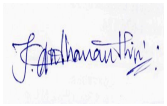
KALASALINGAM
ACADEMY OF RESEARCH AND EDUCATION
(DEEMED TO BE UNIVERSITY)
Under sec. 3 of UGC Act 1956. Accredited by NAAC with "A" Grade

Anand Nagar, Krishnankoil - 626126, Srivilliputtur (via), Virudhunagar District, Tamilnadu.

APPLICATION FOR ADMISSION TO Ph.D. PROGRAMMES

Date of Application:08-07-2021

Department	BIOTECHNOLOGY	Application No.	202110161
Area of Research	PHD	Research Mode	PART TIME



Name :SUBULHANANTHINI.J

Date of Birth / Age :23-04-1998 / 23 Years

Gender :FEMALE

Category :MBC

e-Mail ID :subhananthinijeyamurugan1998@gmail.com

Mobile :8838081252

Father's/Husband's Name	JEYAMURUGAN.S	**Father's/Husband's Occupation**	MANAGER
Family Income	110000	**Residental Type**	RURAL
Birth Place	SIVAKASI	**Mother Tongue**	TAMIL
Religion	HINDU	**Martial Status**	SINGLE
Aadhaar No.	453754756616	**PAN No.**	OHUPS7883E
Physically Challenged	NO	**Type of Disability**	-

Address for Communication:	**Permenant Address:**
1/37A MUDHALIPATTI SIVAKASI VIRUDHUNAGAR DISTRICT TAMILNADU INDIA Pin-626189	1/37A MUDHALIPATTI SIVAKASI VIRUDHUNAGAR DISTRICT TAMILNADU INDIA Pin-626189

Qualification

Degree	Discipline	College/university	Year Passed	AVG/CGPA	Class	Mode
B.SC	GOOD	AYYA NADAR JANAKI AMMAL COLLEGE	2018	78%	FIRST CLASS WITH DISTINCTION	REGULAR
M.SC	GOOD	AYYA NADAR JANAKI AMMAL COLLEGE	2020	88%	FIRST CLASS WITH DISTINCTION	REGULAR

Experience

Organization	Designation	Experience From	Experience TO	Work Nature

Payment Details

Transaction ID	Reference	Date of transaction	Amount	Status
202110161_210710124954	VHD40103754772	10-07-2021	600	SUCCESS

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

J. SUBHANANTHINI

M.Sc. MICROBIOLOGY., DGA

COVERING LETTER

From

J. SUBHANANTHINI

M.Sc. Microbiology, Department of Microbiology,

Ayya Nadar Janaki Ammal College (Autonomous),

Sivakasi- 626 124.

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**
	e-mail address	**subhananthinijeyamurugan1998@gmail.com**
	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

MADURAI KAMARAJ UNIVERSITY

PROVISIONAL CERTIFICATE

Sl. No. : MRK 410651 Date : 16-10-2020

This is to certify that SUBHANATHINI J

has qualified for the Degree of MASTER OF SCIENCE IN MICROBIOLOGY

he/she having studied in AYYA NADAR JANAKI AMMAL COLLEGE, SIVAKASI

(an Autonomous College of this University) and passed the Final Examination held in APRIL 2020 *in* FIRST CLASS WITH DISTINCTION

Reg. No. : 18PY17 Centre Code : 609

ISSUED ON - 21.12.2020
Palkalai Nagar
Madurai-625 021

Asst./Deputy Registrar

Prof. Dr. T. DHARMARAJ
Controller of Examinations

AYYA NADAR JANAKI AMMAL COLLEGE, SIVAKASI

(An Autonomous Institution Affiliated to Madurai Kamaraj University, Madurai, Re-accredited (4th cycle) with 'A+' Grade by NAAC with CGPA 3.48 out of 4 and recognized by UGC as College of Excellence and Star College by DBT)

