Criterion 3
Research, Innovations & Extension

3.1.1: Resource Mobilization and Research

Government Arts and Science College
Kozhinjampara

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3.1.1 RESEARCH GRANTS		
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Kerala State Council for Science, Technology and Environment

Prof (Dr.) K.P. Sudheer
Executive Vice President
KSCSTE, Pattom

29.10.2021

Letter No. 00812/SPS 65/2021/KSCSTE

Dear Mrs.Nimi Narayanan

Sub:-Financial assistance for Student Project scheme of KSCSTE reg.
Ref:-Your application received under Student Project scheme

This is to invite your attention to the reference cited and to inform that the project proposal titled **"In vitro evaluation of cholesterol-lowering potential of lactic acid bacteria and its application in fermented dairy products."** submitted by Mrs.Nimi Narayanan as PI and Jasheena P as student investigator(s) has been approved. An amount of **₹10000/-** is sanctioned by the Council. The budget estimate of the project is as detailed below.

SL.NO.	ITEMS	AMOUNT(₹)
1	Consumables	4000
2	Minor equipments	3000
3	Travel	500
4	Research Literature & Documentation	1500
5	Others (for analysis)	1000
	Total	**10000**

The PI has to submit the signed Terms and Conditions (as per the guidelines) and the date of start of the project within two weeks to the undersigned. The project should be completed within six months and submit the certified soft copy of the final report (in pdf to **sed.kscste@kerala.gov.in**), audited Statement of Expenditure and Utilization Certificate counter signed by the Head of the Institution for releasing the grant. **The format for final report, SE and UC can be downloaded from** www.kscste.kerala.gov.in.

Thanking you,

Yours sincerely,

Prof (Dr.) K.P. Sudheer

To

The Principal, Govt. Arts And Science College, Kozhinjampara, 678554

Copy to:

Mrs.Nimi Narayanan, Assistant professor,Microbiology, Govt. Arts And Science College, Kozhinjampara-678554
Jasheena Pstudent(s), Govt. Arts And Science College, Kozhinjampara-678554

Sasthra Bhavan, Pattom P.O., Thiruvananthapuram - 695 004, Kerala State, India
Tel : 0471 - 2548200-09, EVP - 2543557, 2548222, MS - 2534605, 2548220, CoA - 2543556, 2548248
Fax : 0471 - 2540085, 2534605 e-mail : kscste@gmail.com, www.kscste.kerala.gov.in

UTILIZATION CERTIFICATE

Certified that out of Rs. 10,000/— sanctioned by Kerala State Council for Science, Technology and Environment towards financial assistance for the student project titled 'In vitro evaluation of cholesterol-lowering potential of lactic acid bacteria and its application in fermented dairy products',

(File No./Letter No... 00812/SPS65/2021/KSCSTE), an amount of Rs.16,948/-was utilized for the purpose for which it was sanctioned, leaving a balance of Rs. -6948/-at the close of 16,948/- as shown in the Statement of Expenditure annexed.

Sreelatha Menon

SREELATHA MENON B Com, FCA
Chartered Accountant
M. No: 213380
2nd Floor, Prime Complex
Kalmandapam, Palakkad- 678 013

Name & Signature of Principal Investigator

NIMI NARAYANAN
Assistant Professor
Dept. of Microbiology
Govt. Arts & Science College, Kozhinjampara
Nattukal (P.O), Palakkad - 678 554

Name & Signature of Head of the Institution

20/12/2021

GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Seal & Signature of Chartered Accountant

UDIN - 22213388BDOHYA3464

Office Seal

Govt. Arts & Science College ★ Kozhinjampara, Palakkad-678 554 ★

STATEMENT OF EXPENDITURE

Project Title: In vitro evaluation of cholesterol-lowering potential of lactic acid bacteria and its application in fermented dairy products.

File No./ Letter no. 00812/SPS 65/2021/KSCSTE

Name of Institution: Government Arts and Science College Kozhinjampara Nattukal P.O, Palakkad

Receipts	Amount (Rs.)	Payments	Amount (Rs.)
1. Amount sanctioned from KSCSTE (to be receivable)	10,000	1. Consumables	4,168
		2. Minor equipments	4,000
2.		3. Travel	500
3.		4 Research Literature & Documentation	2,280
		5. Others (Analysis)	6,000
Total	10,000	**Total**	16,948/-

Certified that I have exercised all kinds of checks to see that the grant has been utilized for the purpose for which it was sanctioned by KSCSTE.

Sreelatha Menon

Name & Signature of Principal Investigator

NIMI NARAYANAN
Assistant Professor
Dept. of Microbiology
Govt. Arts & Science College, Kozhinjampara
Nattukal (P.O), Palakkad - 678 554

7/4/2022

Name & Signature of Head of the Institution

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Seal & Signature of Chartered Accountant

SREELATHA MENON B Com, FCA
Chartered Accountant
213386
Kalmandapam, Palakkad-678 013

Office Seal

Govt. Arts & Science College

ELECTRONIC CLEARING SERVICE FACILITY FOR RECEIVING PAYMENTS

Details of Account Holder

Name of the Institution/ Person	Govt. Arts and Science College Kozhinjampara
Contact Address	Nattukal P.O, Palakkad-678554 Ph:
Mobile number of Co-ordinator *(mandatory)* Telephone No./ Fax No.	9249570187
E-mail ID of the DIR/ REG/ AO/ FO	principalgasck@gmail.com.

Bank Account Details

Institution/ Individual Account Name (As per Bank record)	Smt. Amala (Principal-in-charge)
Account No. (SB/CC)	67011515002
IFS Code	SBIN0070185
Branch Name	SBI, Kozhinjampara
Branch Address	Kozhinjampara, Palakkad
MICR No.	678002922

Certified that the Institute's /person's account is in NEFT /RTGS enabled branch. I hereby declare that the particulars given above are correct and complete.

Name, Address & Signature of the
PI/ Coordinator

NIMI NARAYANAN
Assistant Professor
Dept. of Microbiology
Govt. Arts & Science College, Kozhinjampara
Nattukal P.O, Palakkad

Date: 07/12/2022

Name, Address & Signature of the
Competent Authority AO/ FO

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Institution Seal

Govt. Arts & Science College ★ Kozhinjampara, Palakkad-678 554 ★

KERALA STATE COUNCIL FOR SCIENCE TECHNOLOGY AND ENVIRONMENT

FORMAT OF STUDENT PROJECT REPORT (max. 15 pages)

1. Project Title : In vitro evaluation of cholesterol-lowering potential of lactic acid bacteria and its application in fermented dairy products.
2. File No : **Letter No. 00812/SPS 65/2021/KSCTE**
3. Name & Address of student : **Jasheena P.**
   2 nd year MSc. Microbiology
   Department of microbiology
   Government Arts and Science College
   Kozhinjamapra
4. Name & Address of the Principal Investigator &
   Co- Investigator with mobile No. : **Nimi Narayanan**
   Assistant Professor
   Department of microbiology
   Government Arts and Science College
   Kozhinjamapra
5. Broad area of research : Microbiology
6. Specific area : Food microbiology
7. Date of Start : 03-01-2022
8. Total cost of Project : RS. 10,000/-
9. Approved objectives of the proposal:

   - To isolate lactic acid bacteria from different fermented foods.
   - To evaluate the potential of different strains of lactic acid bacteria to reduce cholesterol during in vitro growth.
   - To assess the probiotic properties of best performing strains
   - To use the best performing strain with high cholesterol-lowering and probiotic potential for the production of fermented dairy products like yoghurt with low cholesterol level.

- To determine the physicochemical properties and viability of probiotics in inoculated yoghurt.

10. Methodology: (500 words)

Collection of samples

Fermented food samples like curd, yoghurt, pickle and idli batter were collected from various sources. Homemade fermented foods with no added preservatives were selected in order to isolate diverse species of lactic acid bacteria. Samples were collected in sterile containers and delivered to the laboratory within 15 minutes.

Isolation of lactic acid bacteria

The primary isolation of lactic acid bacteria was carried out using deMan Rogosa Sharpe (MRS) agar medium, the standard medium accepted by the International Dairy Federation for lactic acid bacteria IDF 1983. Food samples were serially diluted up to 10^{-7} and 0.1 ml of 10^{-4}, 10^{-5} , 10^{-6} and 10^{-7} dilutions were plated on MRS agar (de Man et al., 1960). After at 37 °C for 48 h, thirteen bacterial isolates were selected from MRS agar plates and were further purified by streak plate method. The isolates were grouped as lactic acid bacteria after examining their Gram reaction, cell morphology, catalase reaction and non- motility.

Determination of cholesterol removal from culture medium *in vitro*

The isolates were inoculated (1% (v/v) inoculums with 0.1 $O.D_{610\ nm}$) into freshly prepared MRS broth supplemented with 0.3 % of oxgall as a bile source and cholesterol with a final concentration of 100 µg/ml. Uninoculated MRS broth with cholesterol served as control. After incubation at 37 ◦C for 48 h, growth was determined by measuring optical density at 610 nm in a colorimeter (Aimil, India). Samples were centrifuged at 10000 rpm for 20 minutes (Remi, India) and the amount of cholesterol in the cell free supernatant was determined colorimetrically by Zak's method (Zak and Ressler, 1955). 1 ml cell free supernatant was mixed well with 5 ml with ferric chloride acetic acid reagent ($FeCl_3$, 0.05 g ; aldehyde free acetic acid, 100ml) and 3 ml of concentrated H_2SO_4. After 30 min incubation at room temperature, the absorbance of this solution was measured at 580 nm using ferric chloride acetic acid reagent alone as blank using colorimeter (Aimil, India). The OD was then compared with the standard graph prepared with known concentrations of cholesterol solution (8, 16 24, 32 and 40 µg/ml) to determine the concentration of cholesterol present in

the given sample. The ability of each strain to reduce the amount of cholesterol was calculated with the following equation: % of cholesterol reduction = $[(C_0 - C)/ C_0] \times 100$, where C_0 and C are the cholesterol content in the uninoculated control and inoculated cultures after 48 h. Based on cholesterol removal efficiency, three isolates, labeled as S_1P_1, S_1P_2 and S_1I_2 , exhibited higher percentage of cholesterol removal were selected for the production of probiotic dairy products.

Identification of selected lactic acid bacteria

Biochemical identification

The selected LAB were identified as per Bergy's Manual of determinative bacteriology (2000) based on its morphological and biochemical characteristics

Molecular Identification

DNA from isolate was amplified by PCR, using the primers 16S-RS-F (5′-CAGGCCTAACACATGCAAGTC-3′) and 16S-RS-R (5′-GGGCGGWGTGTACAAGGC-3′) and the 16SrRNA genes in the PCR products were sequenced. PCR products were checked by agarose gel electrophoresis and then the sequencing reaction was done in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol. The sequence of 16s rRNA gene was aligned using using BLASTn (Basic local alignment search tool) homology search tool. Phylogeny tree was constructed using the Maximum Likelihood method (Tamura and Nei, 1993). On genomic level identification, both S_1P_1 and S_1P_2 were confirmed as the same organism and therefore, only two isolates, S_1P_2 and S_1I_2 were screened for their probiotic potential.

Screening of lactic acid bacteria for probiotic potential

The selected strains with high cholesterol removing percentage are further screened for their probiotic properties such as resistance to low pH, tolerance to bile salts and NaCl, arginine hydrolysis, gelatinase activity, antimicrobial activity and antibacterial susceptibility test.

Tolerance to low pH

10 mL MRS broth which was adjusted to different pH, viz, 2, 3, 4, 5, 6 and 7 with 4 N HCl or 4 N NaOH was inoculated with 100 µL of overnight culture of the isolates and incubated for 24 h at 37 °C and growth was measured at 610 nm in a colorimeter (Aimil, India). The survival percentage of each isolate at different pH was calculated by : Survival (%) = (N_1/ N_0)

× 100 where, N_1 is the absorbance of cultures (OD at 610 nm) at pH 2.3.4.5 or 6, N_0 is the absorbance of cultures (OD) at pH 6.5 (Abdelazez et al., 2018).

