International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 03 Issue: 10 | Oct -2019 ISSN: 2590-1892

CHARACTERIZATION OF ANTIBIOTIC PRODUCING ACTINOMYCETES FROM SOUTH EAST COASTAL REGION OF CHENNAI.

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ABSTRACT:

Antibiotic is one of the most important commercially exploited secondary metabolites produced by bacteria, fungi and Streptomyces employed in a wide range. Most of the antibiotics used today are from the microbes. In present study, 5 soil samples were collected, serially diluted and spread on actinomycetes isolation agar with chlromphenical and fluconozole for inhibition of bacteria and fungi, respectively. In primary screening, isolated microorganisms were tested against *Staphylococcus aureus* (MTCCB 733), *Pseudomonas aeruginosa* (MTCCB 741), *Escherichia coli* (MTTB 82) and *bacillus cereus* (MTCCB 1272) by cross streak method. After primary screening, submerged state fermentation was used for the production of antibiotic for selected strains. Agar well diffusion was used for antimicrobial activity of crude extracts against test organisms. This study suggests that AM07 have the potential to produce antibiotics.

Keywords: actinomycetes, soil sample, antibiotic production, cross streak method, antibacterial activity.

1. INTRODUCTION

Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. While many antibiotics are known to exist, efforts to discover new antibiotics still continue. Therefore, many species such as Streptomyces, Bacillus and Penicillium have been studied continuously for their ability to produce antibiotics (Brock and Madogan, 1991). It was not until 1940 with the discovery of penicillin, the first, best-known and most widely used antibiotic in 1928 by an English Bacteriologist, late Sir Alexander Fleming that the first clinical trials of penicillin were tried on humans. This antibiotic was obtained from a blue green mould of the soil fungi called Penicillium notatum. Penicillin was discovered accidentally in 1928 by Fleming, who showed its efficacy in laboratory cultures against many disease producing bacteria (Schlegel, 2003). This discovery marked the beginning of the development of antibacterial compounds produced by living organisms. Another antibiotic, streptomycin was isolated in 1944 by

Waksman, a Microbiologist, from a species of soil bacteria, called Streptomyces griseus, particularly tubercle bacilli, and has proved to be very valuable against tuberculosis. A vigorous search for more antibiotics was on at that time and in 1947, another antibiotic, chloromycetin was discovered by Burkholder (Sommer *et al*., 2006; Cupp *et al*., 2004). Actinomycetes are Gram positive bacteria which occupy a large proportion of the soil microbial biomass and play an important role in the production of bioactive metabolites. Streptomyces spp. represents at least 90% Actinomycetes isolates, which differ significantly in their morphological and physio-chemical properties (Khaliq *et al*., 2013). This group of microorganisms is responsible for the production of over 6000 types of bioactive secondary metabolites obtained from different species of Streptomyces. Many of these compounds are extracellular enzymes, antibiotics, antimicrobial, anticancer, insecticides, herbicides, etc. (Newman *et al*., 2003; Iznaga *et al*., 2004). 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 in the isolation of certain agents, which have found application in combating a variety of human infections (Retinowati, 2010). That is why more than 70% of naturally occurring antibiotics have been isolated from different genus of actinomycetes (Khanna *et al*., 2011). Out of these different genus, Streptomyces is the largest genus known for the production of many secondary metabolites (Maleki *et al*., 2011), which have different biological activities, such as antibacterial, antifungal, antiparasitic, antitumor, anticancer and immunosuppressive action (Jemimah *et al*., 2011; Nonoh *et al*., 2010). Although thousands of antibiotics have been isolated from Streptomyces, these represent only a small fraction of the repertoire of bioactive compounds produced. Therefore, isolation of new Streptomyces from natural resources and characterization of their secondary metabolites is a valuable endeavour(Berdy, 1995; Watve *et al*., 2001).

MATERIALS AND METHOD

1.ISOLATION OF ACTINOMYCETES

During the study, five soil samples were collected aseptically from five sites at ECR, Chennai, India. The debris from soil samples were removed before collection. The site was digged into 10–15 cm and approximately 10 g of the marine soil was collected in a sterile tube and transported into laboratory and stored at 4°C. From each sample, 1g of soil sample was added in test tube containing 10 ml distilled sterile

water and shaken well using vortex mixer. This was then serially diluted by using standard serial dilution methods up to 10-6. These test tubes were considered as stock cultures for different soil samples. And from each tubes 0.1 ml of sample was taken and spread plate technique was performed and the plates were incubated at 37 °C for 3-4 days for the isolation of actinomycetes. The colonies of actinomycetes were identified and the basis of musty odour and powdery colony and simple staining was performed to visualize hyphae and mycelial structure under microscope.

