REVIEW OF RESEARCH

SOIL AS A NOBLE WEAPON FOR *ACTINOMYCETES OF ANTIBIOTICS* ACTIVE AGAINST HUMAN PATHOGENS IN SANGLI DISTRICT, MAHARASHTRA

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ABSTRACT:

A total of 112 actinomycetes strains were isolated from the non agricultural land soil and agriculture land soil samples of the Sangli district and screened for their anti-bacterial activity. They were evaluated for their inhibitory activities on four test microorganisms. Fifteen actinomycetes isolate which exhibited antimicrobial activity against at least two of the test organisms and were characterized by conventional methods. The cultural characteristics of isolates were also studied in different culture media. The results indicated that six isolates were highly active against Staphylococcus aureus strains. Seven isolates were highly active with an inhibition zone more than 20 mm in diameter. Most of the isolates inhibited growth of the Gram negative bacteria that were tested. All the antibiotic producing actinomycetes were isolated at different temperatures from non agricultural land soil and compost rich agriculture land soil. Fifteen isolates showed activity against bacteria in which most of them from non agriculture land soil where it is less utilized by human neither for agriculture nor for other purpose. These microorganisms may appear to have immense potential as a source of antibiotics active against human pathogens and adopt to improve antibiotic yields.

KEYWORDS: *Soil weapon of Actinomycetes for antibiotics, Sangli district.*

INTRODUCTION

Actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. These researches have been remarkably successful and approximately two thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes[11]. They are the most widely distributed group of microorganisms in nature which primarily inhabit the soil[12]. Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora*[13]. According to the World Health Organization, over-prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens. It is noticed that, the drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics. Because of this, many scientists and pharmaceutical industry have actively involved in isolation and screening of actinomycetes from

different untouched habitats, for their production of antibiotics[12]. Serious infections caused by bacteria have become resistant to commonly used antibiotics and become a major global healthcare problem in the 21st century[1]. *Staphylococcus aureus*, for instance, a virulent pathogen that is responsible for a wide range of infections, has developed resistance to most classes of antibiotics[6]. Goodfellow and Haynes reviewed the literature on isolation of actinomycetes and suggested that only 10% of the actinomycetes are isolated from nature, Clinicians and public health officials have faced hospital acquired drug resistant *S. aureus,* which also bears resistance to many antibiotics. Hence there is need to rediscover new drugs active against these drug resistance pathogens. Majority of the actinomycetes in soil that are potential drug sources remain uncultivable, and therefore inaccessible for novel antibiotic discovery[7]. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi[4,10]. Actinomycetes can be isolated from soil and marine sediments. Although soils have been screened by the pharmaceutical industry for about 50 years, only a small fraction of the surface of the globe has been sampled, and only a small fraction of actinomycetes taxa has been discovered[2,3]. The present study was undertaken to isolate actinomycetes from the soil samples of non agriculture land and agriculture land from Sangli district to assess their anti-bacterial properties. The resistance problem demands that to discover new antibacterial agents effective as a novel weapon against pathogenic bacteria resistant to current antibiotics. Hence we need to screen a large amount of actinomycetes from various habitats for antimicrobial activity in order to get some actinomycetes strains that produce new antibiotics active against drug resistant pathogens.

MATERIALS AND METHODS

Soil sample collection:

Soil samples were collected from different non agricultural and agricultural soil samples of the Sangli district of Maharashtra. Each collection was made from 10-15 cm depth of the soil[14]. These were air-dried for 1 week[17], crushed and sieved. The sieved soils were then used for further actinomycete isolation.

Isolation and culture condition:

For each collected sample, 1g of the soil were suspended in 100 ml of physiological saline water then incubated in an orbital shaker incubator at 28^0C with shaking at 200 rpm for 30 min. Mixtures were allowed to settle, and serial dilutions up to 10^5 were prepared using sterile physiological saline water and agitated with the vortex at maximum speed. An aliquot of 0.1 ml of each dilution from 10^2 to 10^5 was taken and spread evenly over the surface of actinomycetes isolation agar and starch casein agar medium. The both media are added to Rifampicin 2.5 mg/ml and amphotericin B 75 mg/ml to inhibit bacterial and fungal contamination, respectively. Plates were incubated at 28^0C and 37^0C, and monitored at different interval of time like 48hr, 72hr, and 96hr. repeated streaking on starch casein agar plates led to purify bacterial colonies that showed actinomycetes like appearance. The isolated strains are preserved at 4^0C during two months and maintained for longer period by serial subculture.

