Jurnal Perlindungan Tanaman Indonesia, Vol. 18, No.1, 2014: 1–12

BIOLOGICAL CONTROL OF PLANT DISEASES CAUSED BY BACTERIA

PENGENDALIAN BIOLOGI PENYAKIT TUMBUHAN YANG DISEBABKAN OLEH BAKTERI

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ABSTRACT

Bacterial diseases in plants are difficult to control. The emphasis is on preventing the spread of the bacteria rather than curing the diseased plant. Integrated management measures for bacterial plant pathogens should be applied for successfull control. Biological control is one of the control measures viz. through the use of microorganisms to suppress the growth and development of bacterial plant pathogen and ultimately reduce the possibility of disease onset. The study of biological control of bacterial plant pathogen was just began compared with of fungal plant pathogen. The ecological nature of diverse bacterial plant pathogens has led scientists to apply different approach in the investigation of its biological control. The complex process of entrance to its host plant for certain soil-borne bacterial plant pathogens need special techniques and combination of more than one biological control agent. Problem and progress in controlling bacterial plant pathogens biologically will be discussed in more detail in the paper and some commercial products of biological control agents (biopesticides) will be introduced.

Key words: bacterial plant pathogen, biocontrol

INTISARI

Penyakit tumbuhan karena bakteri sulit dikendalikan. Penekanan pengendalian adalah pada pencegahan penyebaran bakteri patogen dan bukan pada penyembuhan tanaman yang sudah sakit. Untuk suksesnya pengendalian bakteri patogen tumbuhan diperlukan cara pengelolaan yang terpadu. Pengendalian secara biologi merupakan salah satu cara pengendalian dengan menggunakan mikroorganisme untuk menekan pertumbuhan dan perkembangan bakteri patogen tumbuhan dengan tujuan akhir menurunkan kemungkinan timbulnya penyakit. Sifat ekologi bakteri patogen tumbuhan yang berbeda-beda mengharuskan pendekatan yang berbeda pula dalam pengendaliannya secara biologi. Masalah dan perkembangan dalam pengendalian bakteri patogen tumbuhan secara biologi didiskusikan secara detail dalam makalah ini.

Kata kunci: bakteri patogen tumbuhan, pengendalian biologi

INTRODUCTION

Bacterium (bacteria, pl) is a unicellular procaryotic organism or simple associations of similar cells based upon growth habit, planes of division and cell separation (Murray, 1984). Most bacteria have cell walls and the shape of bacterium are round (cocciform), spiral shaped, and rod-shaped or bacilliform (Murray, 1984). Plant associated bacteria may be beneficial or detrimental (Vidaver & Lambert, 2004; Beattie, 2006; Arwiyanto, 2008) and most of plant-pathogenic bacteria are bacilliform (Goto, 1990). Bacteria can be found almost everywhere, in soil, water, food; inside and on the surface of human, animal, and plant but almost all plant pathogenic bacteria develop mostly in the host plant as parasites, on the plant surface, especially buds, as epiphytes, and partly in plant debris or in the soil as saprophytes (Agrios, 2005).

TJ Burril (1839–1916) was best known for discovery of the first bacterial disease of plants-bacterial blight of pear tree. He isolated the pathogen and gave name *Micrococcus amylovorus*, later changed to *Erwinia amylovora* (Tanner & Tanner, 1948). Since then, the bacterial plant pathology develop rapidly.

The importance of plant disease caused by bacteria is varied depending on the region because the economic of crops vary in each region. However, the available statistical data on yield loss caused by bacteria are very limited. Data from 1976 (Table 1) showed that bacterial leaf blight of rice was not in the list, indicated the minor importance in USA but, actually was very destructive and caused severe losses in Asia (Mew *et al.*, 1993). Even after more than three decades, there was no more statistical data about yield loss, additional data does not mention the number of losses (Table 2).

