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ISOLATION AND IDENTIFICATION OF SEDIMENT DERIVED ACTINOMYCETES THROUGH MOLECULAR CHARACTERIZATION OF 16S RRNA TECHNIQUE

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ABSTRACT

To investigate, isolate and identification, Characterizations of Bioactive potential cytotoxic effect of actinomycetes collected from turmeric crop plantation in Erode, District.The soil samples were air dried under *in vitro* lab condition for 3 – 4 days. After air drying it has been washed two times with the help of distilled water to remove the unwanted dust particles from the soil sample. The soil samples was subjected into the basic microbiological techniques such as, serial dilution, pour plate and spread plate methods, that is to identify the specific growth of actinomycetes and its identified that it is present in the 10^{-3} and 10^{-4}. Followed by the microbiological procedure, finally the samples were undergone for identifying the biochemical characterization and using various parameters of the biochemical test like Methyl red, Vogues-Proskauer, Nitrate Reduction were clearly performed for the identification. After the isolation procedure is completed the sample was subjected to the 16s rRNA sequencing analysis of selected actionomycetes. Isolated actinomycetes culture was identified by 16s rRNA sequencing and it was confirmed through the bioinformatics tools as BLAST.

Key Words: Turmeric soil, Actinomycetes, BLAST, 16s rRNA, MCF-7 cell line.

INTRODUCTION

Actinomycetes are the group of Gram positive filamentous bacteria which are primarily recognized as organism of academic curiosity, potential degraders and also as a potential source for antibiotics. Although actinomycetes are well exploited for antibiotics and other high value metabolites, they are less exploited in terms of nanoparticles [1]. Actinomycetes are the most economically and biotechnologically valuable prokaryotes able to produce wide range of bioactive secondary metabolites, such as antibiotics, antitumor agents, immunosuppressive agents and enzymes [2]. Acti nobacteria arecommonly known as producers of antibiotics and other metabolites [3]. Actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds [4]. Taxonomic identification was done by physiological conditions, biochemical tests, chemo taxonomic investigations and molecular characterization. Morphological observation through macroscopic based on growth pattern on different media [5]. In terms of number and variety of identified species, *Streptomyces* represents one of the largest taxonomic units of identified *Actinomycetes* [6]. Many researchers are working towards isolating actinomycetes which have the ability to degrade harmful chemicals and also those with ability to act as biocontrol agents [7]. From all the genera of actinomycetes, the genus *Streptomyces* is represented in nature by the largest number of species and varieties [8]. These groups of actinomycetes are referred to as the biological antagonistic types. They are of special

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interest since they are the ones that are exploited and their metabolites used in the manufacture of antibiotics. Microbial metabolites are a source of life saving environments for bacterial and fungal infections [9]. Taxonomic characterization was carried out based on 16S rRNA sequence analysis in combination with morphological, physiological and biochemical data [10]. The 16S rDNA sequences are used to compare the percentage of similarity of the target strain with the sequences of other strains deposited in the GenBank/EMBL database and to construct phylogenetic tree which helps in the determination of evolutionary development.

MATERIALS AND METHODS

Collection of Soil Sample

The soil samples were collected from Erode, turmeric plantation crop is enormous. The soil sample was air dried for 3 to 4 days under *in vitro* lab condition. Serial dilution techniques were carried out followed by the pour plate method was done. Morphology shows that the colonies were present in 10^{-3} and 10^{-4} and finally the biochemical methods were done.

Isolation of Actinomycetes

0.5g of soil samples was suspended in 9.5 ml of sterile distilled water and was 1000-fold diluted ,0.1ml of the dilutions was spread on humic acid +modified B vitamins agar (HV) medium, pH 7.2, supplemented with cycloheximide. The plates were incubated at 28ºC for 2 weeks. Marine environment represents one such largely untapped ecosystem from which rare actinomycetes genera having a potential for producing novel metabolites have been discovered.

16 S RNA Sequencing

DNA preparation method

The isolated pure culture of Actinomycetes was inoculated in the sterilized Starch casein broth and kept for incubation for 4 days at 30ºC in rotary shaker at 180 rpm. After 4 days of incubation the culture was centrifuged at 10,000 rpm for 10 minutes in order to separate the mycelium. 0.1 g of mycelium was taken and placed in a clean sterilized dish. It was crushed with the help of liquid nitrogen and added with 50 µl TE buffer supplemented with the lysozyme 20mg/ml. Then the setup was incubated in the water bath for 30 minutes at 37º C. After the incubation period 20 µl of 10% SDS and 20 µl of proteinase K was added and incubated at 55º C for 30 minutes. The lysate was cooled down and extracted once with equal volume of phenol and chloroform solution in 1:1 ratio at 10,000 rpm for 5 mins. The aqueous phase of the solution was transferred to a fresh tube. Precipitation was carried out in order to separate the DNA. 70% ethanol was added as a precipitating agent and kept for 30 minutes in -20º It was centrifuged at 10,000 rpm for 10 minutes in order to separate the pellet. The pellet was washed twice with 90 % ethanol and dissolved in TE buffer. The 20 µl RNase solution was added and then incubated in at 37º C for 1 hour. It was once again extracted with phenol chloroform at 1:1 ratio.

