

Full Length Research Paper

Antifungal activity of extracts obtained from actinomycetes

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In the present investigation an attempt has been done on isolation of actinomycetes from the soil, extracting the antifungal compounds from these isolated actinomycetes and then testing the extract against the growth of *Alternaria* sps, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sps, and *Rhizopus stolonifer*. During the investigation it was found that nearly all the extracts were effective against the test fungi and the mycelial growth of fungi is inversely proportional to the concentration of extract.

Key words: Antifungal activity, actinomycetes extracts, plant pathogenic fungi.

INTRODUCTION

Fungal phytopathogens pose serious problems worldwide and cause a number of plants and animal diseases such as ringworm, athlete's foot, and several more serious diseases. Plant diseases caused by fungi include rusts, smuts, rots, and may cause severe damage to crops. Fungi are some of the world's largest and possibly oldest individuals. Some species of fungi produce mycotoxins that are very toxic to humans. For example, the fungus *Claviceps purpurea* causes the ergot poisoning. An individual infected with the mycotoxin experiences hallucination, gangrene, and blood flow restrictions in his limbs. Humans usually get infected with the fungus after eating cereal grains contaminated with *C. purpurea* (Bauman, 2007).

Excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicide. Because of these problems in fungal disease control, a serious search is needed to identify alternative methods for plant protection, which are less dependent on chemicals and are more environmentally friendly. Microbial antagonists are widely used for the biocontrol of fungal diseases. Actinomycetes are the main source of antifungal, hence, highly used pharmacologically and commercially. These are the secondary metabolites of actinomycetes. The antagonistic activity of actinomycetes to fungal pathogens is usually related to the production of antifungal compounds against *Fusarium oxysporum*,

Sclerotinia minor, and *Sclerotinia rolfsii* (Lim et al., 2000). Biological control of plant diseases has received worldwide attention in recent years mainly as a response to public concern about the use of hazardous chemicals in the environment. Soil actinomycetes particularly *Streptomyces* sp enhances soil fertility and have antagonistic activity against wide range of soil-borne plant pathogens (Aghighi et al., 2004).

In the present investigation the ability of extracellular antifungal metabolites of Actinomycetes against *Rhizopus stolonifer*, *Aspergillus flavus*, *F. oxysporum* and *Alternaria* sp has been reported. This study investigated the antifungal activity of the cell-free culture filtrate of this antagonist to determine secondary antifungal compounds. The antifungal potential of extracellular metabolites produced by soil-borne Actinomycetes could be exploited for its future use as an antifungal compounds (Figure 1).

MATERIALS AND METHODS

Collection of soil sample

The soil samples were collected from various locations from Lovely Professional University, Phagwara, from farms in Hoshiarpur (Punjab) and from the farms in Patiala. Several diverse habitats in different areas were selected for the isolation of Actinomycetes. These habitats included the Rhizosphere of plants, agricultural soil. The samples were taken up to a depth of 20 cm after removing approximately 3 cm of the soil surface (Vijaya et al., 2007). The samples were placed in polyethylene bags, closed tightly and stored in a refrigerator. Heat treatment and CaCO₃ treatment of soil