Bile salt tolerance

In this study, 100 µL of overnight culture were inoculated in MRS broth supplemented with 0.05 %, 0.1 %, 0.15 % and 0.3 % concentrations of bile salt. The pH was adjusted to 6.5 with 4 N HCl or 4N NaOH and incubated for 24 h at 37 °C. The growth of isolates was monitored at 610 nm in a colorimeter (Aimil, India). The survival percentage was calculated with formula, Survival (%) = (N_1/ N_0) × 100, where, N_1 is the absorbance of cultures (OD at 610 nm) cultures containing 0.05 %, 0.1 %, 0.15 % or 0.3 % bile salts, N_0 is the absorbance of cultures without bile salts (Bao et al., 2010).

Growth at different NaCl concentrations

For the determination of NaCl tolerance of isolated LAB, 100 µL fresh overnight culture of isolates were inoculated in MRS broth with different concentrations (1, 2, 3, 4, 5 and 6 %) of NaCl. After 24 h of incubation, their growth was determined and survival percentage at different NaCl concentrations was calculated by: Survival (%) = (N_1/ N_0) × 100,where, N_1 is the absorbance of cultures (OD at 610 nm) containing 1, 2 ,3 ,4, 5 or 6 % NaCl, N_0 is the absorbance of cultures without NaCl (Bao et al., 2010).

Growth at different temperature

10 mL of sterile MRS broth was inoculated with 100 µL of overnight culture of each isolate was incubated at different temperatures, 4 °C, 25 °C, 37 °C, 40 °C and 60 °C. After 24 h, the cell growth was monitored and survival percentage was calculated by the formula, Survival (%) = (N_1/ N_0) × 100, where, N_1 is the absorbance of cultures (OD at 610 nm) at 4 °C, 25 °C, 40 °C and 60 °C, N_0 is the absorbance of cultures at 37 °C (Al-Otaibi et al., 2016). .

Arginine hydrolysis

The ability of the isolated LAB to produce arginine decarboxylase enzyme was checked by inoculating 100 µL of overnight culture into 10 mL MRS broth without beef extract and ammonium chloride supplemented with 0.3 % L- arginine hydrochloride. After incubation of 24 h, the production of ammonia from arginine hydrolysis was indicated by the development of bright orange colour on addition of Nessler's reagent (K_2HgI_4). A negative control without arginine was used as control (Soltan Dallal et al., 2017).

Gelatinase activity

Nutrient gelatin medium was stab inoculated with a heavy inoculum of 18 – 24 h old culture of isolates and incubated for 3 – 4 days at 25 °C. After keeping the tubes 4 °C for 1 h, Afterwards, tubes were tilted to observe the liquefaction of the medium even after exposure to cold temperature. The production of gelatinase by the isolates is indicated by the liquefaction of the medium and its persistence after refrigeration (hydrolysis of gelatin) (Dela Cruz and Torres, 2012).

Antimicrobial activity

The selected isolates were examined for their antimicrobial activity against pathogenic microorganisms, *Bacillus* sp., *Micrococcus* sp., *Escherichia coli*, *Pseudomonas* sp., *Proteus* sp., *Klebsiella* sp., and *Staphylococcus sp.* by ditch plate method (Gaanappriya et al., 2013). Broth culture of each pathogen was swabbed over nutrient agar and 80 mm wide wells were cut on the agar plates, after the inoculum was absorbed. The culture supernatant of each of LAB isolates was filtered by passing through 0.22 µM membrane filter and 100 µL of the culture supernatant (pH adjusted to 7.00 by sterile 0.1 N NaOH) was pipetted into the wells and incubated in the upright position for 24 h at 37°C. Antibacterial activity of each of the isolates was checked against all the seven pathogenic organisms by measuring the diameter of the clear zone.

Antibiotic susceptibility test

Petri dishes containing 15 m1 of MRS agar, were overlaid with 6 ml of soft non nutrient agar (1.5 %) at 45°C, seeded with 200 µL of an overnight culture of each isolate and different antibiotic discs, erythromycin (15 µg), vancomycin (30 µg), ampicillin (10 µg) and ciprofloxacin (5 µg) were placed over agar with sterile forceps. After incubation at 37°C for 24 h, the diameter of the zone of inhibition was measured and the results were expressed as sensitive (S), Intermediate (I) and resistant (R) according to the National Committee for Clinical Laboratory Standards, NCCLS (NCCLS,1997).

Assimilation of cholesterol under simulated intestinal conditions

The survival and cholesterol-lowering property of selected isolates in simulated intestinal fluid were done as per the method by Tokatlı et al., (2015). Simulated intestinal fluid (NaCl, 0.85 % (w/v); bile salt, 3 g/L; pancreatin, 1 g/L; pH 8.0) was supplemented with cholesterol (100 µg/ml) and inoculated with overnight culture (1%, v/v) of selected isolates. The growth

and the amount of cholesterol remaining in the medium was measured. Survival percentage was calculated by:Survival (%) = (N_1/ N_0) × 100,where, N_1 is the absorbance of cultures (OD at 610 nm) in simulated intestinal fluid and N_0 is the absorbance of cultures in MRS broth.

Bile Salt Hydrolase (BSH) activity of selected strains

Overnight broth cultures of isolates (10 µL) were added in wells cut on MRS agar supplemented with 0. 5 % sodium taurodeoxycholic acid and 0.37 % calcium chloride and incubated at 37 °C for 72 h. The formation of deconjugated bile acid precipitation zones (opaque halos) around the wells indicated BSH activity (Hernández-Gómez et al., 2021).

Biocompatibility test

For determination of biocompatibility of the two selected isolates, MRS agar plates were prepared and one strain was streaked as straight line while test strain line was drawn perpendicular of it, then the plates were incubated for 24–48 h at 37° C. (Mukhammadiev et al., 2021).

Production of yoghurt

Production of yoghurt was done by inoculating the boiled and cooled buffalo milk with : a) selected probiotic strains alone b) commercial starter cultures alone c) probiotic strains and starter culture. The selected probiotic strains, S_1P_2 and S_1I_2, in different combinations were also tried. In each case, the cholesterol content, viability of probiotic strains and physico-chemical properties of yoghurt were analyzed. Seven experimental set ups were conducted for yoghurt production which varied in the nature of inoculum. They were: (a) S_1P_2 (b) S_1I_2 (c) S_1P_2 and S_1I_2 (d) a traditional yoghurt starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. (e) S_1P_2 and starter culture (f) S_1I_2 and starter culture and (g) S_1P_2, S_1I_2 and starter culture. All experimental set ups were inoculated with 2 % (v/v) overnight broth cultures and incubated at 42°C for 24 h.

Determination of physicochemical properties of yoghurt

Determination of pH and titratable acidity

pH was measured by a pH meter (Hanna, India) before and after fermentation. Total acidity expressed as percent of lactic acid, was determined by titrating with 0.1 N NaOH using phenolphthalein as indicator (Sadler and Murphy, 2010).

Determination of syneresis, moisture content and total soilds

2 mL of yogurt (for each of the studied formulations) was weighed and centrifuged at 5000 rpm for 5 min and the separated serum was then weighed. Syneresis is calculated using the following equation, syneresis (%) = $(W_s/W_y) \times 100$. where, W_s is the supernatant weight after centrifugation and W_y is the weight of the yogurt in tube (Mani-López et al., 2014). To determine moisture content, each yoghurt sample (5 g) was kept in an oven at 105 ℃ for 3 h. Reading was taken until a constant weight was obtained. The moisture content was expressed as the percentage (%) of the dry weight of sample (Association of Official Analytical Chemists method AOAC, 1995). The weight of the residue obtained from moisture content analysis was expressed as percentage total solids using the formula (Joseph et al., 2011),

$$\text{Total solids (\%)} = \frac{(\text{weight of the dish} + \text{dry yoghurt}) - (\text{weight of the dish})}{\text{Weight of the sample}} \times 100$$

Determination of cell viability

The viability of probiotic isolates in different types of yoghurt produced was checked by serially diluting different yoghurt formulations (up to 10^{13}) and plating on MRS agar. After incubation at at 37 ℃ for 48 h, the total number colonies were counted and compared.

Determination of cholesterol content in yoghurt

For the determination of cholesterol content in the yoghurt formed, 1 g of yoghurt was saponified with 5 ml of alcoholic KOH (30 %) solution and incubated in a water bath at 37-40 °C for 55 minutes. After cooling to room temperature, 10 ml. of petroleum ether was added, mixed well, followed by the addition of 5 ml water and shaken vigorously. Samples were centrifuged at 5000 rpm for 10 min to separate the layers and the petroleum ether layer alone was transferred to the 25 mL dry beaker and placed in a water bath at 60 °C to evaporate the solvent. After cooling, cholesterol content was determined colorimetrically by Zak's method (Adu et al., 2019).

11. Salient Research Achievements:
    a. New observations:

       The current research indicated that isolated lactic acid bacteria possess significant level of cholesterol reduction in culture medium. The isolates were able to survive in simulated intestinal condition, in the presence of pancreatin, indicating their ability

to colonize the intestinal habitat. Bile salt hydrolase activity was detected in two probiotic strains, suggesting that deconjugation of bile acids by bile salt hydrolase enzyme as the possible mechanism to reduce cholesterol. In addition to their cholesterol-lowering potential, the selected isolates possessed many properties needed for ideal probiotic such as tolerance to low pH, growth at different bile and NaCl concentration and varying incubation temperatures, antagonistic activity against food born pathogens and sensitivity to different antibiotics. Low-fat yoghurt could produce by the inoculation of milk with consortium of selected isolates with significant level of cholesterol reduction. The physico-chemical properties of yoghurt produced in all formulations, pH, titratable acidity, syneresis, moisture and total solid content were found within the acceptable limit. The isolates were also able to grow in fermented yoghurt as evidenced by their increase in viable count in all types of yoghurt fermentations after one month of storage. The two isolates, *Lactobacillus plantarum* strain LPYO175 and *Lactiplantibacillus plantarum* strain 3-1, can be used as ideal probiotics for the production of functional dairy products with low fat content.

b. Innovations/Technologies generated:

Lactic acid bacteria isolated from fermented foods were capable of reducing cholesterol content in the culture medium. Among them, two isolates, *Lactobacillus plantarum* strain LPYO175 and *Lactiplantibacillus plantarum* strain 3-1, possessed higher cholesterol-lowering potential (68.18 %) in culture medium, can be used as prospective candidates for the production for low-fat dairy products like yoghurt. Yoghurt with desirable physicochemical characteristics could produce by their individual and combined inoculation, with a higher cholesterol removal (78.01 %) in yoghurt resulted from the inoculation of consortium. The isolates were able to survive in simulated intestinal condition also. These isolates were found to possess different probiotic properties like tolerance to low pH, growth at different bile and NaCl concentration and varying incubation temperatures. In addition, they possessed antagonistic activity against different food born pathogens, *Bacillus* sp., *Micrococcus* sp., *Escherichia coli*, *Pseudomonas* sp., *Proteus* sp., *Klebsiella* sp., and *Staphylococcus sp.* They also exhibited varying level of sensitivity to common antibiotics. These results suggest that the isolated LAB, *Lactobacillus plantarum*

strain LPYO175 and *Lactiplantibacillus plantarum* strain 3-1, can be used for the production of natural low-fat foods as a potential alternative to the expensive chemical and physical processes of cholesterol reduction. Their probiotic properties can be considered as an added advantage which confers additional health benefits.

c. Application potential:

Milk and dairy products are rich sources of saturated fats which increase the cholesterol level in blood, thereby are regarded as unacceptable food item for people with cardiovascular diseases. A possible association between high dairy consumption and cardiovascular related diseases is often raised because of their cholesterol contents. It is important then to screen and regulate the cholesterol content of dairy products on the market. Cholesterol lowering ability of lactic acid bacteria is very well documented both in vitro and in vivo. In this context, the isolates in this study can also be introduced to reduce cholesterol level in foods such as dairy products. They can be used for the production of natural low-fat foods as a potential alternative to the expensive chemical and physical processes of cholesterol reduction. To date, only a few studies are available on the cholesterol-lowering activity of LAB in dairy products, mainly in cheese (Belviso et al., 2009). In this scenario, the ability of two isolates, *Lactobacillus plantarum* strain LPYO175 and *Lactiplantibacillus plantarum* strain 3-1, to reduce cholesterol in yoghurt up to 78 % becomes a promising approach for the production of functional food. The low-cholesterol yoghurt can be consumed by persons suffering from different health problems, like diabetics and cardiovascular disease. Probiotics can help to keep heart health by lowering LDL cholesterol and blood pressure. Moreover, the probiotic inclusions provide additional health benefits also. Probiotic based products do not produce alteration or negative impact on the food chain. The unique feature of probiotics is that they do not possess potential for infectivity or in situ toxin production, unlike other food or drug ingredients used to reduce the fat content. Other future prospects include *in vivo* studies to evaluate the effect of the probiotic strains on serum cholesterol level using animal models and to use them for the commercial production of functional foods.