PCR amplification, sequencing and restriction analysis

PCR amplification of the 16S rDNA of the Actinomycetes and automated sequencing was performed using primers 27F-5' AGAGTTGATCMTGGCTCAG 3' and 1492R-5' TACGGYTACCTTGTTAGCGACTT 3' , Final volume of the reaction mixture was to be maintained at 25 µl containing 1X PCR buffer, 1.5 mM of $MgCl_2$, 200 µM of each dNTP, 20 pico-moles of each primer, 2.5U of Taq DNA polymerase and 100 ng of template DNA. The amplification was performed in an Eppendorf Thermo-cycler 96. The following profile was set such as initial denaturation step at 94º C for 2 minutes, then 30 amplification cycles of 94º C for 1 minute, 55º C for 1 minute and 72º C for 2 minutes. The PCR product was detected by agarose gel electrophoresis and was visualized by ultraviolet florescence upon straining by ethidium bromide. Before sequencing the amplified products were purified using HiPurA^{TM} PCR product purification spin kit. Sequencing reactions were performed using the ABI PRISM® BigDye® Terminator version cycle 3.1 Cycle sequencing Kit using the appropriate primer.

Basic Local Alignment Search Tool (BLAST)

From the 16S rRNA studies, sequence of nucleotide was obtained. From the obtained sequence the corresponding organism was to be known, hence the BLAST tool was being used. The obtained sequence was compared with the database of NCBI so that we can able to know the exact organism. In the NCBI website nucleotide BLAST has to be selected where they obtained nucleotide sequence has to be entered in the FAST format and then the database has to be selected to 16S ribosomal RNA sequence. After entering the job title the data has to be submitted by pressing submit button.This has redirect to another where we can find the list of organism, based upon the maximum score among the other organism the organism was identified.

MTT Assay

The cells were plated separately in 96 well plates at a concentration of 1×10^5 cells/well. After 24 h, cells were washed twice with 100 µl of serum-free medium and starved for an hour at 37°C. After starvation, cells were treated with different concentrations of test compound for 24 h. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37ºC in a CO_2 incubator. The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of

DMSO and this was mixed properly by pipettting up and down. Spectrophotometrical absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graph pad prism5 software [11].

