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Actinomycete from coal fields of Andhra Pradesh and their antibiotic production

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ABSTRACT

Diversified actinomycete species were isolated from coal fields of Andhra Pradesh and dominant genera were separated. A total of eleven antibiotic microbial producers have been isolated from the coal soil, five of them have shown activity against both Gram-positive and Gram-negative bacteria. On the other hand, six isolates were only active against Gram-positive bacteria. They exhibited resistance to ampicillin, tetracycline, rifampicin, methicillin, streptomycin, streptothricin and chlorotetracycline and taken HIV-drugs like Lamostad-N-30, Trimune-30, zidovudine contain L = Laminarin, S = Stavudine and n = nevirapine but slight resistance factor identified by HIV-drugs against mandamarri strain of coal site of Andhra Pradesh. Metabolites produced by the strain showed good antimicrobial activity against Gram-positive and Gram-negative bacteria.

Key words: Actinomycetes, *Streptomyces* strains, basal medium, antibiotics, pure cultures.

INTRODUCTION:

The term ‘actinomycete’ now encompasses a wide range of bacteria. *Streptomyces* genus of the order actinomycetales constitute a distributed group of bacteria. They have many properties that favour their predominance among other saprophytic microorganisms. They are best known for their economic importance as producers of antibiotics, vitamins, carbon, nitrogen, amino acid utilizations and enzymes and are certain to have a significant role in future microbiology **[1]**. During the last 40 years, more than 1000 substances and preparations which posses antibiotic properties, i.e., have the capacity to inhibit the growth of and even to destroy various microorganisms, in dilute solutions, have been isolated from culture of an actinomycetes may, therefore, be said to have been isolated in 1940. It was designated as actinomycin by **[2]**. This research was aimed to isolate bacterial strains from coal soil able to produce antibiotic and we think any useful strain to help for HIV-patients and then to study the different conditions affecting its productivity of all the known microbes, actinomycetes represent a rich source of

biologically active metabolites such as antibiotics, enzymes, anticancer agents **[3, 4]**. Majority of the antibiotics so far reported are obtained from *streptomyces*, which are common inhabitants of soil **[5]**.

MATERIAL AND METHODS

The specimens (actinomycetes) used in this study were isolated from the coal soils of Andhra Pradesh. Coal soils from different places were brought to the laboratory in aseptic condition. Actinomycetes from the coal soil had been isolated by pour plate technique or starch-case in gar and glycerol-aspergine agar after serial dilution in distilled water. Dry colonies of actinomycetes were selected and isolated. The culture of streptomyces sp. MM-1 of which antimicrobial activity was studied in the basal medium consisting of yeast extract 10.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; $CaCl_2.2H_2O$, 0.04 g; $FeSO_4.7H_2O$, 0.05 g; $ZnSO_4.7H_2O$, 0.005g and distilled water 1000 ml. The pH was adjusted to 7.5 and incubated at 28^oC for 7 to 14 days. For studying the antimicrobial antibiotic production, minimum inhibitory concentration and to perform bioautography.

RESULTS

The physiological characteristics of the isolates are present in Table 1, out of eleven isolates OC-1, OC2 show gram-negative and no morphological and physiological characteristics of D-xylose, Mannitol, D-glucose, sucrose, ammonium hydrogen ortho phosphate BPA, BPA-S, OC-1, OC-2 showed negative characters against potassium nitrate and ammonium sulphate. Glycerol showed positive reaction against all eleven sites.

Table 1: Morphological and physiological characteristics of eleven isolates

Characteristics	MM-KK2	MM-KK5	GDK-1	KGM	BP-1	BP-2	BP-3	BPA	BPA-S	OC-1	OC-2
Straining for actinomycetes	GP	GP	GP	GP	GP	GP	GP	GP	GP	-	-
D-Xylose	+	+	+	+	-	+	-	-	+	-	-
Mannitol	+	+	+	+	+	-	-	-	-	-	-
D-glucose	+	+	+	+	+	-	-	-	+	-	-
Sucrose	+	-	+	+	-	-	+	-	-	-	-
Glycerol	+	+	+	+	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	-	-	+	-	-
Sodium nitrate	+	+	+	+	+	+	+	+	-	-	-
Ammonium hydrogen ortho phosphate	+	+	+	+	+	+	+	+	+	-	-
Potassium nitrate	+	+	+	+	+	+	-	-	-	-	-
Ammonium sulphate	+	+	+	+	+	+	+	-	-	-	-

MM-KK2 = Mandamarri Kalyani Khami 2 ; MM-KK5 = Mandamarri Kalyami Khami 5 ; GDK-1 = Godavarikhani ; KGM = Kothagudem; BP-1 = Bhupalpally-1; BP-2 = Bhupalpally-2 ; BP-3 = Bhupalpally-3; Bpa = Bellampalli; BPA-s = Bellampalli-Shanthighani ; OC-1 = Open cast 1 ; OC=2 = open cast 2; + = positive ; - = Negative ; GP = Gram positive.

