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BIOLOGICAL CONTROL OF BACTERIAL WILT IN SOUTH EAST ASIA

PENGENDALIAN HAYATI PENYAKIT LAYU BAKTERI DI ASIA TENGGARA

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ABSTRACT

Bacterial wilt disease caused by *Ralstonia solanacearum* destroys many crops of different plant families in South East Asia despite many researches about the disease, and the availability of developed control method in other parts of the world. There is no chemical available for the bacterial wilt pathogen and biological control is then chosen as an alternative to save the crops. Most of the biological control studies were based on antagonism between biological control agent and the pathogen. The biological control agents were intended to reduce the initial inoculum of the pathogen. The effort to minimize the initial inoculum of the pathogen by baiting with the use of hypersensitive host-plant was only reliable when conducted in the greenhouse experiments. Various microorganisms have been searched as possible biological control agents, for instance avirulent form of the pathogen, soil or rhizosphere bacteria (*Bacillus* spp. and fluorescent pseudomonads), actinomycetes (*Streptomyces* spp.), yeast (*Pichia guillermondii*, *Candida ethanolica*), and a consortium of microorganisms known as effective microorganisms (EM). None of these biological control agents has been used in field application and they need further investigation in order to effectively control bacterial wilt. Opportunities and challenges in developing biological control to combat bacterial wilt are discussed in the paper.

Key words: bacterial wilt, biological control, *Ralstonia solanacearum*

INTISARI

Penyakit layu bakteri yang disebabkan oleh Ralstonia solanacearum *menghancurkan banyak tanaman dalam famili yang berbeda di Asia Tenggara meskipun telah banyak penelitian tentang metode pengendaliannya. Penyakit ini sulit dikendalikan karena banyaknya variabilitas patogen dan belum tersedianya sumber ketahanan yang mapan. Di samping itu, sampai saat ini belum ada bahan kimia yang tersedia untuk patogen layu bakteri ini sehingga pengendalian biologi kemudian dipilih sebagai cara alternatif untuk menyelamatkan tanaman. Sebagian besar penelitian pengendalian biologi didasarkan pada antagonisme antara agen pengendalian biologi dan patogen. Agen pengendalian biologi tersebut dimaksudkan untuk mengurangi inokulum awal patogen. Upaya untuk meminimalkan inokulum awal patogen dengan umpan dengan menggunakan tanaman inang sangat rentan hanya dapat diandalkan ketika dilakukan dalam percobaan rumah kaca. Berbagai mikroorganisme telah diteliti kemungkinannya sebagai agensia pengendalian biologi seperti bentuk avirulen dari patogen, bakteri tanah atau bakteri rizosfer (*Bacillus *spp. dan pseudomonad fluorescen), actinomycetes (*Streptomyces *spp.), khamir (*Pichia guillermondii*,* Candida ethanolica*), dan konsorsium mikroorganisme yang dikenal sebagai EM (*Effective Microorganisms*). Meskipun demikian tidak satupun agensia pengendalian biologi ini sampai pada taraf aplikasi lapangan sehingga diperlukan penelitian lebih lanjut. Peluang dan tantangan dalam mengembangkan pengendalian biologi untuk memerangi penyakit layu bakteri dibahas pada tulisan ini.*

Kata kunci: layu bakteri, pengendalian biologi, Ralstonia solanacearum

INTRODUCTION

There are many bacterial wilt diseases of plant. This paper discuss bacterial wilt caused by *Ralstonia solanacearum* and mostly on Solanaceous crops. Bacterial wilt caused by *R. solanacearum* is one of the most devastating disease of crops. It attacks more than 200 species of plants, most of them are cash crops (Hayward, 1994). Bacterial wilt occurs in tropical countries with the moderate to severe crop losses have been reported (Arwiyanto, 1995; Elphinstone, 2005). Due to their numerous number of host plants and highly diverse of the pathogen (Sequeira, 1993) make the disease difficult to control by cultural practices, especially crop rotation. The diversity of *R. solanacearum* strains is shown by five races based on host range (Hayward, 1994), five biovars based on physiological and biochemical characteristics (Hayward, 1991), and four phylotypes roughly corresponding to geographic origins (Fegan & Prior, 2005). The use of chemical for controlling this pathogen only visible in the greenhouse production. In addition, by using chemical, there is a potential destruction of soil microflora; many beneficial microorganisms will be killed by this method. The chemical effective against *R. solanacearum* is highly biocide and it is not always available in South East Asian

countries. Therefore development of alternative method of control which is environmentally sound is preferable. Among them, biological control has been investigated with an increased interest and has been reviewed (Trigalet *et al*., 1998). In the last decade, numerous studies have been devoted to bacteria showing a capacity to control *R. solanacearum* either by producing antibiotics or bacteriocins which inhibit growth of the pathogen within the rhizosphere or by inducing host plant resistance. These bacteria are distributed among different genera and species including *Pseudomonas fluorescens*, *Pseudomonas glumae*, *Pseudomonas cepacia* (Aoki *et al*., 1991), *Bacillus* spp., *Erwinia* spp., and spontaneous avirulent mutants of *R. solanacearum* (Arwiyanto *et al*., 1994). Bacteriophage have been reported as one of biological control agent, however, there is no paper describing the work in South East Asian countries.

