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ANTIBACTERIAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM *CAPSICUM ANNUUM* L.

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ABSTRACT

A total of 72 actinomycetes strains were isolated from *Capsicum annuum* L. rhizosphere soils of Warangal, Khammam, Karimnagar and Mahabubnagar from Telangana State, India. Among these 25 showed antifungal activity against *Colletotrichum capsici* and *Fusarium oxysporum*. Among these 25 strains, three (OUA17, OUA18 and OUA27) were grown in starch casein broth for 6 days, centrifuged and powdered samples were extracted by rotary evaporator and were screened for antibacterial activity against bacterial pathogens viz., *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*. All the three strains showed growth inhibition against these pathogenic bacteria. Among the three strains OUA27 showed highest inhibition against *E. coli*. These actinomycetes strains were identified by 16S rRNA sequence as *Pseudonocardia* sp. OUA17TAVKSP, *Streptomyces* sp. OUA18TAVKSP and *Streptomyces graminearus* OUA27TAVKSP, deposited in NCBI database and the accession number given were KP013381, KP013382 and KP013383 respectively. The phylogenetic tree was constructed by using phylogeny.fr online phylogeny programs.

KEYWORDS: **Actinomycetes, antibacterial activity, bacterial pathogens, *Pseudonocardia*, *Streptomyces*.**

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INTRODUCTION

Actinomycetes are gram-positive bacteria frequently filamentous and sporulating with DNA rich in G+C from 55-75%[1]. Most of the actinomycetes are unparalleled sources of bio-active metabolites including antibiotics, plant growth factors and other substances[2]. Actinomycetes have been well known for the production of secondary metabolites. Many of these used as antibiotics such as streptomycin, gentamicin, rifamycin and erythromycin are the products of actinomycetes. Actinomycetes are important sources of new bioactive compounds such as antibiotics and enzymes[3,4,5,6] which have diverse clinical effects and are active against many organisms (bacteria, fungi, parasites etc.). Human pathogenic bacteria cause serious infections. For example *Staphylococcus aureus* causes various infections including bacteremia, pneumonia, endocarditis, osteomyelitis and pimples. *S. aureus* is a virulent human pathogen and due to the misuse of antibiotics, they have become resistant to various groups of antibiotics[7]. Actinomycetes represent a high proportion of the soil microbial biomass and have the capacity to produce a wide variety of antibiotics and extracellular enzymes[8]. Moreover, these are important source for novel antibiotics and hence having a high pharmacological and commercial interest including control of infectious diseases[9]. Actinomycetes produce a large number of important secondary metabolites such as antibiotic compounds including streptomycin, actinomycin, and tetracycline[10]. Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora*[11]. Among the actinomycetes occurring *Streptomyces* spp. has been recognized as major producer of bioactive metabolites with broad spectrum activities such as antibacterial and antifungal agents[12,13,14]. *Streptomyces,* soil-dwelling filamentous bacteria are profile producers of a wide range of antimicrobial agents[15]. *Streptomyces* continue to be a rich source of vitamins, carbon, nitrogen, amino acids utilization and enzymes. These bacteria, like *Pseudomonas aeruginosa*, are common organisms which were found in the environment, which act as opportunistic pathogens in clinical cases where the defense system of the patient is compromised[16]. Both human pathogens and fungal phytopathogens are prone to develop ''drug'' resistances. The effectiveness of the older types of antibiotics can decrease substantially. There is an urgent need to work towards the invention of safer antifungal agents which are expected to be renewable, non-petrochemical, naturally eco-friendly, and easily obtainable[17,18]. The aim of the present study is to develop antibiotics produced by the selected *Streptomyces* spp. and *Pseudonocardia* sp. against bacterial pathogens.

MATERIALS AND METHODS

Collection of rhizosphere soil samples

Capsicum annuum L. rhizosphere soil samples were collected from chilli cultivating areas of Warangal, Khammam, Karimnagar and Mahabubnagar, Telangana State, India. These samples were taken from the growing roots up to a depth of 5 cm after removing approximately 3cm of the surface soil. These samples were placed in sterile polythene bags and analysed for actinomycetes.

Isolation of actinomycetes

The actinomycetes were isolated by serial dilution plate method[19] on chitin medium[20]. 1ml aliquots were added to cool and solidified agar medium. The plates were incubated at 28±2°C for 8 days and sub cultured on starch casein agar medium[21].

