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Isolation and antimicrobial activity of some actinomycetes from the industrial soil of Solapur region, Maharashtra

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ABSTRACT

110 actinomycetes were isolated from 50 soil samples of industrial area of Solapur region and were tested for antimicrobial activity against six bacteria namely, *Bacillus subtilis, Staphylococcus aureus, E.coli, Shigella dysentriae, Vibrio cholera, Klebsialla pneumonae* and four fungi namely, *Candida albucance, Aspergililus niger, Aspergillus flavus* and *Microsporium gypseum*. Among the actinomycetes tested, threeteen isolates showed antagonistic activity against bacteria and seven isolates against fungi. Out of these twenty isolates, seven Few selected strains with good antibacterial activity were further used for morphological, physiological and biochemical studies.

INTRODUCTION

Large number of microorganism like bacteria, actinomycetes and fungi are explored from different habitats and are utilized for different purposes .Actinomycetes comprises an extensive and divers group of gram positive, aerobic and mycelial bacteria that play an important role in soil cycle. Many are well known for their economic importance as a producer of biologically active substances such as, antibiotics, vitamins and enzymes[1-3,5,10]. These products are commercially important and utilized for human welfare. In fact more than 50% of known natural antibiotics produced are from actinomycetes[11,18]. New antimicrobials are permanently needed due to the increase in microbial resistance for antibiotics, evolution of novel diseases and toxicity of currently used compounds[4,7].Fungi are eukaryotic and have machinery for protein and nucleic acid synthesis similar to that of higher animals .It is therefore very difficult to find out the compounds inhibiting fungal metabolism without exhibiting any toxicity to human[9,17]. As the pathogenic fungal diversity is increasing in nature, there is an argent need to identify new, effective and safer antifungal antibiotics[12].

Solapur district is situated in the southern part of Maharashtra prevails hot and dry conditions during most of the months in a year. The place is known for textile industries in the country and is completely unexploited for microbial diversity. Therefore, the present work aimed to isolate the actinomycetes from different soil samples of the region and exploit them for the production of antibiotics against bacteria and fungi.

MATERIAL AND METHODS

Sample collection

Soil samples from different industrial areas (sugar, textile) of solapur region were collected within the range

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TABLE 1 : Antibacterial activity of active isolates (mm)

Isolates	Activity against(mm)						
	B.subtilis	*S.aureus*	*E.coli*	*V.cholerae*	*S.dysentrae*	*K.pneumoniae*	*C.albucans*
4	22	25	35	33	14	10	30
15	25	-	21	25	18	-	-
27	25	25	27	37	30	25	27
28	14	30	25	22	23	30	22
39	17	30	-	20	24	23	21
55	28	30	28	26	15	15	-
57	10	-	15	15	-	-	17
63	32	23	30	28	22	23	13
78	24	28	21	20	20	22	27
79	22	-	28	12	-	-	-
90	-	30	25	30	28	33	-
92	26	33	27	30	35	32	20
95	-	33	-	-	-	-	8

of depth 5-10cm, brought to laboratory in sterile plastic bags and dried in oven.

Isolation of actinomycetes

Actinomycetes were isolated using soil dilution plate technique. Starch casein agar (Starch -10.0g, casein-0.3g, KNO_3-2.0g, K_2HPO_4-2.0g, $MgSO_4$-0.05g, $FeSO_4$-0.1g, $CaCO_3$-0.2g, agar-20.0g, Distilled water-1000ml, pH-7.2) and glycerol aspargine agar (L-Aspargine-1.0g, glycerol-10.0g, K_2HPO_4-1.0g, Trace salt solution-1.0ml, Distilled water-1000ml, agar-20.0g, pH-7.0-7.4 [trace salt solution-$FeSO_4$,$7H_2O$-0.1g, $MnCl_2.4H_2O$-0.1g, $ZnSO_4$, $7H_2O$-0.1g, Distilled water-100ml]) media supplemented with nystatin (50g/ml) were employed[16]. One gram of dried soil sample was taken in 9ml of distilled water, agited vigorously and preheated at 50°C for 0.5h. Serially diluted soil samples (10^{-5}, 10^{-6}, 10^{-7}) were agited with maximum speed and 0.1 ml of aliquot was spread on the plates containing glycerol aspargine agar and starch casein agar. Plates were incubated at 30°C for 6 days. The actinomycetes colonies grown in the plates were again inoculated on fresh media to get pure colonies and finally stored the cultures at 4°C in refrigerator.

