A METAGENOMIC APPROACH TO SCREEN BIOACTIVE COMPOUNDS AGAINST MULTI DRUG RESISTANT ORGANISMS

STUDENT PROJECT PROPOSAL

Submitted to

Office of Research and Development

Kalasalingam Academy of Research and Education

Submitted by

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COVERING LETTER

From

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To

Office of Research and Development,

Kalasalingam Academy of Research and Education,

Krishnankovil.

Respected Sir,

Sub: Submission of project proposal- biology Sector

I hereby wish to submit the research proposal with detailed work plan and budget for the proposed research work. The proposal has been framed under "Biology Sector". The project proposal entitled "**A metagenomic approach to screen bioactive compounds against Multidrug resistant organisms**". Kindly consider this research proposal and do the needful.

Thanking you, Sir,

Sivakasi

10.07.2021

Yours sincerely,

(**J.SUBHANANTHINI**)

Office of Research and Development,

Kalasalingam Academy of Research and Education,

APPLICATION FOR STUDENT PROJECT PROPOSAL

1	Name of the student	**SUBHANANTHINI.J**
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	Phone No. & Mobile No.	8838081252
3	Project Title	**A Metagenomic approach to screen Bioactive compounds against Multidrug resistant organisms**
4	Sector in which your Project proposal to be Considered	**BIOLOGY**
5	Project Details	AS PER THE FORMAT-ENCLOSED

Introduction:

Metagenomics is the study of metagenomes, genetic material recovered directly from environment samples. The broad field may also be referred to as environmental genomics, eco genomics or community genomics. Metagenomics is also described as environmental and community genomics which involves genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms in most environments on earth such as water or the soil.

Early studies on Metagenomics focused on 16S ribosomal RNA sequences which are relatively short, often conserved within a species. PCR was used to the study diversity of ribosomal RNA sequences. The genomes can be usually isolated from the environment and fragmented and then cloned into an organism by means of their plasmid in to a vector which has capacity to replicate and expressed.

Metagenomics entails extraction of DNA from a community so that all of the genomes of organisms in the community are pooled. These genomes are usually fragmented and cloned into an organism that can be cultured to create 'metagenomic libraries'. These libraries are then subjected to analysis based on DNA sequence or on functions conferred on the substitute host by the metagenomic DNA.

Metagenomic studies can be grouped into four categories based on different screening methods:

(a) Shotgun analysis using mass genome sequencing;

(b) Genomic activity-driven studies designed to search for specific microbial functions;

(c) Genomic sequence studies using phylogenetic or functional gene expression analysis;

(d) Next generation sequencing technologies for determining whole gene content in environmental samples.

Objectives:

- To isolate the DNA from the soil samples collected from diversified fertile areas.
- To amplify the DNA using 16SrRNA primers and sequence the amplified DNA.
- To digest the amplified DNA using selective restriction enzymes.
- To clone the DNA fragment and transform them into expression vector BL21.
- To screen the expressed protein and characterization of the structure through MS analysis.
- To study the effect of the protein against multidrug resistant bacteria.

Methodology

DNA extraction

Isolation or extraction of environmental DNA is a primary step for these metagenomic approaches. The DNA can be extracted and purified on the basis of charge using electrophoresis. Extraction of DNA is done by adding extraction buffer contains Tris – HCL (pH-8.0), 100mM sodium EDTA (pH- 8.0), 1.5 M NaCl. Which is then incubated for 1hour at 65ºC by adding sodium dodecyl sulphate. After incubation, the sample is centrifuged at 1400rpm for 5 mins. Lysate is then transferred to the centrifuge tube and isopropanol was added. Tubes are kept at 4ºC for 2hrs. The sample is then centrifuged again and the pellet is washed with 70% ethanol air dried and stored in Tris Buffer. Presence of DNA is confirmed by the gel electrophoresis and purified.

Function-Based Metagenomic Analysis

Functional metagenomics involves identification of clones that express activities conferred by the metagenomic DNA. Activity-based metagenomics provides an opportunity to circumvent culturing and to survey a community's functions. Function-based metagenomics, offers the opportunity to add functional information to the nucleic acid and protein databases.

Amplification

To perform targeted metagenomics, the environmental DNA is extracted and the gene of interest is PCR amplified using primers designed to amplify the greatest diversity of sequences for the gene of interest. Further, these amplified genes are sequenced, which results in the thousands of small subunit rRNA reads per sample and can probe hundreds of samples simultaneously.

Cloning and Transformation

The vector BL21, is used for transferring the fragments of extracted DNA, and they are transformed into a suitable host (*E. coli*). This allows the DNA that originated from environment sample will be expressed. The DNA within the vector changes into the cells of the model organism.

Transformation occurs when DNA is inserted into a cell. Then the DNA will produce stable proteins. To determine which method of transformation to use (chemical, electrical, or biological), analyze the type of sample under investigation.

Screening

The isolating DNA from the environmental sample, after cloning in to a suitable vector, transforming the clones into a host bacterium and screening the resulting transformants. Screening involve in analyzing the expression of any antibiotic production by the transformants.

Antibiotic production is tested by testing the efficacy against multidrug resistant organism through disc diffusion method and well diffusion method.

Antagonistic study using well diffusion method

Extracts obtained by various processes were evaluated for their potential antibacterial activities by the standard agar well diffusion assay. All extracts membrane syringe filter. Petridises (100mm) containing 18 ml of Mueller Hinton Agar (MHA) were seeded with inoculum of bactericidal strain. Media was allowed to solidify. Wells of 6 mm diameter were cut into solidified agar media using a sterilized cup-borer. 100µl of each extract was poured in the respective well and the plates were incubated at 37ºC overnight. The experiment was performed in duplicates under strict aseptic conditions to ensure consistency of all findings. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.

Any other details

Nil