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POTENTIAL OF MICROBIAL ANTAGONISTS AS BIOCONTROL AGENTS AGAINST PLANT FUNGAL PATHOGENS

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ABSTRACT

A variety of fungi are known to cause important plant diseases, resulting in a significant lost in agricultural crops. The plant diseases need to be controlled to maintain the level of yield both quantitatively and qualitatively. Farmers often rely heavily on the use of synthetic fungicides to control the plant diseases. However, the environmental problems caused by excessive use and misuse of synthetic fungicide have led to considerable changes in people's attitudes towards the use of synthetic pesticides in agriculture. Today, there is an increase in the awareness of the people about the healthy food and healthy environment. In response to this need, some researchers have focused their effort to develop alternative measures to synthetic chemicals for controlling plant diseases. Among these, is that referred to as biological control using microbial antagonists. Many microbial antagonists have been reported to posses antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis, B. cereus*, *B. amyloliquefaciens*, *Trichoderma virens*, *Burkholderia cepacia*, *Saccharomyces* sp, *Gliocadium* sp. Three species of rhizobacteria isolated from rhizospheres of rice grown in Bali, *i.e. Enterobacter agglomerans*, *Seratia liquefaciens* and *Xanthomonas luminescens* were found to effectively suppressed the growth of *Pyricularia oryzae* Cav. the cause of rice blast disease. Understanding the mechanism by which the biocontrol of plant diseases occurs is critical to the eventual improvement and wider use of biocontrol method. These mechanisms are generally classified as competition, parasitism, antibiosis, and induction of host resistance. Over the past forty years, research has lead to the development of a small commercial sector that produces a number of biocontrol products. The market share of biopesticides of the total pesticide market is less than three percent. However, significant expansion is expected over the next decades due to the expanded demand for organic food, and increased demand for safer pesticides in agriculture and forestry. The challenge is to develop a formulation and application method that can be implemented on a commercial scale, that must be effective, reliable, consistent, economically feasible, and with a wider spectrum. Continual laboratory works followed by field experiments are needed to establish excellent biocontrol agents particularly against plant fungal pathogens.

INTRODUCTION

Agricultural crops are exposed to approximately 70,000 species of pests, but of these only 10% are considered serious pests (Pimentel, 1997). Pest insects, plant pathogens, and weed potentially cause the reduction of world food production more than 40% if pesticides are not applied. Pre-harvest pest losses are approximately 15% for pest insects, 13% for diseases, and about 12% for weeds. After harvest, another 20% of the food is lost to another group of pests (Pimentel, 1997). Plant diseases caused by a variety of fungi may cause significant losses on agricultural crops. All plants are attacked by some kinds of fungi, and each of parasitic fungi can attack one or many kinds of plants. More than 10,000 species of fungi can cause disease in plants (Agrios, 2005).

Fungal diseases may be minimized by the reduction of the inoculums, inhibition of its virulence mechanisms and promotion of genetic diversity in the crop (Strange and Scott, 2005). The use of chemical fungicides in agriculture has been proven to bring about various benefit such as reducing the fungal infection that may rob water and nutrients from crop plants or may cause spoilage while the products are transported to the market. Fungicides may also prevent the growth of fungi that produce toxins, such as aflatoxins. In 1997, worldwide, 5.7 billion pounds of pesticides were used, of which 0.5 billion were fungicides (Goldman, 2008). There are numerous classes of fungicides, with different modes of action as well as different potential for adverse effect on health and environment. Milne (2004) indicated 311 compounds are registered and used as fungicides to control various plant fungal diseases. Of these, seven agents are antagonistic microorganisms and only one agent is derived from plant extract, i.e., extract of *Reynoutria sachalinensis* (Giant Knotweed).

