Bharath
INSTITUTE OF HIGHER EDUCATION AND RESEARCH
(Declared as Deemed-to-be University under section 3 of UGC Act, 1956)
(Vide Notification No. F.9-5/2000 - U.3, Ministry of Human Resource Development, Govt. of India, dated 4th July 2002)
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Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2018-O-01

Date: 16.08.2018

TO

Mr. R. Srikumar
Assoc. Professor/Microbiology,
BIHER.

Thro: Concern Head of the Department

Greetings!!!

We are happy to announce that the Research Advisory Committee has approved your proposal for Seed Money Scheme-2018 which was presented by you. You are requested to complete the proposal and send the progress report to the Dean Research in the prescribed time period.

Title of the Project: Molecular detection of Candida species by Restriction Fragment Length Polymorphism (RFLP) analysis of PCR from HIV infected persons

Seed Money Amount: Rs.1, 00,000/- (Rupees One Lakh Only)

Approved on: 06.08.2018

Payment details:

Voucher No.46 **Dated: 22.08.2018**

With Regards

Dean-Research

Bharath University

SELAIYUR, CHENNAI - 600 073, TAMIL NADU, INDIA.

CASH / PAYMENT VOUCHER

Date 22/08/2018

V.No. 46

Debit _____ Amount

Rs.

PAID TO Dr. R. Srikumar

RUPEES one lakh only

TOWARDS Seed Money Scheme - 2018

BHARATH UNIVERSITY CHENNAI 600 073. INDIA.

Authorised by **Finance Manager** **Cashier/Accountant** **Payee's Signature**

PROPOSAL SUBMISSION

1. **Details of Principal Investigator**

Name	: Dr. R. Sri Kumar
Designation	: Associate Professor
Highest Qualifications	: Ph.D.
Department	: Microbiology
E-mail	: rsrikumar_2003@yahoo.in
Contact no	: 9442500300
Date of Joining	: 02.01.2012

2. **Details of Co-Principal Investigator**

Name	: Dr. Sandhya Rani. T
Designation	: Assistant Professor
Highest Qualifications	: M.Sc
Department	: Microbiology
E-mail	: sandhya.micro86@gmail.com
Contact No	: 8098572603
Date of Joining	: 08.04.2013

Technical details

1. Introduction:

Oral Candidiasis a standout amongst the most widely recognized astute mycological infections in HIV patients. The range of Candida infection is starting from symptomless colonization to oropharyngeal candidiasis (OPC), esophagitis, onychomycosis, vulvovaginitis, cutaneous candidiasis and systemic candidiasis inclusive of candidemia. It has been seen that just about all HIV tainted individuals are colonized with Candida (Brandolt et al., 2017). Oropharyngeal candidiasis (OPC) is the First sign of HIV infection. It can spread from the mouth through the pharynx to the oesophagus. Candida albicans is as often as possible segregated species as a pathogen of the oral mucosa, Non albicans species are ensnared with more prominent recurrence of entrepreneurial pathogens related with disorders especially in immunocompromised hosts (Repentigny et al., 2002). OPC mainly occurs when the patients having CD4 counts less than 200 and additionally increased plasma HIV RNA levels. Which is undoubtedly correlated among oral Candida carriage just as with oral Candidiasis in HIV patients (Liu et al., 2006). These results may propose that a decline in oropharyngeal Candida carriage and Oral Candidiasis in HIV can be accomplished by starting patients on exceptionally dynamic antiretroviral therapy (HAART) without the requirement for explicit antifungal treatment. Oral Candidiasis is usually complicated with oesophagal Candidiasis which may restrain sustenance utilization and result in weight loss, undermining the overall health of the HIV infected persons. Nowadays, oral Candidiasis may cause the improvement of azoles resistant because of delayed utilization of antifungal agents and may incline to a move in non-albicans species related with unmanageable and repetitive diseases (Hamza et al., 2006). Moreover, various procedures dependent on phenotypic qualities, for example, segregation dependent on colony colour on CHROM agar Candida medium, assimilation profiles and spore formation for identification of Candida species, they are not fully reliable. During this study, we have tendency to determine in Candida species by sequence of Restriction Fragment Length Polymorphism (RFLP) analysis of PCR from HIV infected persons and furthermore we demonstrated that phenotypic and genotypic identification method was necessary to work out the species.

