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STUDIES ON ANTIFUNGAL ACTIVITY OF A BACTERIAL STRAIN ON SOME FOOD SPOILAGE FUNGUS

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ABSTRACT

Due to the development of increased resistance power of large number of fungal and bacterial pathogens to common antimicrobial agents, there is an urgent need to survey for new novel antimicrobial agents. In the present study the bacterial strain capable of producing antimicrobial agent was isolated from soil collected from the organic waste dump site. The isolated bacterial strain was further screened for their antifungal activity. The identified antifungal bacteria were *Azomonas sp*. DS. The metabolites of the isolated bacteria were screened for antifungal activity against ten different fungal species. Screening was done by using paper disc containing 20µl of culture supernatant placed on nutrient agar plate seeded with test organisms. The activity was determined by measuring the inhibition zone diameter (in cm) both for crude and dilution with 0.1 (M) phosphate buffer (pH-7.0) in the ratio of 1:1 (Crude extract:Buffer) . The *Azomonas sp*. DS showed inhibition zone diameter of 6.3 cm against *Beauveria sp*. with the application of crude metabolite and dilution of crude metabolite with 0.1 (M) phosphate buffer (pH-7.0) in the ratio of 1:1 (Crude metabolite: Buffer) showed inhibition zone diameter of 4.0 cm. From this work it might be concluded that *Azomonas sp*. DS can be used for the production of novel antifungal agent. Further study will be needed for characterization of the metabolites.

KEY WORDS: Bacteria, Fungi, Antifungal bacteria. Antifungal agents, Bacterial metabolites.

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INTRODUCTION

There is an urgent need to survey for new a novel antimicrobial compound against large number of fungal and bacterial pathogens. These pathogens are extremely difficult to control due to its complex epidemiology and virus pathogen of aerial infection [1]. Isolation of bacterial strains directly from soil could be a good alternative for the selection of bio control agent, since these cultures from nature are already well developed to survived in the field in contrast to laboratory strains [2].With a view to isolate novel bacterial culture, having antifungal characters, soils collected from organic waste dump site and then screened their antimicrobial activities against several fungal species.

MATERIALS AND METHODS

Sample collection

The soil samples used for this work were collected from different locations in and around Bangalore, Karnataka. The samples were labelled according to the site of collection as BANG 1, BANG 2, BANG 3, BANG 4, and BANG 5 samples. The samples were transported in sterile polyethylene bags to the laboratory. When samples could not process immediately, they were stored at 4°C for no longer than 18 to 24 h.

Isolation of bacterial strain

The soil sample was mixed and a suspension of 1 g (dry weight equivalent) in 10 ml of sterile water was prepared. One ml of the soil suspension was then diluted serially (ten-fold) and used in the estimation of aerobic heterotrophic bacterial populations by standard spread-plate dilution method described by Ogunmwonyi et al. (2008) [3] in triplicate. Nutrient agar containing 0.015% (w/v) nystatin (to inhibit fungi growth) was used for bacteria isolation and incubation was at 35^{o}C for 48 h. Pure isolates of representative communities were maintained on agar slant at 4^{o}C.

Screening and identification of antifungal bacterial isolates

Dual culture method was performed to screen the antifungal activity of bacterial strain. Briefly, One 10-mm disk of a pure culture of test fungi was placed at the center of a Petri dish containing PDA. A circular line, made with a 6 cm diameter Petri dish dipped in a suspension of bioantagonistic bacteria (5 x 10^{9}-cfu mL^{-1}), was placed surrounding the fungal inoculum. Plates were cultured for 27±3°C for 72 h and growth diameter of the pathogen (fungal growth) was measured and compared to control growth where the bacterial suspension was replaced by sterile distilled water. Identification of bacterial isolates showing antifungal properties were based on cultural, microscopic, and biochemical characteristics with reference to Holt et al. (1994)[4].

Separation of antifungal metabolites

Cell free supernatant of these cultures was collected, by growing them on nutrient broth (NB) for 48 h on a rotary shaker at 200rpm at 35±2^{0}C, centrifuged at 10,000rpm for 20 min. at 4^{0}C. Antibacterial compound was recovered from the filtrate by solvent extraction method following the process described by Westley *et al*, 1979 [5] . Ethyl acetate was added to the filtrate in the ratio of 1:1(v/v) and shaken vigorously for 1 hr for complete extraction. Antibiotic was present in ethyl acetate phase ,so it was separated from the aqueous phase. Then it was evaporated to dryness by keeping it in water bath at 60^{0}C-70^{0}C. After completely evaporation, the leftout residues were taken for further studies.

Detection of antifungal activity

The antifungal activity was determined by agar well method [6,7,8]. The partially purified extract obtained by the evaporation of the ethyl acetate extract was dissolved in 1 ml 0.2 M phosphate buffer (pH 7.0). Then 100µl of it was loaded into well bored and test organism swabbed Potato Dextrose agar plates. The plates were incubated at 27±3°C for 72 h and examined. The diameter of the zones of complete inhibition was measured in centimeter.