200791

M.Sc. Degree Examinations, April 2020

ANJAC

MARKS CUM GRADE STATEMENT

NAME : SUBHANANTHINI, J

REGISTER No. : 18PY17

MAJOR : MICROBIOLOGY

ADMITTED IN : JUNE 2018

SEM	SUBJECT CODE	TITLE OF THE PAPER	CREDIT	MAXIMUM MARKS I	E	T	MARKS SECURED I	E	T	GRADE POINT	GRADE	R	M/Y
I	CY101	Principles of Microbiology	4	50	50	100	43	42	85	8.5	D++	P	N18
	CY102	Microbial Biochemistry and Physiology	4	50	50	100	41	38	79	7.9	D	P	N18
	CY103	Cell and Molecular Biology	4	50	50	100	43	43	86	8.6	D++	P	N18
	CY104	Practical-I [Principles of Microbiol., Microbial Biochemistry and Physiol., Cell and Molecular Biology]	3	50	50	100	48	44	92	9.2	O	P	N18
	CY105	Comprehension & *viva voce*-I	1		100	100		63	63	6.3	A	P	N18
	EY101	Genomics and Proteomics	4	50	50	100	46	42	88	8.8	D++	P	N18
	EO102	Elements of Bioinformatics	3	50	50	100	43	41	84	8.4	D+	P	N18
	SG101	Spoken English	1	100		100	78		78	7.8	D	P	N18
II	CY206	Microbial Genetics	5	50	50	100	40	44	84	8.4	D+	P	A19
	CY207	Immunology	5	50	50	100	44	38	82	8.2	D+	P	A19
	CY208	Soil and Agricultural Microbiology	4	50	50	100	42	45	87	8.7	D++	P	A19
	CY209	Practical-II [Microbial Genetics]	3	50	50	100	50	48	98	9.8	O+	P	A19
	CY210	Practical-III [Immunology & Soil and Agricultural Microbiology]	3	50	50	100	48	46	94	9.4	O	P	A19
	CY211	Comprehension & *viva voce*-II	1		100	100		69	69	6.9	A+	P	A19
	EY203	Biostatistics and Techniques in Microbiology	3	50	50	100	41	39	80	8.0	D+	P	A19
	SG202	Soft Skills	1	100		100	82		82	8.2	D+	P	A19
III	CY312	Medical Microbiology	5	50	50	100	47	45	92	9.2	O	P	N19
	CY313	Food and Pharmaceutical Microbiology	4	50	50	100	43	44	87	8.7	D++	P	N19
	CY314	Environmental Microbiology	4	50	50	100	45	41	86	8.6	D++	P	N19
	CY315	Practical-IV [Medical Microbiology, Food and Pharmaceutical Microbiology & Environmental Microbiology]	3	50	50	100	48	41	89	8.9	D++	P	N19
	CY316	Comprehension & *viva voce*-III	1		100	100		59	59	5.9	B+	P	N19
	EZ304	Bioinstrumentation Technology	4	50	50	100	46	45	91	9.1	O	P	N19
	EY305	Concepts in Genetic Engineering	3	50	50	100	43	43	86	8.6	D++	P	N19
IV	CY417	Project and *viva voce*	22		100	100		95	95	9.5	O+	P	A20

PERFORMANCE

CREDITS EARNED			CGPA	: 8.81
	CORE PAPERS	: 76 / 76	OVERALL GRADE	: D++
	ELECTIVE PAPERS	: 17 / 17	CLASS	: FIRST (D)
	SUPPORTIVE PAPERS:	2 / 2		
	TOTAL	: 95 / 95		

23.04.1998
DATE OF BIRTH

Signature of the Candidate

CONTROLLER OF EXAMINATIONS A.N.J.A COLLEGE, SIVAKASI. OFFICE SEAL P.G. COURSES

16 OCTOBER 2020
DATE OF ISSUE

Dr.C.Ashok
PRINCIPAL

Dr.P.Sivasamy
CONTROLLER OF EXAMINATIONS
(PG COURSES)

Any alteration or overwriting invalidates this Statement of Marks Cum Grade.

AYYA NADAR JANAKI AMMAL COLLEGE

[Autonomous, affiliated to Madurai Kamaraj University, Madurai, Re-accredited (4th Cycle) with 'A+' Grade (CGPA 3.48 out of 4) by NAAC, Recognized as College of Excellence & Mentor Institution by UGC]

SIVAKASI - 626 124

VIRUDHUNAGAR DISTRICT - TAMIL NADU

TRANSFER AND CONDUCT CERTIFICATE

Admission No: S785/18 Book No: 113 T.C. No: 23552

1. Name of the Student : SUBHANANTHINI, J
2. Date of Birth as entered in the Admission Register (in words) : 23/04/1998 (TWENTY THIRD APRIL NINETEEN NINETY EIGHT)
3. Name of the Father of the Student : JEYAMURUGAN
4. Nationality, Religion & Caste : INDIAN HINDU THOTTIYANAICKER
5. Community to which he/she belongs : DNC
6. Gender : FEMALE
7. Date of Admission to that Class : 08/06/2018
8. Class in which the student was studying at the time of leaving the College : II M.Sc. MICROBIOLOGY
9. Whether the Student has paid all the fees due to the College : YES
10. Whether he/she is qualified for promotion to a higher class : REFER MARK STATEMENT
11. Date on which the student left the College : 23/09/2020
12. Whether the student was in receipt of any Scholarship : NO
13. Date of Application for Transfer Certificate : 16/10/2020
14. Identification Marks
    a) A MOLE ON THE RIGHT LEG FOOT
    b) A SCAR ON THE RIGHT ELBOW
15. The Conduct and Character of the Student : Good

