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Screening of antagonistic actinomyctes from soils of shimoga

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ABSTRACT

About 18 actinomycetes isolates were isolated from soils of Shimoga characterized by morphological and biochemical methods as Nocardia, Streptomycetes, and Streptosporangium.When screened for antimicrobial activity by cross streak method, isolate kss1 completely arrested growth of S.typhi, isolate kss2 inhibited B.subtilis. S.aureus was inhibited by isolate kss4, and isolate kss3 restricted fungi *A.niger*.

KEYWORDS

Actinomycetes;
Antimicrobial activity;
Cross streak.

INTRODUCTION

Actinomycetes are branching filamentous bacteria comprise a significant proportion of bacterial population in forest and agricultural soils[12]. The diversity of actinomycetes secondary metabolites is unmatched in medical significance. Numerous bioactive compounds have been isolated from actinomycetes. Among which antibiotics are substances essential for health. More than 55% of therapeutically useful antibiotics are yielded by Streptomycetes spp only[8]. Screening of novel actinomycetes can be useful in the discovery of new antibiotics and novel species of actinomycetes.

The present study was carried to isolate, screen and characterize naturally occurring soil actinomycetes antagonistic to bacterial and fungal pathogens from local soils of shimoga.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from different forest areas of Thirhahalli in separate sterile polythene bags. Within selected area 4 samples were collected which are equidistant from one another(1-2 km).

Isolation of actinomycetes

Collected soil samples were dried in aseptic condition for1-2 days. 1g of dried soil sample was subjected to serial dilutionupto10^{-5}dilution[4,6]. One ml of 10^{-3} and 10^{-4} dilutions aseptically transferred to sterile Petri plates,along with this Starch casein agar, Arginine glycerol salt agar, Modified albumin agar and Malt extract yeast extract agar media were added by pour plate method[9]. The media were supplemented with antibacterial (Pencillin) and antifungal (Flucanazole) antibiotics to provide a condition for profuse growth of actinomycetes[7], the plates were incubated at $30+2^0$C for 10-14 days[8]. Selected colonies of actinomycetes were transferred from mixed cultures of plates onto respective agar plates to get pure cultures and preserved at 4^0C until further examination (photo no1).

Characterization

Morphological characterization was done by grams

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TABLE 1 : Biochemical tests

Sl no	Isolates	Starch hydrolysis	Gelatin hydrolysis	H2S production	Casein hydrolysis	Sugar fern\mentation		
						Sucrose	Dextrose	Glycerol
1	Kss1	-	-	-	-	-	+	-
2	Kss2	-	-	-	-	+	+	+
3	Kss3	-	-	-	-	-	+	-
4	Kss4	-	+	-	-	-	+	+

TABLE 2: Antagonistic activity of active isolates

Sl no	Isolates	*B.subtilis*	*S.typhi*	*A.niger*	*C.alb*	*S.aureus*
1	Kss1	-	+	+	-	-
2	Kss2	-	+	-	-	-
3	Kss3	-	-	+	-	-
4	Kss4	-	-	-	-	+

staining and cover slip method[5,11]. Selected isolates were subjected to biochemical tests like starch hydrolysis, Casein hydrolysis, gelatin hydrolysis, H_2S production and sugar fermentation[1,10] (TABLE 1).

Antimicrobial activity

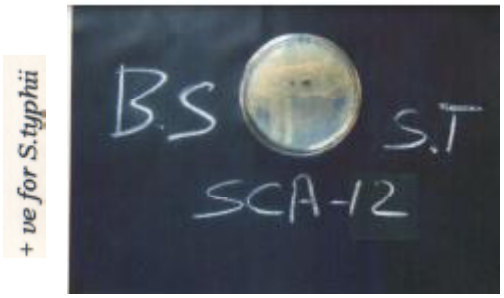
Screening of antimicrobial activity was conducted by Cross streak method[8,13] where suspected antibiotic producers streaked on one side of the plate and these plates were incubated at30+-20C for 2-3 days to permit and antibiotic production. Then the test bacteria *S.typhi, B.subtilis*[15] and fungi *C.albicans*, *A.niger* cross streaked perpendicular to the actinomycets and incubated at ambient temperatures[2] TABLE 2 (photo no 2,3,4,5)

RESULTS

About 48 actinomycetes were isolated. Morphological examination of 18 isolates clearly indicates that they belong to genera Streptomycetes, Nocardia, Thermoactinomycetes and Streptosporangium. The isolate kss 1 belonging to Nocardia genera completely arrested the growth of *S.typhi* and *A.niger*. But was

Isolate no 1 (kss1)

Isolate no 2 (kss 2)



Isolate no 3 (kss 3)

ineffective against *B.subtilis* and *C.albicans*. (Photo no 1and 2). Kss 2 isolate belonging to Nocardia genera also completely inhibited S.typhi and just restricted the growth of B.subtilis. Where as it was not inhibiting

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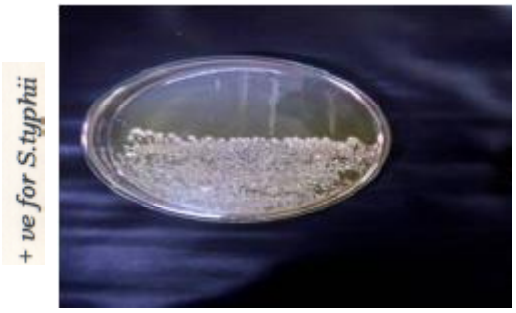

Isolate no 4 (kss 4)

Isolate no 1

Isolate no 2

Isolate no 3

Isolate no 4

A.Niger and *C.albicans* (photo no3). Isolate kss 3 belonging to streptomycetes genera showed no inhibition against the tested bacteria and fungus *C.albicans*, where as it was inhibiting the *A.niger* (photo no 4). Isolate kss4 was thermoactinomycetes inhibited *S.aureus* completely, but not *C.albicans* and *A.niger* (photo no5).

DISCUSSION

Actinomycetes could be isolated from a number of soils,on dilution plates, employing a suitable media that supports the growth of actinomycetes. These are slow growing with specific colony characters. Since 1937 many thousands of actinomycetes were isolated and screened in different research laboratories all around the world, whose out come is that though small quantity of antibiotics are produced by different microorganisms but their effectiveness against broad range of microorganisms is a rare event[8].

Nearly100 soil samplings from West Bengal resulted in 450 isolates, among which 12 isolates were chosen for screening of antibiotics, finally 2 of them were found to be potent antibioc producers[8]. From 120 soil samples 68 isolates were obtained among which 42 were characterized[6].

The present study also investigated 48 isolates among which 18 isolates belonging to 4 different genera were characterized belonging to 4 different genera two of them found to be potent antagonistic. These isolates may be of high commercial value as they are antagonistic against human pathogens

Summary

The malnad region of Karnataka is a thick evergreen forest which was opted for this screening of antagonistic actinomycetes. Totally 48 isolates were obtained from 8 soil samplings, belonging to the genera Streptomycetes, Nocardia, Thermoactinomycetes and streptosporangium. Among which three potent actinomycetes were chosen having maximum inhibitory effect against tested pathogenic microorganisms.

CONCLUSION

The difference in color, heterogeneous morphology and biochemical activity of isolates reveal that there is a high degree of diversity among the isolates of actinomycetes. Among the characterized genera two of them were found to be effective antagonistic against tested pathogens.

Also the presence of bioactive actinomycetes in the soil of Malnad region, Karnataka (INDIA) indicates that, it is an eminently suitable ecosystem for screening of actinomycetes and their bioactive compounds.

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