ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 10 | October - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ISOLATION AND SCREENING OF SOIL ACTINOMYCETES AND THEIR ANTIFUNGAL ACTIVITIES AGAINST SOME FUNGI CAUSING OPPORTUNISTIC MYCOSIS

Botany

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ABSTRACT

Background: Actinomycetes are gram-positive, aerobic, endospore producing filamentous bacteria exhibiting high G+C content in their DNA. They are well known for their ability to produce antibiotics as secondary metabolites. Fungi are causal pathogens of human mycoses. Mycoses caused by opportunistic fungi are called opportunistic mycoses. **Aim and Objective:** To isolate and characterize the actinomycetous bacteria from grass land soil of Patna and to evaluate the antifungal activities against fungi causing opportunistic mycoses in human beings. **Methods:** Actinomycetous flora were isolated by serial dilution technique and identified on the basis of morphological features. The Actinomycetous isolates were in vitro screened for their antifungal activity against the fungi causing opportunistic mycoses viz. Aspergillus niger and Candida albicans by spot inoculation method. **Results:** Out of eleven Actinomycetous isolates, only six viz Actinomicrobium, Sphaerobacter, Actinomyces, Streptomyces, Microbispora and Actinobispora showed antifungal activity against Aspergillus niger and Candida albicans. **Conclusions:** It can be concluded that the Actinomycetous isolates can be useful for the production of antifungal drugs for treatment of mycoses.

KEYWORDS

Actinomycetes, Antifungal activity, Opportunistic mycoses, Ray fungi

INTRODUCTION

Actinomycetes, commonly known as ray-fungi are gram-positive, aerobic, endospore producing filamentous bacteria exhibiting high G+C content in their DNA with the GC percent of 57-75 (Ho et al[1]). They form branching network of hyphae on a solid substratum. The hyphae are septate, and the hyphal cells contain several nucleoid. Hyphal cells are about 20 µm long. Many Actinomycetes have an aerial mycelium that extends above the substratum and forms asexual, thin walled spores, called conidia on conidiophores. Actiniomyces, Actinoplanes, Micromonospora, Microbispora, Nocardia, Streptomyces, Thermoactinomyces etc. are most common soil Actinomycetes. The species of Streptomyces produce an odour-causing compound called geosmin, which gives soils their characteristic earthy odor. Actinomycetes are well known for their ability to produce antibiotics as secondary metabolites (Baltz[2], Demain and Sanchez[3], Kekuda et al[4], Naine et al.,[5] Newman and Cragg[6]). Streptomycin, Gentamycin, Erythromycin, Chloramphenicol, Rifamycin, Actinomycin etc. are presently used antibiotics produced by Actinomycetes (Atta et al[7], Duraipandiyan et al[8], Gallagher et al[9], Zotchev[10]).

Fungi are causal pathogens of a number of human diseases viz. phycomycosis, Candidiasis, Actinomycosis, Detmatomycosis, Aspergillosis, Penicillosis, Otomycosis (Mycotic Otitis) etc. These diseases are known as mycoses. Mycoses caused by opportunistic fungi are called opportunistic mycoses. An opportunistic organism is generally harmless in its normal environment but becomes pathogenic in a compromised host. A compromised host is seriously debilitated and has a lowered resistance to infection. This condition occurs due to malnutrition, alcoholism, cancer, diabetes, leukemia, or another infectious disease, trauma from surgery or injury, an altered microbiota from the prolonged use of antibiotics (e.g., in vaginal candidiasis), and immunosuppression by drugs, viruses (HIV), hormones, or genetic deficiencies. The most important opportunistic mycoses include systemic aspergillosis, candidiasis, and pneumocystis carinii pneumonia. The major portal of entry for Aspergillosis is the respiratory tract. Inhalation of conidiospores can lead to several types of pulmonary aspergillosis. Similarly, Candidiasis is the mycosis caused by the dimorphic fungus Candida albicans.

Antifungal bacteria have been isolated by JavedIqbalQazi et al[11], Catherine[12], Chakrabarti and Shivarakash[13], Richardson[14] etc. Antimicrobial activities of microbial strains have been studied by several workers viz. Vineeta et al[15], Okami and Hotta[16], Bikova and Treimanis[17]etc. Similarly, antibiotic producing micro-organisms have been isolated and characterized by Atul Pratap et al[18], Singh et al[19], Mishra et al[20], Kolawole et al[21] etc.

The present investigation is aimed to isolate and characterize the actinomycetous bacteria from grass land soil of Patna and to evaluate the antifungal activities against fungi causing opportunistic mycoses in human beings.

