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ISOLATION OF BIOACTIVE ACTINOMYCETES FROM AGRICULTURAL SOILS OF WARANGAL DISTRICT.

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ABSTRACT

As agriculture is main occupation in India which is not only affected by natural calamities such as draughts and floods but also by disease causing microorganisms. To control the damage caused by pathogenic microorganisms, previously chemicals were used but now microbes are widely used as biocontrol agents.Out of various groups of microbes, some of the microbial communities will offer a wide range of securities to the host plant includes pathogen avoidance,pests and insects repelling and growth promotion.Therefore, in present study we investigated on microbiome of agricultural soils.Soil samples were collected from different fields like Paddy, Cotton, Maize, Red gram and Mirchi. A number of actinomycetes isolates were obtained from various soil samples.The isolates were identified based on the morphological study, microscopic observation and biochemical profile. Antimicrobial activity is carried out by several test organisms:*Staphylococcus aureus* (ATCC 25923), *Escherichia coli*(ATCC 25922), *Klebsiellapneumonia*(ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853). The isolated actinomycetes were further inoculated on Pikovskaya's agar media and on Carboxy Methyl Cellulose agar media to study their phosphate solubilizing and cellulolytic activity.

Keywords: Phosphate solubilisingactinomycetes, antimicrobial activity, cellulolytic.

1.0 INTRODUCTION

Agriculture is the main occupation of India. India's economy and its pride also depends on agriculture. It is estimated that Global agriculture has to double food production by 2050 in order to feed the growing population of world (1). Soil microbes are the most abundant of all the biota in soil and responsible for driving nutrient and organic matter cycling, soil fertility, soil restoration, factory health and ecosystem primary product.Microorganism are constantly present in soil, ordure and decaying factory napkins which are suitable to degrade wastes that

are identified with the substrate organic matter (2). Agriculture soil is a dynamic medium in which a large number of pathogenic and nonpathogenic bacterial and fungal foliage live in close association. Soil microflora directly participates in nutrient cycles.Microorganisms produce some useful composites that are salutary to soil health, factory growth and play an important part in nutritive chains that are important part of the natural balance in the life in our earth (3,4). Along with bacteria and fungi actinomycetes are also important soil microorganisms (5). Actinomycetes are the most abundant organisms that form thread-like fibers in the soil and are responsible for characteristically" earthy" smell of lately turned healthy soil. Actinomycetes aren't only useful for husbandry but also used in other sectors i.e., medicinal, drugetc., In attempts to develop marketable biocontrol and factory growth promoting products using rhizobacteria, it's important to fete the specific challenges they present. To begin with, the commerce between PGPR species and their factory symbionts appears to be specific, indeed within a crop or cultivar (KIoepper 1996). Therefore webbing and insulation of promising strains of actinomycetes with implicit antibiotics is a thrust area of hunt since numerous times. As there's a geographic variation in Indian soil type and their contents, hence it is relatively likely that the distribution of antibiotic producing actinomycetes is also variable.Thus, disquisition of agrarian ecosystems for bacteria, fungi and actinomycetes is necessary to understand agrarian microbiome and their relation to plant growth and yield.Inaddition phosphate solubilising and cellulolytic activity of actinomycetesgreatly contributes to the agricultural sector.Phosphate solubilizers supply the demand of soluble phosphate to the crop thereby increasing productivity and cellulolytic properties can help use these organisms to degrade organic cellulose, from agricultural waste, into simple sugars and make it available to plant for nutrition.

2.0 MATERIALS AND METHODS:

2.1 Soil sample collection:

All Soil samples were collected from 5-15 cms below the surface of the soil. Soil samples were collected from various agricultural fields of Warangal District like paddy, cotton, maize etc., The collected soil samples were then packed in ziplock bag and stored.

2.2 Determination of physiochemical properties of soil:

Freshly collected soil samples were analyzed for physiochemical properties. The moisture content of the sample was removed by placing in hot air oven at 105°C to maintain constant weight. The physiochemical properties i.e.,humidity,pH, temperature and aw were determined according to methods suggested by Pramer and Schmidt, (1964) (6).

