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EVALUATION OF ANTAGONISTIC ACTIVITY OF SOIL BACTERIA AGAINST PLANT PATHOGENIC FUNGI

Ibatsam Khokhar, Muhammad Saleem Haider, Amna Ali, Irum Mukhtar and Sobia Mushtaq
Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
Author email: ibatsamk@yahoo.com

ABSTRACT

The purpose of this research was to evaluate antagonistic activity of some bacteria isolated from soil. Bacteria are able to synthesise a wide range of secondary metabolites with fungicidal capabilities. The antagonistic potential of five soil bacterial strains (*E. coli, Baccillus fortis, B. faragiris, Pseudomonas fluorescens* and *P. malophilia*) was assessed in vitro against *Alternaia alternate, A. citri, Aspergillus aculatus, A. japonicus, Dreshlera biseptata*. The result indicated that tested bacterial species exhibited varying degree of antagonistic potential against all pathogenic fungi. *Pseudomonas malophilia* showed maximum inhibitory potential against all tested fungi with reduction of up to 60% in fungal colony diameter. Results also showed that *Pseudomonas fluorescens* and *Bacillus fortis* exhibited almost similar biocontrol potential against three pathogenic fungi viz. *Aspergillus aculatus, A. japonicus* and *Dreshlera biseptata*. On other hand, *Escherichia coli* showed least effective biocontrol prospective against *A. citri, Aspergillus aculatus* and *Dreshlera biseptata,* where it reduced the fungal growth from 5 to 12 %. Results indicated that tested bacterial species can be used as promising biocontrol agent.

Key words: Antagonistic bacteria, *Aspergillus, Alternaia, Pseudomonas.*

INTRODUCTION

A significant high number of fungal diseases have an influence on crop plants throughout the year when a farmer fails to take proper preventative measures. Plant disease control, therefore has become heavily dependent on fungicides to combat the wide variety of fungal diseases. Plant protection is an important area, which needs attention due to hazardous inputs of chemicals control (Goud and Muralikrishnan, 2009). Therefore the search for new antimicrobial agents is a field of utmost importance in disease management. Biological control may be an alternative to chemicals in the control of some pathogenic fungi, or in order to reduce environmental pollution (Frommell and Pazos, 1993). It has been described as a non-hazardous strategy to reduce crop damage caused by plant pathogens (Weller, 1988; O'Sullivan and O'Gara, 1992; Cook *et al*., 1995). A large body of information has been accumulated regarding antagonism between bacteria and fungi, and its possible role in the biological control of plant pathogenic fungi (Gowdu and Balasubramanian, 1988). Production of antimicrobial compounds seems to be a general phenomenon for most bacteria. A broad-spectrum of classical antibiotics, metabolic by-products, lysozyme and several types of protein exotoxins, and bacteriocins has been reported in bacteria (Riley and Wertz, 2002; Yeaman and Yount, 2003). These biological substances are remarkable in diversity and natural abundance, since some substances are restricted to some bacterial groups while other are extensively produced (Riley and Wertz, 2002; Parret *et al.,* 2003). Antifungal metabolites produce by bacteria like *Pseudomonas* spp. *Bacillus* spp., have been investigated for their antifungal properties (Moita *et al.,* 2005; Siddiqui *et al.,* 2005; Nourzian, 2006). The aim of this research was to determine, the antagonistic effectiveness of some soil bacteria against important phytopathogenic fungi *in vitro*.

MATERIALS AND METHODS

Bacterial cultures: Six soil samples were randomly collected from field areas of the University of the Punjab, Lahore, Pakistan, in sterilized plastic bags. For bacterial isolation, a soil dilution plate method was used (Waksman, 1922). One gram of soil sample was suspended in 10ml of sterilized distilled water, course particles were removed by filtration through a layer of gauze. One ml of filtrate was used to make serial dilution of soil samples up to 10^{-5}. One ml of the 10^{-5} soil dilution was added onto surface of solidified Loury Burmti (LB) medium in plates. The dilution was spread with sterilized spreader on the medium and the plates were placed in an incubator at 37°C for 24 hours. Distinct individual bacterial colonies were purified by streaking on a new nutrient agar plate. Pure cultures were identified according to the literature (Bergy and Holt, 1993). Selected bacterial species were: *Escherichia coli, Bacillus*

fortis, B. farraginis, Pseudomonas flourescens and *P. malophilia*

Isolation of Identification of pathogenic fungi: Phytopathogenic fungal species were isolated from specific infected samples (Table. 1) by direct plating method (Nazim *et al.,* 2008). Small pieces of sample were sterilized in 1% sodium hypochloride for two minutes, washed three times with sterile distilled water and placed on Petri dishes 2% Malt Extract Agar (Malt extract 20g, agar 15g, distilled water 1L). Plates were incubated at 25^{o} C for 4 days. The cultures were further purified by single spore isolation technique (Choi *et al.,* 1999). Purified fungal species were identified according to literature (Ellis, 1971, 1976; Pitt, 1979; Raper and Fennell, 1965; Domsch, *et al*., 1980).