2. PRIMARY SCREENING

20 Actinomycetes stains are isolated and they were screened for antibiotic production. The test bacteria used for primary screening were *Escherichia coli* MTCCB 82 (E. coli), *Staphylococcus aureus* MTCCB 737 (S.aureus), *Pseudomonas aeruginosa* MTCCB 741(P.aeruginosa). Plate containing Mueller Hinton agar was streaked with isolated colonies of actinomycetes in the centre along the length and then, fresh sub cultured test organisms were streaked perpendicular to the actinomycetes isolates. Then the plates were incubated at 37°C for 24 hours. After incubation observation for zone of inhibition was made and recorded.

3. SECONDARY SCREENING

3.1. ANTIBIOTIC PRODUCTION OF ACTINOMYCETES

Based on the result of primary screening each isolates of actinomycetes were subject to submerged state fermentation. For antibiotic production, Yeast Malt Broth was prepared according to the composition using 50% sterile Sea water, sterilized by autoclave. The AM01, AM02, AM07, AM09 and AM15 were been inoculated separately into the Yeast Malt Broth. The inoculated broth was been kept in an orbital shaker for incubation for10days to obtain for maximum growth, followed by filtration. The filtrate was been collected and equal volume of ethylacetate was added. After 24 hours of incubation, the organic layer was collected and kept in rotary evaporator at 40ºC in round bottom flask. The concentrated extracts was carefully transferred to sterile eppondroff. The crude extracts thus obtained was used for further study.

3.2. AGAR WELL DIFFUSION METHOD

In these method inoculums was prepared as that of disc diffusion method. The well was prepared in the plate by using sterile cork borer (6mm in diameter). A volume of 50 µL of 10 mg/mL of crude extracts of AM01, AM02, AM07, AM09 and am15 were carefully dispensed into each well and incubated at 37°C for 24hr. DMSO used as a negative control. After

24hr of incubation, zone of inhibition around each well was recorded and the experiment was repeated for three times

Figure: 1 Antimicrobial activity of selected strains

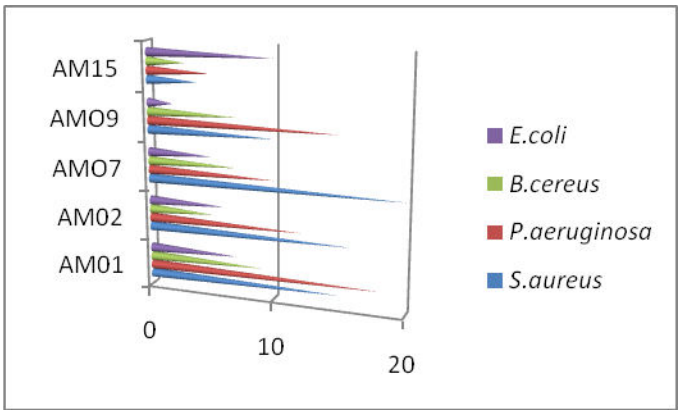

Table: 1 zone of inhibition (mm) against bacterial strain

STRAINS	*S.aureus*	*P.aeruginosa*	*B.cereus*	*E.coli*
AM01	15 mm	18mm	9mm	7mm
AMO2	16mm	12mm	5mm	6mm
AM07	20mm	10mm	7mm	5mm
AM09	10mm	15mm	7mm	2mm
AM15	4mm	5mm	3mm	10mm

4. RESULT AND CONCLUSION

The result of the present study revealed that marine soil actinomycetes of southern east costal region appear to have immense potential as a source of antibacterial compounds. Totally 20 actinomycetes included in this study, from that 5 were shown to be capable of antibiotic production using cross streak method. Out of 20 isolated actinomycetes, five namely AM01, AM02, AM07, AM09 and AM15 showed antimicrobial activity against selected strains. However, isolates AM07 showed maximum inhibition against *s.aureus* and the isolates AM01 and AM09 against *P.aeroginosa* with highest scores. The crude extract showed higher inhibition zone against gram positive bacteria than gram negative bacteria. For the present study, a range of inhibition zone was recorded for the crude extract of AM07 against test organism were 5 to 20 mm. The inhibition zone of crude extracts from five selected isolates against test microorganism ranged from 0 to 20 mm. Therefore, the further purification process is significant to get pure antibiotic substance for the application of treatment of different pathogenic microorganism.

In the present study, the effect of the crude extracts of promising isolate AM07 has shown higher antimicrobial activity. The result of the present study were interesting and encouraging because the crude extracts from the isolates may have promising antibiotics.

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