Although such studies frequently gave promising results under *in vitro* or controlled conditions, the use of bacteria antagonistic towards *R. solanacearum* in naturally infested soils has met with limited success. Poor competition ability and poor edaphic adaptation of the introduced organism that is in competition with the indigenous microflora, particularly within the rhizosphere, are cited as the most probable causative explanations.

Reducing initial inoculum (potential inoculum) is important in the management of soilborne disease such as bacterial wilt. A highly susceptible of tomato cultivars were used for extracting *R. solanacearum* from soil. It was successful in reducing the amount of initial inoculum but the incidence of the disease was still high. When 30 days old seedlings were used as a bait and the plants were pulled out at 7 days after transplanting (L treatment) , the result obtained was comparable with antibiotic treatment (control) (Figure 1). The use of younger seedlings did not succeed in reducing the bacterial inoculum in the soil (treatment A−H).

MICROORGANISMS USED AS BIOLOGICAL CONTROL AGENTS

There are many crops have been reported abandoned by *R. solanacearum* in South East Asian countries; tomato, eggplant, tobacco, potato, chili pepper, peanut, sesame, ginger, most of them are cash crops. Many microorganisms belong to several genus have been explored as biological control agent against *R. solanacearum* (Table 1). Exploring the biological control agent is started by isolation of the candidates mostly from rhizosphere of host or non-host plants. Antibiosis in vitro is then performed in double layer agar followed by greenhouse experiments and only

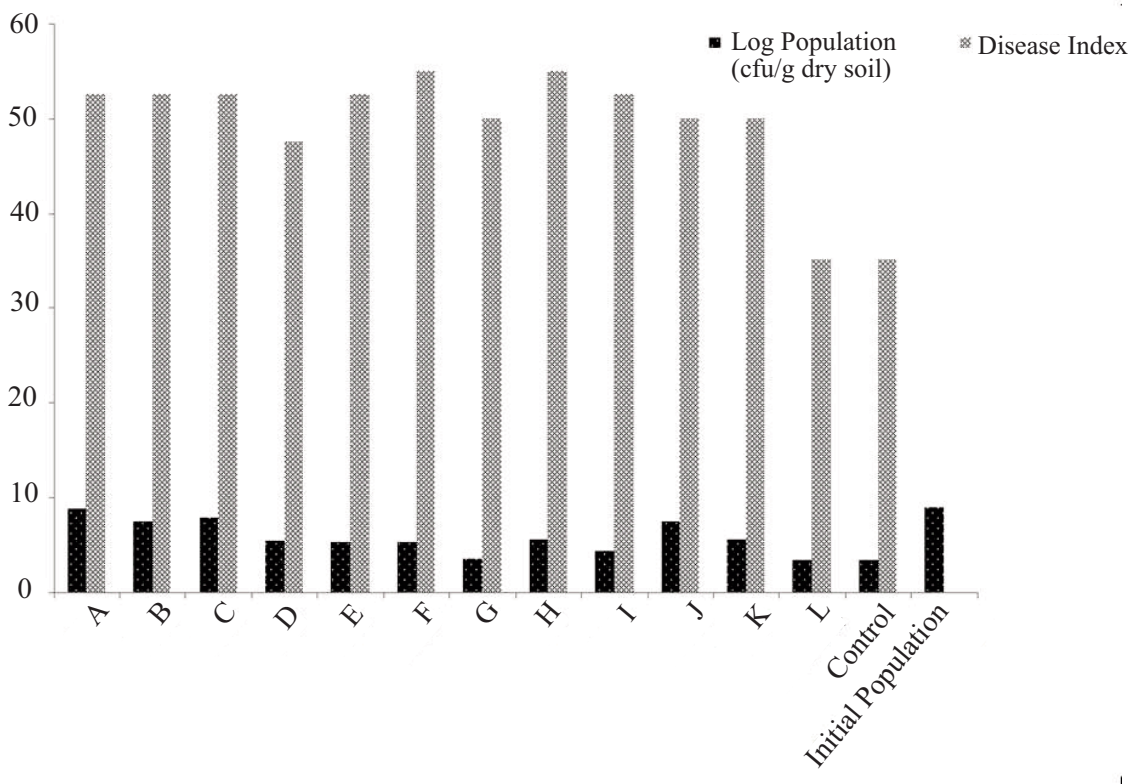

Figure 1. Disease index of bacterial wilt and population of *Ralstonia solanacearum* insoil treated with susceptible cultivar (Arwiyanto *et al*., 2011)