Test bacteria

The selective bacterial pathogens used for antibacterial activity were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae* collected from the department of Microbiology, Osmania University, Hyderabad.

Antibacterial activity by disc diffusion assay

The potent antifungal strains of actinomycetes[22] were screened against selected pathogenic bacteria. Actinomycetes strains were inoculated and grown in 100 ml of sterilized starch casein broth in 250 ml flasks, incubated in shaker (150 rpm) at 28ºC for 6 days and centrifuged at 10,000 rpm for 15 min. The clear supernatant was taken and added to an equal volume of butanol solvent. After extraction by rotary evaporator, the compound was taken and tested for antibacterial activity against pathogenic bacteria by disc diffusion method[23]. In this method PDA (Potato Dextrose Agar) media with p^H 7 was poured in petriplates, later 100µl suspension containing 10^8 CFU (Colony Forming Units)/ml of test pathogenic bacterial broth cultures were swabbed on the media surface. The sterilized filter paper discs (6mm diameter) were impregnated with the compound and placed on PDA plates. The diameter of inhibition zones were measured after incubation for 24 h at 28ºC. Three replicates were maintained for each treatment. Each assay was repeated three times.

RESULTS

Three potent antifungal strains were selected from 25 strains that showed antifungal from among 72 actinomycetes strains and screened for antibacterial activity against five pathogenic bacteria viz., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae*. These three strains (OUA17, OUA18 and OUA27) showed antibacterial activity against five bacteria (Figure 1). The zone of inhibition was measured in mm (Table 1 and Figure 2). OUA27 showed maximum inhibition zone (16.33 ± 0.57), OUA17 and OUA18 showed minimum inhibition zone (0.83 ± 0.28) against *E. coli*. OUA18 showed maximum inhibition zone (4.33 ± 0.57) followed by OUA27 (3.66 ± 0.57) and OUA17 (1.00 ± 0.00) against *S. aureus*. OUA27 showed 2.00 ± 0.00 mm inhibition zone against *P. aeruginosa*. However, OUA17 and OUA18 showed inhibition zone of 1.00 ± 0.00 mm surrounding the discs. OUA27showed maximum inhibition (5.00 ± 0.00) whereas OUA18 (4.00 ± 0.00) and OUA17 (3.33 ± 0.57) recorded minimum inhibition against *B. subtilis.* OUA18 showed 7.33 ± 0.57 mm inhibition zone followed by OUA17 (6.00 ± 1.00) and OUA27 (5.33 ± 0.57) against

K. pneumoniae. All the three actinomycetes strains showed antibacterial activity against five pathogenic bacteria. Among the three actinomycetes strains OUA27 showed highest inhibition (16.33 ± 0.57) against *E. coli*.