Date: 16/10/2020

AYYA NADAR JANAKI AMMAL COLLEGE (AUTONOMOUS) SIVAKASI 626 124

PRINCIPAL

PRINCIPAL
Ayya Nadar Janaki Ammal College
SIVAKASI

MADURAI KAMARAJ UNIVERSITY

PROVISIONAL CERTIFICATE

Sl. No. : MRK 410651 Date : 16-10-2020

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he/she having studied in AYYA NADAR JANAKI AMMAL COLLEGE, SIVAKASI

(an Autonomous College of this University) and passed the Final Examination held in APRIL 2020 *in* FIRST CLASS WITH DISTINCTION

Reg. No. : 18PY17 Centre Code : 609

ISSUED ON - 21.12.2020
Palkalai Nagar
Madurai-625 021

Asst./Deputy Registrar

Prof. Dr. T. DHARMARAJ
Controller of Examinations

आयकर विभाग
INCOME TAX DEPARTMENT

भारत सरकार
GOVT. OF INDIA

स्थायी लेखा संख्या कार्ड
Permanent Account Number Card

OHUPS7883E

नाम / Name
SUBHA NANDHINI J

पिता का नाम / Father's Name
JEYAMURUGAN

जन्म की तारीख / Date of Birth
23/04/1998

J. Subhanandhini

हस्ताक्षर / Signature

इस कार्ड के खोने/पाने पर कृपया सूचित करें/लौटाएं:

आयकर पैन सेवा इकाई, एन एस डी एल
5 वीं मंजिल, मंत्री स्टर्लिंग,
प्लॉट नं. 341, सर्वे नं. 997/8,
मॉडल कालोनी, दीप बंगला चौक के पास,
पुणे - 411 016.

If this card is lost / someone's lost card is found, please inform / return to :

Income Tax PAN Services Unit, NSDL
5th Floor, Mantri Sterling,
Plot No. 341, Survey No. 997/8,
Model Colony, Near Deep Bungalow Chowk,
Pune - 411 016.

Tel: 91-20-2721 8080, Fax: 91-20-2721 8081
e-mail: tininfo@nsdl.co.in

இந்திய தேர்தல் ஆணையம்

Election Commission of India

வாக்காளர் புகைப்பட அடையாள அட்டை ELECTOR PHOTO IDENTITY CARD

ZAY1212455

வாக்காளரின் பெயர்: சுபாநந்தினி

Elector's Name : SUBHA NANTHINI

உறவினரின் பெயர் : ஜெயமுருகன்

Relation's Name : JAYAMURUGAN

இனம் / Sex : பெண் / Female

பிறந்த தேதி / வயது / DOB / Age : 23/04/1998, 20

முகவரி: 1-37A, வி.சொக்கலிங்கபுரம் (வ.கி), வி.சொக்கலிங்கபுரம் (ஊ) , வார்டு 1 சொக்கலிங்கபுரம் பெத்துலுபட்டி, விருதுநகர், 626189

Address: 1-37A, V.Chokkalingapuram(R.V.),V.Chokkalingapuram (P) , Ward 1 Chokkalingapuram Pethulupatti, VIRDHUNAGAR, 626189

Date: 22/03/2019

வாக்காளர் பதிவு அலுவலர்
Electoral Registration Officer

தொகுதி எண் மற்றும் பெயர் : 206, விருதுநகர்

பாகம் எண் மற்றும் பெயர் : 252, ஊராட்சி ஒன்றிய நடுநிலைப்பள்ளி , North Building West Side -Pethulupatti

AC NO & Name : 206, Virudhunagar

Part No. & Name : 252, Panchayat Union Primary School , North Building West Side -Pethulupatti

குறிப்பு / Note :

1. வாக்காளர் புகைப்பட அடையாள அட்டை வைத்திருப்பது மட்டுமே தற்போதைய வாக்காளர் பட்டியலில் நீங்கள் வாக்காளராக இடம்பெற்றிருக்கிறீர்கள் என்பதற்கு உத்தரவாதமல்ல.ஒவ்வொரு தேர்தலுக்கு முன்பும் நடப்பிலுள்ள வாக்காளர் பட்டியலில் உங்களுடைய பெயர் உள்ளதா என்று சரிபார்க்க வேண்டும்

1. Mere Possession of Elector photo identity Card is no guarantee that you are elector in the current electoral roll. Please check your name in the current electoral roll before every election.