Control of bacterial plant pathogens can be achieved by means of exclusion, eradication/ sanitation, and crop protection. Since curing the diseased plants is difficult to obtain, preventing

Table 1. Loss estimate for plant pathogenic prokaryotes

Prokaryote	Disease Name	Loss (millions USD)
Pseudomonas solanacearum	Bacterial wilt of tobacco and tomato	9.4
P. syringae pv *glycinea*	Bacterial blight of soybean	65
P. syringae pv *syringae*	Bacterial leaf blight of wheat	18
X. campestris pv. *malvacearum*	Bacterial blight of cotton	15
Agrobacterium tumefaciens	Crown gall of fruit and nut	23
Erwinia amylovora	Fire blight of pear	4.7
E. carotovora subsp. *carotovora* and/or subsp. *atroseptica*	Soft rot and/or blackleg of potato	14
Clavibacter michiganensis subsp. *insidiosus*	Bacterial wilt of alfalfa	17
C.m. subsp. *nebraskensis*	Goss's bacterial wilt and blight of corn	3
C. xyli subsp. *xyli*	Ratoon stunt of sugarcane	10
Xylella fastidiosa	Phony peach,	20
	Pierce's disease of grape	3
Spiroplasma citri	Stubborn disease of citrus	1
MLO	Pear decline	1.6
	Lethal yellowing of coconut	3

Source: Kennedy & Alcorn (1980)
Note: current scientific name of procaryote in the table could be consulted in Bull *et al*. (2010)

Table 2. Losses of yield caused by plant pathogenic bacteria

Prokaryote and disease	Location	Comments
Citrus canker (*Xanthomonas citri* pv. *citri*)	Asia, Africa, Brazil, USA	Caused eradication of millions of trees in Florida in 1910s and again in the 1980s and 1990s
Fire blight of pome fruits (*Erwinia amylovora*)	North America, Europe	Kills numerous trees annually
Soft rot of vegetables (*Erwinia carotovora* subsp. *carotovora*)	Worldwide	Huge losses of fleshy vegetables
Bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae*)	Asia	Destructive in Japan and India; spreading
Bacterial wilt of banana	Worldwide	Destructive in the Americas; spreading elsewhere
Citrus greening disease	Asia and spreading to other region	Severe in Asia; spreading

Adapted from Agrios (2005)

the spread of the pathogen is more reliable method to reduce disease incidence. Integration of various compatible methods will minimize yield losses, minimize environmental pollution, and keep crop production stable.

Biological control is one of the crop protection methods which is relatively new in the field of bacterial plant pathology. However, this field of study gain much more interests recently. Two specific symposium on biological control of bacterial plant diseases with great papers attended by many plant pathologists around the globe indicating the growing interest of this field (Zeller & Ulrich, 2005; Anonymous, 2008).

PRINCIPLES OF BIOLOGICAL CONTROL OF BACTERIAL PLANT PATHOGENS

Biological control of plant pathogens is a reduction of inoculum or disease producing activity of a pathogen accomplished by one or more organisms other than man (Cook & Baker, 1983). The definition, however, do not accommodate virus particle as a biological control agent, since virus is not an organism. The interrelationships of many environmental variables can result in multiple interactions among organisms and their environment, several of which might contribute to effective biological control. Furthermore, natural products and chemical compounds discovered as a result of basic research into the molecular mechanisms of

pathogenesis and biological control have led to the development of "biorational" pesticides. Here, the term biological control is used in the broader sense.

Bacteria that reduce the incidence or severity of plant diseases are refered as biocontrol agents and if they exhibit antagonistic activity toward a pathogen, it is called an antagonist. Recent advances in microbial and molecular techniques have significantly contributed to new insights in underlying mechanisms by which introduced bacteria function. Thus, biocontrol agents reduce disease incidence of bacterial plant diseases by competition of (space, nutrient, gas, oxygen), antibiosis, induced resistance, and interference with their life.

Survival of plant pathogenic bacteria in nature occurs most commonly in plant debris left on the soil surface, in and on seeds, in soil, and in association with perennial hosts (Vidaver & Lambrecht, 2004). Knowledge of their survival is usually essential to manage the disease and to control biologically. Aerial bacterial plant pathogens survived temporarily on the plant surface before infection while the soil-borne plant pathogenic bacteria could survive for long time in the soil. Accordingly, the control of aerial plant pathogenic bacteria is easier to be accomplished. However, the fact is not always the case. Occurrence of low number of plant pathogenic bacteria in the leaves of symptomless resistant hosts is a factor of greater significance in the epidemiology of foliar plant pathogens. Bacteria may survive as an internal resident in the resistant plants (Hayward, 1974).