Most fungicides can cause acute toxicity, and some cause chronic toxicity as well (Goldman, 2008). The use of chemical pesticides has been known to cause various environmental and health problems. The International Labor Organization (ILO) estimates that as much as 14 per cent of all occupational injuries are due to exposure to pesticides and other agrochemical constituents (ILO, 1996). The World Health Organization (WHO) and the United Nations Environment Programme estimates that each year, three million workers in agriculture in developing world experience severe poisoning from pesticides, about 18,000 of whom die (Miller, 2004). Appropriate technological improvement, which results in more effective use of natural resources is required in agriculture. One of them is the use of microbial antagonists.

Many microbial antagonists have been reported to posses antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis*, *B. cereus*, *B. amyloliquefaciens*, *Trichoderma virens*, *Burkholderia cepacia*, *Saccharomyces* sp, *Gliocadium* sp. The successful control by these antagonists mainly against the diseases caused by following genera of pathogens : *Alternaria*, *Pythium*, *Aspergillus*, *Fusarium*, *Rhizoctonia*, *Phytophthora*, *Botrytis*, *Pyricularia* and *Gaeumannomyces* (Pal and Garderner, 2006). This paper describes briefly the biocontrol potential of microbial antagonists particularly against plant fungal pathogens.

Fungal Diseases on Agricultural Crops

More than 10,000 species of fungi, can cause disease in plants (Agrios, 2005). Classes of fungi that commonly cause diseases in agricultural crops are Plasmodiophoromycetes (cause clubroot of crucifers, root disease of cereals, and powdery scab of potato), Oomycetes (cause seedling damping-off, late blight, downy mildews, and white rust disease), Zygomycetes (cause soft rot of fruit), Ascomycetes and Deuteromycetes (cause leaf spots, blights, cankers, fruit spots, fruit rots, anthracnoses, stem rots, root rots, vascular wilts, soft rot), and Basidomycetes (cause rust and smut diseases (Agrios, 2005).

Apple scab, caused by the fungus *Venturia inaequalis* is the most important diseases of apples worldwide, and it very likely occurs in every country where apples are grown. In some circumstances, the losses from apple scab can be 70% or more of the total fruit value (Agrios, 2005). *Macrophomina phaseolina*, is important root pathogen and causes dry root rot or stem canker, stalk rot or charcoal rot of over 500 plant species (Sinclair, 1985; Shahzad et al., 1988). The genus *Fusarium* contains a number of species, which have been recognized for a long time as being important plant pathogens (Booth, 1971; Nelson *et al*., 1983). *Rhizoctonia solani* exists in the soil and attacks more than 2000 species of plants (Parmeter, 1970). *Phytophthora capsici* causes foot rot disease in black pepper (*Piper nigrum* L.) and other crops in India and tropical countries (Dinu et al., 2007). This fungus affects all parts of black pepper plants namely roots, stems, shoots, leaves and spikes.

Sudana et al. (1999) reported that 10 millions of banana plants were died because of wilt disease caused by fungus *Fusarium oxysporum* f.sp. *cubense.* Within two years (2007-2009) the banana wilt disease caused the decrease of banana production more than 60% (Sudana et al, 1999). *Phytophthora palmivora* the cause of black pod disease on cocoa has become an important obstacle in cocoa production in Bali. Survey done by Suprapta et al. (2006) indicated that the disease incidence during rainy season in three main cocoa growing areas in Bali was relatively high, varying from 78 to 88%. Stem rot disease caused by *Fusaium oxysporum* f.sp. *vanilae* has been known as one of important constraints for the vanilla cultivation and responsible for the decreasing of vanilla production (Semangun, 2000; Suprapta et al., 2006; Jayasekar et al., 2008, Sulistyani, 2004). Rice blast disease is one of the most important fungal disease on rice that may resulted in apparent yield loss. The disease caused by *Pyricularia grisea* Sacc., (synonym *Pyricularia oryzae* Cav.) (Kato, 2001). The yield losses resulted from the outbreak of blast disease varied according to the geographical condition. In Japan the disease may cause the yield loss up to 100% (Kato, 2001), in China up to 70% (Chin, 1975) and in Bali, Indonesia varied from 21% to 37% (Suprapta and Khalimi, 2012).