Gram staining:

A smear of culture was taken in a clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1 min and washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 1 min and washed with tap water. Alcohol was used to decolorize the smear until the violet color ceased to flow away. The slide was washed with water and counter stain safranin was flooded over the smear for 2 min, then the slide was washed, drained, air dried, and viewed under microscope. The culture retaining the violet color indicated that it was Gram-positive organism.

In vitro screening of isolates:

In vitro screening of isolates for anti-bacterial activity was done; Morphologically distinct actinomycete isolates were selected for anti-bacterial activity screening against the pathogenic test

organisms by conventional spot inoculation method[15] and single line streak method[5] on agar medium. In spot inoculation method, pure actinomycetes isolates were spot inoculated on starch casein agar medium. The plates were incubated at 28^{0}C for six days, and then inverted for 40 min over chloroform in fume hood. Colonies were then covered with a 0.6% agar layer of nutrient agar medium, previously seeded with one of the test organisms.

The antimicrobial activity was observed after 24hr incubation at 37^{0}C. In single line streak method, pure actinomycetes isolates were inoculated in a single streak down the middle of a plate of screening media and incubated at 28^{0}C for 4 days for the production of any antibiotics. A single streak of each test organism was added perpendicular to the actinomycetes streak. Test organisms were placed perpendicular to culture streak. The plates were incubated for 24hr at 37^{0}C.

Antibacterial activity:

Antibacterial activity of Isolates for the present study Gram positive bacteria *Staphylococcus aureus, Bacillus subtilis,* and Gram negative bacteria *Escherichia coli* and *Pseudomonas putida,* were used to test antibacterial activity of all the 15 isolates.

RESULTS

Table no. 1 Table 1- Total number of actinomycetes isolates with antibacterial activity isolated at different temperatures.

Soil sample	Temperature Isolated at ^{0}C	Isolated Strains	No. of Isolated strains *bacteria	% of active Isolates*Bacteria
Non agriculture soil	28	47	6	12.76
	37	24	3	12.5
	Total	**71**	**9**	**12.68**
Agriculture soil	28	22	5	22.73
	37	19	1	5.26
	Total	**41**	**6**	**14.63**
TOTAL		**112**	**15**	**13.39**

Table 2- Antibacterial activity of isolates

Cultures of	Gram positive bacteria		Gram negative bacteria	
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas putida***
NAS 1	NA	NA	A	NA
NAS 2	NA	A	NA	A
NAS 3	NA	NA	A	A
NAS 4	NA	NA	A	NA
NAS 5	A	A	NA	A
NAS 6	A	NA	A	NA
NAS 7	A	A	NA	A
NAS 8	NA	NA	A	A
NAS 9	NA	A	NA	A
AS 1	A	NA	A	A
AS 2	NA	A	A	NA
AS 3	A	NA	NA	A
AS 4	NA	NA	A	NA
AS 5	A	NA	NA	A
AS 6	NA	NA	A	NA

NAS= Non agriculture soil, **AS**= Agriculture soil, **A**= Activity; **NA**= No activity

Table 3- Culture characteristics of selective isolates on starch casein agar medium

Soil sample	Culture code	Colour	Mycelium type	Pigment production	Gram's reaction
Non agriculture	NA 1	Grey	Substrate	Yellow	+
	NA 2	Yellow	Aerial	Red	+
	NA 3	White	Substrate	Red	+
	NA 4	White	Substrate	Yellow	+
	NA 5	Green	Aerial	-	+
	NA 6	White	Substrate	-	+
	NA 7	White	Aerial	-	+
	NA 8	White	Aerial	Red	+
	NA 9	Grey	Aerial	-	+
Agriculture	A 1	White	Substrate	Red	+
	A 2	Green	Aerial	Yellow	+
	A 3	White	Aerial	-	+
	A 4	Grey	Substrate	Yellow	+
	A 5	White	Aerial	-	+
	A 6	Yellow	Aerial	Red	+

