Antifungal Activity and Phosphate Solubilizing Ability of Fungi Isolated from Rice Rhizosphere in Low Country Wet Zone of Sri Lanka

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Abstract
Rhizosphere is the soil adjacent to plant root system and is a region with intense microbial activity. The role and importance of rhizosphere microorganisms in sustainable crop production have been reviewed by several researchers. Considering the negative impacts of agrochemicals, it is urgently needed to reduce the use of agrochemicals and use eco-friendly technologies to increase rice production. The objective of this study was to isolate fungi with antifungal activity and phosphate solubilizing ability from rice rhizosphere. In this study, fungi were isolated from rhizosphere*s* of Bw 367, Bw 372 and Bw 272-6B rice varieties grown under drained and water logging conditions of paddy fields in the low country wet zone of Sri Lanka. The spread plate technique was used to isolate and purify all the isolates on Potato Dextrose Agar medium. A total of 41 and 32 fungal isolates were recorded in the rice rhizosphere under drained and water logging conditions, respectively. Antifungal activity of all the isolates was determined against *Rhizoctonia solani, Bipolaris oryzae* and Curvularia sp. using dual culture method. Two fungal isolates from rhizospheric soils of drained paddy field and three isolates from water logging paddy field showed more than 70% inhibition of the three pathogens by competition. All the isolates were screened for phosphate solubilisation on Pikovskaya's agar medium and nine were identified as phosphate solubilising0 isolates. This study reveals that the rice rhizosphere is a rich source of fungi with antifungal activity and phosphate solubilizing ability.

Keywords: Antifungal Activity, Phosphate solubilisation, Rhizosphere
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Introduction

Rhizosphere is the soil in association with plant roots and root produced materials. Microorganisms in the rhizosphere play important roles in growth and ecological fitness of the host plant including plant protection, solubilisation of unavailable forms of phosphorous and production of growth promoting hormones (Kent and Triplett, 2002). Antifungal activity against plant pathogens and phosphate solubilising ability of rhizosphere microorganisms has been published by many researchers (Manivannan *et al.*, 2012; Shyamala and Sivakumar, 2012). Use of agrochemicals in paddy cultivation exerts adverse impacts on environment as well as on human health. Considering the negative impacts of agrochemicals, it is urgently needed to use eco-friendly agro technologies to increase crop production. Bio fertilizer, bio pesticides and bio fungicides have emerged as important research priority areas globally in view of growing demand for safe and healthy food, long term sustainability and concerns on environmental pollution associated with agrochemicals. Exploring the potentials of beneficial microorganisms in soil is important for opening avenues for sustainable agriculture. Since the microorganisms are environment specific, to understand the microbial diversity and their functions, investigation on occurrence of microorganisms from different environments is essential.

The objectives of this study were to isolate phosphate solubilising fungi and fungi with antifungal activity against the pathogens of rice rhizosphere.

Materials and methods

Isolation of fungi from rice rhizosphere

Rhizosphere soil samples were collected from two month old plants of Bw 367, Bw 372 & Bw 272-6B rice varieties grown in a water logging paddy field and in a drained paddy field in low country wet zone. Plants were carefully removed from the soil and shaken to remove excess soil. Soil still clinging to roots was separated using a sterilized spatula. One gram of separated rhizosphere soil was dissolved in 100 ml of sterilized distilled water. Soil dilution series was prepared using the sterilized distilled water and 10^{-3}, 10^{-4} and 10^{-5} dilutions were used to isolate fungi. Using the spread plate technique, 0.1 ml of each dilution was plated on potato dextrose agar (PDA) medium and incubated at room temperature (29 ± 2 °C) for 7 days. After incubation, all morphologically different fungal colonies were sub cultured to obtain pure cultures. Two biological replicates were performed for each dilution.

Determination of in vitro antagonistic activity of fungal isolates

All the isolates were evaluated for antifungal activities against three rice plant pathogens using dual culture method on PDA medium. Plates were incubated at room temperature (29 ± 2 °C) for seven days. Three biological replicates were performed for each isolate against each pathogen. Rice plant pathogens used for the study were *Rhizoctonia solani*, *Curvularia* sp. and *Bipolaris oryzae*. The percentage inhibitions of pathogens were measured using the formula described by Shyamala and Sivakumaar, 2012.

$$I = 100 (C - T) / C$$

I = Percentage inhibition of pathogen growth
C = Mycelial growth of pathogen in control plate
T = Mycelial Growth of pathogen in dual culture

Determination of phosphate solubilizing ability of fungal isolates

Fungal isolates were inoculated on Pikovskaya's agar medium which contains insoluble calcium phosphate as sole P source and incubated at room temperature (29 ± 2 °C) for 7 days (Pikovskaya, 1948). Three biological replicates were performed for each isolate. Phosphate solubilizing ability of microorganisms was visually detected by formation of clear zone around the microbial colonies.