Biocontrol of Fungal Pathogens

Biological control of soil borne pathogens by introduced microorganisms has been studied over 80 years, but most of the time it has not been considered commercially feasible. However interest and research in this area increased steadily. There is a shift toward the important role of biological control in agriculture in the future. Several companies now have programs to develop biocontrol agents as commercial products.

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line defense for root against attack by pathogens. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection and also during secondary spread in the roots. In the suppressive soil to pathogens, microbial antagonism of the pathogen is especially great, leading to substantial disease control. Although pathogen-suppressive soils are rare, those identified are excellent examples of the full potential of biological control of soil borne pathogens (Weller, 1988).

Isolates of *Pseudomonas* were evaluated for antifungal activity against five fungal plant pathogens, i.e. *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Erysiphe cruciferarum* (Singh *et al*., 2011). All fungal strains tested showed significant reduction in terms of radial diameter after the treatment with *Pseudomonas* cultures, in comparison with the controls. Out of the five fungal pathogens studied, *Fusarium oxysporum* showed maximum extent of inhibition (% control inhibition = 51.76%) followed by *Aspergillus niger* (50.14%), and least by *Erysiphe cruciferarum* (22.27%). The antagonistic effect of *Pseudomonas* might be explained on the basis of its antifungal secondary metabolites that are capable of lysing chitin which is the most important component of fungal cell wall (Singh et al., 2011). Treatment with the antagonist *Pseudomonas aeruginosa* strain 950923-29 in combination with DL-β-amino-*n*-butyric acid (BABA) effectively suppressed the *Phytophthora* blight disease of pepper caused by *Phytophthora capsici* (Lee et al., 1999).

An isolate of *Trichoderma harzianum* capable of lysing mycelia of *Sclerotium rolfsii* and *Rhizoctonia solani* was isolated from a soil naturally infested with those pathogens (Elad *et al*., 1980). Under green house conditions, incorporation of the wheat-bran inoculums preparation of *T. harzianum* in pathogen-infested soil reduced significantly bean diseases caused by *S. rolfsii*, *R. solani*, or both, but its biocontrol capacity was inversely correlated with temperature. The wheat-bran preparation of *T. harzianum* increased growth of bean plants in a non-infested soil and its controlled *S. rolfsii* more efficiently than a conidial suspension of the same antagonist. In naturally infested soils, wheat-bran preparation of *T. harzianum* inoculums significantly decreased diseases caused by *S. rolfsii* or *R. solani* in three field experiments with bean, cotton, or tomato, and these increased significantly the yield of beans (Elad et al., 1980).

Patale and Mukadam (2011) tested the antagonistic activities of three *Trichoderma* species, i.e. *T. viride*, *T. harzianum*, and *Trichoderma* sp. against seven pathogenic fungi, namely *Aspergillus niger*, *A. flavus*, *Phytophthora* sp., *Fusarium oxysporum*, *Rhizoctonia solani*, *Penicillium notatum*, and *Alternaria solani*. They found that all three species of *Trichoderma* suppressed effectively the growth of seven pathogenic fungi. Atifungal activity of *Pseudomonas fluorescens* was tested against *Pythium ultimim*, *Macrophomina phaseolina* and *Pyricularia oryzae* (Goud and Muralikrishnan, 2009). All three pathogenic fungi were inhibited by *P*. *fluorescens* with inhibitory activities ranging from 50% to 80%.

The asbuscular mycorrhizae fungi (AMF) are the symbiotic fungi that predominate in the roots and soils of agricultural crops. The AMF play an important role in the reduction of plant pathogens, such as *Rhizoctonia solani* (Yao et al., 2002), and *Phytophthora* species (Trotta et al., 1996; Cordier et al., 1996). The antagonistic interaction between AMF with various soilborne plant pathogens is the reason for their use as biocontrol agents.