Lindow and Brandl (2003) noted that compared to other habitats, such as the soil, rates of plasmid transfer on leaves are very high and may make the genetic and phenotypic stability of inocula introduced onto plants unpredictable with time. It is tempting to speculate that the nutrient-rich but localized leaf sites that support cell aggregates and at which bacteria at least transiently retain high levels of metabolic activity are also the sites at which gene transfer occurs. If so, this would suggest that leaves are at least transiently less oligotrophic than other habitats, such as soil.

The rhizosphere is commonly perceived as a site where there are high levels of microbial activity and large numbers of bacteria (Foster, 1988). This is true since young roots themselves are so nutritious and because they secrete a wide range of metabolites into the soil; root surfaces are the main locations for soil organisms of all types (Rovira 1965). Thus, rhizosphere has been a point of entry for most plant pathologist working in biological control (Cook & Baker, 1983) even until today.

Plant pathogenic bacteria do not form resting spores or structures comparable to fungi or nemathodes; they remain dormant during the period in association with: seeds, perennial plant hosts or parts, insect, epiphytes, plant residues, soil, and other non host material (Schuster & Coyne, 1974). By understanding the nature of survival site and infection process, one can design the delivery method of a biological control agent and introduce a desire trait that improve their competitiveness and capability in producing antimicrobial or inducing resistance in plant. Biological control agents used for bacterial plant pathogen include *Bacillus*, *Erwinia*, *Streptomyces*, Pseudomonads especially fluorescent pseudomonad, avirulent form of the pathogen, bacteriophage, protozoa, and bdellovibrio (Goto, 1990).

BIOLOGICAL CONTROL OF BACTERIAL DISEASES OF FOOD CROPS

Bacterial diseases of food crops develop rapidly when the environmental condition conducive for disease development. Control of the disease should be performed as soon as possible due to its short disease cycle. Thus, scouting for the first symptom to individual plant in a crop plantation is mandatory. Seed dressing/coating with a biological control agent, dipping the seedling in a bacterial suspension are the visible methods to deliver a biological control agent onto plant surface effectively.

RICE BACTERIAL LEAF BLIGHT (*Xanthomonas oryzae* pv. *oryzae*)

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the destructive diseases in rice (Figure 1). Whenever susceptible rice varieties are grown in environments that favor bacterial blight, very high yield losses over 70% may be happened (Anonymous, 2011). Nowadays, however, yield losses of 1% or less are the norm, as resistant varieties have been deployed in the main rice-producing zones of Asia (Savary *et al.*, 2000). However, in areas of high disease pressure, like tropical sub-Saharan Africa, new crop varieties that are released with single sources of genetic resistance are frequently overcome either before or soon after poor farmers gain access to the improved varieties. Although some farmers do apply chemical herbicides and pesticides, access is not always accompanied with training, which results in ineffective and unsafe use. Thus, alternative of control other than the use of resistant varieties should be investigated.