d. Any other:

11. Details of publications (including paper/poster presentation in seminar/symposium)
12. Reference:

- Abdelazez, A. Abdelmotaal, H., Evivie, S.E., Melak, S., Jia, F., Khoso, M.H., Zhu, Z., Zhang, L., Sami, R., & Men, C. (2018). Screening potential probiotic characteristics of *Lactobacillus brevis* strains in vitro and intervention effect on Type I diabetes in vivo. BioMedical Research Int.1-20.
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- National Committee for Clinical Laboratory Standards. (1997). Approved Standard M2- T7. Methods for antimicrobial susceptibility testing of aerobic and facultatively anaerobic bacteria. National Committee for Clinical Laboratory Standards, Villanova, Pa.
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- Tamura, K. & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution, 10,512-526.
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Name of the Institution/ Person	Govt. Arts & Science College, Kozhinjampara Nattukal, Palakkad - 678554
Contact Address	Ph: 04923 - 272883
Mobile number of Co-ordinator *(mandatory)* Telephone No./ Fax No.	9495658052
E-mail ID of the DIR/ REG/ AO/ FO	principalgasck@gmail.com

Bank Account Details

Institution/ Individual Account Name (As per Bank record)	Smt. Amala A K (Principal in charge)
Account No. (SB/CC)	67011515002
IFS Code	SBIN0070185
Branch Name	SBI, Kozhinjampara
Branch Address	Kozhinjampara Panchayath
MICR No.	678002922

Certified that the Institute's /person's account is in NEFT /RTGS enabled branch. I hereby declare that the particulars given above are correct and complete.

Name, Address & Signature of the PI/ Coordinator

DR. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal.P.O,
Palakkad-678 554

Date:

Smt. Amala. A.K
Name, Address & Signature of the Competent Authority (DIR/ REG/ AO/ FO)

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Govt. Arts & Science College ★ Kozhinjampara, Palakkad-678 554 ★ Institution Seal

STATEMENT OF EXPENDITURE

Project Title: Isolation, Identification and Characterization of Amylase producing bacteria from Mangrove Environment of Kerala

File No./ Letter no. 00519/SPS 65/2021/KSCTET dtd: 29.10.2021

Name of Institution: Govt. Arts & Science College, Kozhinjampara Nattukal, Palakkad, Kerala - 678554.

Receipts	Amount (Rs.)	Payments	Amount (Rs.)
1. Amount sanctioned from KSCSTE (to be receivable)	10,000	1. Consumables	500.00
2. —		2. Minor equipment	4000.00
3. —		3. Travel	500.00
—		4. Analysis.	4500.00
		5. Documentation.	1570.00
Total	10,000	Total	11,070.00

Certified that I have exercised all kinds of checks to see that the grant has been utilized for the purpose for which it was sanctioned by KSCSTE.

Name & Signature of Principal Investigator

Dr. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal.P.O,
Palakkad-678 554

Name & Signature of Head of the Institution

Smt. Amala. A.K

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL P.O
PALAKKAD-678 554.

Seal & Signature of Chartered Accountant

CA AJITH SIVADAS
B.Com, ACA, ACMA, ACS, DISA
PROP. AJITH S & COMPANY (FRN: 018164S)
CHARTERED ACCOUNTANTS
ANUGRAHA, VANIYAR STREET
NALLEPILLY, PALAKKAD-678553
MEMBERSHIP No: 234833

Office Seal

Govt. Arts & Science College ★ Kozhinjampara, Palakkad-678 554 ★

UTILIZATION CERTIFICATE

Certified that out of Rs. ..10,000/-..... sanctioned by Kerala State Council for Science, Technology and Environment towards financial assistance for the student project titled 'Isolation, identification and characterization of amylase producing bacteria from mangrove environment of Kerala...' (File No./Letter No. 005(a)/SPS65/2021 KSCSTE), an amount of Rs..11070.....was utilized for the purpose for which it was sanctioned, leaving a balance of Rs. -1070 at the close of ...11070. as shown in the Statement of Expenditure annexed.

Smt. Amala. A.K
Asst. Professor

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL (PO)
PALAKKAD-678 554.

Name & Signature of Principal Investigator

Dr. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal P.O,
Palakkad-678 554

Name & Signature of Head of the Institution

Seal & Signature of Chartered Accountant

C.A. AJITH SIVADAS
B.Com., F.C.A., Adv. Diploma M.A. CIMA (UK)
PROP. AJITH S & COMPANY (FRN: 018164S)
CHARTERED ACCOUNTANTS
ANUGRAHA, VANIYAR STREET
NALLEPILLY, PALAKKAD-678553
MEMBERSHIP No. 201833

Office Seal

Govt. Arts & Science College
Kozhinjampara, Palakkad-678 554

KERALA STATE COUNCIL FOR SCIENCE TECHNOLOGY AND ENVIRONMENT

FORMAT OF STUDENT PROJECT REPORT (max. 15 pages)

1. Project Title: **Isolation, identification and characterization of amylase Producing bacteria from mangroove environment of Kerala**
2. File No: **00519/SPS 65/ 2021/ KSCSTE/ Dtd: 29.10.2021**
3. Name & Address of student: **Aiswarya R**
4. Name & Address of the Principal Investigator &

   Co- Investigator with mobile No. : **Dr. Robert Antony A, Assistant Professor, Department of Microbiology, Govt. Arts and Science college, Kozhinjampara, Palakkad, Kerala – 678554, Mobile: 8344147637.**

   Email ID: robertmicrobiology@gmail.com
5. Broad area of research: **Microbiology**
6. Specific area: **Identification of different microbial communities from mangroove environment of Kerala for Industrial application.**
7. Date of Start: **1st January 2022**
8. Total cost of Project: **Rs. 10,000/- (Rs. Ten Thousand Only)**
9. Approved objectives of the proposal:

   Mangrove ecosystems provide important ecological benefits and ecosystem services, including carbon storage and coastline stabilization, but they also suffer great anthropogenic pressures. Microorganisms associated with mangrove sediments and the rhizosphere play key roles in this ecosystem and make essential contributions to its productivity and other applications. Microorganisms from mangrove environments are a major source of antimicrobial agents and also produce a wide range of important medicinal compounds, including enzymes, antitumor agents, insecticides, vitamins, immunosuppressant's and immune modulators. However, the phylogenetic and functional description of microbial diversity in mangrove ecosystems has not been addressed to the same extent as for other environments. Here we will trying to explore the biodiversity of microbiome in mangrove ecosystem and finding the metabolic products and application of that products in various fields.

Dr. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal.P.O,
Palakkad-678 564

10. Methodology:

Sample Collection

The water, soil and root samples were collected from the mangrove forest in Venmenadu, Chavakkad, Thrissur District of Kerala State, India. The samples were collected in sample bottles from two different locations at a depth of 10 centimeter each. The samples were collected at 11.00 a.m during summer season. Then, the samples are stored aseptically to decrease the bacterial contamination from the air and soil surface. The sample bottles were given the location information and taken to the laboratory. In the laboratory, the samples were stored in the refrigerator with a temperature of 5-10°C until it is used (Triyanto *et al.*, 2008).

Isolation of Microorganisms from Mangrove Samples

The isolation was performed using spread plate method (Dubey and Maheshwari, 2005). The soil samples were weighed as much as 10 grams and then suspended in 90 ml of sterile distilled water and homogenized using a shaker. 10 ml of water and root (crushed) sample was taken and put into 9 ml of sterile distilled water in the test tube. Next, 1 ml of the suspension was taken and put into 9 ml of sterile distilled water in the test tube so that dilution factor of 10^{-1} was obtained. After that, a dilution of 10^{-2} to 10^{-8} was made. Then, 0.1 ml of each dilution of 10^{-6}, 10^{-7} and 10^{-8} was spreaded using a L-rod into an aseptic petri dish that contain solidified nutrient agar media and incubated at 37°C for 24 hours until colonies were grown on the media. For the isolation of fungi, 0.1 ml of the suspension from 10^{-3}, 10^{-4} dilution was spreaded on sabourad's dextrose agar media (Marista E *et al.*, 2013).

Enzyme Analysis

Amylase

The screening of amylase producing bacteria was done by using a starch agar medium. Bacterial isolates were spotted on top of the agar medium and incubated at 37 °C for 48 hours to observe hydrolysis rates. After incubation, Lugol's iodine solution was poured into agar medium and kept undisturbed for 5 minutes, until clear zones were formed around bacterial colonies. The blue color indicated negative hydrolysis of amylases while clear zones indicated positive hydrolysis (Ahmed *et al.*, 2017).

Protease

The screening of proteolytic bacteria was conducted by using casein agar medium. Bacterial isolates were spotted on top of the agar medium and incubated at 37 °C for 48

hours to observe hydrolysis rates. Clear zones around bacterial colonies indicated proteolysis positive results (Mamangkey *et al*., 2019).

Cellulase

The screening of cellulolytic bacteria was performed by using Carboxy-methyl cellulose medium. Bacterial isolates were spotted on top of the agar medium and incubated at 37°C for 48 hours to allow maximum growth. After incubation, the Gram's iodine was poured into the agar medium and settled for 10 min. The agar medium was that contain clear zones formed around colonies, indicated cellulolytic positive results (Singh *et al*., 2014).

Gelatinase

The screening of gelatinase producing bacteria was performed by using gelatine medium. Bacterial isolates were spotted on top of the agar medium and incubated at 37°C for 48 hours to allow maximum growth. Clear zones around bacterial colonies indicated a gelatinase positive result (Saima *et al*., 2013).

Phosphatase

The screening of phosphatase producing bacteria was performed by using Pikovskayas agar medium. Bacterial isolates were spotted on top of the agar medium and incubated at 37°C for 48 hours to allow maximum growth. Clear zones around bacterial colonies indicated a gelatinase positive result (Pikovskaya 1948).

Enzyme assay to determine enzyme activity

Amylase

The Dinitro Salicylic acid (DNS) method was used to determine the amylase activity of each bacterial isolate. The substrate solution was made by dissolving 1% starch solution in citrate buffer. To each test tube, 1 ml substrate solution was added, followed by 1 ml citrate buffer (pH 5) and 1 ml crude enzyme. The test tubes were incubated at 50°C for 30 minutes in a water bath. 3 ml of DNS solution was added to each test tube to stop the reaction and left undisturbed at room temperature for 10 minutes. The test tubes were then incubated in boiling water for 10 minutes in the water bath and then cooled down to room temperature. The colour intensity of the solution was observed by measuring the optical density using a spectrophotometer at 540 nm. The reading was compared to a prepared blank solution. (Ghose 1987).

Cellulase

Cellulase activity was measured following the method of Miller (Miller 1959). A reaction mixture composed of 0.2 mL of crude enzyme solution plus 1.8 mL of 0.5%

carboxymethyl cellulose (CMC) in 50 mM sodium phosphate buffer with a pH of 7 was incubated at 50°C in a water bath for 30 min. The reaction was terminated by adding 3 mL of DNS reagent. The colour was then developed by incubating the mixture in boiling water for 5 min. Optical density of samples was measured at 575 nm against a blank containing all the reagents minus the crude enzyme. The concentration of glucose produced for each solution was obtained from a glucose standard curve.

Protease

0.5 mL of crude enzyme was mixed with 1 mL of 1% casein solution in 1 mL 0.05 M potassium phosphate buffer pH 7.5. The samples were incubated at 37°C for 30 min. After incubation, 3 mL of 110 mM trichloroacetic acid (TCA) was added to each sample to stop the reaction and then centrifuged at 10,000 rpm for 15 min. The clear supernatant (1 mL) was mixed with 2 mL of 0.5 M sodium carbonate solution and 0.5 mL Folin's reagent. The mixture was recorded at 660 nm using a spectrophotometer (Folin *et al.*, 1927). Protein was measured by using of Lowry method (Lowry *et al.*, 1951) with bovine serum albumin (BSA) as standard. The absorbance at 660 nm was recorded after the reaction and compared with the standard curve plotted against the standard protein (BSA) according to the standard curve.

