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Screening and Isolation of Antibiotic producing Microorganisms from Soil

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Abstract- Diverse microorganisms are known to produce a wide variety of Antibiotics as secondary metabolites that are being developed and are used in treating various infections in humans, animals and plants. Microorganism has always been the primary source for production of antibiotics and still continues to maintain its significance. Antibiotics are produced by several group of microorganisms such as bacteria, fungi and actinomycetes. Abuse of antibiotics in various fields resulted in development of resistance in various bacteria which is a major threat in today's scenario. Hence the screening of various antibiotics with effective antimicrobial activity is still continuing though thousands are available in today's market.
The present study was undertaken to screen various organisms from different sources for their antimicrobial activity. Samples were screened by crowded plate technique. Out of 10 samples screened only one soil sample from rhizosphere region has shown zone of inhibition. The organism was purified and tested for antimicrobial activity against E.coli sps, Pseudomonas sps, Bacillus, Klebsiella and Staphylococcus sps. The organism showed zone of inhibition only on gram positive bacteria. However maximum zone was observed with staphylococcus alone. Bacillus did not show significant inhibition. Further tests are being done to know the sensitivity of various staphylococcal strains of different origin with isolate.

Keywords: *Actinomycetes, Antimicrobial avtivity, Staphylococcus spp, Agar well diffusion method.*

I. INTRODUCTION

Soils are the reservoirs of many antibiotic producing microorganisms due to its diverse nature. There are many microorganisms such as fungi, bacteria, actinomycetes which are potent in producing antibiotics which are widely used in today's scenario [1]. Genera from actinomycetes remain at the top of the natural antibiotic producers [2]. Actinomycetes are filamentous bacteria, basically aerobic and wide spread in soil.
These are unique microbes which comprises high G+C content of 57-75% in their DNA the major significance of actinomycetes lies in their ability to produce antibiotic [3]. Many of the currently known antibiotics such as streptomycin, gentamycin, rifamycin, erythromycin, novobiocin, amphotericin, vancomycin, neomycin, chloramphenicol, tetracycline, and nystatin come from actinomycetes [3]. Actinomycetes also produce enzymes, vitamins and pigments that can be put to other uses .They are also useful in cancer treatment, bioremediation, used as plant growth promoting agents (help to produce plant growth hormone Indole3-acetic acid), bio control tools, biopesticide agents, anti-fungal compounds and biocorrosion [4]. Abuse of antibiotics in various fields resulted in development of resistance in various bacteria which is a major threat in today's scenario. Hence the screening of various antibiotics with effective antimicrobial activity is still continuing though thousands are available in present market. Microorganisms has always been the primary source for production of antibiotics and still continues to maintain its significance.

II. RELATED WORK

Avilala Janardhan *et al.,* (2014) isolated the actinomycetes from mangrove soil collected from Nellore region of Andhra Pradesh, India, and screened for its ability to produce bioactive compounds. Identification was done based on cultural,Morphological and biochemical features and isolated strain was found to be *Nocardiopsis alba*. The bioactive compounds produced by this strain were purified and based on spectral data, the structure of the isolated compound was predicted as "(Z)-1-((1-hydroxypenta=2, 4-dien-1-yl) oxy) anthracene-9, 10-dione." [5]. JS Naina *et al.,* 2012 evaluated the potential actinomycetes producing bioactive compounds amended in Amirthi forest, Vellore, Tamil nadu, India. The maximum activity was found against *Pseudomonas aeruginosa*. The maximum inhibition was found at 5.0mg/ml concentration with 90.7%.Identificatiuon of species was done by 16S rRNA sequencing and found to be *Streptomyces spp* VITNSJ2. [6].

III. MATERIALS AND METHODS

i. Soil sample collection:

Total 10 samples were collected from different areas of sainikpuri, Secunderabad.

Soil samples were collected from depth of 11-15cm below the surface and kept in clean plastic bags until further analysis.

ii. Isolation and identification:

Crowded plate technique was employed to screen antibiotic producing microorganisms. 1g of each sample was mixed with 9ml of normal saline,vortexed and serially diluted up to 10 in a series of test tubes. From the selected dilution tube 0.1ml of the sample was transferred to nutrient agar and incubated at 37^{o}c for 24hrs.Identification was done by colony morphology and microscopy.

iii. Microorganisms used in the study:-

Escherichia coli,*Staphylococcus sps*,*Bacillus sps, Klebsiella sps, Pseudomonas sps* and isolated culture ARK. Three strains of animal origin from veterinary hospital belonging to *Staphylococcus aureus* were used in the study.

iv. Screening for antimicrobial activity:-

The isolated organism ARK was tested for antimicrobial effect against various bacteria by agar well diffusion method[7][8].