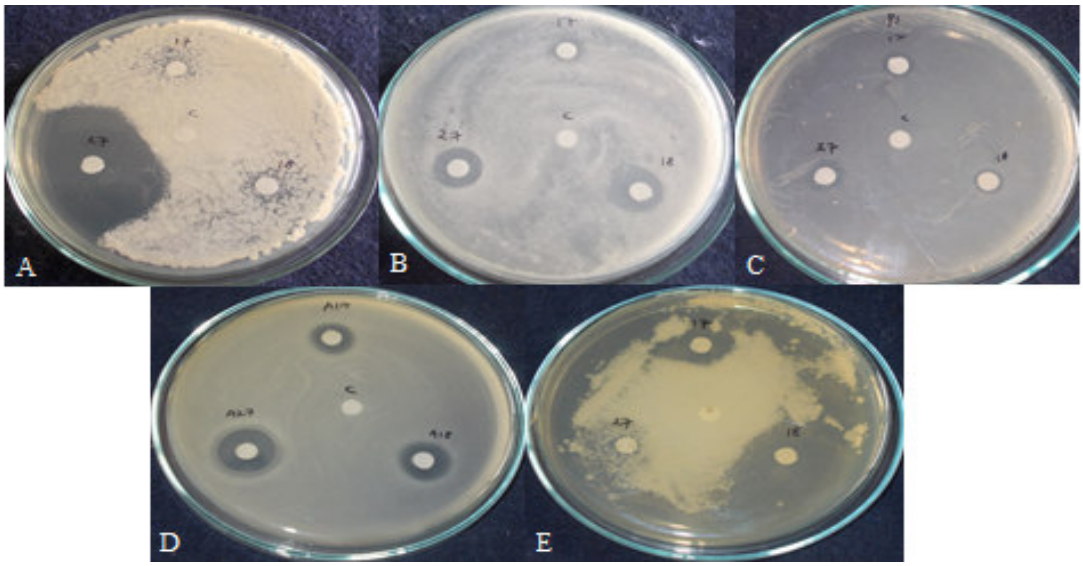

Figure 1
In vitro antibacterial activity of actinomycetes extracts against pathogenic bacteria; A) Escherichia coli; B) Staphylococcus aureus; C) Pseudomonas aeruginosa; D) Bacillus subtilis; E) Klebsiella pneumoniae.

Table 1
Actinomycetes extracts showed inhibition zone (Mean ± Standard deviation) against pathogenic bacteria.

Bacterial pathogens	Inhibition zone (mm)		
	OUA17	OUA18	OUA27
E. coli	0.83±0.28	0.83±0.28	16.33±0.57
S. aureus	1.00±0.00	4.33±0.57	3.66±0.57
P. aeruginosa	1.00±0.00	1.00±0.00	2.00±0.00
B. subtilis	3.33±0.57	4.00±0.00	5.00±0.00
K. pneumoniae	6.00±1.00	7.33±0.57	5.33±0.57

