, 2019). So that the inhibition of antimicrobial compounds can be done by damaging the cell wall, changing the permeability of the cell membrane, and finally, cell membrane damage produces the inhibition of cell growth and cell death (Djereng *et al*., 2017)

Rhizobacteria consortia as biocontrol of Ralstonia solanacearum

Some of the antagonistic bacteria in consortia are effective in inhibiting the pathogen of *R. solanacearum*, such as *Pseudomonas fluorescent, P. cepacia* and *Bacillus subtilis.* They can reduce the percentage of disease incidence from 47% to 7% (Mulya *et al.,* 2000). Several consortia of antagonistic bacteria used to control bacterial wilt disease in Table 3.

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Table 3. Consortia of antagonistic bacteria as biocontrol of *R. solanacearum*

Antagonistic Bacteria	Host	Inhibition Result		Reference
		Inhibition *In Vitro*	Disease Incidence *In vivo*	
Trichoderma viride + Bacillus thuringiensis + P. fluorescens	Hydroponic Vegetables	70.27%		Khan *et al.* (2018)
T. viride + B. thuringiensis		63.83%		
T. viride + P. fluorencens		59.84%		
P. fluorescens + Trichoderma	Ginger	55%	20%	Nandish *et al.* (2019)
P. fluorescens + T. harzianum	Ginger	34%	15.63%	Bora *et al.* (2016)
T. harzianum + B. subtilis	Potato	13.5 mm	10.14%	Hanudin *et al.* (2012)
T. harzianum +P. fluorescens		13.5 mm	10.14%	
T. harzianum + B. subtilis + P. fluorescens		14.5 mm	35.27%	
P. fluorescens + Bacillus sp.	Ginger	18.8-15.3 mm	50.5%	Shanmugam *et al.* (2013)
Bacillus subtilis + Burkholderia cepacia		15.2-14.8 mm	87.8%	
P. fluorescens + P. cepacia	Ginger		27%	Mulya *et al.* (2000)
P. cepacia + Bacillus sp.			27.1%	
P. fluorescens + Bacillus sp.			27.12%	
P. fluorescens + P. cepacia + Bacillus sp.			31.25%	
P. fluorescens +Trichoderma sp.	Potato		31%	Istifadah *et al.* (2019)
B. amyloliquefaciens + B. subtilis	Potato		32%	Ding *et al.* (2013)

The study of the inhibition of the pathogen *R. solanacearum* showed that the consortia of antagonistic bacteria *in vitro* was able to inhibit pathogens from 34 to 70% with 10-30% disease incidence (Table 3). The use of a consortia of antagonistic bacteria has advantages, such as providing better crop yields, increasing plant resistance, and suppressing the development of pathogens (Khan *et al.,* 2018). The existence of synergism and a combination of roles (nutritional competition, antibiotics, induction of plant resistance) simultaneously as a biocontrol and bio-stimulant in plants is a factor that causes the consortia of antagonistic bacteria to be effective in controlling plant pathogens (Mulya *et al.,* 2000). The ability of bacteria found in the rhizosphere has mechanisms i.e. competition and direct antibiosis against pathogens. The mechanism of antibiosis is by producing toxin compounds and secondary metabolites that can inhibit pathogen growth and nutritional competition (Nandish *et al*., 2019). Synergism between antagonistic bacteria in the synthesizing antibiotics can produce the high inhibition of pathogen growth by producing antimicrobial substance. (Yuliar *et al.,* 2015). Therefore, the antagonistic bacteria *P. fluorescens, P. cepacia, B. subtilis, B. amyloliquefaciens,* and *B. thuringiensis* have a great ability to inhibit the growth of the pathogen *R. solanacearum* and increase the plant resistance.

Effectivity analysis of biological agents and bactericides against Ralstonia solanacearum

Bacterial wilt disease caused by the pathogen *Ralstonia solanacearum* is reported to attack various types of plants as a whole, causing quite high yield losses of up to 90% and causing plants death (Arwiyanto, 2014). Therefore, it is necessary to control the pathogen *R. solanacearum* which is effective, safe, environmentally friendly and can reduce the loss of control costs in plant cultivation. Analysis of the effectiveness of control of biological agents and bactericides against the pathogen *R. solanacearum* showed in Table 4.

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Table 4. Analysis of control effectiveness against inhibition of *R. solanacearum*

Parameter	Control Techniques		
	Bactericide	Single Bacteria	Bacteria Consortium
Inhibition Effectiveness	*In vitro* 99% & 60% incidence rate (James & Sally Mathew, 2015) Fast inhibition, application is carried out when the plant appears sick symptoms (Reddy, 2014)	Disease incidence 33% (Zhang *et al.,* 2018) Long time inhibition, application pre-emptively so inhibition is effective (Singh & Jagtap, 2017)	*In vitro* 25-70% & Disease incidence 10% (Hanudin *et al.,* 2012) Long time inhibition, application pre-emptively so inhibition is effective (Singh & Jagtap, 2017)
Economic Impact	Quickly run out (evaporate) and intensity of use is not controlled so that production costs are high (Raini, 2015)	Sustainable, Research, and expertise costs (skills) (Zamrodah, 2015)	Sustainable , Research, and expertise costs (skills) (Zamrodah, 2015)
Environmental Impact	Resistance to pathogenic bacterial strains, Human health, and Environmental pollution (Raini, 2015)	Safe and environmentally friendly, The balance of soil ecosystem (Zamrodah, 2015)	Safe and environmentally friendly, The balance of soil ecosystem (Zamrodah, 2015)

Bactericidal control showed a higher inhibitory effectiveness compared to control using antagonistic bacteria (James & Sally Mathew, 2015). However, it cannot be said to be effective in inhibiting the pathogen *Ralstonia solanacearum*. This is because the rate of bacterial wilt disease in plants by applying bactericidal control is still high, more than 50% compared to control with the use of antagonistic bacteria (rhizobacteria) ranging from 10 to 32% (Table 4)**.** So that, the best alternative solution in suppressing the development of pathogens of *R. solanacearum* is biological control based on consortia of antagonistic bacteria by considering the effectiveness of inhibition of the pathogen *R. solanacearum* from the efficiency of cost losses and the impact on the environment of each control to maintain the stability of potential microorganisms in soil and sustainable.

Conclusion and Suggestion

Three recommended antagonists are found viz. *Pseudomonas fluorescens, Bacillus subtilis,* and *Bacillus* thuringiensis which have the potential to control bacterial wilt disease. Rhizobacterial consortia are more effective as a sustainable, safe and environmentally friendly control of bacterial wilt with 10% disease incidence and 3 and 6 times more effective than single antagonistic bacteria and bactericide. Further, research is needed regarding the synergy between antagonistic bacteria, feasibility tests and their potential as an alternative control for bacterial wilt disease.

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