2.3 Isolation of Actinomycetes:

Isolation of actinomycetes was done by using Pour plate method, Spread plate method, Membrane filter technique and Direct inoculation technique (7, 8, 9). One gram of soil sample wastaken and serially diluted up to 10^{-6} using distilled water as adiluent. The mixture was shaken vigorously using a vortex;0.1 ml of each dilution was placed on starch casein agar(composition: soluble starch: 10 g, K_2HPO_4 : 2 g, KNO_3: 2 g,casein: 0.3 g, $MgSO_4.7H_2O$: 0.05 g, $CaCO_3$: 0.02 g, $FeSO_4.7H_2O$: 0.01 g, Agar: 15 g, and filtered sea water: 1000 mland pH: 7.0 ± 0.1), and the inoculum was spread properlyusing a sterile glass spreader. The inoculated plates wereallowed to stand at room temperature for 5–10 minutes toallow the liquid to be absorbed and were incubated at 28° Cfor 7 days.

2.4. Identification:

Identification of the actinomycetes wasdone on the basis of macroscopic and microscopic examination and physiological tests as suggested by Bergey’sManual of Systematic

Bacteriology, 2nd Edition, Vol 5, The Actinobacteria, Part A.The isolates were confirmed based on microscopic observation and biochemicalprofile. (10).

2.5 Macroscopic Characterization:

The isolated actinomycetes were observed for aerial mycelium, sub-merged mycelium, colour, and diffusible pigments. They were preliminarilyidentified by the morphology of the cells and spore chain morphology.

2.6 Microscopic Observation:

Microscopic examination was performed by coverslip and Gram Staining method.Cover slip was inserted into the solidified starch casein agarmedium plate at an inclination of 45° with the agar surface.Actinomycete isolate was inoculated along the surface of themedium that meets the surface of the buried cover slip. Itwas incubated at 28° C for four days. The cover slip wasremoved using sterile forceps and placed on an individualclean glass slide, which were then observed at oil immersionobjective [11]

2.7 Cellulolytic Actinomycetes

This is based on the hypothesis that if the microorganisms are allowed to grow in a medium containing cellulose as the only carbon source, then it should produce cellulase enzyme to utilize cellulose for their growth and the metabolic needs[12]. After 72 h colonies characteristic of actinomycetes were picked and spot inoculated on Carboxy Methyl Cellulose (CMC) Carboxymethylcellulose (a soluble form of cellulose), 0.5g, $NaNO_3$, 0.1 g, K_2HPO_4, 0.1 g, KCl, 0.1 g, $MgSO_4$, 0.05 g, Yeast Extract, 0.05 g, Glucose 0.1 g and Agar 17 g in 1000 ml distilled water. For visualization of clear zones Congo red solution and Gram's Iodine were used.

2.7. 1Congo red visualization method

Plates were fluddedwith Congo red solution for 15 minutes and then de-stained with the salt solution (1M sodium chloride solution) for 10–15 minutes. The cleared zonesindicates the cellulose degradation into simple sugars by the enzymatic activity .Unstained areas indicate that the CMC has been broken down to β 1→4 glucans that contain seven or fewer glucose residues. The diameter of the clear zones can be measured [12].

2.7.2Visualization of zones using gram's Iodine

An improved method for the detection of extracellular cellulase production by microorganisms is by plate assay. In this method, CMC plates were flooded with Gram's iodine instead of congo red. Gram's iodine formed a bluish-black complex with cellulose giving a sharp and distinct zone around the cellulase-producing microbial colonies within 3 to 5 minutes. According to Kasana et al. (2008) this is more rapid and efficient method [13].

2.8Phosphate solubilizing Actinomycetes

Pikovskaya's agar plates inoculated with different cultures of actinomycetes and the plates showing clear zone after 48 hrs or more of incubation at 28 ± 2 °C were considered to solubilize inorganic phosphate. In order to aid visualization of solubilizing zone we added 0.1% (W/V) Methyl red in the media. Colonies showing solubilization were surrounded by red zone as methyl red is red at pH 4.4 and yellow at pH 6.2 and the phosphate solubilizing isolates produce acid which helps in solubilizing phosphate.

2.9 Antimicrobial activity

Agar well diffusion method:

Five wells of 6 mm diameter were made on the agar plate with the help of sterile cork borers. The test organism was swabbed on the agar surface and 100 μl of crude extract was poured in the wells. Ethyl acetate and antibiotic discs (oxacillin, nitrofurantoin, and ciprofloxacin) were used for negative and positive control, respectively. The plates were allowed to stand for few minutes and incubated at 37°C without inverting for 24 hours.

3.0RESULTS AND DISCUSSION:

This study revealed that the agricultural soil is quite rich in microbial flora.The physiochemical properties of soil also play an important role in the growth of microorganisms. Diversified microflora was observed.The isolation of various actinomycetes species showed that In agriculture process, soil microorganisms such as bacteria, fungi and actinomycetes may play important roles in soil fertility and in the form of loss and gain in the production of grains, fruits, vegetables (14). Further, we need to study how these microbes are influencing plant growth and yield. In conclusion the goal of this research was to focus on sustainable agriculture i.e., to identify agriculture friendly microorganisms.