2. Review of status of Research and Development in the subject

Thanyasrisung P, Kesakomol P, Pipattanagovit P, Youngnak-Piboonratanakit P, Pitiphat W, Matangkasombut O. Oral Candida carriage and immune status in Thai human immunodeficiency virus-infected individuals. J Med Microbiol. 2014; 63(5):753-759

The prevalence of fungal infections has been increased, especially in immunocompromised hosts such as consumers of corticosteroids and antibiotics, the patients with diabetes, malnutrition, severe malnutrition, alcoholics, HIV/ AIDS, cancer, and certain genetic disorders. 1 Candidiasis is considered as the most important opportunistic fungal diseases

worldwide and remains a clinical problem, predominantly among the immunocompromised patients. 2, 3 Oral candidiasis (OC) is the commonest human fungal infection presented in the oral cavity among the HIV/ AIDS patients. 4 The different manifestations of OC in the immunocompromised patients especially among the HIV/ADIS patients involved oral thrush (pseudomembranous candidiasis), denture stomatitis, median rhomboid glossitis, hyperplastic candidiasis, erythematous candidiasis, linear gingival erythema, perleche or angular cheilitis, salivary gland swellings, sore formation in the oral cavity, and oral hairy leukoplakia. 5 Many people with OC can stay without any specific clinical symptoms for a long time. However, several symptoms in these people may include burning sensation and pain in the mouth, changes in taste sensation, and difficulty in swallowing liquids or solids, and or white creamy or creamy plaques in different parts of the oral cavity. 6, 7

2.1. International Status:

High-resolution melt analysis (HRMA) is a powerful molecular method to detect mutations, polymorphisms, epigenetic information, and also to identify species of different organisms by comparing relative positions and shapes of melting curves. 16 Real-time PCR followed by HRMA is a basic, fast, accurate, and closed-tube technique with the susceptibility for the single nucleotide. 17 Phylogenetic analysis is essential for obtaining a better understanding of the evolution of the genus Candida and detection of the relative degree of Candida species as well as infer evolutionary pathways of some of gene families. 18 Therefore, the aim of this study was molecular identification of Candida isolates by Real-time PCR high-resolution melting analysis and investigation of the genetic diversity of Candida species.

2.2. National Status:

NIL

3. Progress/ achievement so far, if any

a). Reference papers was collected.
b). Literature survey was studied.
c). Materials and methods were designed.

4. Work plan

4.1 Methodology

The present study has been endorsed by the Research and Ethical Committee of Medical Microbiology and Immunology Department, Faculty of Medicine at Sri Lakshmi Narayana Medical College Pondicherry. Patients who have utilized antifungal medications were prohibited

from the study. Every one of the patients signed an informed consent form to participate in the study. Specimen collection 10 ml of sterile phosphate-buffered saline was given to the patients and asked them to wash their mouth for sixty seconds and then expectorate into the given container. Every sample was instantly taken to the laboratory; vortex mixed followed centrifugation at 6000 rpm for 10 min. The pellet acquired from the rinse sample was re-suspended in 1 ml of sterile phosphate buffered saline. A hundred microliters of the re-suspended specimens were cultured on Sabouraud Dextrose Agar and incubated at 37◦C for 72 h. Separates on SDA were distinguish as Candida by colony morphology, 10%KOH and Gram staining.

Phenotypic identification Candida isolates were speciated phenotypically by Germ Tube Test (GTT). For determination and identification of multiple species used CHROME-agar Candida on the reported of colony colour for 48h at 37C and other discriminative tools chlamydospore formation on cornmeal agar. The isolated Candida was put away in glycerol broth at 700C for additional process by molecular techniques. Molecular detection

Restriction Fragment Length Polymorphism (RFLP) examination of PCR was one of the highest quality level techniques for identification of Candida species in the present study (Santos et al., 2010).

DNA Extraction: DNA eradication was performed utilizing the conventional bead beater method. A loopful of fresh pure colonies of Candida was suspended in 100 l STES buffer [200 mM Tris–HCl (pH 7.6), 100 mM EDTA (Ethylenediaminetetraacetic acid), 0.1% SDS(sodium dodecyl sulfate)] and 40 ±l of TE (Tris–EDTA) buffer [10 mM Tris–HCl (pH 8), 1 mM EDTA], 120±l phenol: chloroform mixture (1:1 V/V) and 0.3 g sterile zirconium beads (0.1 mm diameter; Bio Spec-Products)were added. The samples were homogenized using a mini bead beater (model 3110BX; Bio Spec Products) at 480 rpm for 5 min. The upper aqueous phase (100 l) was transferred to a sterile microcentrifuge tube and DNA was precipitated in the presence of 220 l cold ethanol (100%) and 10 l of 3 M sodium acetate at -20 ◦C for 18 h. The solution was centrifuged at (13,000 rpm)15,493g for 12 min and the DNA pellet was air dried and dissolved in 30 l TE buffer. Extracted DNA samples were stored at -20 ◦C until used.