Among soil microorganisms, yeasts have received little attention as biocontrol agents of soil borne fungal plant pathogens in comparison to bacterial, actinomycetes, and filamentous fungal antagonists. The ability of certain species of yeasts to multiply rapidly, to produce antibiotics and cell wall-degrading enzymes, to induce resistance of host tissues, and to produce plant growth regulators indicates the potential to exploit yeasts as biocontrol agents. *Saccharomyces* sp. was proven to be effectively suppressed the growth of *Sclerotium* sp. (the cause of stem rot disease on vanilla seedlings), *Fusarium oxysporum* f.sp. *vanillae* (the cause of stem rot disease on vanilla in the field), *F. oxysporum* f.sp. *cubense* (the cause of *Fusarium* wilt on banana) and *Phytophthora palmivora* (the cause of black rot disease on cocoa) on PDA medium. The *Saccharomyces* sp. was formulated in compost containing saw dust, maize flour, sucrose and rice bran. This formulation has been patented in Indonesia with patent number IDP 0024496.

Biological control of plant diseases is a result of many different types of interaction among microorganisms and can occur through different mechanisms, which are generally classified as : parasitism/predation, antibiosis, competition, lytic enzymes, and induced resistance (Pal and Gardener, 2006). The most effective biocontrol active microorganisms studied appear to antagonize plant pathogen employing several modes of actions. For example, *Pseudomonas* known to produce the antibiotic 2, 4-diacetylphloroglucinol (DAPG) may also induce host defenses. Additionally, DAPG-producers bacterial antagonists can aggressively colonize root, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of plant through competition for organic nutrients (Heydari and Pessarakli, 2010). A study on the development of bioagents to control rice blast disease on rice caused by *Pyricularia oryzae* Cav. found three potential species of rhizobacteria isolated from the rhizospheres of rice grown in Bali, namely *Enterobacter agglomerans* (isolates Ch2D, Gg14D and Ch4Bdak), *Xanthomonas luminescens* (isolate Ch3D) and *Seratia liquefaciens* (isolate Gh13Da) (Suprapta and Khalimi, 2012).

Challenge for the Success of Field Application

In general, the potential antagonistic microorganisms selected from *in vitro* tests often fail to effectively control plant disease in greenhouse or field trials, particularly to soil borne pathogens. Several factors such as the type and the content of organic matter, pH, nutrient level, and moisture level of the soil influence the efficacy of the biocontrol agents. Due to the variations in environmental factors from one place to other places, sometimes, a good biocontrol agent under *in vitro* conditions fails in *in vivo* conditions. To achieve the success, the environmental factors should be similar to those from which the biocontrol agents were isolated. Likewise, the method of application can influence the success of field trials. In general, there are three means of applying the antagonists for biocontrol, namely seed inoculation, vegetative part inoculation and soil inoculation.

A field trial done by Lee and co-workers (1999) showed that the control efficacy of antagonist *Burkholderia cepacia* strain N9523 against *Phytophthora capsici*, the cause of *Phytophthora* blight of pepper was higher by soil-drenching than by wounded stem inoculation. Dawar et al. (2008) tested the biocontrol potential of different microbial antagonists, i.e. *Bacillus thringiensis*, *Rhizobium melilot*i, *Aspergillus niger* and *Trichoderma harzianum* by coating the seeds with gum Arabic, glucose, sucrose and molasses. This method reduced successfully the infection of root rot fungi on okra and sunflower, i.e *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. The highest suppression capacity was shown by seed treatment with *T. harzianum* using 2% of glucose.

CONCLUDING REMARKS

Plant diseases caused by pathogenic fungi may result in significant yield losses of agricultural crops. Farmers, in general still rely on the use of synthetic fungicides to control plant diseases. However, the misuse of these chemicals may cause serious environmental and health problems. Microbial antagonists are potential agents that can be explored to provide effective and safe means to manage plant diseases. Several microorganisms have been tested and proven to posses antagonistic properties against plant pathogenic fungi. Our recent study showed that three species of rhizobacteria *i.e*. *Seratia liquefaciens, Enterobacter agglomerans* and *Xanthomonas luminescens* apparently suppressed the growth of *Pyricularia oryzae*, the cause of rice blast disease. The potential of these agents can be improved by continual improvement in isolation, formulation and application methods, particularly in the field.

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