As shown in Table 1 a total of 14 isolates out of 10 soil samples were identified as actinomycetes species based on morphology and microscopic observation (Fig 1). All isolates were slow growing, aerobic, gram-positive nature with white to cream and greyish colour colonies.

Table 1: Number of Actinomycetes Isolated from different soil samples

Soil sample	**Location**	**Physical characteristics of soil**			**No of actinomycetes isolates**
		pH	**colour**	**consistency**	
A	KADIPIKONDA(Maize)	7.5	BLACK	MOIST	4 (A1 to A4)
B	RAMPET (Mirchi)	6.0	BROWN	MOIST	3 (B1 to B3)
C	SHAYAMPET (Cotton)	5.9	GREY	DRY	3 (C1 to C3)
D	KAMALAPUR (Paddy)	5.5	GREY	DRY	2 (D1 to D2)
E	THARALAPALLY (Rsd Gram)	5.9	BROWN	DRY	2 (E1 to E2)

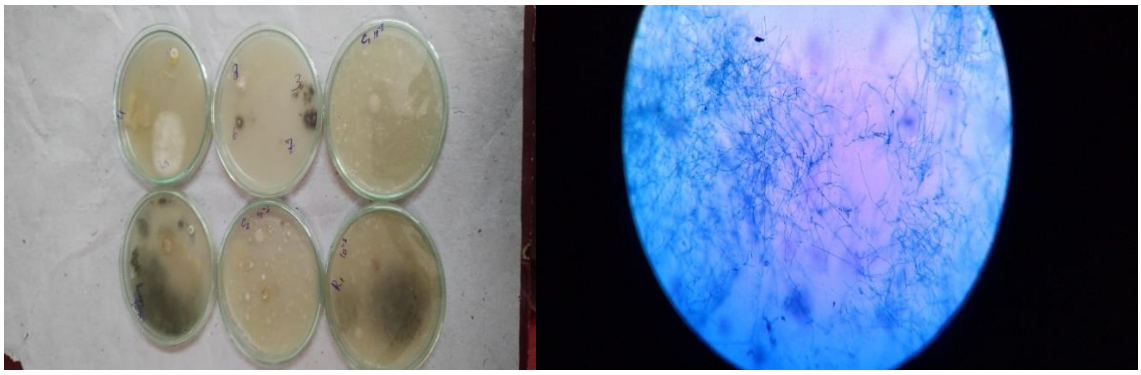

Fig 1: a) Actinomycetes colonies b) Microscopic view

Out of which 8 isolates have shown good cellulolytic activity. Two isolates failed to show activity and the remaining with less activity. A2 and E2 isolates showed highest activity. 13 isolates exhibited phosphate solubilizing activity only one isolate failed to show the activity. E2 isolate followed by C3 isolate has highest phosphate solubilising activity. A2, D2, E1 and E2 isolates exhibited good antimicrobial activity (Table 2).

Table 2: PGP activities of Isolated Actinomycetes

Actinomycete Isolate No	**Cellulolytic activity (Zone mm)**	**Phosphate solubilising activity (Zone mm)**	**Antibacterial activity (Zone in mm)** *S.aureus (ATCC 25923)*	*E.coli (ATCC 25922)*	*K.pneumoniaP.aeruginosa (ATCC 700603)*	*(ATCC 27853)*
A1	10	8	0	0	0	0
A2	35	12	16	0	4	0
A3	5	10	4	0	0	2
A4	0	6	0	0	0	0
B1	20	10	8	4	0	2
B2	15	12	5	0	0	0
B3	10	6	4	0	0	2
C1	8	12	0	0	0	0
C2	12	10	6	2	0	2
C3	22	13	2	0	0	4
D1	0	0	0	0	0	0
D2	30	10	12	6	4	4
E1	25	7	10	8	2	6
E2	35	14	4	10	2	8

3.1 CONCLUSION:

As most of these isolates are cellulolytic and have phosphate solubilizing capacity with antimicrobial activity, adding these to soil will help increase the quality of soil. Hence, these isolates can be exploited in agriculture to increase the yield.In conclusion, our results suggest that simultaneous screening of actinomycetes for plant growth promoting activities is a good

tool to select effective PGP actinomycetes for biofertilizer development.The use of such PGP actinomyceteseither singly or in consortium can act as efficient bioinoculants which may be an effective approach to reduce the usage of synthetic fertilizers and pesticides for sustainable agriculture.

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