Identification of Potential Isolate

Biochemical Test

The selected bacterial isolate was identified presumptively through morphological, phenotypical and biochemical tests including catalase test, oxidase test, MRVP test, citrate utilization test, starch hydrolysis test, gelatine hydrolysis test, nitrate reduction test, fermentation of sugar: maltose, sucrose, and fructose (Holt 1994).

Molecular Identification

Isolation of bacterial genome

Colonies from a single streak on the agar plate were scraped and suspended in PBS and centrifuged. The pellet obtained was dispersed. 600 µl of cell lyses buffer such as SDS, Tris-EDTA was added and mixed by inverting the vial for 5 min and incubated for 10 min with gentle mixing till the suspension looked almost transparent. 600 µl of isopropanol was layered on top of this solution. The two layers were mixed gently till white strands of DNA were seen and until the solution is homogenous. The strands of DNA were spooled with the help of a pipette tip and transferred into a new vial. 500 µl of 70% ethanol was added to the spooled DNA. The spooled DNA was spun to precipitate DNA at 10,000 rpm for 10 min, the supernatant was discarded. The pellet

was air-dried. 50 µl of 1X TE was added and the pellet was suspended (incubated for 5 min at 55–60°C to increase the solubility of genomic DNA). Electrophoresis was carried out in 0.8 % agarose gel, stained with ethidium bromide, and DNA was visualized under ultraviolet (UV) light (Ausubel *et al.*, 1987)

16S rRNA gene sequencing and phylogenetic analysis

Amplification of the 16S rRNA gene was performed using the universal primers sets of 16S rRNA forward primer 27F 5′-GAGAGTTTGATYCTGGCTCAG-3′ and reverse primer 1492R 5'AAGGAGGTGATCCARCCGCA -3′. The final products were analyzed through electrophoresis on 1% agarose gel and stained with 0.5μg mL−1 ethidium bromide (Weisburg et al., 1991).

Purified PCR products were sequenced, and sequence search similarities were conducted using basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast). The Phylogenetic tree was constructed using Muscle method for sequence alignment. All 16S rRNA partial sequences of the amylase-producing isolates were deposited on NCBI database (Altschul *et al.*, 1990)

Determination of the Optimal Conditions for Amylase Production

The optimum temperature and pH of the crude enzyme were determined using the DNS method as described previously. The reading was taken after the incubation at 24, 48 and 72 hours.

Optimal Temperature

The optimum temperature was determined by incubating the enzyme at different temperatures starting from 35°C, 40°C and 45°C at a pH of 7 (Das *et al.*, 2004)

Optimum pH

The optimum pH was determined by incubating the enzyme at a pH of 6, 7, 8 and 9. The pH was adjusted by using HCL and NaOH (Ramesh 1989).

Optimum Substrate

The optimum amount of starch for the growth of bacteria was determined by incubating enzyme at different substrate concentration. The concentration of the substrate was made upto 1 to 4 percentage. One millilitre of enzyme is added and the suspension was incubated at 37 °C The reading was taken at 24, 48, 72 hours (Deb P *et al.*, 2013).

Purification of Enzyme

Purification of amylase enzyme was achieved by ammonium sulphate precipitation. 100 ml of cell-free extract was saturated with centrifuged at 1000 rpm for 15 min. The supernatant was collected and saturated up to 0-5 % with ammonium sulphate. Then,

the content was centrifuged at 7000 rpm for 15 min and the pellet was collected for further analysis (Asad *et al.*, 2011).

Fourier Transform Infrared (FT–IR) Analysis of Purified Enzyme Sample

Culture supernatant was dissolved in ethanol and the FT-IR spectra were recorded on a Perkin Elmer, Spectrum MRX-1 model. The spectra were scanned in the range of 400 to 4000cm.

Gas Chromatography–Mass Spectrophotometry Analysis

Purified extracts (1mg) and amino acid standards (1µg) were dissolved in 0.5ml of 2.5N HCl in 1-propanol and heated at 100°C for 1hr. Excess reagents were removed in a stream of dry nitrogen. Dichloromethane (200ml) and 50µl penta fluoropropionic anhydride were added and the mixtures heated at 100°C for 20 min. Reagents were then removed in a stream of nitrogen and the residues dissolved in 200µl dichloromethane. Samples were injected split less onto a 25×25mm fused silica column on a Perkin-Elmer Tr10 1000 Mass spectrometer (Perkin-Elmer, Wellesley, MA) with a scan range of 45–650nm. The carrier gas was nitrogen at 1ml min^{-1} and the chromatograph was programmed from an initial temperature of 80 to 200°C at 10°C min^{-1}

11. **Salient Research Achievements:** New observations: The present study focuses on the isolation of amylase producing bacteria from mangrove ecosystem located at Chavakkad, Thrissur, Kerala Mangrove Soil, water and root samples were serially diluted and plated to enumerate their microbial load. Maximum amount of growth was seen in water sample (TNTC) and least number in root sample ($2*10^6$ CFU/ml). A total of 12 isolates were isolated from the Mangrove Soil, water and root samples. Primary screening of these 12 isolates by inoculating these in starch agar medium and iodine solution used as indicator of starch hydrolysis a total of 5 bacterial strains which produced clear zone in the starch agar medium were isolated and purified. Among the 5 bacterial strains, MR A1 strain showed maximum activity on assay test done using DNS method (2.79 µg/ ml) and was selected as best amylase producer and identified as strain of *Bacillus flexus* (*Priestia flexa* strain GASCKMS0) based on the, morphological, biochemical, and molecular sudies. The enzymatic assay result reveals that maximum enzyme production was shown after 48 hours of incubation which declined after 72 hours of incubation. The optimum temperature and pH for the activity of the amylase obtained from this strain were 37°C and 7.0, respectively.

    a. Among various carbon sources 4% starch gave maximum production of amylase and starch act as an inducer for amylase production. Purification of enzyme was

done using ammonium sulphate precipitation method and maximum precipitation was seen at 20 % saturation of ammonium sulphate. Further confirmation of the enzyme was done by GC MS analysis and FT-IR methods. Amylase activity was tested by taking corn starch and potato starch as substrates. The result of corn and potato starch degradation revealed that degradation of corn starch was faster when compared of that potato starch which indicates that the maximum activity of amylase produced by the isolate was in corn starch compared to potato starch. This study has shown that these Gram positive, rod shaped, bacteria are able to synthesize amylase that is evidenced in the hydrolysis of starch which is very important in biotechnology.

b. **Innovations/Technologies generated:** Varieties of microorganisms isolated from the mangroove environment. Due to short period of project time only bacterial isolates were analysed for the production of industrial applicable enzymes. A lot of actinomycetes and fungal isolates were stored for further studies.

c. **Application potential:** Applications Trials for the isolated amylase was done using corn starch and potato starch. Amylase activity in both the starch substrate showed similar result but it was higher in corn starch when compared to potato starch. Maximum amylase activity was seen in corn-starch at 72 hours of incubation. This is supported by the study of Fean D. Sarian *et al* (2012), who reported that potato starch granules were degraded slowly compared to wheat and tapioca and corn starch granules. Previous study report of Monika Klisowska *et al* (2003) has also revealed that Corn starch was the most susceptible and potato starch the least after 72 hours of incubation. In addition, potato starch and other tuber starches have a typical B-crystalline structure, and contain more water, thicker and larger growth rings (crystalline-amorphous portion) and longer average amylopectin branch chain length than other types (Buttrose 1963). It has been reported that this crystalline form is more resistant to enzymatic hydrolysis than the A-type (Gérard *et al*. 2001)

d. Any other: **NIL**

12. Details of publications (paper/poster presentation in seminar/symposium): **NIL**

13. Reference:

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Dr. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Arts & Science College
Nattukal.P.O,
678 554

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- Taniguchi H, F Odashima, M Igarashi, Y Maruyama and M Nakamura. 1982. Characterization of a potato starch-digesting bacterium and its production of amylase. Agric Biol Chem 46: 210 – 2115.
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- Widmer, F; Seidler, R; Gillevet, PM; Watrud, LS; Di Giovanni, GD. A highly selective PCR protocol for detecting 16S rRNA genes of the genus *Pseudomonas* in environmental samples. *Applied and Environmental Microbiology*., 1998, 64(7), 2545-53.

KERALA STATE COUNCIL FOR SCIENCE TECHNOLOGY AND ENVIRONMENT

FORMAT OF STUDENT PROJECT REPORT (max. 15 pages)

1. Project Title: Synthesis of silver nanoparticles using yeast flavanoids and their antibacterial effects on common food pathogens

2. File No.: **Letter No: No.00510/SPS 64/2019/KSCSTE**

3. Name & Address of student: **Princy P.K, 2nd year M.Sc Microbiology,**
   Department of Microbiology, Govt. Arts and Science College, Kozhinjampara, Nattukal, Palakkad – 678554.

4. Name & Address of the Principal Investigator &
   Co- Investigator with mobile No. : **Dr. Robert Antony. A, Assistant Professor**
   Department of Microbiology, Govt. Arts and Science College, Kozhinjampara, Nattukal, Palakkad – 678554.
   Phone: 8344147637, Email ID: robertmicrobiology@gmail.com

5. Broad area of research: Microbiology

6. Specific area: Antibacterial activity by using nanotechnology

7. Date of Start: 1st February 2020

8. Total cost of Project: Rs. 10000/- (Rupees Ten thousand only)

9. Approved objectives of the proposal:
   - ❖ To extract the flavanoids from the yeast by using different solvents.
   - ❖ Conform the flavanoids by different analytical methods like NaOH test, ammonia test, TLC analysis, spectrophotometric, HPLC and GSMS analysis.
   - ❖ Conform the antibacterial effects of the flavanoids on common food pathogens.
   - ❖ Chemically synthesis the silver nanoparticles using yeast flavanoids.
   - ❖ Characterize the sysnthesized nanparticles by using UV spectrum, FTIR, XRD analysis.
   - ❖ Once again analysis the antibacterial effects of the silver nanparticles on common food pathogens

10. Methodology: (500 words)

Collection of Food Pathogens

Well studied and identified bacterial foodare pathogens were collected from rhizosphere biology research laboratory, department of microbiology, Bharathidasan University, Tiruchirappalli, Tamilnadu. These isolates were kept in our laboratory for further investigation and usage. Morphological and some biochemical test were conducted on these isolates for the confirmation.

Collection of Yeast Sample and Cultivation

One yeast isolate strain was collected from nearby bakery at Kozhinjampara, Palakkad. The yeast granules were soaked in 10% sugar solution for 2 hours. Then serially diluted the solution. Then 1ml sample was plated on to nutrient agar media. Incubate the plates for 24 hours at 37°C.Yeast isolates are re-cultivated on YMEPG medium (Yeast Extract Malt Extract Peptone Glucose) medium. The medium was autoclaved at 121°C for 20 minutes, cooled to approximately 45°C and adjusted to pH 3.7 (Wickerham, 1951).

Production Media

YMEPG medium the amount of glucose here is 100 g/L, this media prepare in two-step the restis preparing all media content except glucose to dissolved in half a liter of dist. Water and then autoclaved at 121°C for 20 minutes. The second is to dissolve glucose in the other half liter of dist. Water then autoclaved at 115°C for 60 minutes. A loop full of yeast inoculum was taken from a pure culture of the yeast isolate grown on slants and inoculated into 50 ml of sterilized propagation media then incubated for 48 hours at 27oC on a shaker with 140 rpm. Take 15 ml of previous yeast propagation media and transfer it into 150 ml production media then incubated for 72 hours at 28 ± 22oC on a shaker. Centrifuge each broth cultures for 15 min at 5000rpm, the cell mass drying on air and weight (Wickerham, 1951).

Extraction of Flavonoids from Yeast

Centrifuge yeast culture for obtaining their biomass and homogenize with 40 ml different solvents like chloroform, ethanol, butanol, diethyl ether or benzene at 16000rpm for 10 minutes. Mixture was filtered through Whatman No.1 filter paper. Different extracts obtained after filtration were stored separately for further investigation.

Primary Screening of Flavonoids

Sodium Hydroxide Test

The extract was treated with a few drops ofNaOH. Formation of intense yellow color, which becomes color less on addition of few drops of dilute hydrochloric acid, indicates the presence of coumarin and flavonoids (Vimalkumar*et al.*,2014).

