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Original Article

Isolation And Screening Of Antibiotic Producing Actinomycetes From Soils In Gulbarga City

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ABSTRACT

Actinomycetes are fascinating group of microorganisams. Actinomycetes are Gram positive having G + C (>55%) content in their DNA. The aim of present study is to screen potential strain of Actinomyctes and checking against known Gram positive and Gram negative pathogenic bacteria by using cup plate method. The isolates(VPD 14 and VPD 19) could not show zone of inhibition against Gram positive bacteria but the same strains were considerably significant against Gram negative bacteria (*E-coli* and *Pseudomonas*) showing zone of inhibition 0.2cm,0.4cm,0.1cm and 0.3cm respectively.

Keywords:

Acinomyctes, Antibacterial activity, Zone of inhibition, cup plate method

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Introduction:

The name Actinomyctes is derived from Greek 'aktis' (a ray) and mykes(fungus) and given to these organisms from initial observation of their morphology. Actinomycetes are prokaryotes of Gram-positive bacteria but are distinguished from other bacteria by their morphology, DNA rich in guanine plus cytosine (G+C) and nucleic acid sequencing and pairing studies. They are characterized by having a high G+C content (>55%) in their DNA [1-3]. The majority of Actinomyctes are free living saprophytic bacteria found widely distributed in soil, water and colonizing plants. Actinomyctes population has been identified as one of the major group of soil population [4] which may vary with soil type.

Actinomycetes provide many important bioactive substances that have high commercial value. Their ability to produce a variety of bioactive substances has been utilized in a comprehensive series of researches in numerous institutional and industrial laboratories. This has resulted inthe isolation of certain agents, which have found application in combating a variety of human infections [5]. That is why more than 70% of naturally occurring antibiotics have been isolated from different Genus of Actinomycetes [6]. Out of these different genus, *Streptomyces* is the largest genus known for the production of many secondary metabolites [7], which have different biological activities, such as antibacterial, antifungal, anti parasitic, anti tumour, anticancer and immunosuppressive actions [8,9,1 0] .

Antagonism among microorganisms is common phenomenon in soil resulting from the production of antibiotics. In human medicines antibiotics have proved to be great therapeutic value. Actinomyctes are the sources of the antibiotics used in medicines today. Actinomyctes have the capability to synthesize many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides and enzymes like xylanse, cellulase of these compounds antibiotics are predominate in therapeutic and commercial importance.

Looking into the importance of antibiotics and capability of Actinomyctes to synthesize same the present work was to isolate and screen antibiotic producing actinomycetes from soil samples in Gulbarga City. The outcome of this finding may be important to give direction for researchers and for future treatment of multidrug resistant human pathogens.

2. Materials And Methods

2.1 Source

The soil samples were collected in sterilized container from various places like Public garden, Hospitals, School ground, Agricultural land etc.,

2.1.1 Collection of samples

Various soil samples collected from the place of availability in a sterilized container and brought to the laboratory for the isolation of Actinomycetes. Location of the samples collected, nature of the sample and the pH were recorded.

2.2 Media

The media used for the isolation of Actinomyctes are Ken Knight Meuniers medium (pH -7) containing g/l Dextrose-1; KH_2PO_4-0.10; $NaNO_3$-0.10; KCl-0.10; $MgSO_4.7H_2O$-0.10; Agar-15, Starch Casein agar (pH-7) containing g/l Soluble starch-10; K_2HPO_4-2;KNO_3-2; NaCl-2; Casein -0.3; $MgSO_4.7H_2O$-0.05; $CaCO_3$-0.02; Ferrous Sulphate-0.01; Agar-15, Asparagine mannitol agar (pH-7.4) containing g/l K_2HPO_4-1; KNO_3-0.5; $MgSO_4.7H_2O$-0.2; $CaCl_2.6H_2O$-0.1; NaCl-0.1; $FeCl_3.6H_2O$-trace; Asparagine-0.5; Mannitol-1.0; Agar-15 and Muller- Hinton agar (pH-7,4) containing g/l Beef infusion- 300, casamino acids acid hydrolysate of casein- 17.5; starch-1.5; agar 17.

