



Original Research Article

Efficacy of *Pseudomonas* as biocontrol agent against plant pathogenic fungi

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A B S T R A C T

A variety of fungi are known to cause important plant diseases, resulting in a significant loss in agricultural crops. The plant diseases need to be controlled to maintain the level of yield both quantitatively and qualitatively. Farmers often use the common 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. Many microbial antagonists have been reported to possess antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis*, *B. cereus*, *B. amyloliquefaciens*, *Trichoderma viride*, *Trichoderma harzianum*, *Burkholderia cepacia*, *Saccharomyces* sp, and *Gliocadium virens*. Three species of rhizobacteria isolated from rhizospheres of Rice grown in Bihar region, i.e. *Enterobacter agglomerans*, *Serratia 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. The present communication deals with the antagonistic effect of *Pseudomonas* on six fungi viz. *Pyricularia oryzae*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Erysiphe cruciferarum*. Overall the culture of *Pseudomonas* showed the maximum inhibition on the growth of *Pyricularia oryzae* which is responsible for the blast disease in rice crops and other fungi also.

Keywords

Efficacy,
Pseudomonas,
Biocontrol,
Agriculture,
Antagonist

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 several 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 inoculum, 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 benefits 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 which 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, 1996) 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 possess antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtilis*, *B. cereus*, *B. amyloliquefaciens*, *Trichoderma viride*, *Trichoderma harzianum*, *Burkholderia cepacia*, *Saccharomyces* sp and *Gliocadium virens*. The successful control by these antagonists mainly against the diseases caused by different pathogenic genera viz. *Alternaria*, *Pythium*, *Aspergillus*, *Fusarium*, *Rhizoctonia*, *Phytophthora*, *Botrytis*, *Pyricularia* and *Gaeumannomyces* (Pal and Gardener, 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 Basidiomycetes (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 an 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 *Fusarium 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 diseases on rice that may result in apparent yield loss and the disease is 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). Although pathogen-suppressive soils are rare, those identified are excellent examples of the full potential of biological control of soil borne pathogens (Weller, 1988).

Materials and Methods

Antagonist activity of isolates of *Pseudomonas* against six fungi viz. *Pyricularia oryzae*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Erysiphe cruciferarum* were tested using dual culture technique (Dhingra and Sinclair, 1995). Seven days old culture of antagonists and test pathogen were taken into consideration.

Culture disc of equal size were transferred aseptically to 90 mm Petriplates on opposite side. As *Pseudomonas* sp. grows faster, the test fungal disc was placed on medium two days earlier than antagonist. The distance between the inoculum points of each fungi and potential antagonist was 5 cm. The disc of each fungus was placed at the centre in separate Petriplates alone served as control. The inoculated plates were incubated in a BOD incubator at 28±2°C. Observations on growth of antagonists and the tested fungi from centre of inoculum were recorded after seven days of incubation. The inhibition in radial growth of test fungi over control was

calculated. Nutrient agar medium for *Pseudomonas* and Asthana and Hawkers medium for the growth of fungi was prepared. The sterilized medium was poured in Petriplates allowed to solidify and then was inoculated with 8–10 days old culture of test fungi separately along with the culture of *Pseudomonas* sp. The plates were incubated at 28±2°C in BOD incubator for 7 days. The radius of the developed colonies was measured to estimate the fungal growth.

Results and Discussion

Isolates of *Pseudomonas* were evaluated for antifungal activity against six fungal plant pathogens, i.e. *Pyricularia oryzae*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and *Erysiphe cruciferarum* (Singh et al., 2011). All tested fungal strains showed significant reduction in terms of radial growth after the treatment with *Pseudomonas* cultures, in comparison with the controls. The results were shown in the Table 1.

The growth behaviour of the test fungi with the culture of antagonist bacteria *Pseudomonas* sp varied considerably. The culture of *Pyricularia oryzae* showed minimum colony diameter (08mm) followed with *Aspergillus niger* (14mm), *Alternaria alternata* (16mm), *Fusarium oxysporum* (17mm), *Aspergillus flavus* (20mm) and *Erysiphe cruciferum* (26mm). The maximum inhibition in colony diameter was observed in *Pyricularia oryzae* (89%) followed by *Aspergillus niger* (80%), *Alternaria alternata* (77), *Fusarium oxysporum* (76%) and *Aspergillus flavus*, (71%) and *Erysiphe cruciferarum* (64%) over control. The culture of *Pseudomonas* sp was found more effective for the control of *Pyricularia oryzae* which causes more and more loss in the productivity of rice crops in these areas.

Current scenario and future strategies of biocontrol

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. Antifungal activity of *Pseudomonas fluorescens* was tested against *Pythium ultimum*, *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 arbuscular 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 soil borne 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. Biological control of plant diseases is a result of 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).

The culture of *Pseudomonas* can be used to control the blast diseases in rice crops caused by *Pyricularia oryzae* Cav. So that yield loss can be checked in time.

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 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 *et al.* (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 thuringiensis*, *Rhizobium meliloti*, *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.

Table.1 Showing effect of Pseudomonas on the radial growth of fungi

S.No	Name of the fungi	Colony diameter (in mm)	% inhibition (in mm)
01.	Control	70	00
02.	<i>Pyricularia oryzae</i>	08	89
03	<i>Fusarium oxysporum</i> ,	17	76
04	<i>Aspergillus niger</i> ,	14	80
05	<i>Aspergillus flavus</i> ,	20	71
06	<i>Alternaria alternata</i>	16	77
07	<i>Erysiphe cruciferarum</i>	26	64

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