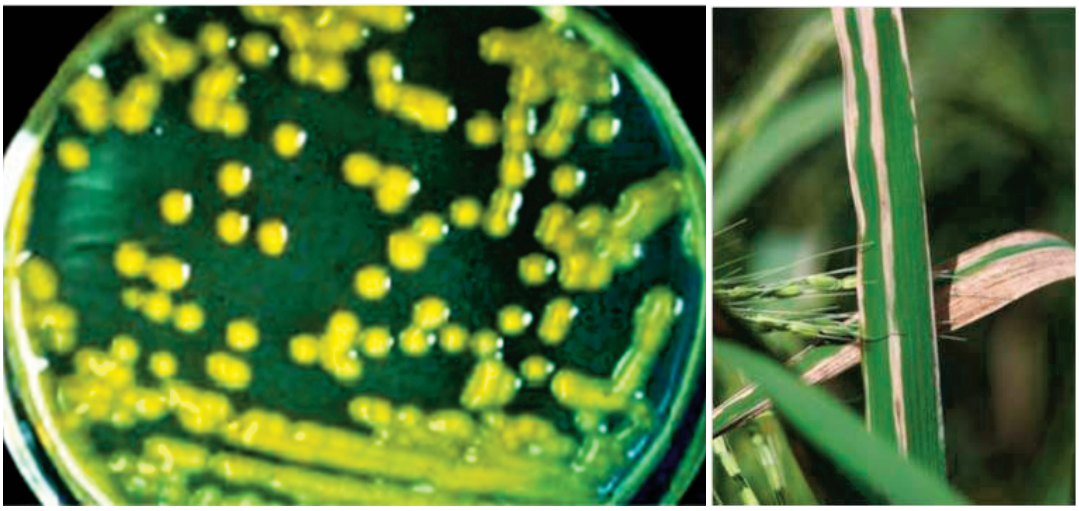

Figure 1. Colonies of *Xanthomonas oryzae* pv. *oryzae* (left), typical symptoms of bacterial blight on leaves (right) (IRRI, 2011)

Phenazine has been known for long time having suppression activity against *X. oryzae* pv. *oryzae* (Oda *et al.*, 1966). Certain members of the fluorescent pseudomonads produce and secrete phenazines (Goto, 1996). *Pseudomonas fluorescens* (strain 2-79) produced phenazine and has been studied intensively for biocontrol of take-all of wheat caused by *Gaumanomyces graminis* var *tritici* (Cook & Rovira, 1976). This antagonistic bacterium have been studied intensively from its basic to molecular aspects for decade until now. There is only one paper concerning with the use of *Pseudomonas fluorescens* for controlling rice bacterial leaf blight and this existing publication is not accessible to scientific community (Arunatha & Gananamanickam, 1987 *cit*. Anonymous, 2011), making difficult to extent the study. The lack of funding and the lack of interest from scientists to this important field might be the reasons. However, from next year Bill and Melinda Gates foundation will donate a sum of money to grant research on crop protection, including biocontrol of this important disease (http://www.grandchallenges.org/Explorations/Topics/Pages/ProtectPlantCropsRound8.aspx).

In rice fields, populations of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* are diverse and dynamic (Adhikari *et al*., 1995). Antagonistic interactions between closely related strains of both gram-negative and gram-positive bacteria are often influenced by the production of bacterial toxins termed bacteriocins (Konisky, 1978). It has been reported that antagonistic interactions occur between several wild-type strains of the rice bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Dardick *et al.*, 2003). The use of bacteriocin producing strain is promising for biocontrol of this important bacterial plant pathogen.

Species of Bacillus have been applied to rice plants as seed treatment before sowing, a root dip prior to transplantion, and two foliar sprays prior to inoculation could suppress 59% of bacterial leaf blight (Vasudevan *et al*., 2002).

Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by using some or all of the host biosynthetic machinery (Mayer, 2007). Phages specific to *X. oryzae* pv *oryzae* are found in the water of rice field, irrigation canal, and rivers. The population density of bacteriophage is correlated with the number of its bacterial host. However, the problem in using of bacteriophage in the biocontrol of this pathogen was its inactivation by UV light, variable bacterial sensitivity, and the rapid development of bacterial resistance to the phage (Okabe & Goto, 1963).

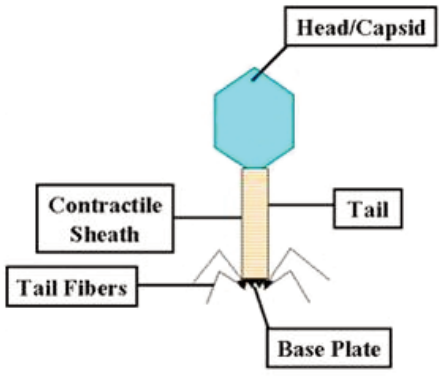

Figure 2. Structure of T4 bacteriophage (http://pathmicro.med.sc.edu/mayer/phage-1.jpg)

For a long time, phages are mainly used for typing bacterial strains and for analyzing the ecological behaviour of pathogenic bacteria. However, after phage has been patented for biocontrol of plant diseases (Jackson, 1989), many reports on the use of phage as a biological control agent could be found anywhere but it is difficult to find the paper of it for rice bacterial leaf blight (Lang *et al*., 2007).