DISCUSSION

Soil samples were collected from non agricultural and agricultural land soil of Sangli district region of Maharashtra, India. One gram of soil sample was dried and taken for isolation of actinomycetes. The suspected 112 actinomycetes were isolated at two different temperatures (28^{0}C & 37^{0}C) in which 71 (63.39%) from non agricultural land soil and 41 (36.60%) from agricultural land soil and inoculated on the starch casein agar medium for purification. And the pure colonies were maintained in the starch casein agar medium, slants at 4^{0}C. All the 112 cultures were screened against bacteria but only the 15 isolates showed the antibacterial activity and were designated such as NAS (1,2,3 4,5,6,7,8,9)& AS (1,2,3,4,5,6) as shown in table no. 2

They were also studied for culture characteristics as shown in table no. Table 3. This study was undertaken with an aim of isolation and screening of actinomycetes in non agriculture soil and agricuture soil of Sangli district region at two different temperatures and selecting the isolates with antibacterial activity.

Using the selective media and cultivation conditions described previously a total of 112 different actinomycetes isolates were recovered from 20 soil samples that were collected from non agriculture land soil and agriculture land soil of Sangli district, Maharashtra. The soil of non agriculture land gives the higher number of actinomycetes isolates (47 and 24 isolates, respectively) as compare to agriculture land soils as shown in Table no.1. All isolates grew on starch casein agar media showing morphology typical of actinomycetes, since the colonies were slow growing, aerobic, glabrous, folded and with aerial and substrate mycelia of different colors. All actinomycetes isolates were Gram-stain positive.

The cultural characteristics Pigment production, morphological characteristics of the different actinomycetes isolates shown in Table no. 3. The colour of the substrate mycelium and aerial spore mass was varied. During screening of new isolates for drug discovery, many potentially interesting microorganisms might be excluded due to their morphological similarities, suggesting similar biochemical behavior; thus, many isolates are lost and only a few of them are finally tested. In this study, the total number of isolated actinomycetes 112 was screened on agar medium and antibacterial activity was observed in 15 (13.39 %) of the isolates and appeared promising shown in Table no. 1.

In previous studies, it was shown that the isolation rate of actinomycetes with antimicrobial activity is higher than 40%[15] and in others less than 10%[16].

These results confirm that the actinomycetes are able to produce a wide variety of antibiotics with antibacterial activity. Thakur et al. also reported that a total of 110 actinomycetes isolates were isolated from the soil samples collected from the protected forest soil from two states in Northeast

India[17]. These were then characterized by conventional methods and assessed for their antagonistic activity preliminary against test microorganisms. Results of the present study also indicate that the higher number of actinomycetes was isolated from non agriculture land soil active against bacteria, where the human activity is very less as compare to agriculture land soil or other purpose and these non agriculture land soil actinomycetes can be useful for many applications such as control of infectious diseases and drug discovery.

CONCLUSION

Fifteen isolates showed activity against bacteria in which most of them from non agriculture land soil where it was utilized less by human for agriculture or other purpose. These microorganisms produce some of the most important medicines ever developed. They are the source of lifesaving treatments for bacterial and fungal infections. In spite of the tremendous success of the past secondary metabolite research, the number of terrestrial antibiotics seems currently to approach a saturation curve with an apparent limit in the near future. The increasing number of duplications and the urgent demand for new leading antibiotics in pharmacology for the treatment of drug resistant infectious pathogens has enforced the search for metabolites in so far untouched habitats where the human activities are very less. Hence the non agriculture land soil is very precious in site of biodiversity which has been amply justified by the richness of microbial diversity. Our studies will establish the rich actinomycete diversity of the region, especially the various niche habitats of Sangli district and also these isolates may be effectively used in large scale production for commercial, industrial and pharmaceutical applications in the coming future. Therefore the present study reveals that non agriculture land soil appear to have immense potential as a source of antibiotics active against human pathogens.

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