The isolates utilized carbon and nitrogen sources such as D-xylose, mannitol, D-glucose, sucrose, glycerol, starch, sodium nitrate, ammonium hydrogen orthophosphate, potassium nitrate and ammonium sulphate served as good nitrogen sources for the isolates.

Isolates had the capability to produce different enzymes such as cellulose, chitinase, protease, catalase and urease (Table 2).

Table 2: Characterization of enzymes of eleven isolates

Characteristics	MM-KK2	MM-KK5	GDK-1	KGM	BP-1	BP-2	BP-3	BPA	BPA-S	OC-1	OC-2
Straining for actinomycetes	GP	GP	GP	GP	GP	GP	GP	GP	GP	-	-
Cellulase	+	+	-	+	-	-	+	+	+	-	-
Chitinase	+	+	+	+	-	-	+	+	+	-	-
Protease	+	+	+	+	+	+	+	+	+	-	-
Catalase	+	+	+	+	-	-	-	-	-	-	-
Urease	+	+	+	+	+	+	-	-	-	-	-

MM-KK2 = Mandamarri Kalyani Khami 2 ; MM-KK5 = Mandamarri Kalyami Khami 5 ; GDK-1 = Godavarikhani ; KGM = Kothagudem; BP-1 = Bhupalpally-1; BP-2 = Bhupalpally-2 ; BP-3 = Bhupalpally-3; Bpa = Bellampalli; BPA-s = Bellampalli-Shanthighani ;OC-1 = Open cast 1 ; OC=2 = open cast 2; + = positive ; - = Negative ; GP = Gram positive.

Out of eleven factors screened were found no influence the enzyme production from OC-1, OC-2 (Table 2) and other isolates have immense potential as a source for commercial application. Protease showed enzyme activity 9 sites except OC-1 and OC-2. Urease showed slight moderate activity and catalase showed less enzyme activity. The exhibited sensitivity to a number of antibiotics and they were resistance to ampicillin, tetracycline, rifampcin, methicillin, streptomycin, streptothricin, chlorotetracycline (Table 3). Approximately 40% (06) of the isolates produced antibiotics, included among these were broad and narrow spectrum. Six isolates produced antibacterial activity against Gram positive bacteria and two against Gram-negative bacteria. They were tested systematically on different media, under different conditions of culture and against different test organisms **[6, 7]**. The study revealed that this *streptomyces* sp. Is a promising for antibacterial antibiotic production. The logical basis for these antibiotics of their antibiotic spectrum or range of activity against different organisms, supplemented by their physical and chemical properties.

From Table-3 showed complete absence of antibacterial antibiotic resistance of HIV-drug against eleven isolates but MM-KK2, MM-KK5, GDK-1, BP-2 BPA showed antibiotic resistance to ampicillin, tetracycline, rifampcin, methicillin, streptomycin, streptothricin, chlorotetracycline, Actinomycin-D.

Determination of the antimicrobial activity

The antimicrobial activity was determined by agar well method, studies on the substance produce by actinomyces which inhibits homologous strain **[8]** and to determine the potency of the antibiotic produced by *streptomyces* sp. Muller-Hinton medium was the culture medium and the test bacteria which included *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 431), *Escherichia coli* (MTCC 40), *Proteus vulgaris* (MTCC 426), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 424 by using cup-plate method). The actinomycete was streaked near the periphery of a plate and incubated, where upon the test organism was streaked at right angles to but not in conduct with it. After an addition 1 to 2 days of incubation zone of inhibition were recorded (Table 4).