BACTERIAL WILT (*Ralstonia solanacearum*)

Bacterial wilt caused by *Ralstonia solanacearum* (Figure 3) is one of the most important bacterial diseases of plants of commercial value in the tropics, subtropics and warm temperature regions of the world. The disease affects plants in more than 35 families (Kelman, 1953) and more than 20 additional families of plants contain hosts of *R. solanacearum* for a period of almost more than four decades (Hayward, 1994). The pathogen is difficult to control due to their genetic variability, its multiple site of infection, and its wide range of host plant.

R. solanacearum while in the host plant, grow within plant tissues in highly selective niches. On such conditions, therefore, the biological control agents should be applied during the early stages of infection when the pathogen is on the host surface. It should be noted that the bacterium can survive in the soil for extended periods of time without a host and enters the plant through any types of wound (Hayward, 1991).

Biocontrol of *R. solanacearum* are mostly based on antagonism (antibiosis) activity and the antagonistic bacteria have been isolated form various sources (Table 3). Antibiosis activity is the easiest to perfom in laboratory by dual test culture, and it can screen thousand candidates efficiently. However, this method will eliminate the candidate of biological control agents that have mechanism other than antibiosis such as induced resistance and competition (Fravel, 1988; Arwiyanto *et al*., 2007a).

Solanaceous crops other than potato and other vegetatively propagated crop were protected biologically from bacterial wilt by dipping the root system of seedlings before transplanting (Figure 5). *Pseudomonas putida* strain Pf-20 (Figure 4) has been developed for management of tobacco and tomato bacterial wilt. The bacterium inhibited the pathogen growth in vitro, suppressed the disease development in the green house and suppressed disease development of cigar-tobacco bacterial wilt in the field.

The dipping method was effectively deliver the biological control agent into the surface of plant root, thus covering the outer layer of root and keep the pathogen away from the plant. This one time application of biological control agent, however, does not give consistent satisfactory control. Population densities of introduced antagonist bacteria in the rhizosphere usually are greatest soon after planting and gradually decline throughout the growing season, often drop below the detection limit (Weller, 2007). This fact point out the importance of adding an amount of the biological control agent into root surface, periodically, which is often not visible in the field condition.

Other method to deliver bacterial antagonist is by seed treatment, either by seed dressing, seed coating, or seed pelleting. Treatment of tomato seed with water suspension of *P. putida* strain Pf-20 suppressed bacterial wilt into some degree (Asrul *et al*., 2004). When the *P. putida* Pf-20 was mixed with solid matrix and used for pelleting the tobacco seed (Figure 6), the bacterium could survive in the coated tobacco seed, colonize root system, but the degree of protection was inferior compare with seedlings treatment (Wuryandari *et al*., 2004).

Figure 3. Colonies of *Ralstonia solanacearum* on CPG medium (left), the wilt symptom on tobacco (center) (Arwiyanto *et al*., 1995); and tomato (right) (Arwiyanto, 2000, unpublished)

Table 3. Some antagonistic bacteria against *R. solanacearum*

Antagonist	Author
Pseudomonas fluorescens and fluorescent pseudomonad	1. Kempe and Sequeira (1983) 2. Ciampi-Panno *et al.* (1989) 3. Gallardo *et al*. (1989) *cit*. Trigalet *et al*. (1994) 4. Anuratha and Gnanamanickam (1990) *cit*. Trigalet *et al*. (1994) 5. Wydra *et al*. (2005) 6. Arwiyanto *et al*. (2007a)
Pseudomonas glumae	1. Wakimoto (1987) *cit.* Trigalet *et al*. (1994) 2. Furuya *et al.* (1991)
Pseudomonas cepacia	1. Aoki *et al*. (1991) *cit*. Trigalet *et al*. (1994)
Pseudomonas putida	1. Arwiyanto dan Hartana (2001) 2. Irawati, Arwiyanto and Widyastuti (2003) 3. Anith *et al*. (2004) 4. Asrul *et al*. (2004) 5. Wuryandari, Arwiyanto, Hadisutrisno, dan Hartana (2004) 6. Arwiyanto and Nurcahyanti (2007) 7. Kurabachew and Wydra (2008)
Bacillus sp.	1. Fucikovsky *et al*. (1989) *cit.* Trigalet *et al*. (1994) 2. Anuratha and Gnanamanickam (1990) 3. Phae *et al*. (1992) 4. Anith *et al*. (2004) 5. Arwiyanto *et al*. (2007b) 6. Kurabachew and Wydra (2008) 7. Nguyen *et al*. (2011)
Avirulent mutants of *R. solanacearum*	1. Kempe and Sequeira (1983) 2. Chen and Echandi (1984) 3. Tanaka *et al*. (1990) 4. Quimio and Ayo (1989) 5. Trigalet and Trigalet-Demery (1990) 6. Hara and Ono (1991) 7. Arwiyanto *et al*. (1994) 8. Arwiyanto and Nurcahyanti (2007) 9. Arwiyanto *et al*. (2010)
Streptomyces	1. Arwiyanto and Bustamam (2010) 2. Arwiyanto *et al.* (2007c)
Bacteriophage	1. Alvarez *et al*. (2007) 2. Yamada *et al*. (2007) 3. Fujiwara *et al*. (2011)