PCR Analysis

The ITS-1 and ITS-2 regions of Candida spp. were amplified using universal primers; ITS- (50- TCC GTA GGT GAA CCT GCG G-30) and ITS-4 (50- TCC TCC GCT TAT TGA TAT GC-30). Initial Denaturation for 5 mins at 94◦C: This progression warms the double stranded DNA template strand to the purpose where ever the strands begin denaturing and the hydrogen bonds are broken between the nucleotide base pairs. Denature thirty seconds at 94◦C: Continuing denaturation of double-stranded DNA. Anneal primers for forty-five seconds at 58◦C.The forward and reverse primers are steady among this temperature vary to strengthen to every one of the single-stranded DNA template strands. The DNA polymerase is likewise steady enough to now tie to the primer DNA sequence. Broaden DNA for one minute at 72◦C: The emerald polymerase has an ideal temperature around 70-75◦C so this progression empowers the

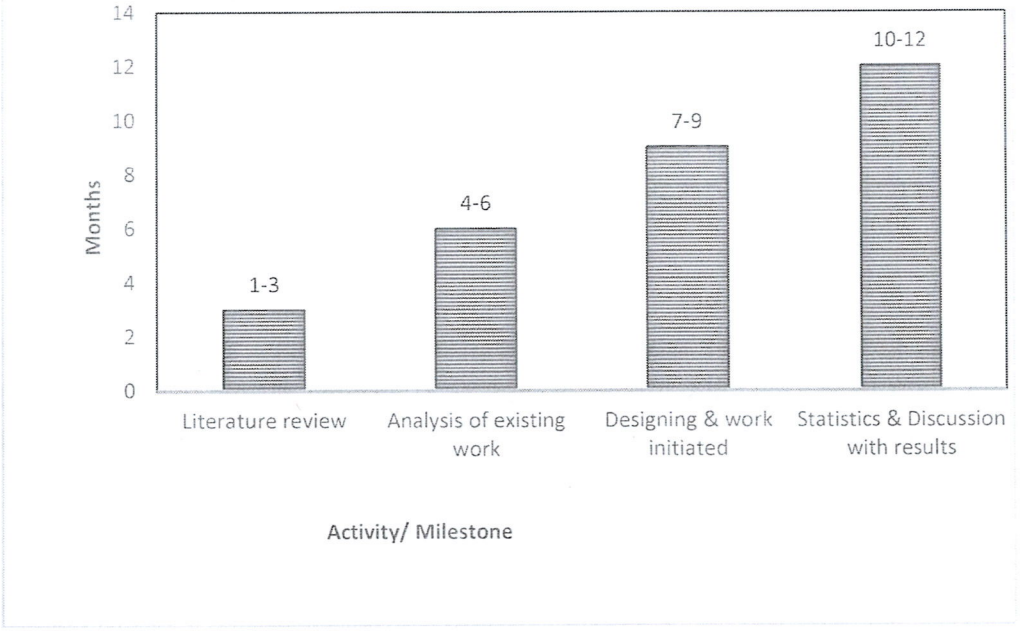
DNA polymerase to synthesize and extend the new target DNA strand precisely and quickly. Rehash stages two to four for forty cycles. Last Extension for seven minutes at 72◦C: The last extension to million any projecting ends of the freshly synthesized strands. Intensified PCR products were kept running on 2% agarose gel electrophoresis and envisioned by UV transilluminator. Presently the DNA polymerase is likewise steady enough to tie to the primer (Mirhendi S et al.,2006).

RFLP analysis:

RFLP is done by using MSP1 restriction enzyme. 5'...CC G G...3',3'...G G CC...5' 1 _L MspI enzyme 5000 units (BioLabs, England) and 2 _L enzyme buffer (NEB buffer 4) were added to 7 _L of each PCR product. Incubation at 370C for 16 h was done. Restriction fragments were separated by 3% agarose gel electrophoresis (Iwen et al., 2002).

4.2 Time Schedule of activities giving milestones through BAR diagram. (Maximum of 1/2 pages)

S. No	Activity/ mile stolen	1st Year			
1	Literature review	**1-3 month**			
2	Analysis of existing work	-	**4-6 month**		
3	Designing & work initiated	-	-	**7-9 month**	
4	Statistics & Discussion with results	-	-	-	**10-12 month**



4.3 Expected outcome within the time period of See Money Scheme

Our study showed that C. albicans and non-albicans like C. tropicalis C. glabrata, C. parapsilosis along with C. krusei were the vital species segregated from HIV-confirmed patients may be due to misuse of drug and lack of sexual discrimination which are the main factors impact the wideranging of dispensation frequency of Candida spp. PCR- RFLP is a high definite, sensitive, quick, reliable technique and also applicable method for fungal detection in clinical laboratory for identification of medically important Candida spp. It is used for detection of mycological examinations in HIV-positive along with other immune compromised patients for epidemiological studies.