2.2.1 Isolation of potential Actinomyctes for the production of Antibiotics

Soils from different sources were subjected to serial dilution technique for the isolation of discrete colonies of Actinomyctes on selective media.

2.2.2 Enumeration of Actinomyctes

All the Actinomycetes based on their respective colony characteristics and staining features [11] grown on selective media were respectively enumerated [12] and calculared using formula as mentioned below

Number of Actinomycetes per gram of sample =

$$\frac{\text{Average colony count X dilution factor}}{\text{Weight of the sample}}$$

2.2.3 Identification of Actinomycetes

Actinomyctes colonies on selective media were subculture and maintained on starch casein agar slants for the identification work. Cover slip method [13] was used for the identification. A sterilized cover slip is carefully inserted at an angle of about 45o C into starch casein agar in a Petri plate until about half of the cover slip is in the medium. Actinomyctes are than inoculated along the line where the medium meets the upper surface of the cover slip. After the suitable incubation, cover slip in the medium facilitates the distinction between substrate mycelium and aerial mycelium. Then the cover slip was stained and observed under microscope and identified.

2.3 Pathogenic Strains

Pathogenic Strains *E.coli, Pseudomonas*, S.aureus and Streptococci were collected from Hospital and Pathogenic laboratory in Gulbarga city.

2.3.1 Screening

Screening of potential strains consists of the two steps i.e. Primary and secondary staining.

2.3.1.1 Primary Screening

In the primary screening antimicrobial activity of isolated Actinomyctes were determined by perpendicular streak method [14] on nutrient agar along with test organisms. The Actinomycetes which produces antibiotics, showed the clear zone of inhibition.

2.3.1.2 Secondary Screening

Secondary Screening was performed to analyze the potentiality among the positive isolates. Cup plate was used for the secondary screening. In this method Muller Hinton medium was used to test the antibiotic susceptibility. Sterilized media was poured in the sterilized Petri plate, lawn culture of pathogenic organisms, *E.coli, Pseudomonas*, Streptococci and S. aureus was done on solidified medium. With the help of sterilized cork borer wells of different diameter was bored and than broth culture of positive Actinomycetes was inoculated into the wells and incubated to check for the zone of inhibition.

3. Results And Discussion

3.1 Occurrences Of Actinomycetes

The Actinomycetes were isolated from the various soil samples of Gulbarga city, their degree of occurrence and source from which they were obtained are shown in table – 1. The occurrence of Actinomycetes population was more in Hospital area followed by Agricultural land and school ground. No Actinomycetes were present in soil sample of public garden.

Table-1 Sources and Occurrences of Actinomycetes

Sr. No	Sources	Actinomycetes (Number of colonies)	Percentage of Occurrences
1	Hospital 1	20	29.8%
2	Hospital 2	21	31.3%
3	Agricultural land	10	14.9%
4	School ground	5	7.4%
5	Hospital 3	11	16.4%
6	Public garden	nil	nil

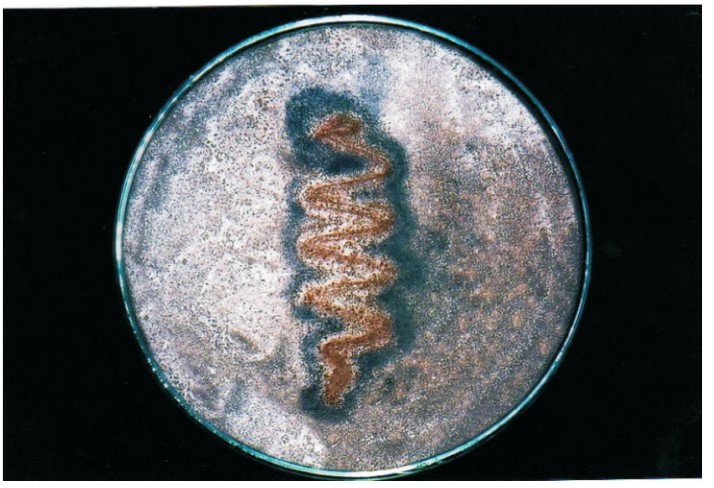
3.2 Primary Screening of Actinomycetes

Among 40 strains isolated from different sources, 11 isolates were able to show good zone of inhibition against test organisms. Zone of inhibition is shown in table 2. Looking into zone of inhibition shown by VPD 14 and VPD 19, they were subjected to further studies