Results and discussion

Isolation of fungi from rice rhizosphere

Fourty one and 32 fungal isolates were isolated from rhizosphere soils of drained paddy field and water logging paddy field respectively.

In vitro antifungal activity of fungal isolates

Rhizoctonia solani which causes the sheath blight disease and *Bipolaris oryzae* and *Curvularia* sp. which cause brown spot disease of rice were used for the evaluation.

Table 1: Antifungal activity of fungi isolated from rice rhizosphere

Location	Pathogen	Mechanism of Antifungal activity	No. of isolates showed antifungal activity				
			5% - 25 % Inhibition (I)	26% - 50% I	51% - 70% I	70% < I	No I
Drained paddy field	*Rhizoctona Solani*	Competition	09	07	01	02	18
		Production of inhibitory zones	03	02	-	-	
	Curvularia sp.	Competition	03	07	03	06	12
		Production of inhibitory zones	06	05	-	-	
	Bipolaris oryzae	Competition	01	04	07	06	11
		Production of inhibitory zones	02	11	-	-	
Location	Pathogen	Mechanism of Antifungal activity	No. of isolates showed antifungal activity				
			5% - 25 % Inhibition (I)	26% - 50% I	51% - 70% I	70% < I	No I
Water logging paddy field	*Rhizoctona Solani*	Competition	02	01	03	03	07
		Production of inhibitory zones	09	07	-	-	
	Curvularia sp.	Competition	04	02	01	06	06
		Production of inhibitory zones	09	04	-	-	
	Bipolaris oryzae	Competition	02	03	03	05	06
		Production of inhibitory zones	09	04	-	-	

There are different modes of action of bio control agents against plant pathogens, such as inhibition of the pathogen by antimicrobial compounds, competition for nutrients, parasitism and induction of plant resistant mechanisms (Shyamala and Shivakumar, 2012). In this study, fungi isolated from rice rhizozphere showed two modes of antagonism against the used pathogens; production of inhibitory substances and inhibition by competition. According to the results, significantly greater inhibition of pathogens was observed by competitive inhibition than inhibition by inhibitory substances or antimicrobial compounds.

Data of antifungal activity of fungal isolates are summarized in Table 1. Two fungal isolates from drained paddy field and three isolates from water logging field showed more than 70% inhibition of all the three pathogens by competition showing a higher potential of these isolates in controlling sheath blight and brown spot diseases of rice.

Phosphate solubilising ability of fungal isolates

Fungi which are capable of solubilizing Tricalcium phosphate produced clear zones around the microbial colonies on Pikovskaya's agar medium (Figure 1). Phosphate solubilizing ability was shown by five and four fungal isolates isolated from water logging paddy field and drained paddy field respectively. The size of the clear zone produced by fungi was varying with the isolate.

Conversion of insoluble phosphates into soluble forms by phosphate solubilizing microorganisms (PSM) involves processes of acidification, ion chelation and exchange reactions. It has been observed that several fungal strains have a higher phosphate solubilizing ability than bacteria (Kumari *et al.*, 2010). Phosphorus use efficiency of plants could be improved through the inoculation of relevant PSMs.

To commercialize these products, investigations on efficiency of identified beneficial microorganisms under field conditions and development of a suitable medium for inoculants, which is cost-effective and that has a good shelf life are needed. These microbial products will ensure a more sustainable solution to increase crop production while reducing or eliminating the need for agrochemicals.

This study reveals that the rice rhizosphere is a rich source of fungi with antifungal activity and phosphate solubilizing ability.

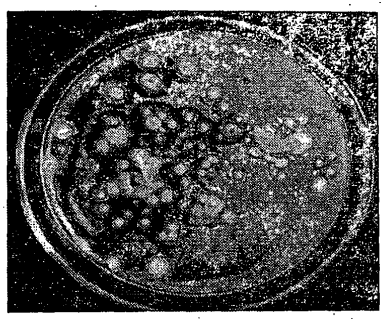

Figure 1: Phosphate solubilizing ability of fungus isolated from rice rhizosphere on Pikovskaya's agar medium.

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