a few go to field test. Although the scheme could screen thousand's isolates in a time, its exclude mechanisms other than antibiosis such as competition (food and space) and synergism which is believed occur in the rhizosphere of plants. Most of the antagonists were used as a singly rather than in a consortium of micro-organisms in the control of the pathogen. Accordingly, a consortium of microorganisms will control pathogen better than single use although it is not always the case. The mixture of fluorescent pseudomonads, *Bacillus* spp. and Streptomycetes could not protect tobacco plants from wilting, however, the use of single antagonistic biocontrol agents gave significant protection (Arwiyanto, 2007). The similar result was reported in Thailand using the mixture of antagonist.

Nevertheless, a consortium of microorganisms called EM was reported could suppress bacterial wilt in pot trials (Lwin & Ranamukhaarachchi, 2006). Four antagonists: *Bacillus megaterium*, *Enterobacter cloacae*, *Pichia guillermondii,* and *Candida ethanolica* were tested and found significantly antagonistic to *R. solanacearum* in *in vivo* conditions with unsterilized soils and hence possess potential to control BWD in tomato. *E. cloacae*, *P. guillermondii* and *C. ethanolica*

Table 1. Saprophytic bacteria and bacteriophages that have been studied as biological control agents against bacterial wilt

Antagonist	Author
Pseudomonas fluorescens and fluorescent pseudomonads	1. Kempe & Sequeira (1983) 2. Ciampi-Panno *et al*. (1989) 3. Gallardo *et al*. (1989) 4. Anuratha & Gnanamanickam (1990) 5. Nasrun *et al*. (2004) 6. Doan & Nguyen (2005) 7. Wydra *et al*. (2005) 8. Arwiyanto *et al*. (2007)
Pseudomonas glumae	1. Wakimoto (1987) 2. Furuya *et al*. (1991)
Pseudomonas cepacia	1. Aoki *et al*. (1991)
Pseudomonas putida	1. Arwiyanto & Hartana (2001) 2. Irawati *et al*. (2003) 3. Anith *et al*. (2004) 4. Asrul *et al*. (2004) 5. Wuryandari *et al*. (2004) 6. Arwiyanto & Nurcahyant (2007) 7. Kurabachew & Wydra (2008)
Bacillus sp.	1. Fucikovsky *et al*. (1989) 2. Anuratha & Gnanamanickam (1990) 3. Phae *et al*. (1992) 4. Anith *et al*. (2004) 5. Prihatiningsih *et al*. (2006) 6. Djatmiko *et al*. (2006) 7. Kurabachew & Wydra (2008) 8. Nguyen *et al*. (2011)
Avirulent mutants of *Ralstonia solanacearum*	1. Kempe & Sequeira (1983) 2. Chen & Echandi (1984) 3. Tanaka *et al*. (1990) 4. Quimio & Ayo (1989) 5. Trigalet & Trigalet-Demery (1990) 6. Hara & Ono (1991) 7. Arwiyanto & Goto (1993) 8. Hertanto *et al*. (1997) 9. Irawati *et al*. (2003) 10. Arwiyanto & Nurcahyanti (2007) 11. Arwiyanto *et al*. (2010)
Streptomyces	1. Arwiyanto & Bustamam (2010) 2. Arwiyanto *et al*. (2007)
Bacteriophage	1. Alvarez *et al*. (2007) 2. Yamada *et al*. (2007) 3. Fujiwara *et al*. (2011)

Source: Arwiyanto (2011)

also increased growth and fruit yield compared to the control. Disease severity varied with antagonist and time of application; compared with the control, disease severity was reduced 41.6–77.1% when antagonists were applied one week prior to transplanting tomato seedlings (Nguyen & Ranamukhaarachchi, 2010). The pot trials should be extended into field experiments before the technology is adopted by farmers. Moreover, as suggested by the authors, rigorous toxicological testing are needed to establish whether strains of *E. cloacae* and *P. guilliermondii* pose a human health risk.

The use of mycorrhizae in the management of bacterial wilt was reported in Malaysia. *Glomus mosseae* suppress tomato bacterial wilt due to better nutrient uptake and thus enhance plant growth (Tahat, 2009). Other author reported that in the greenhouse experiment, the incorporation of tree bark compost in potting mixes of peat:sand:compost in ratio of 5:3:2 added with antagonist Pa II (*Pseudomonas aeruginosa*) and Kts 26 (*Pseudomonas putida*) individually, and Kt8 (*Pseudomonas aeruginosa*) + B333 (*Pseudomonas aeruginosa*) in combination antagonists significantly gave lower disease severity index and disease incidence of bacterial wilt of tomato compared to other antagonists tested. In the field trial using the three antagonists showed that the tree bark compost potting mix added with Kt8+B333 in combination gave 57% reduction of bacterial wilt disease of tomato which was better compared to Pa II (50%) and Kts 26 (50%) individually (Masyitah, 2004).