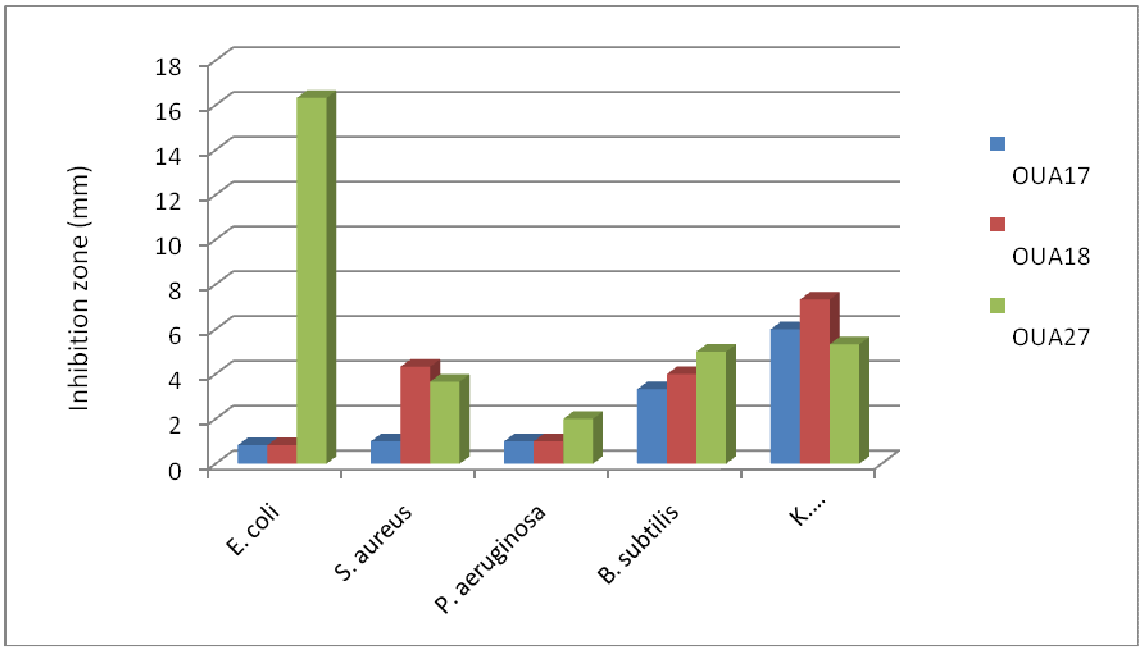

Figure 2
Antibacterial activity of actinomycetes

Molecular analysis and Phylogenetic studies

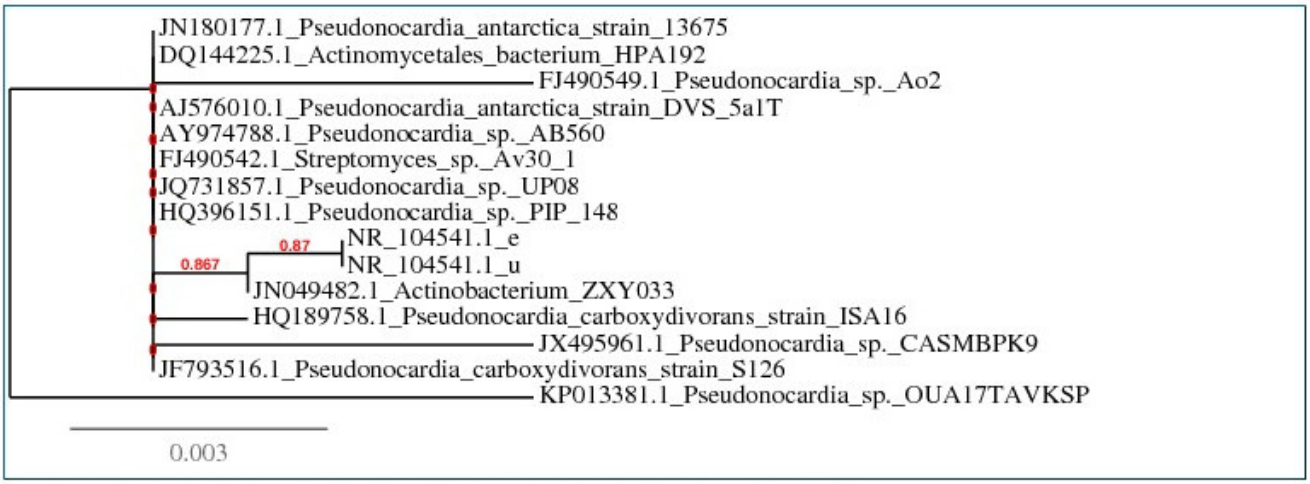

Figure 3
Phylogenetic tree of A17

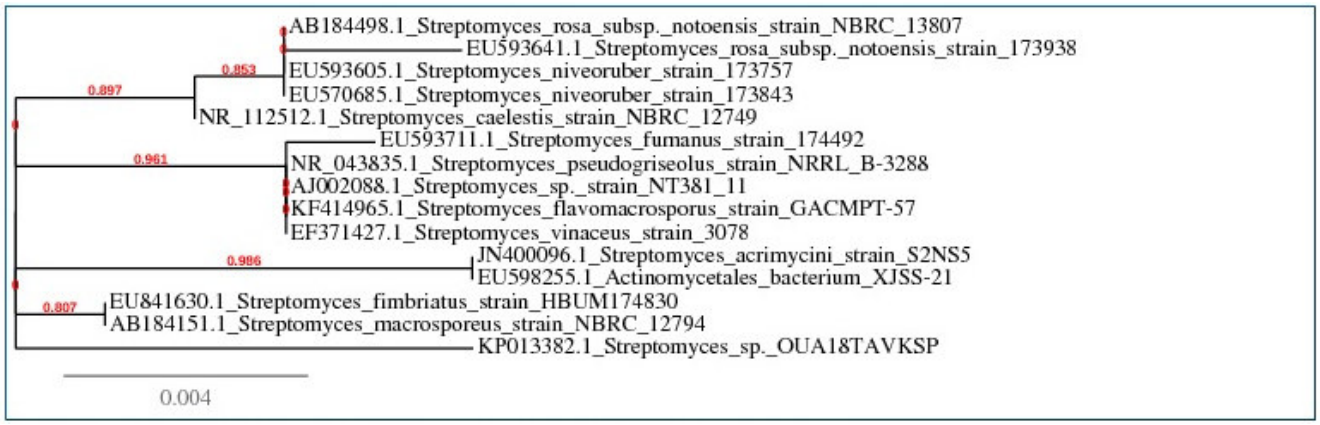

Figure 4
Phylogenetic tree of A18

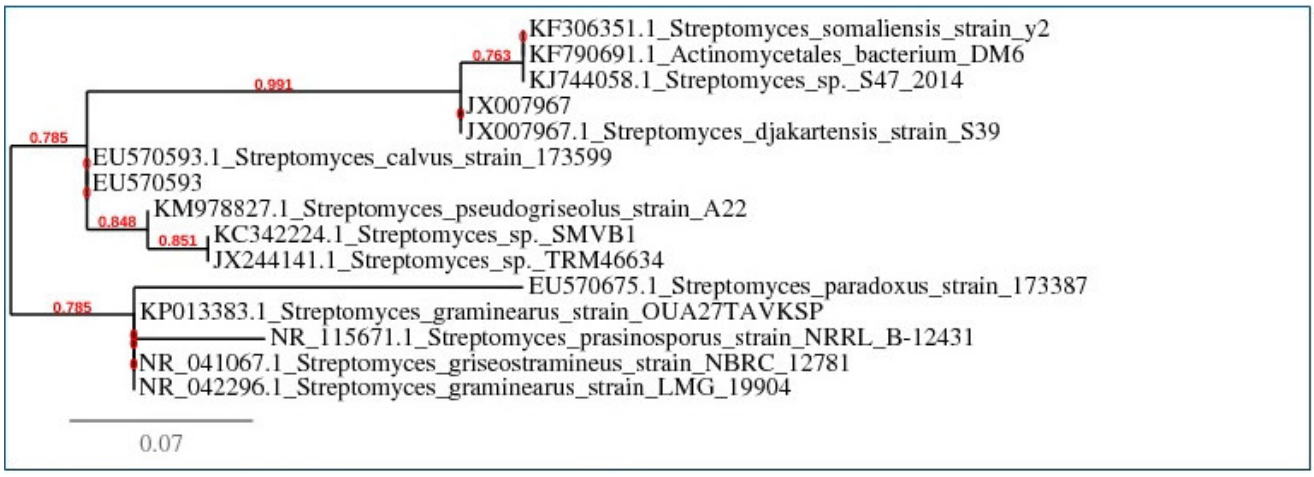

Figure 5
Phylogenetic tree of A27

Actinomycetes strains OUA17, OUA18 and OUA27 were identified by 16S rRNA sequencing analysis which was done with primers, 518F (CCA GCA GCC GCG GTA ATA CG) and 800R (TAC CAG GGT ATC TAA TCC). BLAST analysis for alignment and compared with NCBI database and phylogenic tree analysis was done. The phylogenetic tree was constructed by using phylogeny.fr online phylogeny programs. The numbers at the nodes indicate levels of boot strap support based on neighbour joining analysis of sequence data (Figure. 3, 4 and 5). The 16S ribosomal RNA of OUA17 showed 100% similarity with *Pseudonocardia* sp. ACT-0118, OUA18 showed 99% similarity with *Streptomyces* sp. ZG243 and OUA27 was 100% showed similarity with

Streptomyces graminearus strain LMG19904. 16S rRNA sequence was deposited in NCBI database and the accession number given were KP013381, KP013382 and KP013383 respectively.

DISCUSSION

Actinomycetes are filamentous, gram positive bacteria and are the major producers of biologically active secondary metabolites of economic importance in agricultural, chemical and pharmaceutical industry. The majority of actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Actinomycetes population has been identified as one of the major group of soil population[24] which may vary with the soil type**.