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Antagonisitic Potential of Soil Bacteria Against Food Borne Fungi

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Abstract: Bacteria are able to synthesis a wide range of metabolites with fungicidal capabilities. The antagonistic potential of soil bacterial strains (*E. coli, Baccillus fortis, B. faragiris, Pseudomonas flourescence* and *P. malophilia*) was assessed by the dual culture technique against some food pathogenic fungi, i.e. *Aspergillus flavus*, *A. niger*, *Penicillium italicum* and *P. simplicissmum*. The result indicated that *bacterial* species exhibited varying degree of biocontrol potential against all tested fungi. Out of five bacterial species, *E. coli* showed effective biocontrol potential against all tested fungi with reduction of 82-96% in fungal colony diameter. *Escherichia coli* showed almost complete inhibition against pathogenic fungi such as *Aspergillus niger, A. flavus* whereas *Bacillus fortis* was least effective. The experimental results exhibit the antifungal activity of bacterial species and indicate the possibility of using these bacterial species as antifungal agents against these fungal species.

Key words: Antagonistic · *Aspergillus* · Biocontrol · *Penicillium*

INTRODUCTION

Biocontrol seems to be a reliable alternative to synthetic chemicals, which have raised serious concerns of food contamination. Filamentous Fungi cause serious problems in food by producing mycotoxins and potentially allergenic spores, causing spoilage of food and food products that is costly as well as sometimes dangerous. *Aspergillus* and *Penicillium* species are commonly found as contaminants in foods throughout drying and subsequent storage [1, 2]. Filamentous fungi, mainly *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. produced mycotoxins, secondary metabolites, under the appropriate environmental conditions, are toxic [3].

Antifungal agents produced by microorganisms may be used as biocontrol agent. Antifungal Metabolites produce by bacteria like *Pseudomonas* spp. *Bacillus* spp., have been investigated for their antifungal properties [4-6].

Bacillus species, gram-positive bacteria, are good biological control agents (BCA) for, their ability to produce different types of antimicrobial compounds, such as antibiotics (e.g., bacilysin, iturin, mycosubtilin), siderophores and to induce growth and defense responses in the host plant [7, 8]. However, gram-negative bacteria belonging to Pseudomonas genera have gained significant attention for antagonistic activity [7, 9, 10, 11,].

The aim of this work was to evaluate biocontrol potential of soil bacterial species *E.coli Bacillus,*and *Pseudomonas* spp. against food born pathogenic fungi.

MATERIALS AND METHODS

Soil samples were randomly collected from four different sites near Lahore, Pakistan, in sterilized plastic bags until (Table 1). The samples were processed using the soil dilution plate (12). For soil dilution, one gram of soil diluted in 10ml of sterilized distilled water, course partials 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}. For bacterial isolation, 1ml of the 10^{-5} dilution was added on solidified Loury Burmti medium (g/L) plates. The dilution was spread with sterilized spreader and the plates were placed in an incubator at 37°C for 24 hours. Distinct individual colonies purified by streaking on a new nutrient agar plate. Pure cultures were identified according to the literature [13]. Selected bacterial species were: *E.coli, Bacillus fortis, B. farraginis, Pseudomonas flourescence* and *P. malophilia*.

Pathogens: For fungal isolation different fruit samples were collected from local market Lahore, Pakistan (Table 1). Fungal species were isolated on Malt Extract

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Table 1: List of Microorganisims

Microorganisms	Source	Location
Fungi		
Aspergillus flavus(Link)	Apple	Faisalabad
Aspergillus niger (Tiegh)	Onion	Lahore
Penicillium italicum (Wehmer)	Lemon	Lahore
P. simplicissimum (Oudem.) Thom)	Grapes	Lahore
Bacteria		
Escherichia coli	Agricultural soil	Lahore
Pseudomonas fluorescence	Root nodules (wild pea)	Lahore
Pseudomonas malophilia	Wheat field soil	Lahore
Bacillus fortis	Wheat field soil	Lahore
Bacillus farraginis	Wheat field soil	Lahore

Agar (Malt extract 20g, agar 15g, distilled water 1L) by direct plating method [14]. The cultures were further purified by single spore isolation technique [15]. Pure cultures were identified according to valuable literature [16, 17]. Most frequent fungal isolates were used in experiment.

***In vivo* Evaluation of the Antagonistic Potential of the Tested Bacterial Bioagents:** Antifungal activity of bacterial strains against some food borne fungi, A*spergillus flavus*, *A. niger*, *Penicillium italicum* and *P. simplicissmum* antimicrobial activity was determinate by agar diffusion technique. For testing antimicrobial activity, malt-extract-agar (MEA) medium was used. After solidification of 9cm Petri plate, agar surface was inoculated with 0.3ml suspension (10^6CFU/mL; 0.5 Mac-Farland) of antagonist bacteria. Then small disc from 7 days old fungal culture was taken by cork borer, each of 4 mm diameters. Each disc was cultured in the center of each Petri dish to test the inhibition activity of each isolated bacteria. Each fungal growth was measured after 5days of incubation at 26°C. Fungal growth without bacterial inoculum was used as control. The experiment was carried in triplicate. The cultures were examined for the presence of a clear inhibition zone around the mycelium discs. Percent inhibition was calculated using the following formula:

% inhibition = (1 - (Fungal growth / Control growth)) x 100

RESULTS

Five bacterial species were screened for their antifungal activity against different fungi viz. *Aspergillus flavus*, *A. niger*, *Penicillium simplicissimum* and *P. italicum*. Experimental results showed that all tested bacterial species show varying degree of biocontrol potential against fungal strains (Table 2). *Escherichia coli* showed effective biocontrol potential against all tested fungi with reduction of 82-96% in fungal colony diameter. While in case of *Bacillus fortis,* colony dimatere of *Aspergillus flavus* and *Penicillium simplicissmum* was effectively reduced up to 93% and 76% respectively.