Application of *P. fluorescens* (B 12), *P. aeruginosa* (B 292) and *Trichoderma* sp. (F 196) individually and in combination to control bacterial wilt of tomato was carried out in greenhouse trial. Results showed that combination treatments of B 292 and B 12; B 292 and F 196; B 12 and F 196 and B 292, B 12 and F 196 significantly reduced bacterial wilt compared to the individual treatments of antagonist. However, all the treatments were able to reduce the disease significantly compared to the control (Kwai Hoe, 2004).

Bio-product, EXTN-1 with the greatest efficacy under greenhouse condition was tested for the ability to reduce bacterial wilt, fusarium wilt and foot rot under field condition at Song Phuong and Thuong Tin locations in Ha Tay province, Vietnam. Under field condition, EXTN-1 provided a mean level of disease reduction more than 45,0% against all three diseases compared to water treated control. Besides, EXTN-1 treatment increased the yield in tomato fruits 17.3% than water treated control plants (Figure 2) (Thanh *et al*., 2009).

In Indonesia, the work on biological control of *R. solanacearum* mostly conducted by author. Sumatra tobacco which is famous as a cigar wrapper was devastated by bacterial wilt (Figure 3) and the only effective but not efficient to control was done by choosing a new site, for planting, which do not has history of bacterial wilt. Long crop rotation with forest trees for 3 years followed by twice of sugarcane (3 years) then with *Mimosa invisa* for 6 months do not eliminate the incidence of bacterial wilt. While the wilt

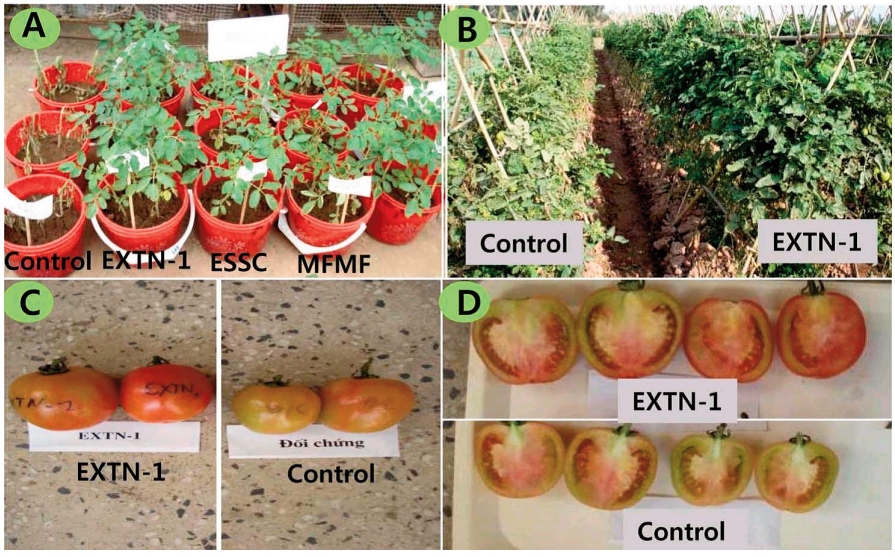

Figure 2. Plant growth promotion and control of disease incidence on tomato by PGPR strains under greenhouse and field conditions (A: control of disease incidence of bacterial wilt caused by *Ralstonia solanacearum* by treatment with PGPR strains compared to water treated control plants; B: enhancement of green pigmentation in leaves; C: enlargement of fruit size; D: increment of fleshy part by EXTN-1 treatment compared to water treated control) (source: Thanh *et al*., 2009)

Figure 3. Deli/Sumatra cigar tobacco plantation infected with bacterial wilt caused by *Ralstonia solanacearum*

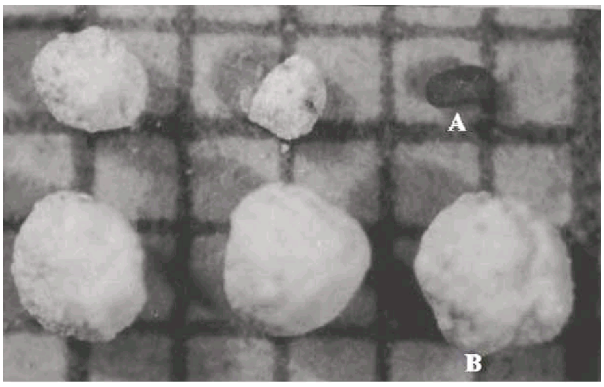

Figure 5. Tobacco seed (A) coated with inorganic matrix plus *Pseudomonas putida* strain Pf-20 (B)

