Research Article

A Study on Antagonistic Potential of Bacteria against Phytopathogenic Fungi

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ABSTRACT

Biocontrol is the most accepted alternative for plant disease management. Among the microorganisms, bacteria are able to produce wide spectrum of antifungal agents. The present study was conducted to determine the antagonistic potential of bacteria against phytopathogenic fungi. Out of eight bacteria were isolated from marine soil, two were appeared potential candidates for production of antifungal compounds. Two isolates are identified as *Klebsiella* sp. AF4 and *Pseudomonas* sp. AF7. The antifungal activities were found strong by both the bacterial strains. Their antimycotic mode of activities can be visualized to be more meaningful against the fungal infections prevailing in this area as compared to chemical antifungal agents.

Keywords: Biocontrol, Antagonistic bacteria, Antifungal activity.

INTRODUCTION

A limiting factor for crop production is the diseases caused by phytopathogenic fungus, affecting seeds from germination and throughout development. Consequently, yield reductions of up to 30% have been reported[1]. Fungicides are the primary means of fungal disease control, but their use is currently controversial because investigation has indicated potentially undesirable environmental side effects[2]. Increased public concern about the accumulation of pesticide residues in the biosphere and the development of resistance among pathogens against conventional antibiotics has led scientists toward the development of alternative strategies for plant disease suppression[3]. Biological control is a nature friendly approach that uses specific microorganisms, which interfere with plant pathogens and pests to overcome the problems caused by chemical methods of plant protection. Among the action mechanisms proposed is mycoparasitism, with concomitant production of enzymes by the microbes that degrade cell walls. Chitinolytic enzymes, together with ß-glucanase or cellulases are the enzymes most frequently considered critical in biocontrol[4]. These antifungal proteins such as chitinases, glucanase are of great biotechnological interest because of their potential use as food and seed preservative agents and for engineering plants for resistance to phytopathogenic fungi[5]. As antifungal activity is most common feature for bacterial species, thus antagonistic bacteria are considered as an ideal biological control agents. The bioactive compound produced by bacteria may act as suppressors or/and inhibitors in the developments of phytopathogens.

Most common fungal pathogens associated with invasive disease in plants are filamentous fungi of *Aspergillus* sp. produced mycotoxins, secondary metabolites, under the appropriate environmental conditions, are toxic[6]. *Aspergillus niger* is a saprophytic and filamentous fungus found in soil, forage, organic debris and food product, causing black mould of onion, Shallot; stem rot of *Dracaena*; root stalk rot of Sansevieria; and boll rot of Cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune[7]. *Aspergillus flavus* is a soil-inhabiting, filamentous fungus that saprophytically utilizes a wide range of organic substrates. Though *A. flavus* is considered a saprophyte, it is also an opportunistic pathogen that can invade agronomically important oil seed crops such as corn, peanut and cottonseed that are under biotic or abiotic stress[8]. Similarly, the genus *Fusarium* contains a number of soil borne species with worldwide distribution which have been known for a long time as important plant pathogens[9]. The most common species, *F. oxysporum*, causes vascular wilt disease in a wide variety of economically important crops[10]. *F. oxysporum* is an anamorphic species characterized by a series of morphological criteria including shape of macroconidia, structure of microconidiophores and formation and disposition of chlamydospores[11].

Therefore, in present work an attempt has been made to isolate and screen potent bacterial species with significant antifungal activity against phytopathogens.

MATERIALS AND METHODS

Collection of soil sample and isolation of bacteria

Soil samples were randomly collected from marine soil, Tamil Nadu. Samples were taken from 2-3 cm depth and collected in sterile polythene bag.

The samples stored at 4 °C. The soil samples were serially diluted up to 10^{-9} dilutions, in distilled water and 1 ml sample from 10^{-8} to 10^{-9} were pour plated in Kings medium B (Hi-Media, Mumbai, India) plates. The plates were kept for incubation at 37 °C for 48 h in an inverted position. The bacterial colonies with different

characteristics were selected and purified by pure culture techniques and refrigerated in nutrient agar slants for further studies.

Isolation and culture conditions of test organisms

The target fungi were chosen to represent potential pathogenic fungi causing plant diseases. Three fungi samples, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* were collected from infected plant sample. Fungi were grown on Potato Dextrose agar (PDA, Hi-Media, Mumbai, India) plates at 25 °C for 5 d, and stored at 4 °C. The cultures were further purified by single spore isolation technique[12].

Identification of fungi

The isolated fungi were identified to the genus and species level on the basis of macro- and micro-morphological characteristics using the most updated keys for identifications[13-15].

Screening of antagonistic bacteria

All isolated bacteria were tested for antifungal activity. A 1-cm^2 fungal plug (any one of targeted fungi) was inoculated in the center of a plate with PDA, each isolated bacterium was streak with a sterile stick at a distance of 2.5 cm from the fungus. The plates were then incubated at 37 °C for 72 h. The strains showed inhibitions of fungal growth were screened for further studies.