TLC Analysis

Secondary screening of flavonoids by Thin Layer Chromatographic analysis (TLC) and using rutin and quercetin as slandered material. All reagents and chemicals were purchased from Sigma-Aldrich. All solvents were HPLC grade and were used as such.The solvents used were freshly distilled before use. Analytical by TLC was carried out on alumina sheets pre-coated with silica gel, Merk, Kiesel gel 60 F254 60 F254 (5×2cm×0.2mm) solvent system (8:2) and (7:3) Dichloromethane: Methanol spraying with H_2SO_4.

Spectrophotometric Analysis

Total flavonoids in yeast methanolic extracts was determined with NaOH reagent using qurcetetin and rutin as a standard the absorbance was measured at 362nm versus blank sample on a spectrophotometer and expressed in terms of equivalent (µg/g DW extract). Yeast extracts were dissolved in a known volume of methanol leave for 10 min.Absorbance (AU) reading was made in Triplicate (Mabry *et al.*,1970, Lombard *et al.*, 2002, Perez-Gregorio*et al.*, 2010).

FT-IR analysis

The dried samples were scrapped out from the petriplates and the fine powder obtained was analyzed in FT-IR spectrophotometer (Bruker Vertex 70) for obtaining the functional groups of these extracts.

GC/MS Analysis

Chemical profile of the flavanoids found in yeast methanolic extract of the highest flavonoids and antibacterial bioactive was estimated by GC/MS analysis.

Antimicrobial Activity Test by using Yeast Flavanoids

Agar Well Plate Method

Antimicrobial activity of the yeast flavonoid was detected using agar well plate method against common pathogens - *Escherichia coli, Pseudomonas, Proteus* and

Staphylococcus aureus. The microorganisms were grown on nutrient broth at 37°C for 24h. The concentrations of microbial inoculums were adjusted to $2.5x10^5$ colony-forming units (CFU) per mL using spectrophotometer at 600 nm. The bacterial suspension was inoculated into entire surface of Muller Hinton Agar (MHA) plates uniformly using a sterile spreader. Three wells were created on the surface of each inoculated solidified MHA plates. 50 microliter of yeast flavonoid were poured on the 1st well of each bacterial strain. 2ndwell was poured with the same quantity of original solvent as positive control and 3rd well with sterile distilled water . The plates were incubated overnight at 37°C and zone of inhibition was measured.

CFU Calculation Method

10 ml of nutrient broth inoculated with food pathogens and different concentration of extracted flavanoid fractions. Here 0.1, 0.2, 0.3…..1ml extractions were added to 10 test tubes containing the pathogens. After 24 hrs intervals of incubation OD was calculated for the each organism at 600nm. CFU also calculated by plating 0.1ml mixed solution from the each test tube. After making spread plate on nutrient agar plates, all the plates were incubated of 24hrs at 37 degree.

Synthesis of Silver Nano Particle by using Yeast Cultures

500ml YMEPG media was prepared, and 5 loopful yeast culture were added and 0.2% silver nitrate ($AgNO_3$) was added. Then incubated on shaker for overnight. After incubation tolerance capacity of the yeast cells were determined by using CFU calculation. After incubation 2% silver nitrate were added and kept under dark for 2 days at room temperature. After incubation the solutions were extracted with different solvent to obtain the AgNPs solutions of yeast.

Preparation of AgNPs using Flavonoid Solutions

1 mM solutions of $AgNO_3$ and 0.02% flavonoid will be used as stock solutions for the preparation of AgNPs. The flavonoids were dissolved in water by slightly increasing the pH using NaOH as they are sparingly soluble in water. After complete dissolution, the pH was neutralized using HCl. 10 mL of individual aqueous flavonoid solution was mixed with 90 mL of 1 mM $AgNO_3$ solution separately. The reaction mixtures were immediately placed in bright sunlight and observed for change in color of the solutions.

Characterization of Synthesized Silver Nanoparticles

The synthesis of AgNPs was monitored using a UV –Visible spectrophotometer in the visible range. For FTIR analysis , the AgNPs solution was placed in glass petriplates and dried at 60 °C in oven. The dried samples were scrapped out from the petriplates and the fine powder obtained was analyzed in FTIR spectrophotometer (Bruker Vertex 70).

Antimicrobial Activity Test by using AgNPs

Agar Well Plate Method

Antimicrobial activity of the synthesized AgNPs was detected using agar well plate method against common pathogens - *Escherichia coli, Pseudomonas, Proteus* and *Staphylococcus aureus*. The microorganisms were grown on nutrient broth at 37° C for 24h. The concentrations of microbial inoculums were adjusted to $2.5x10^5$ colony-forming units (CFU) per mL using spectrophotometer at 600 nm. The bacterial suspension was inoculated into entire surface of Muller Hinton Agar (MHA) plates uniformly using a sterile spreader. Fourwells were created on the surface of each inoculated solidified MHA plates. 50 microliter of synthesized nanoparticles were poured on the 1^{st}well of each bacterial strain. 2^{nd} and 3^{rd}wells were poured with the same quantity of original flavanoids as negative control and $AgNO_3$ as positive control, respectively. Fourth well with sterile distilled water. The plates were incubated overnight at 37 °C and zone of inhibition was measured.

CFU Calculation Method

10 ml of nutrient broth inoculated with food pathogens and different concentration of flavanoid synthesized $AgNO_3$. Here 0.1, 0.2, 0.3…..1ml silver nanoparticle solutions were added to 10 test tubes containing the pathogens. After 24 hrs intervals of incubation OD was calculated for the each organism at 600nm. CFU also calculated by plating 0.1ml mixed solution from the each test tube. After making spread plate on nutrient agar plates, all the plates were incubated of 24hrs at 37 degree.

Determination of MIC for AgNPs Synthesized by using Flavanoids

The antimicrobial activities of flavanoid synthesized $AgNO_3$and silver nitrate (for comparison) were evaluated, antimicrobial activity against *Staphylococcus*

*aureus*performed by serial dilution method according to with some modification in order to determine the minimuminhibitory concentration (MIC) in the culture broth. In the current research, a serial dilution of silver nanoparticles and silver nitrate started from 10 to 150µg ml^{-1} of prepared with each of them (AgNPs and $AgNO_3$) used for determination of the minimum inhibitory concentration (MIC) values. MIC values were taken as the lowest concentration required suppressing the growth of the bacteria in the test tube after incubation (showed no turbidity). 100 µl of the bacterial cells adjusted to ($1x10^6$ ml^{-1}) were added to Mueller–Hinton (MH) medium broth, then 10 µl from each of the serially diluted solutions of the compounds (AgNPs and AgNo3) were added to the bacterial cells. The MIC was defined as the lowest concentration required for inhibiting visible growth of bacteria in the test tube after incubation (showed no turbidity).

11. Salient Research Achievements:
    a. New observations:

       The current research indicated that flavanoid derived AgNPs had noticeable effectiveness compared with native flavanoid against some bacterial isolates tested, the future research may study biochemical or genetics changes occurs in the bacterial cells after treating with flavanoid derived AgNPs, study the effect of Ag NPs with different sizes particularly smaller than 20 nm, study the effect of Ag NPs with different shapes, investigate antibacterial activity in the combination of nano silver and free ions and examine some other pathogenic bacteria species.

    b. Innovations/Technologies generated:

       Flavanoids were extracted from the yeast used to control the food pathogens through nanotechnology mechanism. Butanol extracted flavanoids shows higher activity against the pathogens particularly to *Pseudomonas*. Then the extracted flavanoids confirmed by analysis methods like NaOH test, ammonia test, spectroscopic, FTIR and GCMS analysis. Then these flavanoids used for analysis of antibacterial activity against food pathogens by agar well diffusion and CFU calculation method. After this $AgNO_3$ based nanoparticles were synthesized using flavanoid extractions and whole yeast culture as such. Finally the flavanoid based synthesized nanoparticles were analysed using spectroscopic and FTIR analysis. Then antibacterial activity analysed against the food pathogens. This shows that

the flavanoid based nanoparticles show more good result when compared to crude extract and finally MIC for the AgNO3 was calculated.

c. Application potential:

More researches and studies required related to effect of flavanoid derived AgNPs food pathogensbecause these exhibited extremely highly resistance compared with other bacteria. From the results obtained it is suggested that yeast flavanoid derived silver-nanoparticles could be used effectively against some multidrug resistant and hazardous bacteria also could be used in food preservation particularly in the industries, homeetc. In addition to that, silver nano particles, may be utilized in food fields but certainly in the limitations related to human health and toxicity of nano silver side effects, studies of the American Biotech Labs have concluded that nanosilver products are not toxic to cells, animals or humans with low concentration of nanosilver solutions there is no toxic effects on human's tissues were reported.

d. Any other: No

12. Details of publications (including paper/poster presentation in seminar/symposium).

Presented a paper on this project topic sysnthesis of silver nanoparticles using yeast flavanoids and their antibacterial activity against some common food pathogens at Department of Bitechnology, Karpagam Univerisity, Coimbatore, Tamilnadu on 4th and 5th March 2020.

13. Reference:

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UTILIZATION CERTIFICATE

Certified that out of Rs. 10,000/- sanctioned by Kerala State Council for Science, Technology and Environment towards financial assistance for the student project titled Synthesis of silver nanoparticles using yeast flavanoids and their antibacterial effects against some common food pathogens (File No./Letter No. 005 10/SPS/64/2019 KSCSTE) an amount of Rs. 10,197 was utilized for the purpose for which it was sanctioned, leaving a balance of Rs. -197 at the close of 10,197 as shown in the Statement of Expenditure annexed.

Name & Signature
of Principal Investigator

Dr. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal.P O,
Palakkad-678 554

Name & Signature
of Head of the Institution

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Seal & Signature
of Chartered Accountant

CA AJITH. S B.Com, ACA, ACMA,
MBA(Digital), M.A. CIMA (UK)
PRACTICING CHARTERED ACCOUNTANT
ANUGRAHA, VANIYAR STREET
NALLEPILLY, PALAKKAD- 678553
MEM No: 234833

Office Seal

Govt. Arts & Science College
Kozhinjampara, Palakkad-678 554

ELECTRONIC CLEARING SERVICE FACILITY FOR RECEIVING PAYMENTS

Details of Account Holder

Name of the Institution/ Person	Govt. Arts & Science College, Kozhinjampara
Contact Address	Nattukal, Palakkad - 678554 Ph: 04923-272883
Mobile number of Co-ordinator *(mandatory)* Telephone No./ Fax No.	9495658052.
E-mail ID of the DIR/ REG/ AO/ FO	principalgasck@gmail.com

Bank Account Details

Institution/ Individual Account Name (As per Bank record)	Smt. Amala (Principal in charge)
Account No. (SB/CC)	67011515009
IFS Code	SBIN0070185
Branch Name	SBI, Kozhinjampara.
Branch Address	Kozhinjampara, Panchayath
MICR No.	678002922.

Certified that the Institute's /person's account is in NEFT /RTGS enabled branch. I hereby declare that the particulars given above are correct and complete.

Name, Address & Signature of the
PI/ Coordinator

Dr. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal (PO),
Palakkad-678 554

Date: 22/09/2020

Name, Address & Signature of the
Competent Authority (DIR/ REG/ AO/ FO

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PALAKKAD-678 554.

Institution Seal

Govt. Arts & Science College * Kozhinjampara, Palakkad-678 554 *

STATEMENT OF EXPENDITURE

Project Title: Synthesis of Silvernanoparticles using yeast flavanoids and their antibacterial effects against some common food Pathogens

File No./ Letter no. ..005 10/SPS 64/2019/KSCSTE.....

Name of Institution: Govt. Arts & Science College, Kozhinjampara, Nattukal, Palakkad, Kerala - 678554.

Receipts	Amount (Rs.)	Payments	Amount (Rs.)
1. Amount sanctioned from KSCSTE (to be receivable)	10,000	1. Consumables	4005.00
		2. Minor equipment	2502.00
2.		3. Travel	500.00
3.		4. Analysis	1500.00
		5. Documentation	1690.00
Total	10,000	Total	10,197.00

Certified that I have exercised all kinds of checks to see that the grant has been utilized for the purpose for which it was sanctioned by KSCSTE.