Target strains of bacteria and fungi

Antimicrobial activity of pure actinomycete cultures were performed using six clinical isolates of bacteria such as, *E.coli, Bacillus subtilis, V.cholerae, K.pneumoniae, S. aureus* and *S. dysentriae* type I collected from M.R. medical college Gulbarga., and four fungi namely, *Candida albucans, Aspergillus niger, Aspergillus flavus and Microsporium gypseum.*

TABLE 2 : Antifungal activity of active isolates (mm)

Isolates	Activity against		
	A. niger	*A. flavus*	*M. gypseum*
7	30	12	25
12	25	22	25
24	25	27	25
43	30	32	30
48	35	35	35
82	30	22	-
85	02	18	20

Assessment of antimicrobial activity

Perpendicular streak plate method was used to determine antimicrobial activity of pure actinomycetes. Nutrient agar medium (Peptone-5.0g, Beef extract- 3.0g, Agar-20.0g, Distilled water- 1000ml, pH-7.0) was used for antibacterial activity and Czepekdox agar medium ($NaNO_3$-2.0g, K_2HPO_4-1.0g, $MgSO_4$-0.5g,KCl-0.5g, $FeSO_4$-0.01g, Sucrose-30.0g, Agar-20.0g, Distilled water- 1000ml, pH-6.5) for antifungal activity. Single streak of actinomycete was inoculated at centre of plate and incubated at 30°C for 5 days. After incubation, at 90^0 angles the test organism was inoculated by single streak. Antagonism was measured by the determination of inhibition zone[13].

Biochemical, physiological and morphological studies

The selected actinomycetes were characterized morphologically and physiologically following the direction given by the international streptomyces project[6] and Bergeys manual of systematic bacteriology[17]. Cultural characteristic of pure isolates in various media were recorded after incubation for 7-14 days at 27°C, morphological observation were made with light microscope by using method of Shirling and Gottleib[6]. Utilization of carbon and nitrogen was determined using basal medium containing KH_2PO_4-2.38g, $MgSO_4$-1.0g, K_2HPO_4-5.65g, $(NH_4)_2SO_4$-2.64g, Distilled water - 1000ml, Salt solution-6.25ml, (Salt solution-$CuSO_4$-102mg, $FeSO_4$-176mg, $MnCl_2$-126mg, $ZnSO_4$-24mg, Disttiled water-100ml) and results were recorded after 7 days of incubation. The active isolates were identified

TABLE 3 : Morphological, physiological and biochemical characteristics of seven isolates