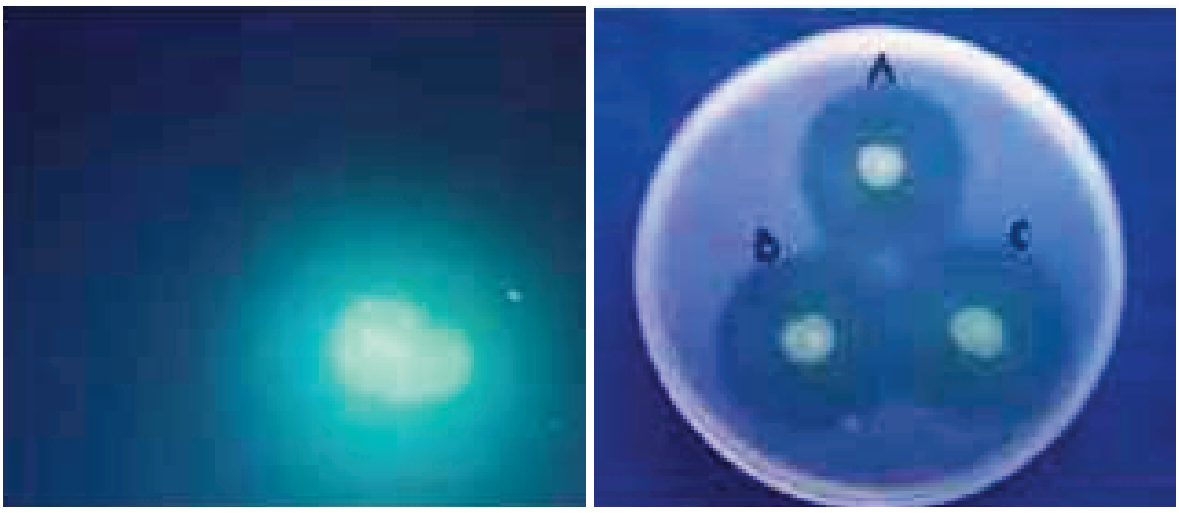

Figure 4. Colony of *Pseudomonas putida* Pf-20 in medium King's B (left) and growth inhibition of *Ralstonia solanacearum* in CPG medium (right) (Arwiyanto, 1997)

Figure 5. Dipping cigar-tobacco seedlings in water suspension of *Pseudomonas putida* strain Pf-20 (left) before planting in the field (right) (Arwiyanto & Hartana, 2001)

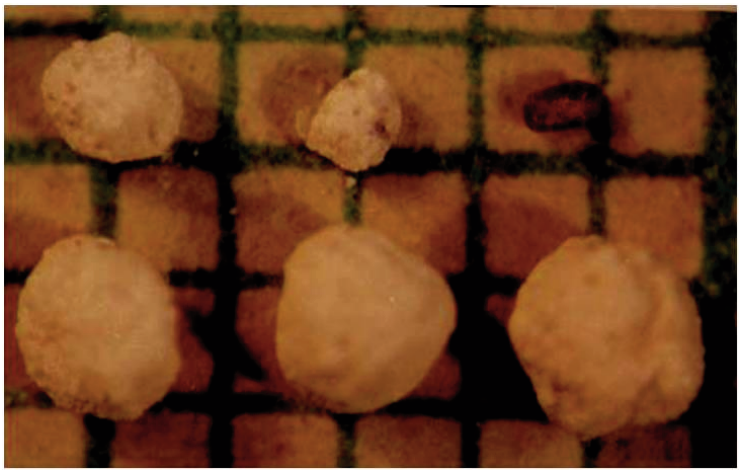

Figure 6. Tobacco seed coated with solid matrix containing *Pseudomonas putida* Pf-20 (Wuryandari *et al*., 2004)