Name & Signature of Principal Investigator

Dr. ROBERT ANTONY.A
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal.P.O,
Palakkad-678 554

22/9/2020

Name & Signature of Head of the Institution

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Office Seal

Seal & Signature of Chartered Accountant

CA AJITH. S B.Com, ACA, ACMA, Adv Diploma (M.A) CIMA (UK)
PRACTICING CHARTERED ACCOUNTANT
ANUGRAHA, VANIYAR STREET
NALLEPILLY, PALAKKAD- 678553
MEM No: 234833

Kerala State Council for Science, Technology and Environment

Prof (Dr.) K.P. Sudheer
Executive Vice President
KSCSTE, Pattom

16.01.2020

Letter No. 00574 /SPS 64/2019/KSCSTE

Dear Mrs.Saranyadevi K

Sub:-Financial assistance for Student Project scheme of KSCSTE reg.
Ref:-Your application received under Student Project scheme

This is to invite your attention to the reference cited and to inform that the project proposal titled **"STUDY ON THE ANTAGONISTIC ACTIVITY OF NEEM ENDOPHYTIC BACTERIA AGAINST RICE SHEATH BLIGHT CAUSING FUNGI RHIZOCTONIA SOLANI"** submitted by Mrs. Saranyadevi K as PI and Anjana M as student investigator(s) has been approved. An amount of **₹10000/-** is sanctioned by the Council. The budget estimate of the project is as detailed below.

SL.NO.	ITEMS	AMOUNT(₹)
1	Consumables	2000
2	Minor equipments	3000
3	Travel	500
4	Research Literature & Documentation	1000
5	Others (for analysis)	3500
	Total	**10000**

The PI has to submit the signed Terms and Conditions (as per the guidelines) and the date of start of the project within two weeks to the undersigned. The project should be completed within six months and submit the certified soft copy of the final report (in pdf to sed.kscste@kerala.gov.in), audited Statement of Expenditure and Utilization Certificate counter signed by the Head of the Institution for releasing the grant. **The format for final report, SE and UC can be downloaded from www.kscste.kerala.gov.in .**

Thanking you,

Yours sincerely,

Prof (Dr.) K.P. Sudheer

To

Mrs. Saranyadevi K, Assistant Professor, Dept. of Microbiology, Government Arts And Science College Kozhinjampara, Nattukal, Palakkad, Kerala - 678554

Copy to:

The Principal, Government Arts And Science College Kozhinjampara, Nattukal, Palakkad, Kerala - 678554

Anjana M, student(s), Master of Science (MSc), Microbiology, Government Arts And Science College Kozhinjampara, Nattukal, Palakkad, Kerala - 678554

UTILIZATION CERTIFICATE

Certified that out of Rs. ...10,000..... sanctioned by Kerala State Council for Science, Technology and Environment towards financial assistance for the student project titled 'Study on the antagonistic activity of neem endophytic bacteria against mice - Rhizobium lolani' (File No./Letter No...005744/SPS64/2019/KSCSTE, an amount of Rs....16622....was utilized for the purpose for which it was sanctioned, leaving a balance of Rs. -6622.. at the close of ..16,622.... as shown in the Statement of Expenditure annexed.

Name & Signature of Principal Investigator	Name & Signature of Head of the Institution	Seal & Signature of Chartered Accountant
Ms. SARANYA DEVI. K Assistant Professor Department of Microbiology Govt. Arts & Science College Kozhinjampara, Nattukal (P.O) Palakkad - 678 554	29/5/2020 PRINCIPAL GOVT. ARTS & SCIENCE COLLEGE KOZHINJAMPARA, NATTUKAL(PO) PALAKKAD-678 554.	CA AJITH. S B.Com, ACA, ACMA, Adv.Diploma (M.A) CIMA (UK) PRACTICING CHARTERED ACCOUNTANT ANUGRAHA, VANIYAR STREET NALLEPILLY, PALAKKAD- 678553 MEM No: 234833

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Details of Account Holder

Name of the Institution/ Person	Government Arts and Science college, Koyhinjampara
Contact Address	Nattukal (Po), Palakkad, - 678554
Mobile number of Co-ordinator *(mandatory)* Telephone No./ Fax No.	9943131010
E-mail ID of the DIR/ REG/ AO/ FO	Principalgasck@gmail.com

Bank Account Details

Institution/ Individual Account Name (As per Bank record)	The Principal (Amala)
Account No. (SB/CC)	67011515002
IFS Code	SBIN0070185
Branch Name	Koyhinjampara
Branch Address	Koyhinjampara (Po), Palakkad
MICR No.	678002922

Certified that the Institute's /person's account is in NEFT /RTGS enabled branch. I hereby declare that the particulars given above are correct and complete.

Name, Address & Signature of the
PI/ Coordinator

Ms. SARANYA DEVI. K
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal (P.O)
Palakkad

Date: 22/9/2020

Name, Address & Signature of the
Competent Authority (DIR/ REG/ AO/ FO

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PALAKKAD-678 554.

Institution Seal

Govt. Arts & Science College
Kozhinjampara, Palakkad-678 554

STATEMENT OF EXPENDITURE

Project Title: 'Study on the antagonistic activity of Neem endophytic bacteria against rice Sheath blight causing fungi Rhizoctonia Solani'

File No./ Letter no. 005744/SPS.64./2019/KSCSTE

Name of Institution: Government Arts and Science College Kozhinjampara, Palakkad

Receipts	Amount (Rs.)	Payments	Amount (Rs.)
1. Amount sanctioned from KSCSTE (to be receivable)	10,000/-	1. Consumables 2. Minor equipment	2005 3094
2.		3. Travel	613
3.		4. Research literature & documention	1050
		5. others (analysis)	9860
Total	10,000/-	Total	16622/-

Certified that I have exercised all kinds of checks to see that the grant has been utilized for the purpose for which it was sanctioned by KSCSTE.

Name & Signature of Principal Investigator

Ms. SARANYA DEVI. K
Assistant Professor
Department of Microbiology
Govt. Arts & Science College
Kozhinjampara, Nattukal (P.O)
Palakkad

Name & Signature of Head of the Institution

PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Seal & Signature of Chartered Accountant

CA AJITH. S B Com, ACA, ACMA,
Adv.Diploma (M.A) CIMA (UK)
PRACTICING CHARTERED ACCOUNTANT
ANUGRAHA, VANIYAR STREET
NALLEPILLY, PALAKKAD-678553
MEM No: 234833

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Govt. Arts & Science College * Kozhinjampara, Palakkad-678 554 *

KERALA STATE COUNCIL FOR SCIENCE TECHNOLOGY AND ENVIRONMENT

STUDENT PROJECT REPORT

1. **Project Title: Study on the antagonistic activity of neem endophytic bacteria against rice sheath blight causing fungi *Rhizoctonia solani.***
2. **File No.:00574/SPS 64/2019/KSCSTE**
3. **Name & Address of student** : Anjana M,
   M.SC Microbiology
   Dept of Microbiology
   Govt Arts and Science College Kozhinjampara
   Palakkad-678554
4. **Name & Address of the Principal Investigator with mobile No.** : Saranya Devi K,
   Assistant Professor
   Department of Microbiology,
   Govt Arts and Science College Kozhinjampara,
   Palakkad-678554
   Mobile No: 09943131010
5. **Broad area of research:** Agricultural Microbiology
6. **Specific area**: Endophytic bacteria and its bio-control and plant growth promoting activity.
7. **Date of Start**: 01/02/2020
8. **Total cost of Project: 10000/-**

9. Approved objectives of the proposal:

Rice (*Oryza sativa*) is the most widely consumed staple food of the human population, particularly in Asia. Ninety percentage of rice is produced in Asia, in which China and India being the lead producers. Diseases caused by various microbes are the significant limiting factors that affect rice production and causing annual yield losses. More than 70 diseases were reported in the rice, caused by fungi, bacteria, viruses, mycoplasma or nematodes. Among these, fungi are the major disease causing agent in the rice field. Rice sheath blight is one of the major rice diseases found worldwide. In India, the estimation of losses due to this disease has been reported up to 54.3 %. Sheath blight is a soil borne disease caused by the fungus *Rhizoctonia solani*. They not only have a supreme role in causing disastrous epidemics in the plant but also play a significant and perpetual role in annual crop yield losses which affect the economy of the country. As a way out for these problems, many methods have been employed.

Using chemical fungicides are the best prevention against fungal disease caused by phytopathogens but it affects the soil fertility, texture and beneficial microbial population. Apart from that, fungus exhibited resistances against chemical fungicides which makes the fungicide ineffective against plant pathogens. Hence the application of chemical fungicides is not an eco-friendly method and it is essential to find a new alternative technique for plant protection which is less dependent on the chemicals and is more eco-friendly.

One of the emerging research areas for the control of phyto-pathogenic agent is the application of endophytic bacteria, which are capable of suppressing the damage caused by phyto-pathogens. It also establish symbiotic relationship with host by means of colonizing plant tissues, which makes them effective biocontrol agents. Several researchers have explored bacterial endophytes as promising biocontrol agents against different plant pathogenic fungi by means of producing several volatile organic compounds with broad

antimicrobial activity against various plant pathogenic microbes. The development of biological products based on beneficial microorganisms can extend the range of options for maintaining the health and yield of crops (Schulz and Boyle, 2006). There is a dearth of information regarding the use of different endophytic microorganisms for the management of soil borne fungal pathogens. Hence, the present investigation was undertaken to tap the endophytic bacterial diversity of neem, it is a divine tree known for its medicinal and insecticidal property. The neem leaf constituents exhibited various antifungal, antibacterial and antiviral activity (Kaushik *et al*., 2002). Based upon this knowledge, the objective of the study is to isolate endophytic bacteria from neem leaves, their identification and investigating bio-control activity against rice sheath blight causing pathogen, *R. solani* under *in-vitro* condition. The effective endophytic bacteria is further subjected to secondary metabolite extraction and identifying its bioactive compounds using GC-MS analysis.

10. Methodology:

❖ **Isolation of bacterial endophytes**

For isolation of neem leaf endophytic bacteria, surface sterilization of leaf sample carried out. The leaf was treated with double distilled water, 0.1% sodium hypochlorite and 70% ethanol. The surface sterilized leaf samples were cut into small pieces and macerated in a sterile pestle and mortar. Tissue extract were then serially diluted and spreaded onto nutrient agar medium and the plates were incubated at 28°C for 24 hours. The bacterial colonies exhibiting different colony morphology were selected, pure cultured and used for bio-control studies (Gupta *et al*., 2015).

❖ **Fungal pathogens used for study**

The rice pathogen *Rhizoctonia solani* (Rice sheath blight) was procured from culture repository of Institute of Forest Genetics and Tree Breeding Institute, Coimbatore, Tamil Nadu, India for present study. Actively growing hyphae were successively transferred to the

new Potato Dextrose Agar (PDA) and the cultures were maintained on slants and stored at 4°C.

❖ **Screening for antifungal activity**

A) Dual culture method

Dual culture plate technique was adopted for testing the antagonistic activity of the endophytes against *R. solani* on PDA plates. The fungal pathogen was inoculated at one side of Petri plate and bacterial endophytes was streaked on the opposite side of the Petri plate. For this, actively growing cultures of both endophytes and pathogens were used. Petri plate inoculated only with the pathogen served as control (Khamna *et al.*, 2009). Percent inhibition of mycelium over control was worked out according to the formula given below:

$$\text{Percent of mycelia inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where, I = Percent inhibition, C = mycelial growth in control (cm), T = mycelial growth in treatment (cm)

B) Antifungal activity in liquid culture

Antifungal liquid culture method was used to test the antifungal activity in liquid broth (PDB). The PD broth inoculated with fungal disc and without bacterial culture was used as control. It was incubated at 28°C for five days. Dry weight of the fungal matt with bacterial strains and control (without bacterial strains) were recorded and compared.

C) Screening for production of volatile antifungal metabolites

The production of volatile substances by selected endophytic bacteria was determined by the method described by Jayaswal *et al.* (1993).

❖ **Screening for production of secondary metabolites**

A) Preparation of inoculum and extraction of cured metabolites

As per the procedure followed by Battu and Reddy (2009), the inoculum preparation and metabolite extraction from selected isolates was carried out.

B) Antifungal property of cured extract

The individual extracts were tested for antifungal activity by well diffusion method described by Perez *et al.* (1990).

C) GC-MS analysis of ethyl acetate extract

Most effective extract was analysed using GC-MS to predict the presence of inhibitory compounds was carried out at SITRA Coimbatore.