2. இந்த அட்டையில் குறிப்பிட்டுள்ள பிறந்த தேதியை வாக்காளர் பட்டியலில் பதிவு செய்யும் நோக்கத்திற்கு அல்லாது வேறு எதற்கும் வயது/பிறந்த தேதி குறித்த சான்றாகக் கொள்ளக்கூடாது

2. Date of birth mentioned in this card shall not be treated as proof of age / D.O.B. for any purpose other than registration in electoral roll.

26 / 206 / 252 / 0034

C.No.: 592 /2010

GCP—26-3—93,200 Cps.—17-6-2005 [P4-10]

R.Dis...../200 dt.

DNC

சான்றிதழ் எண் :
Certificate No. :

1164367

மாவட்டக் குறியீடு எண் :
District Code :

1	7

வட்டக் குறியீடு எண் :
Taluk Code :

0	5

கிராமக் குறியீடு எண் :
Village Code :

0	3	0

சாதிச் சான்றிதழ்
COMMUNITY CERTIFICATE

விருதுநகர் மாவட்டம் விருதுநகர் வட்டம்
வி.சொக்கலிங்கபுரம் கிராமம்/நகரம், திரு. / திருமதி / செல்வி/
செல்வன் சுபா நந்தினி தகப்பனார்/ கணவர்
பெயர் ஜெயமுருகன்
இந்து — தொட்டிய நாயக்கர்
வகுப்பைச் சார்ந்தவர், அரசு ஆணை நிலை எண் 28, பிற்பட்ட மற்றும் மிகவும் பிற்பட்ட பிரிவின் நலத் துறை, நாள் 19—7—1994 வரிசை எண் 242 படி, சீர்மரபினர் பிரிவினைச் சார்ந்தவர் எனச் சான்றளிக்கப்படுகிறது.

This is to certify that SUBHA NANDHINI Son / Daughter of Thiru. S. JEYA MURUGAN of V. CHOCHALINGA-PURAM Village / Town, VIRUDHUNAGAR Taluk, VIRUDHUNAGAR District of the State of Tamil Nadu belongs to HINDU — THOTTIA NAICKER Community, which is recognised as a Denotified Community as per Government Order (Ms.) No. 28, Backward Classes and Most Backward Classes Welfare, dated 19th July 1994 *vide* Serial No. 242.

2. திரு. / திருமதி / செல்வன் / செல்வி சுபா நந்தினி.
என்பவரும் அவருடைய குடும்பத்தினரும் தமிழ்நாட்டில் விருதுநகர்
மாவட்டத்தில் விருதுநகர் வட்டத்தில் வி.சொக்கலிங்கபுரம்
கிராமத்தில் / நகரத்தில் வசித்து வருகிறார்கள் எனச் சான்றளிக்கப்படுகிறது.

2. It is certified that Thiru/Tmt./Selvan/Selvi. SUBHA NANDHINI and his/her family ordinarily reside(s) at V. CHOCHALINGAPURAM Village/Town VIRUDHUNAGAR Taluk VIRUDHUNAGAR District of Tamil Nadu.

முத்திரை
Seal

கையொப்பம் :
Signature :

நாள் :
Date : 22.9.10

பெயர் (தனி எழுத்துக்களில்) :
Name (in Capital Letters) :

பதவி :
Designation :

Zonal Deputy Tahsildar
Virudhunagar

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

STUDENT PROJECT PROPOSAL

Submitted to

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Kalasalingam Academy of Research and Education

Submitted by

J. SUBHANANTHINI

M.Sc. MICROBIOLOGY., DGA

COVERING LETTER

From

J. SUBHANANTHINI

M.Sc. Microbiology, Department of Microbiology,

Ayya Nadar Janaki Ammal College (Autonomous),

Sivakasi- 626 124.

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**
	e-mail address	**subhananthinijeyamurugan1998@gmail.com**
	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