CROWN GALL CAUSED BY *Agrobacterium tumefaciens*

Crown gall, caused by *Agrobacterium tumefaciens*, is distributed worldwide and is responsible for nursery and field losses among a large variety of plants, especially stone fruit trees (Jones *et al*., 1991). A practical way to control of this disease has been developed, initially with strain K84 of *A. radiobacter* (Kerr, 1980). Although the disease has never been reported in Indonesia, this is undoubtedly, as mentioned by Goto in his book (Goto, 1992), the control is one of the most innovative and important advances in biological control of bacterial plant diseases.

The method of control is by inoculation of planting material with non-pathogenic *A. radiobacter* strain K84 immediately before sowing or planting. For over 15 years, crown gall on many different host plants has been successfully controlled by K84 in many countries. The control involving inhibition of the pathogen by Agrocin84 (a bacteriocin produced by K84), biological site competition, and competition of certain nutrient that common these bacteria (Kerr, 1980). This is the only biological control of plant pathogenic bacteria that act in two ways, specific competition and antibiosis.

The biological control of crown gall by K84, however, create a problem by which the pathogen mutate and no longer subject to control. This was happened because strain K84 has a plasmid governing production of agrocin84 and resistance against it (pAgK84) has been transferred to the pathogenic bacteria (see Figure 7).

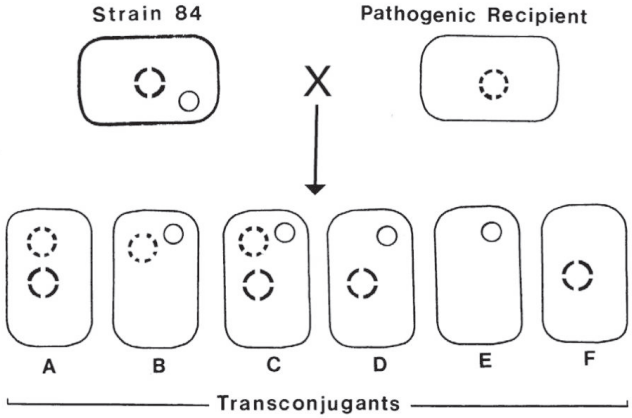

Figure 7. Diagrammatic representation of a cross between strain 84 and a pathogenic recipient of *Agrobacterium tumefaciens*; chromosomes are not shown; strain 84 contains two plasmids, one (solid line) coding for agrocin 84 production and resistance to agrocin 84 and the other (broken line) coding for nopaline catabolism and for conjugation; the pathogen has one plasmid (dotted line) that codes for pathogenicity and for agrocin 84 sensitivity as well as for other characters not discussed in the text; the cross results in six plasmid transconjugants; transconjugants B and C combine (pathogenicity with resistance to agrocin 84 (Kerr, 1980)

A new strain (K1026), a transfer-defficient (tra-) deletion mutant of strain K84 then was constructed and this strain controls crown gall as effectively as strain K84. The strain is commercially available under tradename of NoGallTM, a peat-based formulation containing 109 bacteria per gram (Jones et al., 1991).

Strain K1026 is regarded safe to use in biological control of crown gall because (Jones *et al*., 1991):

1. Strain K84, the progenitor of strain K1026, has been registered as a pesticide and used commercially in many countries for over 15 years with no reports of harm;
2. Strain K1026 is indistinguishable from K84 except it lacks a portion of agrocin84 plasmid and preventing plasmid transfer;
3. No foreign DNA remains in strain K1026;
4. Strain K1026 contains no Ti-plasmid-encoded genes involved in crown gall induction;
5. Strain K1026 can not grow at 37ºC (human body temperature);
6. Agrocin84 is spesifically toxic to agrocinopine-catabolizing agrobacteria, most of which are crown gall pathogens.