- **Morphological, Biochemical and Molecular characterization of effective isolates**

Bacteria selected for the phenotypic and genotypic identification was based on the bio-control and multifactorial PGP traits exhibited under *in-vitro* condition. The 16S ribosomal RNA (16S rRNA) analysis of effective bacteria was carried out at Yaazh Xenomic laboratory, Coimbatore.

- **Screening for Plant growth promoting (PGP) traits of effective endophytic bacterial isolate**

Among 13 endophytic bacteria three showed more than 70% inhibition against fungal pathogens. These isolates were tested for their *in-vitro* plant growth promoting traits.

- IAA Production
- Phosphate solubilization
- Potassium solubilization
- Zinc solubilization
- Ammonia production
- EPS production

- Hydrogen cyanide production

❖ **Evaluation of endophytic bacteria for growth promotion and disease suppression in nursery condition**

I. Treatment model

T1-Control (distilled water)

T2- *Rhizoctonia solani*

T3- *Bacillus haynesii*

T4- *Stenotrophomonas maltophilia*

T5- *Pseudomonas aeruginosa*

T6- *Rhizoctonia solani* + *Bacillus haynesii*

T7- *Rhizoctonia solani* + *Stenotrophomonas maltophilia*

T8- *Rhizoctonia solani* + *Pseudomonas aeruginosa*

❖ **Nursery studies**

Individual isolates were evaluated for the ability to stimulate growth of rice and its biocontrol potential also evaluated. After 3 weeks of growth, plant height and total weight and length of shoots and roots were measured.

11. Salient Research Achievements:

a. **New observations:**

- First report on the isolation of *Bacillus haynesii*, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* from Neem plant located at Palakkad district, Kerala.
- Identified 14 different bioactive compounds from effective bio-control bacterium *Pseudomaons aeruginosa* ethyl acetate extract using GC-MS analysis.

b. Innovations/Technologies generated:

- The sequences of the isolates (***Bacillus haynesii, Stenotrophomonas maltophilia*** **and** ***Pseudomonas aeruginosa***) have been deposited in GenBank and got accession numbers **MN880435, MN880434** and **MN880486** respectively.
- Phylogenetic tree was constructed for all the identified isolates.

c. Application potential:

- Our study indicated that strain *B. haynesii*, *S. maltophilia* and *P. aeruginosa* introduced into the rice rhizosphere might play essential roles in disease control and also involved in plant growth.
- Overall, from the above results, we discovered that strains had direct antagonistic effects against sheath blight causing *R. solani* and allowed beneficial microbes to accumulate more easily in the rice rhizosphere.
- It helps in replacement of chemical fungicides and fertilizer in agricultural farms.

d. Any other:

- Hence, we suggest that these strains had the potential to be used in sustainable agriculture. Although this isolates need further testing under field conditions, we are confident that our findings can be transferred to the field, because the strains were isolated from the same environment in which they are intended to be used.

12. Details of publications (including paper/poster presentation in seminar/symposium)

'Isolation and screening of neem endophytic bacteria for its bio-control and plant growth promotion properties' under communication in **Journal of basic Microbiology** (manuscript number is jobm.202000562).

Reference:

Battu, P. R and Reddy, M. S. 2009. Isolation of secondary metabolites from *Pseudomonas flurescens* and its characterization. *Asian J of Reschem*, 2:26-29.

Gupta, R.M., Prathamesh, S., Kale, Madhuri, L., Rathi and Nikhil N. Jadhav. 2015. Isolation, characterization and identification of endophytic bacteria by 16S rRNA partial sequencing technique from roots and leaves of *Prosopis cineraria* plant. *Asian Journal of Plant Science and Research*. 5(6):36-43.

Jayaswal, R.K., M. Fernandez, R.S., Upadhyay, L. Visintin, M. Kurz, S. Webb, K. Rinehart 2008.Antagonism of *Pseudomonas cepacia* against phytopathogenic fungi. *J Chromatogr A*. 1997;778:363–372.

Kaushik, B., Chattopadhyay, I., Banerjee, R.K., Bandyopadhyay, U. 2002 Biological activity and medicinal properties of neem *(Azadirachta indica). Curr Sci*. 82:1336–1345.

Khamna, S., Yokota, A and Lumyong, S. 2009. Actinomycetes isolated from medicinal plant rhizospheric soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. *World J. Microbiol. Biotechnol*. 25:649–655.

Perez, C., Paul, M and Bazerquw, P. 1990. Antibiotic assay by agar well diffusion method. *Acta Biol med exp*, 15:113-115.

Schulz, B., and Boyle, C. 2006. What are endophytes? *Soil Biol.* 9, 1–14.

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(An Autonomous Org. under the Dept. of Higher Edu.
Ministry of Education, Govt. of India)
ज्ञान-विज्ञान विमुक्तये
विश्वविद्यालय अनुदान आयोग
Block C-20/1A/8, Sector-62, IITK Outreach Centre, Gautam Buddh Nagar 201309, Uttar Pradesh (India) Phone: 0120-6895200

Joint CSIR-UGC Test
JRF AWARD LETTER

Dated: **01.04.2021**

NTA Ref. No.: **201610110404** Roll No.: **KL17600981**

SRUTHI. M
Son/Daughter of **SUNI. P**
and **MANIKANDAN. K.P**
Subject: **LIFE SCIENCES**

Dear Candidate,

I am pleased to inform you that you have qualified for Junior Research Fellowship (JRF) and eligibility for Assistant Professor in the Joint CSIR-UGC TEST conducted in November 2020. The tenure of fellowship is five years and it commences from the date of declaration of NET result, i.e., **04.02.2021** *(or) from the date of admission under M.Phil./Ph.D. (or) from the date of joining M.Phil./Ph.D. programme, whichever is later. The summary of financial assistance offered under the scheme is mentioned at* ***Annexure I*** *available on www.ugc.ac.in/netjrf* along with other Annexures.

The Awardee is required to get admission and registration for regular and full time ***M.Phil./Ph.D.*** *course in a University/Institution/College recognized by UGC at the first available opportunity* ***but not later than three years*** *from the date of issue of this award letter. University/Institution/College is requested to process for award of JRF based on this letter, in accordance with the procedure available on www.ugc.ac.in/netjrf.*

It may be noted that the fellowship amount shall be disbursed through Canara Bank to bank account of the Awardee (any bank) directly. UGC had developed a dedicated web portal (https://scholarship.canarabank.in) for capturing data of the awardee. The Universities/Colleges/Institutions will link the data of the awardee with the master data on the UGC web portal with unique Maker/Checker Ids which have already been provided to them along with the passwords. The Universities/Colleges/Institutions shall update the information in the master data (regarding monthly payment confirmation, HRA, up-gradation, resignation etc.) of the beneficiaries on monthly basis. Based on the data updated on UGC web portal by the concerned Universities/Colleges/Institutions, the payment of the fellowship will be made to the beneficiaries (Detailed process available at https://www.ugc.ac.in/ugc_notices.aspx?id=2153).

*It may also be noted that UGC had proposed to link "**AADHAAR**" with bank account of students so that there can be direct cash transfer and effective disbursal of fellowship into bank account of the student. In this regard, Secretary, UGC had requested the universities to help students in Aadhaar enrolment vide D.O. No. F.14-34/2011 (CPP-II) dated 11.01.2013.*

It may please be noted that the award is liable to be cancelled by Implementing/Awarding agency and it will also attract legal action against the Awardee in the following cases:

i. *If the awardee is found to be ineligible to receive the award at any point during the entire duration of fellowship,*
ii. *Misconduct of Awardee,*
iii. *Unsatisfactory progress of research work,*
iv. *Failure in any examination related to M.Phil./Ph.D.,*
v. *In case any other fellowship is drawn from other source(s),*
vi. *Concealment of facts.*

The e-Certificate of eligibility for Assistant Professor has been uploaded on https://ecertificate.nta.ac.in. The eligibility of the candidate is to be ensured by the concerned institution/appointing authority. The category under which the candidate had appeared may be verified from NTA.

This electronic JRF award letter can also be verified by scanning the QR Code.

With best wishes,

Senior Director, NTA

Note: NTA has issued the electronic JRF award letter on the basis of information provided by the candidate in his/her online application form. The appointing authority should verify the original records/certificates of the candidate while considering him/her for JRF award or appointment, as the NTA will not be liable for any false information provided by the candidate. The NTA is only responsible for the result which can be verified from the repository available in the website of NTA (csirnet.nta.nic.in). The candidate must fulfil the minimum eligibility conditions for NET as laid down in the notification for Joint CSIR-UGC Test.

File Ref.No.130350/RESEARCH-B-ASST-1/2022/Admn

UNIVERSITY OF CALICUT

Abstract

DoR - Ph.D. Programme 2021 - Registration to Ph.D. in Microbiology - Ms. Sruthi M - Department of Microbiology, Govt. Arts & Science College, Kozhinjampara, Palakkad as Research Centre - Granted - Orders issued.

Directorate of Research

U.O.No. 23850/2022/Admn Dated, Calicut University.P.O, 21.12.2022

Read:-(1) Notification No. 204384/DOA-ASST-5/2020/Admn dated 26-04-2021.
(2) The application for Registration forwarded by the Principal, Govt. Arts & Science College, Kozhinjampara, Palakkad in r/o Ms. Sruthi M.
(3) Rules & Regulations for Ph.D. Programme, 2016.
(4) U.O.No.DOR/B3/Ph.D/General/2011 dated 31-05-2012.
(5) Joining Memo No.130350/RESEARCH-B-ASST-1/2022/Admn. dated 17.11.2022.
(6) Joining Report Lr.No.B1/612/2022/GCKZHNBRA dated 23.11.2022.
(7) U.O.No.22979/2022/Admn. dated 02.12.32022.

ORDER

As per papers read above, sanction has been accorded to grant registration to **Ms. Sruthi M** for pursuing Ph.D. Programme in **Microbiology** for a period of six years as a **Full-Time** research scholar with effect from **21.11.2022.** The details of Supervising Teacher, Topic, Category, Mode of Research, etc., are given as under:

Name & Address of the Candidate	Ms. Sruthi M, W/o Deepu T, Dhanya Nivas, E.P Village, Kavassery P.O., Palakkad- 678543
Name & Address of the Guide	Dr. Robert Antony A, Assistant Professor, Department of Microbiology, Government Arts & Science College, Kozhinjampara, Palakkad.
Stream	Full-Time
Subject & Faculty	Microbiology, Science
Period	21.11.2022 to 20.11.2028.
Topic	"PRODUCTION OF POLYHYDROXYALKANOATE (PHA) FROM MARINE BACTERIA BY USING VARIOUS SUBSTRATES"
Centre	Department of Microbiology, Government Arts & Science College, Kozhinjampara, Palakkad.
Fellowship/Sponsorship	UGC-JRF

NB: The scholar shall attend the Research Centre and submit progress report with attendance details to this office once in every six months. The scholar is **not exempted from Course Work and PQE** as per the new regulation for Ph.D. programme.

The University Order read as (7) above stands cancelled.

Orders are issued accordingly.

Suraj Kumar P.M

Assistant Registrar

To

Ms. Sruthi M, W/o Deepu T, Dhanya Nivas, E.P Village, Kavassery P.O., Palakkad- 678543

Copy To:

1. The Principal, Government Arts & Science College, Kozhinjampara, Palakkad.
2. Dr. Robert Antony A, Assistant Professor, Department of Microbiology, Government Arts & Science College, Kozhinjampara, Palakkad (Research Guide)
3. SF/FC.

Forwarded / By Order

Section Officer

ADMINISTRATION

(Directorate of Research)

No. 130350/RESEARCH-B-ASST-1/2022/Admn

Calicut University.P.O,
Dated, 17.11.2022

MEMO

Sub:- DoR - Registration to Ph.D. Programme- 2021- Reg.

Ref:- (1) Application for Ph.D. Programme in respect of Ms. Sruthi. M forwarded by the Principal, Govt. Arts and Science College, Kozhinjampara, Palakkad.
(2) Circular No. 48963/RESEARCH-B-ASST-3/2016/Admn (I) dated 21-03-2017.
(3) Orders of Director, Directorate of Research in the file under reference on 16.11.2022.

With reference to the above, Ms. Sruthi. M is informed that, she is found to be eligible for admission/registration **(Full-Time)** to Ph.D. programme in **Microbiology.** Accordingly, she is directed to report before **the Principal, Govt. Arts and Science College, Kozhinjampara, Palakkad** at the earliest; failing which the application for registration is liable to be rejected without further notice. The candidate has to comply with all the rules and regulations of the Ph.D. programme of the University throughout the course. The candidate is directed to submit the letter of joining issued by the Principal, Govt Arts and Science College, Kozhinjampara, Palakkad after confirming that the candidate has started marking her attendance for the issuance of Ph.D. registration order.