Table 3: Antibacterial antibiotic Resistance

Characteristics	MM-KK2	MM-KK5	GDK-1	KGM	BP-1	BP-2	BP-3	BPA	BPA-S	OC-1	OC-2
Ampicillin	+	+	+	+	-	-	+	+	-	-	-
Tetracycline	+	+	+	-	-	+	-	+	-	-	-
Rifampsin	+	+	+	-	-	+	-	+	+	-	-
Methicillin	+	+	+	+	+	+	+	+	-	-	-
Streptomycin	+	+	+	-	-	+	+	+	-	-	-
Streptothricin	+	+	+	+	-	+	-	+	+	-	-
Chlorotetra cycline	+	+	+	-	-	+	+	-	+	-	-
Lamostad-N-30	+	-	-	-	-	-	-	-	-	-	-
Trimune-30	-	-	-	-	-	-	-	-	-	-	-
Zidovudine	-	-	-	-	-	-	-	-	-	-	-
Actinomycin-D	+	+	+	+	-	-	-	-	-	-	-

MM-KK2 = Mandamarri Kalyani Khami 2 ; MM-KK5 = Mandamarri Kalyami Khami 5 ; GDK-1 = Godavarikhani ; KGM = Kothagudem; BP-1 = Bhupalpally-1; BP-2 = Bhupalpally-2 ; BP-3 = Bhupalpally-3; Bpa = Bellampalli; BPA-s = Bellampalli-Shanthighani ; OC-1 = Open cast 1 ; OC=2 = open cast 2; + = positive ; - = Negative.

Table 4: Antibacterial zone of inhibition against test bacterial species

Code of the sites	Test bacteria inhibition zone (mm)					
	1	2	3	4	5	6
MM-KK2	16	18	17	16	13	14
MM-KK5	12	11	10	15	12	10
GDK-1	15	14	10	11	0	7
KGM	7	6	8	0	7	0
BP-1	0	9	0	0	7	0
BP-2	0	0	0	0	6	6
BP-3	6	7	6	8	6	7
BPA	0	5	0	9	6	5
BPA-S	0	0	5	0	7	0
OC-1	7	0	0	0	0	0
OC-2	0	0	0	6	0	0

MM-KK2 = Mandamarri Kalyani Khami 2 ; MM-KK5 = Mandamarri Kalyami Khami 5 ; GDK-1 = Godavarikhani ; KGM = Kothagudem; BP-1 = Bhupalpally-1; BP-2 = Bhupalpally-2 ; BP-3 = Bhupalpally-3; Bpa = Bellampalli; BPA-s = Bellampalli-Shanthighani ; OC-1 = Open cast 1 ; OC=2 = open cast 2;

Test bacterial : 1. Bacillus cereus; 2. Bacillus subtilis; 3. Escherichia coli; 4. Proteus vulgaris; 5. Staphylococcus aureus; 6. Pseudomonas aeruginosa

From Table 4 showed antibacterial zone of inhibition against six test bacteria, out of eleven sites MM-KK2, MM-KK-5, GDK-1, KGM showed more antibacterial activity but site BP-3 showed moderate amount of activity against test bacteria and zero activity showed by the sites BP-2, OC-1 and OC-2 but moderate antibacterial activity showed by the site BP-2 against test bacteria 5 and 6.

DISCUSSION

A total of 60 different actinomycetes were taken from different coal sites of Andhra Pradesh of which 11 *streptomyces* sp. Isolates producing antimicrobial antibiotic activity and the genus *streptomyces* has been used during the 1940's and 1950's as main source for antimicrobial agents. The present work was conducted to study antimicrobial activity of *streptomyces* sp. Taken from coal mine soil sites and bring to the laboratory in aseptic conditions and did work in Kakatiya University Microbiology laboratory and samples were treated by basal medium and incubated at room temperature (28^{o}C) for seven days. The *Streptomyces* for a of soil samples, collected from different locations and screened for their potentials as a source of antibiotics. All the isolates were tested for their ability to produce inhibitory substances against several test microorganisms. The test microorganisms included gram positive, gram negative bacteria and yeast [9-12]. Morphological, physiological and biochemical characteristics of eleven isolates grew on a range of agar media showing morphological types of *streptomycetes* **[13]**. The mode of action of actinomycin D has revealed that the antibiotic is a patent inhibitor of deoxyribonucleic acid-dependent RNA synthesis. Consequently, the antibiotic has become an extremely useful probe for investigations relating to the synthesis of messenger RNA and protein as well as virus replication. Clinically, actinomycin D tumor, gestational chorio-carcinoma and mixed metastatic carcinoma of the testes.

CONCLUSION

This paper has addressed the problem of antibacterial antibiotic resistance to the coalmine strains variables with known variances. We have shown that thresholding antibiotic is an effective means of solving the problem based on the uniformly most powerful test by the extraction of the strains isolated from coal sites. The HIV- drug Lanostad – N-30 showed slight resistance to MM KK5 strain. We showed that from coal fields of Andhra Pradesh isolated strains produced good antimicrobial activity.

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