On the other hand, *Bacillus faragiris* showed effective biocontrol potential against *Aspergillus flavus* where it reduced the fungal colony diameter up to 87% followed by *Aspergillus niger* (78%) and *Penicillium italicum* (77%). *Pseudomonas flourescence* showed highest antifungal activity against *Penicillium italicum* (94%) and was moderately effective against *Aspergillus niger* (61%). While in case of *Pseudomonas malophilia* colony diameter of *Aspergillus flavus*, *Penicillium italicum* and *Penicillium simplicissmum* was reduced up to 88-98% followed by *Aspergillus niger* (64%).

Table 2: Antagonistic potential of bacteria on the fungal pathogens

	Fungal growth (cm)			
Bacterial strains	*Aspergillus niger*	*Aspergillus flavus*	Penicillium simplicissmum	Penicillium italicum
Control	9.0±0.00	8.3±0.33	2.5±0.00	3.5±0.00
Escherichia coli	1.6±0.08	1.2±0.08	0.1±0.02	0.3±0.06
Bacillus fortis	6.5±0.86	0.6±0.57	0.6±0.08	2.7±2.60
Bacillus faragiris	2.0±0.09	1.1±0.09	1.0±0.12	0.8±0.02
Pseudomonas flourescence	3.5±0.28	4.4±0.26	1.3±0.05	0.2±0.02
Pseudomonas malophilia	3.2±0.14	0.2±0.08	0.3±0.08	0.1±0.02

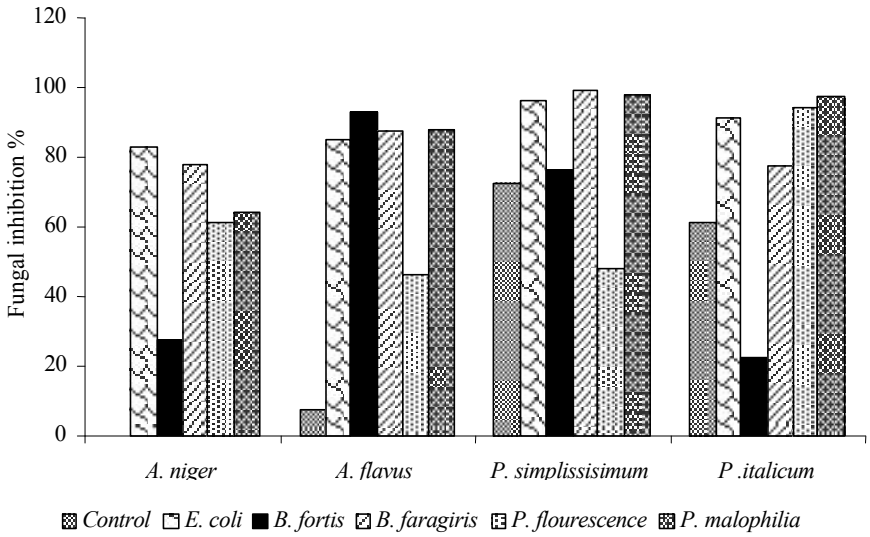

Fig. 1: Inhibition Percentage (%)of fungal species

DISCUSSION

The antagonism between different microbial strains can be expressed by the production of metabolites, competition and direct parasitism, pathogen enzyme activity is also associated with reduction of induced resistance [18]. Several microbial agents have been used for biological control of fresh fruits [19].

In the present study, *Pseudomonas flourescence* showed highest antifungal activity against *Penicillium italicum* (94%) While in case of *Pseudomonas malophilia* colony dimatere of *Aspergillus flavus*, *Penicillium italicum* and *Penicillium simplicissmum* was reduced up to 88-98% while *Pseudomonas flourescence* and *P. malophilia* moderately inhibit Aspergillus *niger* growth, 61% and 64% respectively. Rachna and Shalni [20] also reported the antifungal potential of *Pseudomonas flourescence* against pathogenic fungi, *Alternaria cajani*, *Curvularia lunata*, *Fusarium sp.*, *Bipolaris sp.* and *Helminthosporium sp.* Other studies have already shown the successful control of *Aspergillus flavus* by antagonistic bacteria [21, 22]. In case of *Bacillus fortis, B. faragiris* showed effective biocontrol potential against *Aspergillus flavus* where they reduced the fungal colony diameter up to 93% and 87% respectively while colony diameter of *Penicillium simplicissmum* reduced up to 76% by *Bacillus fortis* and *P. italicum* and *A. niger* was effectively reduced up to 77% by *B. faragiris.* Similar growth inhibitory ability of *Bacilli* species as *Bacillus subtilis B. polymyxa in vitro* have been reported against wood decaying fungi [23]. Previously, antifungal potential of *Bacillus* sp, *Pseudomonas* sp. and *Streptomyces* sp. has also been reported to inhibit the mycelial growth of many species of *Aspergillus, Penicillium* and *Fusarium* [6, 24].

Escherichia coli effectively suppressed colony growth of all tested fungal species. Yadav [25, 26] has reported cytosolic proteins of *Escherichia coli* are responsible for antifungal potential against pathogenic strains of *Aspergillus fumigatus*, *A. flavus*, *A. niger* and *Candida albicans*.

The presented data exhibit the antifungal activity of bacterial species and indicate the possibility of using these bacterial species as a biological agent to control these pathogenic fungi. However, biological agents tested in this study should be investigated extensively for food safety before commercialization.

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