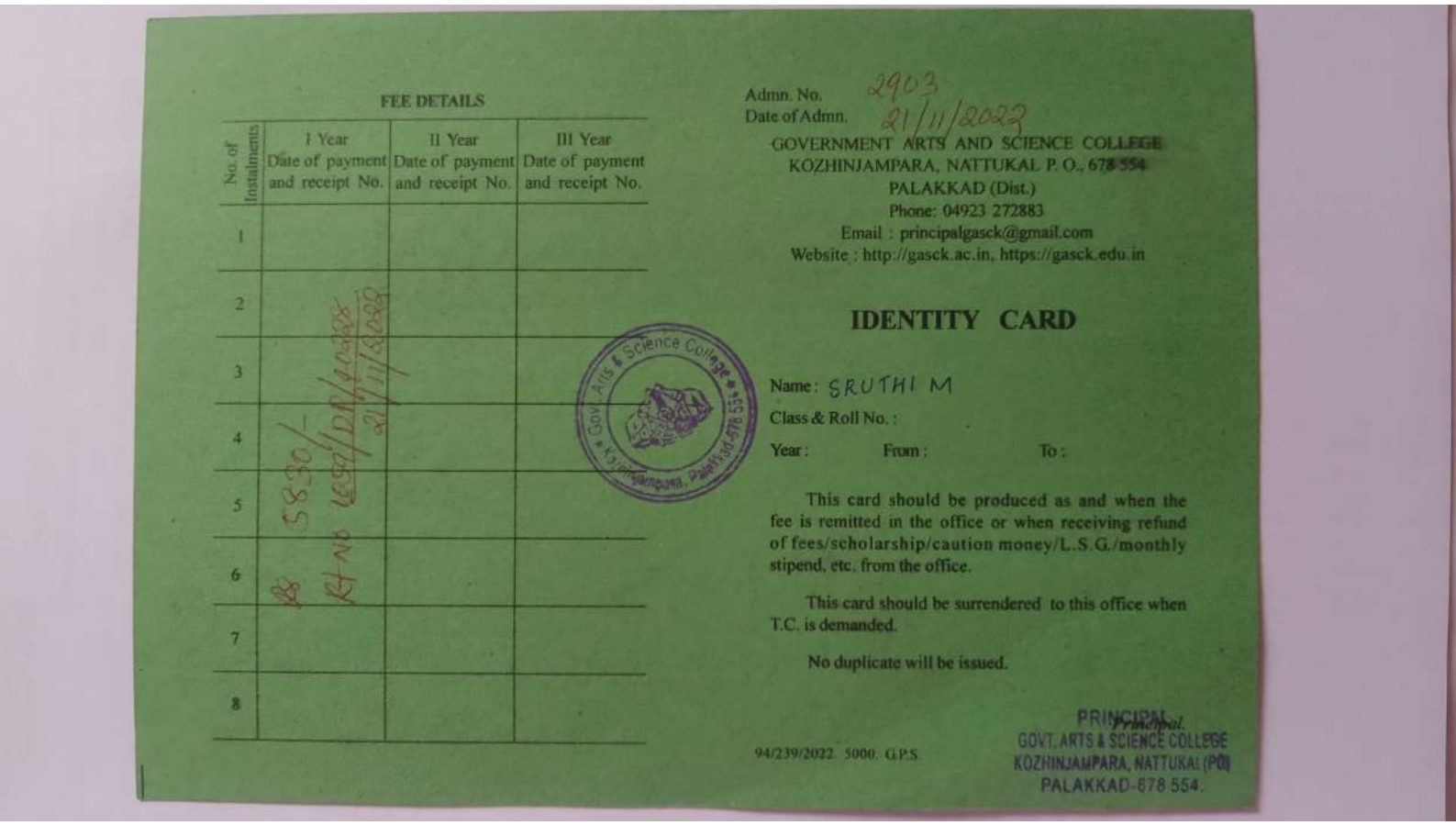
Suraj Kumar P.M
Assistant Registrar

To:

Ms. Sruthi M, W/o Deepu T, Dhanya Nivas, E.P. Village, Kavassery P.O, Palakkad-678543.

Copy To:

1. The Principal, Govt Arts and Science College, Kozhinjampara, Palakkad.
2. Dr.Robert Antony, Assistant Professor, Department of Microbiology, Govt Arts and Science College, Kozhinjampara (Research Guide)
3. SF/FC.



FEE DETAILS

No. of Instalments	I Year Date of payment and receipt No.	II Year Date of payment and receipt No.	III Year Date of payment and receipt No.
1			
2			
3			
4			
5	5830/- Ref No UGD/DN/4038 21/11/2022		
6			
7			
8			

Admn. No. 2903
Date of Admn. 21/11/2022

GOVERNMENT ARTS AND SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL P. O., 678 554
PALAKKAD (Dist.)
Phone: 04923 272883
Email : principalgasck@gmail.com
Website : http://gasck.ac.in, https://gasck.edu.in

IDENTITY CARD

Name : SRUTHI M
Class & Roll No. :
Year : From : To :

This card should be produced as and when the fee is remitted in the office or when receiving refund of fees/scholarship/caution money/L.S.G./monthly stipend, etc. from the office.

This card should be surrendered to this office when T.C. is demanded.

No duplicate will be issued.

Govt. Arts & Science College Kozhinjampara, Palakkad-678 554

PRINCIPAL
Principal
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL (PO)
PALAKKAD-678 554.

94/239/2022. 5000. G.P.S.

IDENTITY CARD

Admission No. : 2903 Date: 21/11/2022

1. Name : SRUTHI · M
2. Guardian's name : DEEPU T
3. Address :

4. Age and date of birth : 26. 2/2/1996
5. Class : PhD
6. Admission No. : 2903
7. Roll No. :
8. Place of residence and distance to the Institution :
9. Duration of the Course : From: To:
10. Signature of student : Sruthi

Certified that Sri/Smt./Kum. SRUTHI · M is a full time student of this Institution and that he/she is not engaged in any other calling or profession.

Principal
PRINCIPAL
GOVT. ARTS & SCIENCE COLLEGE
KOZHINJAMPARA, NATTUKAL(PO)
PALAKKAD-678 554.

Passport size Photo

Details are

Student_ID	Student_Name	Stream	Payment_Month	Component	From_Date	To_Date
201610110404	SRUTHI M	SCIENCE	01-APR-2023	HRA	01-APR-2023	30-APR-2023
201610110404	SRUTHI M	SCIENCE	01-APR-2023	FELLOWSHIP	01-APR-2023	30-APR-2023
201610110404	SRUTHI M	SCIENCE	01-DEC-2022	FELLOWSHIP	01-DEC-2022	31-DEC-2022
201610110404	SRUTHI M	SCIENCE	01-DEC-2022	CONTINGENCY	21-NOV-2022	31-DEC-2022
201610110404	SRUTHI M	SCIENCE	01-DEC-2022	HRA	01-DEC-2022	31-DEC-2022
201610110404	SRUTHI M	SCIENCE	01-FEB-2023	FELLOWSHIP	01-FEB-2023	28-FEB-2023
201610110404	SRUTHI M	SCIENCE	01-FEB-2023	HRA	01-FEB-2023	28-FEB-2023
201610110404	SRUTHI M	SCIENCE	01-JAN-2023	HRA	01-JAN-2023	31-JAN-2023
201610110404	SRUTHI M	SCIENCE	01-JAN-2023	FELLOWSHIP	01-JAN-2023	31-JAN-2023
201610110404	SRUTHI M	SCIENCE	01-JUN-2023	HRA	01-JUN-2023	30-JUN-2023
201610110404	SRUTHI M	SCIENCE	01-JUN-2023	CONTINGENCY	01-APR-2023	30-JUN-2023
201610110404	SRUTHI M	SCIENCE	01-JUN-2023	FELLOWSHIP	01-JUN-2023	30-JUN-2023
201610110404	SRUTHI M	SCIENCE	01-MAR-2023	HRA	01-MAR-2023	31-MAR-2023
201610110404	SRUTHI M	SCIENCE	01-MAR-2023	CONTINGENCY	01-JAN-2023	31-MAR-2023
201610110404	SRUTHI M	SCIENCE	01-MAR-2023	FELLOWSHIP	01-MAR-2023	31-MAR-2023
201610110404	SRUTHI M	SCIENCE	01-MAY-2023	FELLOWSHIP	01-MAY-2023	31-MAY-2023
201610110404	SRUTHI M	SCIENCE	01-MAY-2023	HRA	01-MAY-2023	31-MAY-2023
201610110404	SRUTHI M	SCIENCE	01-NOV-2022	HRA	21-NOV-2022	30-NOV-2022
201610110404	SRUTHI M	SCIENCE	01-NOV-2022	FELLOWSHIP	21-NOV-2022	30-NOV-2022

Scheme: NETJRF Stud ID: 201610110404 Find Student ID Status Approved Fetch

Student Name	Payment Month	From Date	To Date	Component	Sub Component	Amount	Initiated By	Initiated On	Approved By	Approved On	STATUS
SRUTHI M	01-NOV-2022	21-NOV-2022	30-NOV-2022	HRA	HRA	930	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	15-MAY-2023	Approved
SRUTHI M	01-NOV-2022	21-NOV-2022	30-NOV-2022	FELLOWSHIP	JRF	10333	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-DEC-2022	21-NOV-2022	31-DEC-2022	CONTINGENCY	CONTINGENCY	3000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-DEC-2022	01-DEC-2022	31-DEC-2022	FELLOWSHIP	JRF	31000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-DEC-2022	01-DEC-2022	31-DEC-2022	HRA	HRA	2790	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	15-MAY-2023	Approved
SRUTHI M	01-JAN-2023	01-JAN-2023	31-JAN-2023	FELLOWSHIP	JRF	31000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-JAN-2023	01-JAN-2023	31-JAN-2023	HRA	HRA	2790	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	15-MAY-2023	Approved
SRUTHI M	01-FEB-2023	01-FEB-2023	28-FEB-2023	HRA	HRA	2790	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	15-MAY-2023	Approved
SRUTHI M	01-FEB-2023	01-FEB-2023	28-FEB-2023	FELLOWSHIP	JRF	31000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-MAR-2023	01-MAR-2023	31-MAR-2023	FELLOWSHIP	JRF	31000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-MAR-2023	01-MAR-2023	31-MAR-2023	HRA	HRA	2790	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	15-MAY-2023	Approved
SRUTHI M	01-MAR-2023	01-JAN-2023	31-MAR-2023	CONTINGENCY	CONTINGENCY	3000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-APR-2023	01-APR-2023	30-APR-2023	FELLOWSHIP	JRF	31000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-APR-2023	01-APR-2023	30-APR-2023	HRA	HRA	2790	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	15-MAY-2023	Approved
SRUTHI M	01-MAY-2023	01-MAY-2023	31-MAY-2023	HRA	HRA	2790	PRINCIPALGASCK@GMAIL.COM	15-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	15-MAY-2023	Approved
SRUTHI M	01-MAY-2023	01-MAY-2023	31-MAY-2023	FELLOWSHIP	JRF	31000	PRINCIPALGASCK@GMAIL.COM	12-MAY-2023	ROBERTMICROBIOLOGY@GMAIL.COM	12-MAY-2023	Approved
SRUTHI M	01-JUN-2023	01-APR-2023	30-JUN-2023	CONTINGENCY	CONTINGENCY	3000	PRINCIPALGASCK@GMAIL.COM	08-JUN-2023	ROBERTMICROBIOLOGY@GMAIL.COM	08-JUN-2023	Approved
SRUTHI M	01-JUN-2023	01-JUN-2023	30-JUN-2023	FELLOWSHIP	JRF	31000	PRINCIPALGASCK@GMAIL.COM	08-JUN-2023	ROBERTMICROBIOLOGY@GMAIL.COM	08-JUN-2023	Approved
SRUTHI M	01-JUN-2023	01-JUN-2023	30-JUN-2023	HRA	HRA	2790	PRINCIPALGASCK@GMAIL.COM	08-JUN-2023	ROBERTMICROBIOLOGY@GMAIL.COM	08-JUN-2023	Approved