Identification of active isolates

The potent isolates selected were characterized by morphological and biochemical methods. The results of microscopic examination were compared with Bergey's manual of systematic bacteriology[16,17] and the organism was identified upto genus level. Various biochemical tests were performed for the identification of the potent isolates are as follows; Fermentation of sugars, Hydrolysis of starch, Indole production, Methyl red, Voges-Proskauer, Citrate utilization, Nitrate reduction test, Catalase test, Oxidase test.

Antagonistic activity assay

Antagonistic activity assay were performed to assess the potential biocontrol activity of active bacterial isolates *in vitro* by inhibiting the growth of phytopathogenic fungus on PDA media[18].

The bacterial inoculum of the active isolates was picked aseptically and streaked in the center of petridish. Fungal inocula consisted of agar disc (1 cm diameter) punched out with sterilized corkborer from the growing margin of colonies was placed on either side of bacteria inoculated plates. The Petri plates were incubated at 37 °C for three days. The diameter of inhibition zones were measured and experiment was repeated three times.

RESULTS AND DISCUSSION

The production of antifungal substances by bacteria has long been recognized and this knowledge is entering practical life through the use of bacterial antagonists to protect crops against their fungal enemies[19].

Isolation and identification of antagonistic strain

A total of 8 bacteria isolated from various coastal area of Tamil Nadu were screened on the bases of fungal growth inhibition. The clear zones were due to antifungal compound produced by bacterial culture. Out of eight, two isolates AF4 and AF7 were found to be the best antifungal agent and were further analyzed for the rest of the studies. Result of Table 1 revealed that the isolate AF4 is a gram-negative, non-motile rod shaped bacterium with Catalase, Urease, Nitrate reduction positive reactions, Oxidase, Methyl red negative reactions, where isolate AF7 is gram-positive, motile, rod shaped bacterium with Catalase, Oxidase, Voges-Proskauer positive reactions, Methyl red, Indole production negative reactions. Based on the physiochemical, morphological characteristics, the isolates AF4 and AF7 were identified as *Klebsiella* sp. and *Pseudomonas* sp., respectively.

Table 1: Morphological, physiological and biochemical characteristics of the isolated strain AF4 and AF7

Characters	Results	
	AF4	AF7
Morphology	Rod shaped, gram -ve, facultative anaerobic	Rod shaped, gram +ve, Obligate aerobe
Motility	-ve	+ve
Catalase	+ve	+ve
Oxidase	-ve	+ve
Methy red	-ve	-ve
Voges-Proskauer	-ve	+ve
Indole production	-ve	-ve
Citrate utilization	+ve	+ve
Nitrate reduction	+ve	+ve
Hydrolysis of urea	+ve	+ve
Fermentation with Glucose	+ve	+ve
Lactose	+ve	-ve
Sucrose	+ve	-ve

Determination antifungal activity

The antifungal effect of isolated two bacterial cultures, *Klebsiella* sp. AF4, and *Pseudomonas* sp. AF7 was tested against the three plant pathogen fungal strains, such as, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* individually (Figure 1). The performed experiments showed that all the phytopathogenic fungi are very sensible to biocontrol agents. The fungal growth inhibition was recorded after 72 h of incubation. The

evaluation of the antagonism was based on zone of inhibition. The active isolate *Klebsiella* sp. AF4 showed effective biocontrol potential against *F. oxysporum, A. niger* with zone of inhibition of 19 mm, 15 mm, respectively, where it reduced to 10 mm for *A. flavus.* While in case of *Pseudomonas* sp. AF7 have exercised highest antifungal activity against *A. flavus* of 31 mm, and against *A. niger*, *F. oxysporum* of 28 mm, 27 mm inhibition zone, respectively. Among these two isolates, *Pseudomonas* sp. AF7 effectively suppressed the colony growth of all tested phytopathogenic fungi.

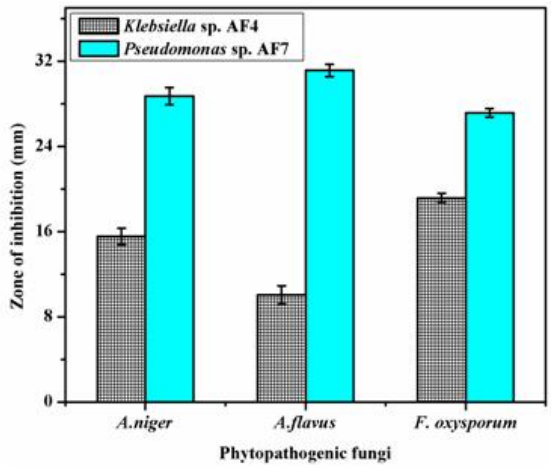

Figure 1: Antagonistic potential of bacterial isolates against phytopathogenic fungi

CONCLUSION

Biocontrol is the new approach for controlling the damage caused by fungal pathogens and reducing the pollution that results from the use of chemical fungicides. Here two promising bacterial cultures isolated from marine environment, which showed a significant *in vitro* antagonistic activity toward different phytopathogenic fungi. These isolates were synthesized antifungal agents which act to protect plants against attack by pathogenic fungi. These observations and further studies will help in developing the *Klebsiella* sp. AF4 and *Pseudomonas* sp. AF7 isolates as a potential biological control agent against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum.*

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