RESULTS AND DISCUSSION

The total bacterial counts (TBC) of soil samples ranged from 5x10^{5}-2x10^{6} colony forming units (cfu) per gram of soil. From the data it is clear

that there were differences in the averages total bacterial counts of the different sampling sites. The microbial population may differ according to the composition of soil at different sampling locations. Moreover, it was observed that the variation of species differed from one sample to another (data not shown). The bacterial strain was subjected for initial screening using test fungal isolates of *Aspergillus niger* and *Trichoderma sp.* These fungal strains were selected for initial screening, as it is the most common soil fungi and *Trichoderma* is used as biocontrol agent against a variety of plant pathogens [7,9,10,11]. The bacterial strain showing antogonostic activity was selected based on the inhibitory activity towards the test fungus. The isolate was designated as DS. The culture DS was identified as *Azomonas* sp. *Azomonas* are typically motile, oval to spherical, and secrete large quantities of capsular slime. They are Gram positive, non-spore forming aerobic bacteria usually found in soil. The spectrum of action of the metabolite against different fungal cultures is recorded in Table 7. *Azomonas* sp.DS showed inhibitory activity towards all the fungal pathogens. The cell free extracts of *Azomonas* sp. inhibited the growth of *Trichoderma* sp. and *Cercospora* sp. with an inhibition zone of 26 cm and 17 cm respectively. However, *Aspergillus* sp., *Cladosporium* sp. and *Rhizotonia* sp. were inhibited by the bacterial extracts with a low degree of inhibition (12-13 cm). The diluted (1:1) extract of the *Azomonas* sp. inhibited the growth of Trichoderma sp. however, the diluted extract inhibited the other pathogens with a low degree of inhibition. The cell free extract of *Azomonas* sp was highly active. However, the other fungal pathogens were inhibited at a minimum level at 1:1 dilution. However, this is the first report on antifungal activity of *Azomonas* sp DS .The control mechanisms used by the bacteria may involve the secretion of fungal cell wall hydrolytic enzymes [10,11,12]. Microbial action cannot be restricted to one niche; so it is necessary to test their adaptation to other sites especially when bacteria have expressed a good antagonistic activity against specific phytopathogenic fungi [8,9,13]. The data reported here indicate that the same antagonistic strains of bacteria that protect from plant pathogens. This strain can be also used as seed inoculants to prevent the seed borne pathogens and their infections.

Table1

Germination of spores in hours at different concentration of culture fluid.

Concentration	*Conc.*	*10^{-2}*	*10^{-4}*	*10^{-6}*	*Type of spores*
Culture No.	Time in hours				
DS	20	12	10	8	
Control	6				*Aspergillus niger*
DS	18	14	10	8	
Control	4				*Trichoderma spp.*

Table 2

ED_{50} values of different cultures for Aspergillus niger.

Culture No.	ED_{50} value (Concentration)
DS	10^{-2}

Table 3

ED_{50} values of different cultures for Trichoderma spp.

Culture No.	ED_{50} value (Concentration)
DS	10^{-6}

Table 4

Morphology of the bacterial cultures.

Culture no.	Gram's reaction	Morphology	Size in µm	Motility
DS	- ve	Cocci	2.75	Non motile

Table 5
Fermentation of carbohydrates by the bacterial cultures.

Culture No.	Glucose	Sucrose	Lactose	Mannitol	Gas production
DS	+	+	+	+	-

Table 6
Biochemical reactions shown by the bacterial cultures.

Culture No.	Amylase production	Gelatin hydrolysis	Casein hydrolysis	Catalase
DS	-	+	-	+

Table 7A
Antimicrobial effect of the cell free supernatant on fungal cultures.

Test organisms	Concentrated Culture fluid DS1**
A. ochraceous	3.0
A. niger	3.0
Trichoderma	5.4
Mucor	2.3
Penicillium	nil
Beauveria	6.3
Cladosporium	5.8
Fusarium	2.9
Ceracospora	3.5
Rhizactonia	2.5

zone of inhibition in cm. Value are average of four reading
*****culture fluid: 24 hrs old culture was centrifuged at 10,000xg for 20 min and used***
******culture fluid was diluted with 0.1M phosphate buffer pH 7.0***

Table 7B
Antimicrobial effect of the cell free supernatant on fungal cultures.

Test organisms	1:1 Diluted Culture fluid DS1 ***
A. ochraceous	3.0
A. niger	3.0
Trichoderma	5.4
Mucor	2.3
Penicillium	nil
Beauveria	6.3
Cladosporium	5.8
Fusarium	2.9
Ceracospora	3.5
Rhizactonia	2.5

zone of inhibition in cm. Value are average of four reading
*****culture fluid: 24 hrs old culture was centrifuged at 10,000xg for 20 min and used***
******culture fluid was diluted with 0.1M phosphate buffer pH 7.0***

Table 8
Antifungal properties of ammonium sulphate precipitated Metabolite on test organisms.

% Ammonium sulphate	0-50	50-60	60-70	70-80	Test organism
Bacterial cultures	Zone of inhibition in cm				
Azomonas DS	1.5	1.9	2.23	2.18	*Aspergillus niger*
Azomonas DS	2.23	2.20	2.00	2.33	*Trichoderma* sp.

Table 9
Antifungal properties of ethyl alcohol precipitated metabolite on test organisms.

Test organism	*Aspergillus niger*	*Trichoderma* sp.
Bacterial cultures	Zone of inhibition in cm	
Azomonas DS	Nil	Nil

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