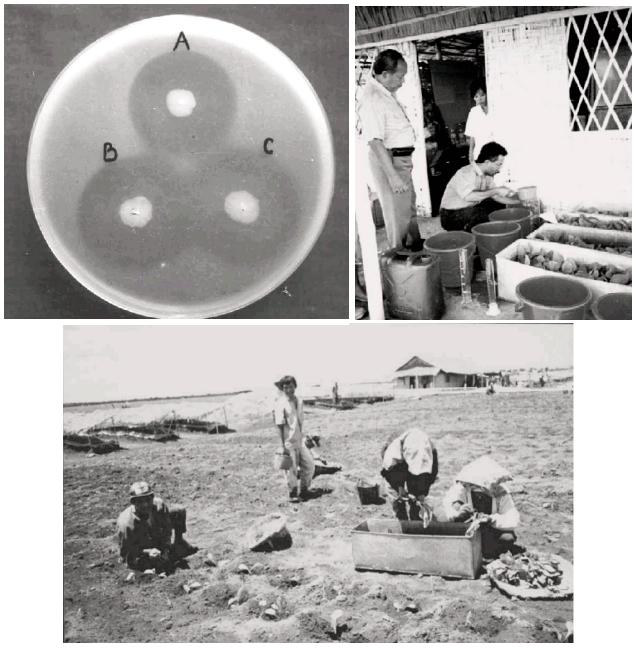

Figure 4. Growth inhibition of *Ralstonia solanacearum* by *Pseudomonas putida* strain Pf-20 in King's B media (top left), dipping tobacco root seedlings (top right) in the water suspension of *P. putida* strain Pf-20 before transplanting (below) (source: Arwiyanto & Hartana, 2001)

incidence could be attributed with the contaminated seedlings, the pathogen could be detected in the rhizosphere of sugarcane, indicated that the pathogen still survived for a long time in the absence of its host plant.

A pseudomonad fluorescens, later identified as *Pseudomonas putida* strain Pf-20, was isolated from the rhizosphere of *M. invisa*. The bacteria inhibited the growth of the pathogen *in vitro* (Figure 4), suppress bacterial wilt development in the greenhouse tests and in the field experiments. In the field application, however, it was very laborious work because the roots of tobacco seedling should be dipped in the water suspension of *P. putida* for 30 minutes before transplanting (Figure 4). Besides, the bacterial suspension was needed in a huge volume per hectare of plantation which is mean costly. To cope the drawback, *P. putida* then was incorporated into the outer space of tobacco seed by seed coating (Figure 5). The bacteria could survive in the coated seed but the degree of protection was inferior compared with root dipping. The development of this technology was hampered by fact that the company replaced the tobacco plant to more profitable commodity, oil palm (the plantation is run by state owned company).

The use of *P. putida* strain Pf-20 by root dipping as well as seed coating, unfortunately, was not able to control bacterial wilt of tobacco in plantation or farmer field in other part of Indonesia, Java Island (unpublished data).

The incidence of tobacco bacterial wilt in some area of Java island (Temanggung district of central Java) is prevalent and the disease intensity worsened due to the existence of *Meloidogyne incognita*. The work on biological control of this disease was started again from the beginning. Streptomycetes was chosen as a biological control agent and isolated from the rhizosphere of healthy tobacco plants which are exist around diseased plants. The methodology of this research, however, was quite different with the current protocol for biological control work. The isolated candidates of biological control agent were tested directly in the field. The results indicated that there are isolates which did not inhibit the pathogen growth *in vitro*, suppress disease development in the field (Table 2). This candidates will be eliminated at early stage when antibiosis is performed to screen the biological control agent. However, culture supernatant of all isolates tested were able to degrade egg mass of *M. incognita* (Figure 6).

Some strain of *R. solanacearum* produced bacteriocin *in vitro* did not inhibit the growth of *P. putida* strain Pf-20 (Arwiyanto & Nurcahyanti, 2007). When *P. putida* strain Pf-20 combined with an avirulent strain of *R. solanacearum* producing bacteriocin, they protected eggplant and chilli from bacterial wilt in a greenhouse experiments (Figure 7, 8; Arwiyanto *et al*., 2012).

Table 2. Disease index of tobacco bacterial wilt bacterial pathogen and inhibition of the pathogen by *Streptomyces* spp.

Isolate Number	Inhibition zone (mm)	Disease Index
Stre4	13.60	40
Stre7	7.40	50
Stre48	0.49	33
Stre61	0.00	40
Stre66	0.00	53
Stre67	0.00	40
Control	-	90

Source: Arwiyanto *et al.* (2007)
Note: *In vitro* test was performed after field testings.