** In this study 72 actinomycetes were isolated from the rhizosphere soil samples of *Capsicum annuum* L. by serial dilution method on chitin medium and sub cultured on starch casein agar medium, screened for antifungal activity against *Colletotrichum capsici* and *Fusarium oxysporum*, out of which, 25 strains showed antifungal activity[22]. Among these 25 actinomycetes three potent strains (OUA17, OUA18 and OUA27) were screened against pathogenic bacteria, which showed good growth inhibition. Among these three strains OUA27 showed highest inhibition zone (16.33 ± 0.57 mm) was formed against *E. coli.* Fifty two actinomycetes strains isolated from the rhizosphere soil of medicinal plants collected from Southeastern Western Ghats of Tamil Nadu[25]. Out of 52 strains, five strains showed high antibacterial activity against all the tested pathogens. Five different extracts of eight actinomycetes showed antibacterial and antifungal activity[26]. S*treptomyces* sp. (A2) showed good inhibition against *S. aureus*, *B. subtilis* and *E. coli* in cross streak method[27].

CONCLUSION

This study clearly proves that the rhizosphere soil samples of *Capsicum annuum* L. derived actinomycetes possess significant antibacterial activity against the various bacteria. It is confirmed from the present study, especially experimental rhizosphere soil samples of Warangal, Khammam, Karimnagar and Mahabubnagar have actinomycetes which are good sources of antibacterial activity.

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