MATERIALS AND METHODS

Soil samples were collected from grass land of Patna at the depth of about 10 cm from the surface. These were air-dried, crushed and sieved. For the isolation of Actinomycetous flora, 5 g of soil sample were suspended in 50 mL of normal saline containing 8.5 g/L NaCl. The soil sample was incubated in an orbital shaker incubator at 28±2^{0}C at the speed of 200 rpm for 30 minutes. Serial dilutions of 10^{-2} to 10^{-5} were prepared using sterile physiological saline and agitated by vortexing. 0.1 mL from each dilution was spread evenly on actinomycetes isolation media viz. Starch casein agar and Water-Yeast extract agar (WYE) media. Rifampicin (2.5 mg/mL) and Amphotericin B (75 mg/mL) were added to both the media to prevent bacterial and fungal growth respectively. The culture plates were incubated at 28 ±2^{0}C for seven to fifteen days and checked for the appearance of colonies.

Actinomycetous flora were identified on the basis of morphological and chemical characteristics following Bergey's Manual of Determinative Bacteriology ed. 9th. The morphological characters of Actinomycetes were performed by Gram staining using iodine crystal violet solution, shape and size. Microscopic characterization was performed by cover slip culture method. Structure of mycelia, their colour and arrangement of conidiophores or arthrospores was studied under oil immersion objective (1000X) of a compound microscope.

The Actinomycetous isolates were in vitro screened for their antifungal activity against the fungi causing opportunistic mycoses viz. Aspergillus niger and Candida albicans by spot inoculation method of Shomurat et al[22]) and single line streak method of Duraipandiyan et al[23] on agar medium. In the spot inoculation method, the actinomycetous isolates were inoculated on starch casein agar medium. The plates were incubated at 28 ±2^{0}C for six days, and then inverted for 40 minutes over chloroform in fume hood. Colonies were then covered with 0.6 % agar layer of nutrient agar medium, previously seeded with one of the test organism. The antifungal activity was observed after 24 hours of incubation at 37^{0}C. In single line streak method, the actinomycetous isolates were inoculated in a single streak down the middle of a plate of screening media and incubated at 28 ±2^{0}C for four days. A single streak of each test organism was added perpendicularly to the Actinomycetes streak and incubated at 37^{0}C for 24 hours.

Aspergillus niger and Candida albicans that cause opportunistic mycoses were used as test organisms.

RESULTS AND DISCUSSION

Actinomycetous floras from soil were isolated using Starch casein agar

and Water-Yeast extract agar (WYE) media. The colonies of Actinomycetous flora showed characteristic powdery appearance with convex, concave or flat surfaces, and the color was white, grey, pink and yellow. All isolates showed filamentous growth on agar media and were Gram positive. Eleven Actinomycetous floras were isolated viz. Actinomicrobium, Rubrobacter, Sphaerobacter, Actinomyces, Brevibacterium, Clavibacter, Actinopolyspora, Streptomyces, Microbispora and Actinobispora. These Actinomycetous isolates were screened for their antifungal activities against Aspergillus niger and Candida albicans which cause opportunistic mycoses in human beings. Out of eleven Actinomycetous isolates, only six viz Actinomicrobium, Sphaerobacter, Actinomyces, Streptomyces, Microbispora and Actinobispora showed antifungal activity against Aspergillus niger and Candida albicans. The zone of inhibition of fungal colonies by actinomycetous isolates was recorded (Table-1).

Table-1: Zone Of Inhibition Of Fungal Colonies In Mm By Actinomycetous Isolates

Actinomycetous isolates	Diameter of colonies of fungal pathogens in control in mm		Diameter of colonies of fungal pathogens after inoculation with Actinomycetous isolates in mm		Percent inhibition of	Percent inhibition
	A. niger	C. albicand	A. niger	C. albicans	A. niger	C. albicans
Actinomicrobium	15	18	4	7	73.34	61.11
Rubrobacter			15	18	0.0	0.0
Sphaerobacter			6	6	60.00	66.67
Actinomyces			3	4	80	77.78
Brevibacterium			15	18	0.0	18
Brachybacterium			15	18	0.0	18
Clavibacter			15	18	0.0	18
Actinopolyspora			15	18	0.0	18
Streptomyces			2	3	86.67	83.34
Microbispora			4	3	73.34	83.34
Actinobispora			3	3	80	83.34

Actinomicrobium, Sphaerobacter, Actinomyces, Streptomyces, Microbispora and Actinobispora caused 73.34 %, 60.00 %, 80.00%, 86.67 %, 73.34 % and 80.00 % inhibition of of growth of Aspergillus niger. Similarly, these Actinobacteria caused 61.11 %, 66.67 %, 77.78 %, 83.34 %, 83.34 % and 83.34 % growth inhibition of Candida albicans. Other actinomycetous isolates did not cause any growth inhibition to Aspergillus niger and Candida albicans. The present findings gain support from the work of Neha Salaria et al[24] who also observed the antibacterial activity of certain Actinomycetes.

CONCLUSIONS

Out of eleven Actinomycetous isolates only six of this viz. Actinomycrobium, Sphaerobacter, Actinomyces, Streptomyces, Microbispora and Actinobispora showed antifungal activity against Aspergillus niger and Candida albicans that cause opportunistic mycoses in human beings. These Actinomycetes might be useful for the production of antifungal drugs for treatment of mycoses.

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