Although there is a potential development of this antagonistic bacteria, the possibility of avirulent strain of *R. solanacearum* to convert into its virulent type force authors to halt this research. However, *P. putida* strain Pf-20 still be a subject of research on the biological control agent for tomato bacterial wilt. A combination of using this bacterium with grafting

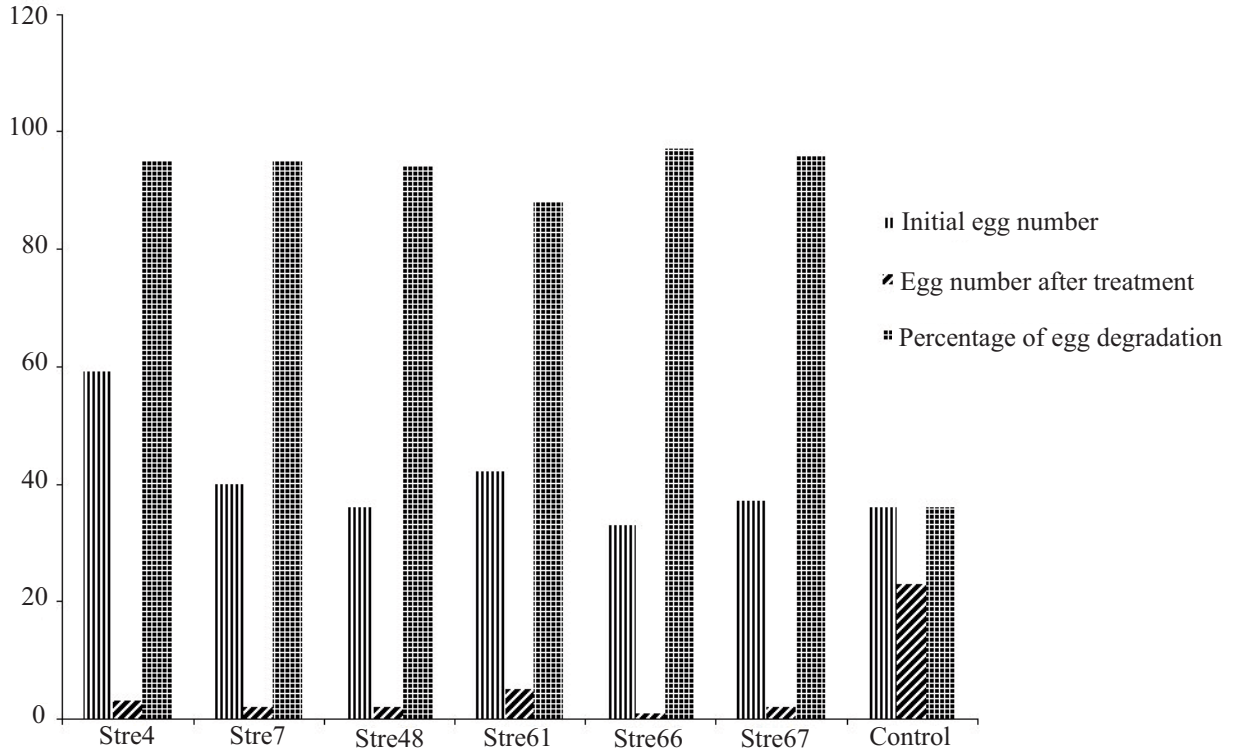

Figure 6. Nematode egg mass degradation by culture supernatant of *Streptomyces* spp. (source: Arwiyanto *et al.*, 2007)