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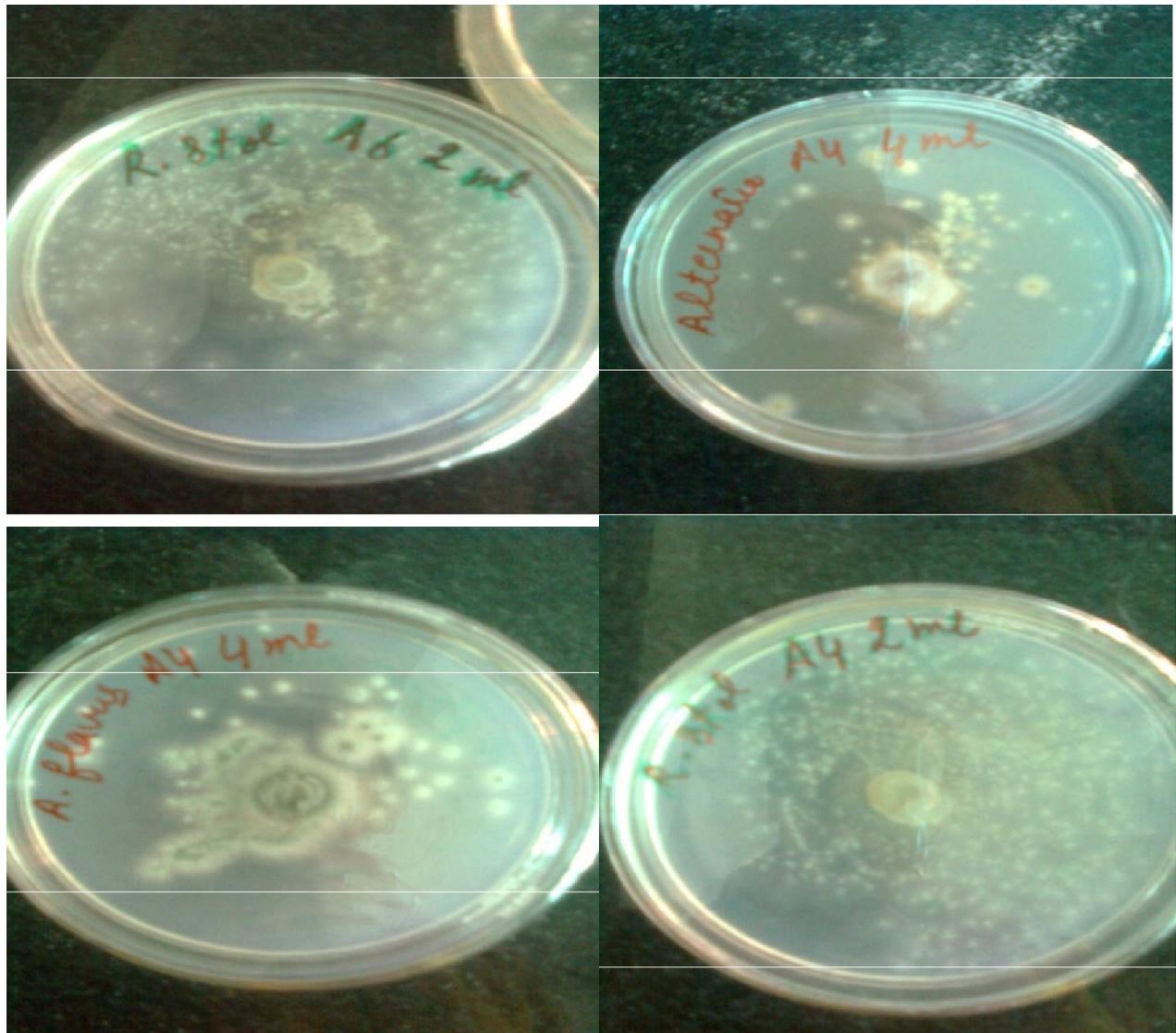


Figure 1. Antifungal activities shown by actinomycetes extract against fungi.

samples were done to isolate actinomycetes.

Media used for the isolation of actinomycetes

Different culture media used for isolation of actinomycetes were Starch casein agar medium, Actinomycetes Hi Veg Agar medium, Actinomycete Isolation Agar medium, Streptomycetes Agar medium

PROCEDURE FOR ISOLATION OF ACTINOMYCETES

Primary isolation

Serial dilution agar plating method is used for the isolation and enumeration of actinomycetes (Aneja, 2003). Prepared Starch casein agar medium is used for the isolation of Actinomycetes. Three starch casein agar media plates with the following dilutions: 1:10,000, 1:100,000 and 1:1,000,000 were used and in these plated 1 ml aliquots of various dilutions was added over cooled and

solidified agar medium. The plates were incubated at 28°C for at least one week.

IDENTIFICATION OF ACTINOMYCETES

The identification of actinomycetes was done on the basis of morphology of spore chain, pigment production, color of aerial mycelium, color of substrate mycelium, consistency, gram's staining, growth on actinomycetes media, growth on streptomycetes media, etc. bio-chemical characterization of actinomycetes was done by esculin hydrolysis, starch hydrolysis, casein hydrolysis, glucose utilization and sucrose utilization.

The potent actinomycetes selected for further studies were characterized by morphological and biochemical methods. The microscopic characterization was done by gram's staining. The mycelium structure, color and arrangement of conidiospore and arthrospore on the mycelium were observed through the oil immersion (100X). The classical method described in the

Table 1. Screening of antifungal activity with extracted antibiotic.

Fungal species	Diameter of Fungal Growth			
	Extract of actinomycetes isolate (2 ml) (mm)	Extract of actinomycetes isolate (4 ml) (mm)	Extract of actinomycetes isolate (6 ml) (mm)	Extract of actinomycetes isolate (10 ml) (mm)
<i>Aspergillus niger</i>	23	18	15	14
<i>Aspergillus flavus</i>	20	No growth	16	15
<i>Alternaria sps</i>	17	15	14	13
<i>Fusarium sps</i>	19	No growth	14	No growth
<i>Rhizopus stolonifer</i>	21	17	15	15

identification key by Nonomura (1974) and Bergey's Manual of Determinative Bacteriology (1957) has been used for the identification of actinomycetes.

plates were inoculated with the agar disc of test fungi in the center of the Petri dish and incubated at 28°C for 4 days. The test fungi used are *Alternaria*, *A. niger*, *A. flavus*, *Fusarium*, *R. stolonifer*.