Table-2 Primary screening of Actimomycetes for antibacterial activity

Sr. No	Actinomycetes isolates	Zone of Inhibition (in cms)
1.	VPD 1	<0.1
2.	VPD 2	<0.1
3.	VPD 4	<0.1
4.	VPD 5	<0.1
5.	VPD 10	<0.1
6.	VPD 12	<0.1
7.	VPD 13	<0.2
8.	VPD 14	<0.4
9.	VPD 16	<0.1
10.	VPD 17	<0.1
11.	VPD 19	<0.3

Fig- 1. Actinomycetes showing zone of inhibition



3.3 Characterization of potential Actinomycetes

The Potential strains VPD 14 and VPD 19 were identified up to genus level as Streptomyces based on cover slip method. Further images were taken under 100X objective

Fig- 2. Cover slip method for identification of Actinomycetes

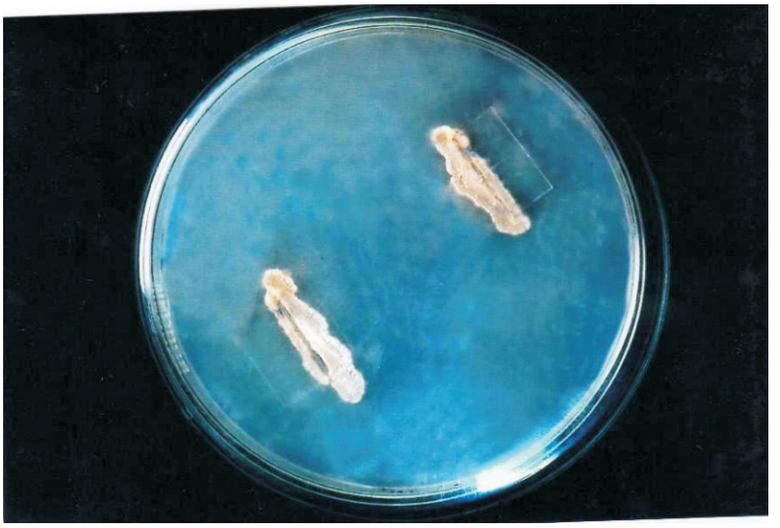


Fig- 3 VPD 14 under 100X objective

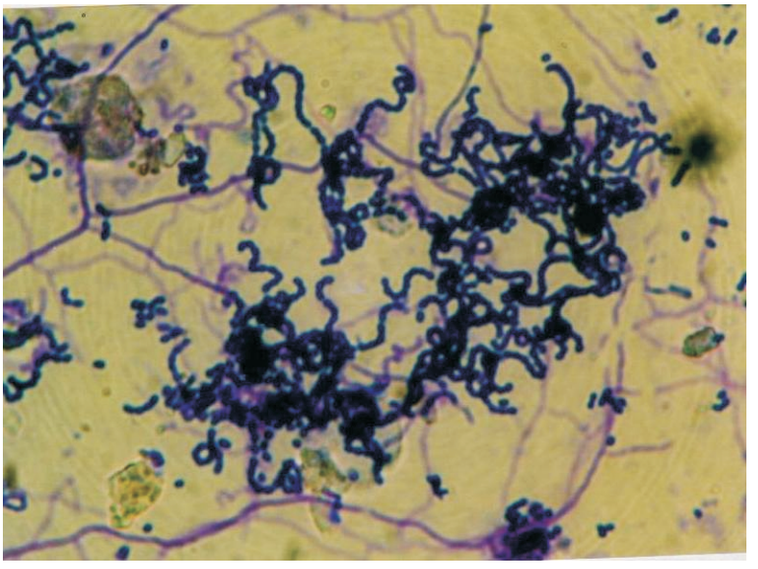
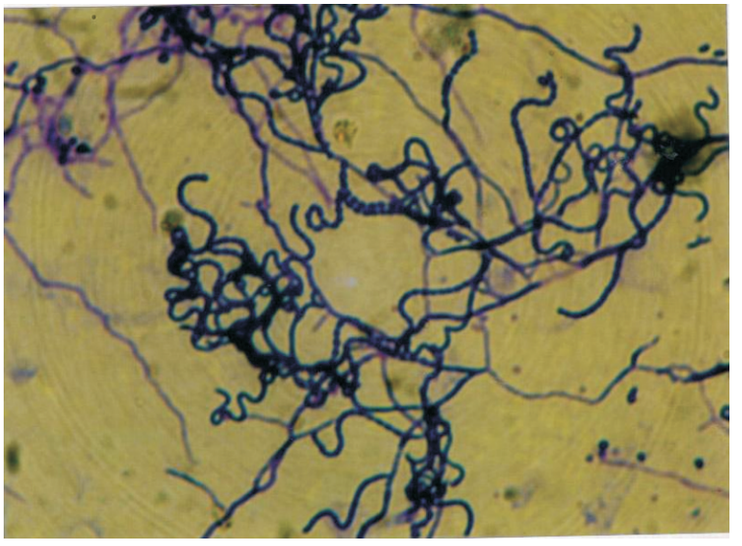