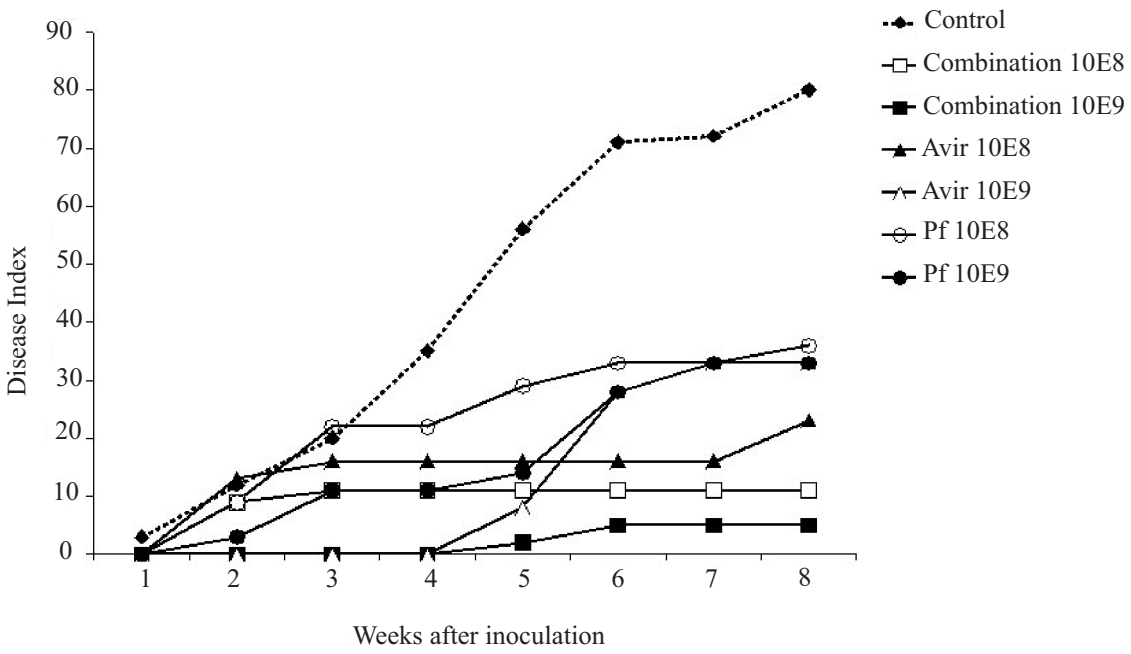

Figure 7. Development eggplant bacterial wilt treated with antagonistic bacteria (source: Arwiyanto *et al*., 2012)

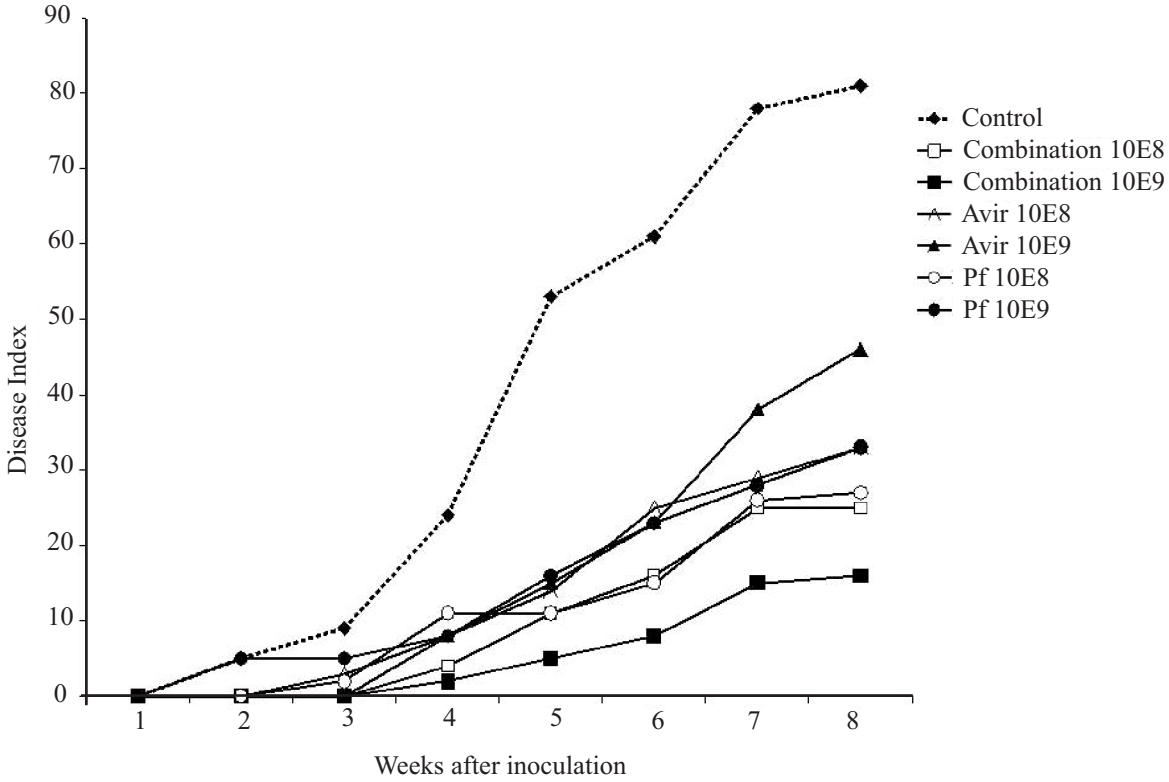

Figure 8. Development chilli bacterial wilt treated with antagonistic bacteria (source: Arwiyanto *et al*., 2012)

of commercial varieties with rootstock to manage bacterial wilt disease is under investigation. Population changing in the rhizosphere of tomato after seedling treatment with *P. putida* strain Pf-20 is investigated also.