EXTRACTION OF ANTIBIOTIC FROM ISOLATED ACTINOMYCETES

Method for fermentation

Preparation of inoculums for fermentation process

Starch casein nitrate broth (100 ml) was prepared and then under aseptic conditions a loop full of purified growth added in the Starch casein nitrate broth. This broth was incubated at 28°C in shaking incubator at 150 rpm for 5 days. After 5 days the inoculums for fermentation process was ready for use.

Fermentation method 1: (In shaking incubator) 100 ml Starch casein broth was prepared and autoclaved. 10 ml of the prepared inoculum was added in the broth. Again incubated at 28°C for 5 days in shaking incubator at 150 rpm. The pH of the medium maintained at 5 - 7 after incubation extraction was done (Atta, 2009).

Fermentation method 2: (In fermenter). Prepared 1500 ml Starch casein nitrate broth and autoclaved. Prepared 150 ml inoculums for the fermentation process. Maintained the pH at 5 - 7. Temperature is maintained at 28°C. Followed the fermenter instructions. After five days take out the fermented broth and extract as explained here.

Method for extraction

After fermentation, the medium was harvested and centrifuged to remove cells and debris. Filtrate is collected in a sterilized screw cap bottle. Filter the fermented broth. The filtrate was mixed with ethylacetate in the ratio of 1:1 (v/v) and shaken vigorously for 1 h in a solvent extraction funnel.

The solvent phase that contains antibacterial compound was separated from the aqueous phase. Solvent phase was evaporated to dryness in water bath at 80 - 90°C and the residue is used to check antibacterial activity (Atta, 2009).

Antifungal activity of actinomycetes

Determination of antifungal activities of pure actinomycetes cultures were performed by using agar disc method. Potato dextrose agar plates were prepared and mixed with Actinomycetes culture extract of different concentrations such as 2, 4, 6 and 10%. Then the

RESULTS AND DISCUSSION

The crude extract of antifungal compounds isolated from Actinomycetes was used to check the antifungal activity against test organism. Different concentrations such as 2, 4, 6, and 10% of extract were used to check antifungal activity and to check the minimum inhibitory concentration. On potato dextrose medium the different fungal strains show different zone of inhibition against crude extract of antifungal compound extracted from Actinomycetes species. The extract obtained from Actinomycetes had the ability to inhibit growth of pathogenic fungi at varying degree. From the Table 1 it has been observed that the growth of fungal mycelium decreases with the increase in the concentration of compound extracted from Actinomycetes species. It has been observed that the extract isolated have inhibited the growth of nearly all the test fungi. Similar results have been investigated by various authors including Khamna (2009) who reported that the crude extract of antifungal compounds was active against *R. stolonifer*, *A. flavus*, *F. oxysporum* and *Alternaria*. Lim et al. (2000) selected 32 Actinomycetes isolates, which showed the inhibitory activity against mycelial growth of plant pathogenic fungi like *Alternaria mali*, *Colletotrichum gloeosporides*, *F. oxysporum*, *cucumerinum*, *Magnaporthe grisea*, *Phytophthora capsici*, and *Rhizoctonia solani*.

In search for soil Actinomycetes having antifungal activity against plant fungal-pathogens, 110 isolates were screened by Aghighi et al. (2004), from which 14 isolates were found active against *A. solani*, *A. alternate*, *Fusarium solani*, *Phytophthora megasperma*, *V. dahlia* and *Sacchromyces cerevisiae*. Bonjar et al. (2005) assayed antifungal Actinomycetes strains for antagonistic activity against *V. dahlia*, *A. solani*, *F. solani* and *G. candidum* four worldwide phytopathogenic fungi. From 110 soil inhabitant strains that have been isolated from soil samples, 10 strains showed antifungal activity as determined through screening and bioassays by agar disk and well diffusion methods. Similar results have

been found by El-mehalawy et al. (2005), Kathiresan et al. (2005), Gebreel et al. (2008), Anitha and Rebeeth (2009) and Kavitha et al. (2010). The Present work has resulted in selective isolation of novel soil Actinomycetes and their antifungal activity against some pathogenic fungi. But more precise work and further development in this field is required to produce more potent bioactive antifungal compounds from Actinomycetes which are easily available in the soil.

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