Characteristics	Isolates						
	4	90	27	57	28	78	95
Aerial mycelium	+	+	+	+	+	+	+
Spore chain morphology							
Rectiflexibles	-	-	-			-	-
Spirals	+	+	+			+	+
Verticillate	-	-	-			-	-
Spore mass colour							
Red	-	-	-	-	-	+	-
Grey	+	+	+	-	-	-	+
Yellow	-	-	-	+	-	-	-
White	-	-	-	-	+	-	-
Mycelial pigment red orange	-	-	-	-	-	+	-
Diffusible pigment produced	-	-	-	-	-	-	-
Mycelial Fragmentation	-	-	-	-	-	-	-
Sub mycelial sporulation	+	+	+	+	ND		+
Enzyme activity							
Protease	+						
Dnase	-	+	+	-	-	+	-
Amylase	+	+	+	+	-	+	+
Catalase	VL	VL	VL	+	+	+	+
H_2S production	-	+	-	-	-	-	-
Nitrate reduction	-	+	+	-	+	+	+
MR	-	+	-	-	ND	+	-
VP	+	+	+	-	-	+	-
Indol production	VL	+	-	-	-	-	-
Urease	+	+	-	-	-	-	-
Lipase	-	-	-	-	-	-	-
Carbon utilization							
Lactose	+	+	+	+	+	-	+
Galactose	+	+	+	+	+	-	+
Fructose	+	+	+	+	+	-	ND
Maltose	+	+	+	+	+	-	+
Growth in 5%NaCl	+	-	-	+	-	-	-
10%NaCl	-	-	-	-	-	-	-
0degree	-	-	-	-	-	-	-
45 degree	-	-	-	-	-	-	-
Utilization of Nitrogen source							
Prolin	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	+	+
Phenyl alanine	+	+	+	+	+	+	+
Tryptophan	+	+	+	+	+	+	+
	S.sp.	S.sp	S.sp	Micro.	S.lav	S.ant	S.rim

+, Positive; -, Negative; ND, Not Determined; VL, Very Less; S. ant, Streptomyces Antibioticus; S. lav., Streptomyces Lavendulae; S. rim., Streptomyces rimosus; Micro., Micromonospora spp; S. sp., Streptomyces species

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up to the species level by comparing their morphology of spore bearing hyphae, entire spore chain and structure of spore chain with the actinomycetes morphologies as described in bergeys manual[8]. This was done by using cover slip method[19]. The other physiological and biochemical Characteristics were determined by the method described by Shirling and gottlieb[6]. All tests were performed at 27°C[14].

RESULT

Isolation of actinomycetes

A total of 110 actinomycete were isolated from 50 soil samples using starch casein agar and glycerol aspargine agar supplied with nystatin (50mg/ml). 58 per cent of the isolates were recovered from glycerol aspargine agar as they utilize glycerol as carbon source easily than the starch.

Antagonistic activity of isolated strains

Thirteen actinomycete strains showed good antibacterial activity against all test bacteria and 7 strains showed antifungal activity against all test fungi.

The result of antibacterial and antifungal activity of active actinomycete strains were given in TABLE 1 and TABLE 2.The utilization of carbon and nitrogen sources, growth characteristics at different temperature and other characteristics are summarized in TABLE 3.

DISCUSSION

The actinomycetes isolated from 50 soil samples collected from industrial area of solapur region and tested their efficacy against bacteria and fungi. Among the isolates, 16 isolates showed antibacterial activity against one bacterial strain and 13 isolates showed antibacterial activity against all the bacterial test organisms. Out of these 13 isolates, isolate number 95 was found affective against only *S. aureus*. Eight isolates showed maximum inhibitory activity against gram negative bacteria than gram positive ones and 5 isolates showed maximum activity against gram positive bacteria. The growth of *Candida albucans* was found inhibited by 9 isolates with a maximum of 30mm inhibition by isolate number 4 and minimum of 08mm inhibition by isolate number 95. Among the test bacteria screened, *S. aureus* and *V.cholerae* were found to be more susceptible to the antibiotics produced by different isolates.

The maximum antifungal activity was recorded in seven isolates against *Apergillus niger* and a dermatophyte namely *Microsporium gypseum*. Comparatively, *A. flavus* was found resistant against the antibiotic produced by these isolates. The morphological and microscopic examinations of these isolates clearly indicated that the 6 isolates are belong to the genera *Streptomyces* and one belongs to *Micromonospora*. Further, comparison of biochemical and physiological characteristics among the isolates indicate that the isolate 78 was closely related to *Streptomyces antibioticus*. Therefore we can say that the isolate 78 as *Streptomyces antibioticus*, 95 as *streptomyces remosus*, 28 as *Streptomyces lavendulae*, 57 as *micromonospora* species and isolates 4, 27, 90 as *streptomyces* species.

The organism will be deposited in Botany Department, Microbial Biotechnology Section, Gulbarga University, Gulbarga.

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