COMMERCIAL BIOLOGICAL CONTROL AGENT FOR BACTERIAL PLANT PATHOGEN

There is a limited product of commercially biological control agent for bacterial diseases of plant compare with those for fungal diseases (Table 4). Mention of trade names or commercial products in this publication is solely for the purpose of providing scientific information. Mention within this article does not imply recommendation or endorsement by the University of Gadjah Mada, nor does it reflect prejudice against other commercial products or ventures that are not described.

FUTURE PROSPECT

There is a growing demand for sound, biologically-based pest management practices suggesting that the market potential of biocontrol products will increase in coming years. The author encourage young plant pathologist in Indonesia to study more and more about biological control of plant diseases. Be a scientist who love of science with an insatiable curiosity. As Louis Pasteur said that "*Let me tell you the secret that has led me to my goal. My only strength lies in my tenacity*" (Beveridge, 1957).

Table 4. Biocontrol product commercially available for bacterial plant diseases

BlightBan A506		Reference
Biocontrol Organism	*Pseudomonas fluorescens* A506	McSpadden Gardener and Fravel (2002)
Target Pathogen/Disease	frost damage, *Erwinia amylovora*, and russet-inducing bacteria	
Crop	almond, apple, apricot, blueberry, cherry, peach, pear, potato, strawberry, tomato	
Formulation	wettable powder	
Application Method	bloom time spray of the flower and fruit	
Manufacturer/Distributor	NuFarm Inc., 1-708-754-3330. www.nufarm.com	
Galltrol		McSpadden Gardener and Fravel (2002)
Biocontrol Organism	*Agrobacterium radiobacter* Strain 84	
Target Pathogen/Disease	crown gall disease caused by *Agrobacterium tumefaciens*	
Crop	fruit, nut, and ornamental nursery stock	
Formulation	petri plates with pure culture grown on agar	
Application Method	bacterial mass from one plate transferred to one gallon of non-chlorinated water; suspension applied to seeds, seedlings, cuttings, roots, stems, and as soil drench	
Manufacturer/Distributor	AgBioChem, Inc. 3 Fleetwood Ct., Orinda, CA 94563, USA; Phone 1-925-254-0789 or 10795 Byrne Avenue, Red Bluff, CA, 90860; Phone 1-530-527-8028. www.crowngall.com	
Nogall		McSpadden Gardener and Fravel (2002)
Biocontrol Agent	*Agrobacterium radibacter* K1026	
Target Pathogen/Disease	crown gall disease caused by Agrobacterium tumefaciens	
Crop	fruit, nut, and ornamental nursery stock	
Formulation	petri plates with pure culture grown on agar	
Application Method	bacterial mass from one plate transferred to one gallon of non-chlorinated water; suspension applied to seeds, seedlings, cuttings, roots, stems, and as soil drench	
Manufacturer/Distributor	Bio-care Technology, Australia/New BioProducts, Inc. 2166 NW Fritz Place,Corvallis, OR 97330, Phone: 541-752-2045; FAX 541-754-3968 FAX. www.newbioproducts.com	
Conguer		Desai *et al*. (2002)
Biocontrol Agent	*Pseudomonas fluorescens*	
Target Pathogen/Disease	*Pseudomonas tolassii*	
Crop	Mushroom	
Formulation	Liquid	
Application Method	Spray	
Manufacturer/Distributor	Mauri Foods, 67 Epping Rd., North Ryde, Australia Sylvan Spawn Laboratory, West Hills Industrial park, Kittaning, PA16201	
Norbac 84C		Desai *et al*. (2002)
Biocontrol Agent	*Agrobacterium radiobacter* strain K84	
Target Pathogen/Disease	crown gall disease caused by *Agrobacterium tumefaciens*	
Crop	-	
Formulation	Aqueous suspension containing bacterial cells, methyl cellulose, and phosphate buffer (refrigerate)	
Application Method	Root, stem, cutting dip, or spray	
Manufacturer/Distributor	New Bioproducts, Inc., 4737 N.W. Elmwood Dr., Corvallis, OR 97330	
Phagus		Desai *et al.* (2002)
Biocontrol Agent	Bacteriophage	
Target Pathogen/Disease	*Pseudomonas tolaasii*	
Crop	Mushroom	
Formulation	Bacterial suspension	
Application Method	-	
Manufacturer/Distributor	Natural Plant Protection, Route d'Artix B.P. 80, 64150 Nogueres, France	

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