Table 1. Phytopathogenic Fungi and their sources

Pathogenic Fungi	**Sources**
Alternaia alternata [(Fr.) Keissl.]	*Aloe vera* leaf spot
Alterneria citri [(Penz.) Mussat]	Citrus twigs, and fruit
Aspergillus aculatus [Iizuka]	Rotted Grapes
Aspergillus japonicus [Saito]	Cotton field soil
Dreshlera biseptata [(Saccardo & Roumeguere) Richardson & Fraser]	Seeds of wheat and rice

In vitro Antagonistic potential of bacterial strains against some pathogenic fungi: Antagonistic activity of bacterial strains against fungal pathogens were studied by agar diffusion technique. For testing for antimicrobial activity, 0.3ml suspension (10 CFU/mL; 0.5 Mac- 6Farland) of antagonist bacteria was inoculated on the surface of Malt-Extract-agar (MEA) medium in 9 cm Petri plate (Mushtaq *et al.,* 2010). Bacterial suspension was spread evenly on the surface of medium. A plug disc of 5 mm diameter received from 7 days old fungal culture was taken by cork borer and was placed in the center of each Petri dish to test the inhibition activity of tested bacterial species. Mycelial growth of each fungal species was measured after 7 days of incubation at 26° C. Fungal growth without bacterial inoculum was taken as control. This experiment was conducted in 3 replicates for each bacterial species. Percentage inhibition was calculated using the following formula:

% inhibition = [1 - (Experimental fungal colony diameter/ Control diameter)] x 100

RESULTS

Screening of the antimicrobial activity of five bacterial isolates was carried out against 5 different pathogenic fungi viz *Alternaia alternate*, *A. citri*, *Aspergillus aculatus, A. japonicus* and *Dreshlera biseptata*. Results showed that bacterial isolates exhibit varying degree of biological potential against pathogenic fungi (Fig 1 & 2) as compare to control. *Pseudomonas malophilia* showed maximum inhibitory potential against all tested fungi with reduction up to 60% in fungal colony diameter. Although, *P. fluorescens* and *B. farraginis* was most effective against *A. citri*, where these bacteria reduced fungal growth to 46-56 % (Fig. 2). On other hand, *E. coli* showed least effective *biocontrol* prospective against *A. citri*, *A. aculatus* and *D. biseptata*, where it reduce the fungal growth from 5 to 12 % (Fig 1& 2). Results also showed that *P. fluorescens* and *B. fortis* exhibited almost similar biocontrol potential against three pathogenic fungi viz. *A. aculatus*, *A. japonicus* and *D. biseptata* (Fig. 2).

DISCUSSION

This study showed variation in antimicrobial potential among different soil bacterial isolates. All bacterial isolates exhibited antimicrobial activity against tested pathogenic fungi. The high proportion of antimicrobial producing strains may be associated with an ecological role, playing a defensive action to maintain their niche, or enabling the invasion of a strain into an established microbial community (Motta *et al.,* 2004).

Results indicated that *P. malophilia* exhibited significant inhibitory activity against all tested fungi. Whereas *P. fluorescens* was least effective as compare to *P. malophilia*. Although different studies reported the antifungal potential of *P. flourescencs* against pathogenic fungi, like *Alternaria cajani*, *Curvularia lunata*, *Fusarium sp.*, *Bipolaris sp.* and *Helminthosporium sp.* (Srivastava, and Shalni, 2008) Results also showed that *P. fluorescence* and *B. fortis* exhibited almost similar biocontrol potential against three pathogenic fungi viz. *A. aculatus*, *A. japonicus* and *D. biseptata*. Previously, antifungal potential of *Bacillus* sp, *Pseudomonas* sp. and *Escherichia* sp. has also been reported to inhibit the mycelial growth of many species of *Aspergillus*, *Penicillium* and *Fusarium* (Nourozian *et al.,* 2008; Munimbazi and Bullerman, 1998; Mushtaq *et al.,* 2010).