Fig- 4 VPD 19 under 100X objective



3.4 Secondary Screening of Actinomyctes for antibacterial activity

The Potential strains VPD 14 and VPD 19 were tested for their antibacterial activity against various Gram positive and Gram negative bacteria recorded in table 3 and 4

Table-3 Antibacterial activity of VPD 14 and VPD 19 against Gram positive bacteria

Sr. No	Strains	Gram Positive bacteria	Zone of inhibition (in cms)
1	VPD 14	*Streptococci*	Nil
		S. aureus	Nil
2	VPD 19	*Streptococci*	Nil
		S. aureus	Nil

The strains VPD 14 and VPD 19 could not show zone of inhibition against Gram positive bacteria *Streptococci* and *S. aureus*. How ever our results match with observations made by [15 and 16]. Gram positive bacteria were more susceptible as compared to Gram negative bacteria.

Table-4 Antibacterial activity of VPD 14 and VPD 19 against Gram negative bacteria

Sr. No	Strains	Gram Negative bacteria	Zone of inhibition (in cms)
1	VPD 14	*E.coli*	0.2
		Pseudomonas	0.4
2	VPD 19	*E.coli*	0.1
		Pseudomonas	0.3

The strains VPD 14 and VPD 19 could show zone of inhibition against Gram negative bacteria *E.coli* and *Pseudomonas*. Zone of inhibition exhibited by VPD 14 was good compared to VPD 19.

The strains of Actinomycetes VPD 14 and VPD 19 behaved differently against Gram positive and Gram negative strains. The result reveals that Actinomyctes were active against Gram negative bacteria compared to Gram positive bacteria. The reason may be different sensitivity between Gram positive and Gram negative bacteria could be due to morphological differences

.

4. Conclusions

Actinomycetes are the most widely distributed groups of micro organisms in nature which primarily inhabit the soil.They are the most economically and biotechnologically valuable prokaryotes. They are responsible for the production of about more than half of the discovered bioactive secondary metabolites notably antibiotics [17] .

Screening of Microorganisms for the production of novel antibiotics has been intensively perceived for many years by scientists. Actinomyctes have capability to synthesize many different antibiotics. Investigations can possibly reveal Actinomycetes species can produce novel antibiotics. It is anticipated that isolation, characterization and study of

Actinomycetes can be useful in the discovery of antibiotics and novel species of Actinomycetes. Actinomycetes are diverse group of microorganisms. In the present study an approach has been given to isolate Actinomyctes from various places/habitats of Gulbarga city.

Actinomycetes are well known prolific antibiotic producers. The present work was undertaken to exploit Actinomyctes for antibacterial activity against Gram positive and Gram negative bacteria by employing primary and secondary screening method. The Actinomyctes were also characterized up to Genus level.

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