Ginger is another important crop which the production also abandoned by bacterial wilt. In Indonesia, the disease cause mild to severe losses and reduce produce up to 90%. The disease has been controlled by cultural techniques such as the use of land which is free from *R. solanacearum*. Nevertheless, it is becoming more difficult for ginger farmers in Indonesia to find suitable planting areas that are not contaminated by the ginger wilt bacteria. An attempt to control ginger bacterial wilt was done by isolating Streptomycetes from the rhizosphere of healthy ginger. As much as 71 *Streptomyces* spp. were isolated and most of them inhibited the growth of the pathogen *in vitro* (Table 3). In this investigation, again it was proven that the inability of a biocontrol agent in inhibiting the growth of the pathogen *in vitro* does not mean that it could not suppress the disease caused by the pathogen (Table 4, Figure 9). The works on potato bacterial wilt are ongoing with *Bacillus* spp. Patchouli oil plant (*Pogostemon cablin*) is also devastated by bacterial wilt (*R. solanacearum*) and an attempt to control biologically did not work successfully as expected (Nasrun *et al*., 2005).

OPPORTUNITIES AND CHALLENGES

Bacterial wilt disease still occurs in many areas of the Southeast Asian countries and significantly reduced the production of cash crops and plantation crops. The complexity of *R. solanacearum* and many points of entry of the pathogen to the host plant, make it more difficult to be controlled. Understanding the biology of the pathogen is one of the keys to success for controlling this notorious plant pathogenic bacteria. However, research on the biology and epidemiology of *R. solanacearum* in the SE countries is very rare so it is likely that bacterial wilt caused still needs a long way to successfully controlled.

Table 3. Growth inhibition of *Ralstonia solanacearum* by *Streptomyces* spp.

Inhibition zone(mm)	The number of isolates of *Streptomyces* that inhibit *R. solanacearum*	Percentage (%)
0	22	30.96
> 0–5	20	28.17
> 5–10	10	14.08
>10–15	7	9.86
>15–22.5	12	16.90

Figure 9. Ginger bacterial wilt suppressed by *Streptomyces* spp. in pot trials (K+ control plot without *Streptomyces* spp., K-control plot without *Streptomyces* spp., and *Ralstonia solanacearum*) (source: Bustamam, 2011)

Table 4. Performance of *Streptomyces* in the control of ginger bacterial wilt

Isolate number	Percentage of Infected planta	Number of shoot	Rhizome weight (g/plant)	Zone of inhibition in vitro (mm)
S4	13.33a–c	9.00a–d	202.44c–e	0.00
S14	26.67bc	8.73a–d	206.83c–e	23.00
S21	13.33a–c	9.33a–d	289.69a–d	20.50
S24	6.67ab	8.87a–d	190.50de	3.50
S27	13.33a–c	9.00a–d	203.30c–e	13.75
S32	20.00a–c	10.20ab	350.67a	3.50
S34	26.67bc	9.60a–c	205.33c–e	7.50
S38	26.67bc	10.07ab	190.38de	28.00
S39	6.67ab	8.40b–d	189.03de	1.00
S40	26.67bc	9.67a–c	191.46de	1.00
S42	6.67ab	11.47a	266.75a–e	5.00
S45	6.67ab	8.93b–d	260.54a–e	0.00
S47	13.33a-c	10.27ab	203.50c–e	21.75
S48	6.67ab	8.53b–d	145.50e	9.50
S49	20.00a–c	10.13ab	198.39c–e	5.50
S50	26.67bc	7.27c–d	172.17de	18.00
S51	13.33a–c	11.47a	201.33c–e	18.00
S53	6.67ab	9.80a–c	315.00a–c	2.00
S57	20.00a–c	9.20a–d	292.25a–d	19.75
S59	6.67ab	9.33a–d	263.71a–e	2.00
S60	13.33a–c	8.53b–d	228.56b–e	0.00
S64	26.67bc	10.60ab	218.21b–e	11.50
S65	26.67bc	6.80d	179.31de	19.50
S67	20.00a–c	10.80ab	327.33ab	11.50
Control	100.00d	9.07a–d	144.67e	

Biological control which has been studied so far mostly based on the antagonism between candidate of biological control agent and bacterial wilt pathogen. In the future, it is necessary to explore biological control agent which is also has capability in competing of space and food in the rhizosphere and rhizoplane of host plant (rhizosphere competent). Other traits needed are competitive saprophytic ability, sensitivity to chemicals, induction of resistance, production of antibiotic or other inhibition substance, ability to growth on different substrates (Cook & Baker, 1983; Desai *et al*., 2002).

Biological control is only one of the measures in the management of bacterial wilt disease. Therefore, in dealing with the disease in the field, it needs a combination of various other control methods such as exclusion, cultural control, use of resistant varieties, and soil treatments. While it has not been possible to accelerate the sanitizing effects of the soil microbiota by adding biological control agents directly to the soil, it is relatively easy to accelerate the process by adding compost, banyard manure, or other organic materials (Cook, 2000).

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