In the present study, *Escherichia coli* showed least effective biocontrol prospective against *A. citri*, *A. aculatus* and *D. biseptata*, where it reduced the

fungal growth from 5 to 12 %. But other studies have reported cytosolic proteins of *Escherichia coli* are responsible for antifungal potential against pathogenic strains of *Aspergillus fumigatus*, *A. flavus*, *A. niger* and *Candida albicans* (Yadav *et al.*, 2007& 2010; Mushtaq *et al.*, 2010). Results of this study indicated that the potential of these microorganisms

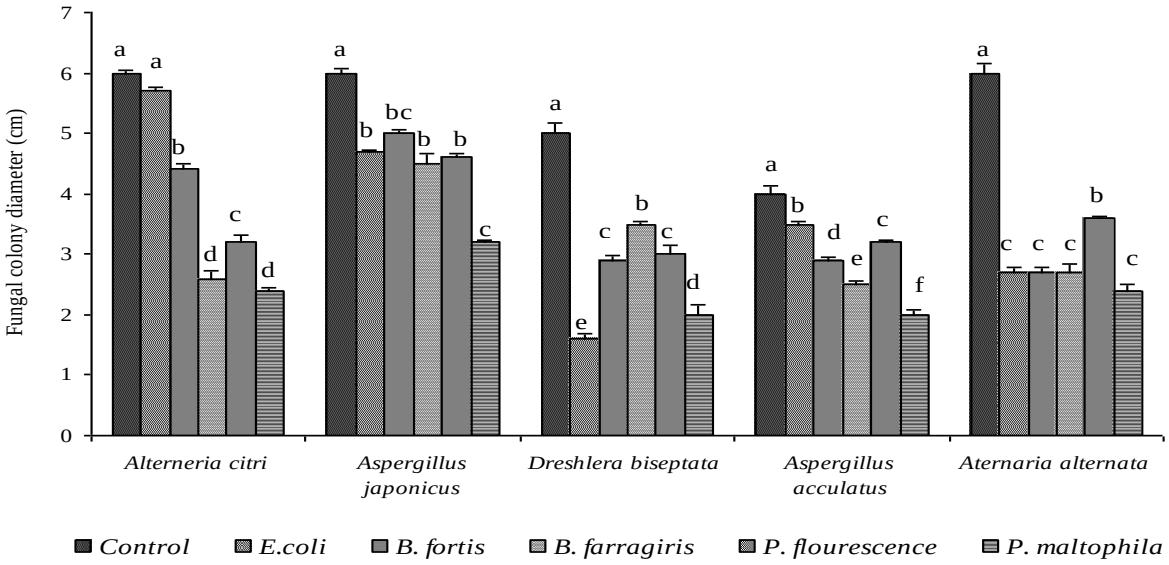

Fig 1. Inhibitory effects of (*E. coli, B. fortis, B. farragiris, P. fluorescence* and *P. maltophila*) on reduction of mycelial growth of (*A. citri, A. japonicas, D.biseptata, A.aculatus* and *A.alternata*).

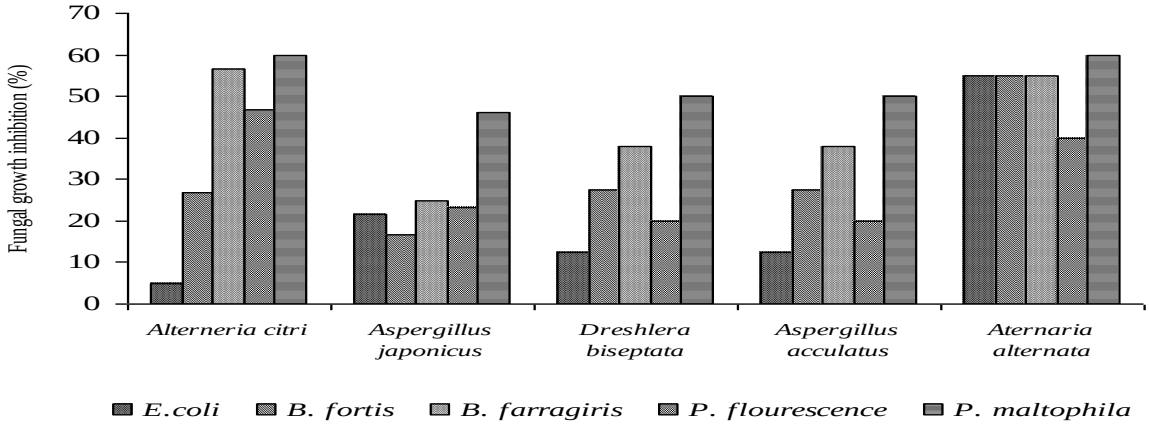

Fig. 2: **Percentage of mycelia growth inhibition of the tested fungi (*A. citri, A. japonicas, D.biseptata, A.aculatus* and *A.alternata*).**

to produce antimicrobial compounds that can be useful for many applications is great and must be better explored in future.

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