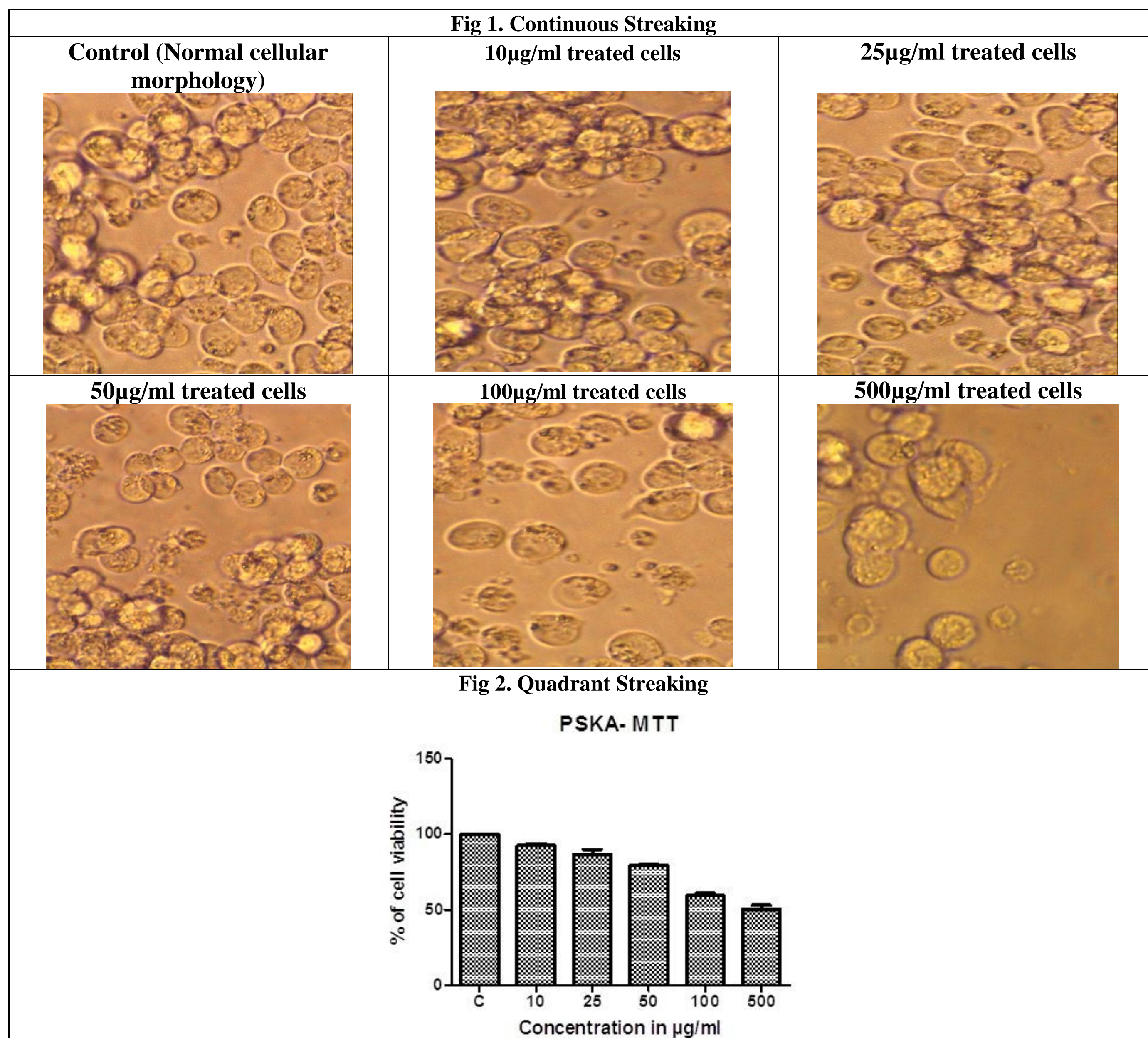

Fig 1. Continuous Streaking

Fig 2. Quadrant Streaking

RESULT AND DISCUSSION

Isolation of Actinomycetes Culture

The Actinomycetes culture was isolated by using Starch casein nitrate agar medium. Antifungal (Nalidixic acid) and Antibacterial (Cycloheximide) chemicals are used to avoid the contamination while during the isolation process. Starch Casein Nitrate agar media is the specific media to isolate the actinomycetes. It is also evident that different physiological characteristics are influencing the growth rate of the actinomycetes. In the present study, the assessment of physiological characteristics of the isolated strain shows that could grow well at 30 and 40ºC temperature, pH 7.0 to 9.0 respectively.

16S rRNA SEQUENCING

An average amount of DNA was extracted and it was to be amplified. The extracted genomic DNA and the PCR product with the help of 1kb DNA ladder was allowed to run in 1% Agarose gel and it is found that they are in undegraded condition. The PCR amplification of the

product yielded the molecular size of DNA 1.5 kb length. Using the automated sequence the sequence was retrieved with the help of suitable primers. The obtained sequence was entered in the form of FASTA format and the corresponding organism was identified using the Basic Local Alignment Search Tool (BLAST) a similarity searching tool.

MTT Assay

Mitochondrial function-based on the MTT conversion to formazon crystals (MTT assay) is often used as cell viability test in cell culture system .in the present study the potential growth of actinomycetes compounds cytotoxic effects on human breast cancer cell line (MCF-7) at the different concentration of 0, 10, 25, 50, 100, 500 (µg/ml) for period of 24h, 48h, and 72h. The cytotoxic activity of this cell line was determined based on the concentrations of the compound required to reduce the survival of cells by 50% (IC_{50}). Actinomycetes were highly cytotoxic to the human brast cancer cell line (MCF-7) were at very low concentrations and IC_{50} dosage is differ in time and dose dependent manner.

SUMMARY AND CONCLUSTION

High temperatures in composting helps to kill viruses, pathogenic bacteria, e.g. coliforms, and weed seeds present. Actinomycetes live predominantly aerobically, i.e. they need oxygen for their metabolism. The compost material, should therefore be well aerated [2]. In growth habit, many Actinomycetes resemble fungi but are smaller, and terms common to both are used to describe morphological features More than one-half of the antibiotics used in human medicine, including aureomycin, chloromycetin, kanamycin, neomycin, streptomycin, and terramycin, come from soil Actinomycetes. The smell of freshly turned soil is due to metabolic end products called geosmins that are produced by these organisms and move through soil as unseen volatiles [11].

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Nil

CONFLICT OF INTEREST
No interest

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