5. Suggested Plan of action stating the name of funding agency where the project will be communicated for financial support within the time period of project.

Nil

6. Bibliography: Nil

Nil

7. List of Projects submitted/implemented by the Investigators (Separate for Pi and Co-PI)

7.1 Details of Projects submitted to various funding agencies:

S.No	Title	Cost in Lakhs	Month of Submission	Role as PI/Co-PI	Agency	Status
1	NA	NA	NA	NA	NA	NA

7.2 Details of Projects under implementation

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
1	NA NA	NA	NA	NA	NA

7.3 Details of Projects completed during the last 5 years

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
1	NA	NA NA	NA	NA	NA

8. List of publications published by the Investigators, if any:

a) Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1.	E. Kavitha a, b **R. Srikumar c**	High-Level Mupirocin Resistance in Staphylococcus spp. among Health Care Workers in a Tertiary Care Hospital	Pharmacology	103	320–323	2019
2.	S Latha, R Venkataramanan, **R Srikumar**, RV Kumar	Effect of Triphala on noise stress induced alteration in glucocorticoid and carbohydrate metabolism.	International Journal of Pharma and Bio Sciences	6(2)	1-15	2015
3.	Manikandan Sundaramahalingam, **Srikumar Ramasundaram**, Sheela Devi Rathinasamy, Ruvanthika Pulipakkam Natarajan, Thangam Somasundaram	Role of Acorus calamus and alpha-asarone on hippocampal dependent memory in noise stress exposed rats.	Pakistan journal of biological sciences: PJBS	16(16)	770-778	2013

b). Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1.	**Sandhya Rani T** and Jayaranjani K K. Jayarani*	Intestinal parasitic infections in pre school and school going children from rural area in Puducherry	Current Research in Microbiology and Biotechnology	2(4)	406-409	2014
2.	Jayarani.K and 5Naveenkumar.C 1***Sandhya rani T,** 2Balasubramanian 3B.Sai Ravi Kiran	Incidence of malaria and typhoid in acute fever in tertiary care hospital around pondicherry	International Journal of Recent Scientific Research	6(6)	4378-4381	2015

9. Budget

SI. No	**Head**	**Amount (Rs.)**
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	45000
2	Consumables (gels bottles, cotton, sprit, testing charges, tools, etc.)	10000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Others consumables	10000
	Total	**1,00,000**

*In case of any joint proposal for purchasing a same equipment, each of the associated PLs is also required to give separate budget (without any clubbing) to avoid any ambiguity, if all the associated projects are not awarded by committee.

10. Name of at least two subject experts from the Institute and one from the outside Institute with their contact details:

1. **Dr. Dr. G. Muthu** Research Scientist, ICMR, Thirunelveli **Mobile No**: 98843 04202 **E-mail id:** gopalmuthukrishnan@gmail.com	2. **Dr. Manikandan** Associate Professor in Physiology Tagore Medical College and Hospital, Chennai **Mobile No**: 9444434725 **E-mail id:**manikandanphysio@gmail.com

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Molecular detection of Candida species by Restriction Fragment Length Polymorphism (RFLP) analysis of PCR from HIV infected persons

It is certified that

1. I do hereby agree to submit a complete proposal for financial support to the external funding agency within the time period of SMS-2018.
2. I undertake that spare time on equipment procured in the project will be made available to other users.
3. I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in to.
4. I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involved field trails/experiments/exchange of specimens, human & animal materials etc.
5. I agree to abide by the terms and conditions of SMS-2018, BIHER, and Chennai.

Name and signature of
Principal Investigator

Name and signature of
Co-Principal Investigator

Date: 06.07.2018
Place: Pondicherry

Forwarded by Head of the Department

Signature of the Head

DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE,
KOODAPAKKAM POST,
PUDUCHERRY - 605 502

PROJECT EVALUATION FORMAT

Recommendation sheet

Name of the Principal Investigator	Dr.R.Srikumar
Name of the Co-Principal Investigator	Dr. Sandhya Rani. T
Name of the Department	Microbiology
Title of project	Molecular detection of Candida species by Restriction Fragment Length Polymorphism (RFLP) analysis of PCR from HIV infected persons
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended.
Financial allocation recommended	Rs. 1,00,000/-

SI. No.	Head	Amount
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	45000
2	Consumables- Gel bottles, cotton, sprit, testing charges, tools, etc.	10000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Others consumables	10000
	Total	**1,00,000**

Name and Signature of the Research Advisory Committee members with date.

Internal Quality Assurance Cell IQAC Pondicherry-02 SLIMS

Dr. A. Suguna