IV. RESULTS AND DISCUSSION

Screening and isolation of Antibiotic producing organisms:

Basing on the zone of inhibition observed in crowded plate technique, antibiotic producing organisms were screened. Of all the three different soil samples screened for antibiotic producers, only one plate from garden soil showed colonies with zone of inhibition. The culture was purified and used for further analysis. The colony morphology was found to be dry, crumbly,and white to creamish coloured and stained gram positive .Basing on the colony morphology and gram staining characters the isolate was identified as member of Actinomycetes and was designated as isolate ARK(Figure 1 and Figure 2)

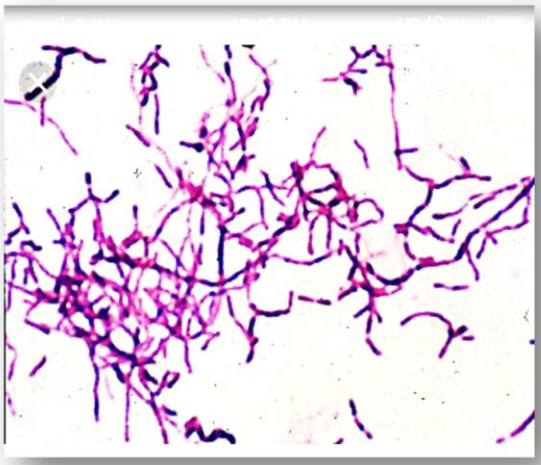

Figure 1: Gram staining of isolate ARK

Figure 2: Cultural characteristics of isolate ARK on Nutrient agar medium

Antimicrobial activity of Isolate ARK:

Agar well assay was done to know the spectrum of activity of isolate ARK against five bacterial cultures which included both gram positive and gram negative.The isolate ARK inhibited only gram positive bacteria but not gram negative.Hence it may be producing a narrow spectrum antibiotic which may be cellwall inhibitor. Among the two gram positive cultures tested ie *Bacillus sps* and *Staphylococcus sps,* significant zone of inhibition was observed only with *Staphylococcus sps* but not with *Bacillus sps* (Table 1)

Table 1:Antimicrobial activity of Isolate ARK on test organisms

Nature of organism	Test cultures	Zone of inhibition(24hrs)
Gram –ve	E coli	No inhibition
	Klebsiella	No inhibition
	Pseudomonas	No inhibition
Gram +ve	Bacillus	7mm
	Staphylococcus	19mm

The isolate ARK was tested with 5 different *staphylococcus* isolates from various sources, to know the patterns of sensitivity . The results indicated that the antibiotic produced by ARK could show significant inhibition only in *staphylococcus* lab isolate 1 and *staphylococcus* lab isolate 2 but not animal pathogens this may be due to the changes in the gram positive cell walls of different *staphylococcus* isolates tested. (Table 2)

Table 2:Antimicrobial activity of Isolate ARK against different *Staphylococcus aureus* strains

S.No	*Staphylococcus aureus* strains	Zone of Inhibition in mm
1	Lab isolate 1	27
2	Lab isolate 2	35
3	Animal pathogen 1	No inhibition
4	Animal pathogen 2	No inhibition
5	Animal pathogen 3	No inhibition

Impact of Incubation time on antibiotic production of isolate ARK

Since only two *Staphylococcus* lab isolates showed significant zone of inhibition, these two isolates were further tested for time line studies on antibiotic production. Maximum antibiotic production was observed at 48 hours of incubation for both *Staphylococcus* isolate 1 and *Staphylococcus* isolate 2. However the antimicrobial activity is reducing at 72 hrs and 96 hrs respectively. This indicates that the antibiotic produced may not be stable after 48hrs which needs to be explored further.

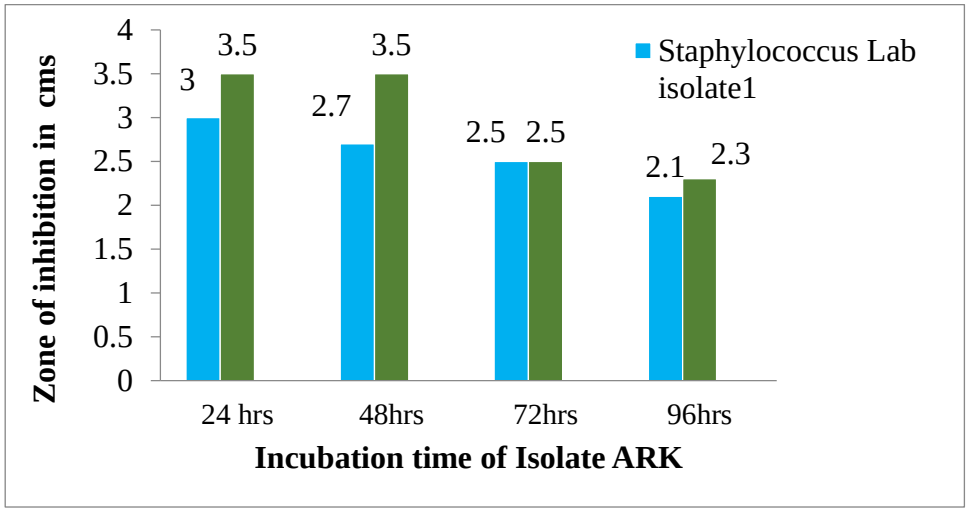

Fig 3:- Impact of incubation time on antibiotic production

Conclusion and Future studies:

The results of present study indicates that the soil contain great diversity of antibiotic producing Actinomycetes. The isolated strain ARK exhibited narrow spectrum of antimicrobial activity as it inhibited gram positive bacteria. When tested with animal pathogens ,strains of *Staphylococcus* were relatively resistant. As the isolate is specifically inhibiting *Staphylococcus sps* but not other bacteria, strain improvement of this bacteria may yield a better results infighting against Methicillin resistant

Staphylococcus aureus(MRSA) strains. Also development of resistancecan be reduced to a greater extent as it is not a broad spectrum amtibiotic.Future work will be based on identification of structure using NMR,characterization of purified antimicrobial compounds and extraction of bioactive compounds of isolate ARK.Characterization of isolate ARK by 16S rRNA sequencing.

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