KERALA STATE COUNCIL FOR SCIENCE, TECHNOLOGY AND ENVIRONMENT

Scheme for the Augmentation of Research & Development. (SARD)

PROJECT COMPLETION REPORT

1. **Title of the Programme:** Study of Microbial Diversity, Study of Primitive Bacteria, Isolation of microorganisms producing enzymes, Evaluating their Application in Waste Management.
2. **Name of Co-ordinator & address:** Dr.Valsa A. K., Associate Professor
   Department of Microbiology,
   Sree Sankara College, Kalady, Ernakulam Dt, 683574
3. **Implementing Institution(s) and other collaborating Institution(s):**
   Department of Microbiology, Sree Sankara College
4. **Date of commencement**: 5th March 2014
5. **Planned date of completion:** 4th March 2017
6. **Actual date of completion**: 31st March 2017
7. **Objectives as stated in the Research Proposal:**

   1. To study and document the microbial diversity from different sites of the Western Ghat region - Malayatoor range, using advanced molecular biological tools.
   2. To study about primitive bacteria and exploit them as source of enzymes as well as models for understanding biological interactions and evolutionary history.
   3. To isolate microorganisms producing enzymes and to study their industrial applications
   4 To isolate microorganisms and screen them for the production of microbial enzymes capable of waste degradation, there by evaluating their application in waste management

8. **Deviation made from original objectives if any, while implementing the programme and reasons thereof:** Attempts were made to isolate halophilic archaea from samples collected from salt pans and tannery effluent. However few salt tolerant, marine bacteria and fungi were isolated and studied. Efforts are still going on in this regard.
9. **Abstract of the Research Programme (Not more than 500 words)**

Studies were carried out on the soil microbial diversity of the malayattoor range , Amini island of Lakshadweep, enzyme production by bacteria and studies on waste degradation by a consortium of organisms.

Analysis of soil bacterial diversity of five different samples including three Western Ghats sites were carried out in the study. A total of 110 bacteria were isolated and characterized using biochemical and molecular biological tools. Selected bacteria from the first sample and all the bacteria from the third sample were tested for enzyme production and found that many bacteria from the Western Ghats are multienzyme producers. Some bacteria produce large quantities of certain enzymes and may be utilized for industrial applications after further characterization. These studies only identified omly the culturable bacteria. Detailed metagenomic studies and are going on for the identification of highly potent and novel molecules of economic, industrial and medicinal importance.

Bacteria and fungi were isolated and identified from inland and sea shore soil of Amini island of Lakshadweep. A total of twenty bacteria and six fungi were identified.

Lipase producing bacteria were isolated from the soil in the premises of a dairy. A bacterial consortium comprising *Bacillus coagulans, Bacillus simplex* and *Trichococcus* sp. was prepared and its efficiency in the degradation of lipid rich dairy effluent was studied. When the dairy effluent was treated with the bacteria individually and as a consortium, there was a reduction in Biological oxygen demand (BOD) and lipid content. . There was a positive correlation between the lipase secretion and the reduction in lipid content which might have contributed to the reduction in the BOD.

In another study thirteen pectin degrading bacteria were isolated by serial dilution plate technique and identified by microbiological techniques and 16S rRNA gene sequencing. Further studies on the optimization of culture media and the cultural conditions and pectinase production using immobilized cells were carried out using *Bacillus subtilis* whose halo zone was greater than the other isolates. Pectinase production by immobilized cells higher than that of free cells and could be used for five cycles.

Based on the different characteristics like antagonistic activity, enzyme production, 10 potent bacterial strains were used for the study of waste degradation. Two sets of microbial consortium was used in two formulations one as broth and another as husk previously adsorbed by microbial consortia. Composting study was conducted in earthen pots, by the application of consortium as broth or husk. Cow dung was used as a natural starter. Different parameters were studied during the course of composting, at intervals of 2nd, 7th, 14th and 21st day. pH and temperature range were 6-8.5, and 25°C to 35°C, respectively, degradation time was 21 days resulting in compost with dark brown color, even textured and having an earthy aroma. Results of enzyme activity during composting process showed that the two sets of compost were active when compared to control. Of the 2 sets studied set II showed more enzyme activity On the basis of parameters studied, among the 2 sets of microbial consortia, 2nd gave promising result. Among the broth and husk, more stable form was husk formulation

10.Key words (Not exceeding ten): Microbial diversity, Microbial enzymes, Waste degradation, microbial consortium, Western Ghats, Lakshadweep.

10. Achievements:

i. List of Research publications

1. Shelvin T Kuriakose, Achamma Thomas and Mohan S. (2015) Soil microbial diversity of Mallana Forest in the Malayattoor division of Western Ghats, Proceedings of International Conference on Biodiversity and Evaluation, Perspectives and Paradigm Shifts [ISBN: 978-93-80095-70-7] pp: 277-280.
2. Remya S Menon and **Mohan S.** (2016) Diversity of Soil Microbes in Amini Island of Lakshadweep, Proceedings of the 25th Swadeshi Science Congress [ISBN: 978-81-928129-3-9] pp: 382-386.

3. Nayana Krishnan and Valsa A K (2016) Biodegradation of Lipid Rich Dairy Effluent by Bacterial Consortium. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 9, 16-20 (e-ISSN: 2319-2402, p- ISSN: 2319-2399.)*
4. Anju Joseph, Geedhu Lowrance and Valsa A K, (2015) Diversity of Pectin degrading bacteria, Proceedings of the International Conference on Biodiversity and Evaluation – Perspectives and Paradigm shifts, ISBN 978-93-80095-70-7 pp 320-322.
5. Remya K R, Achamma Thomas and Mohan S. (2017) Soil Microbial Diversity of Knacheri Site of the Western Ghats, Proceedings of the 26th Swadeshi Science Congress (Accepted for publication).
6. Anju Joseph, Geedhu Lowrance, Devi V D and Valsa A K (2017) Optimisation of the cultural conditions for the production of extracellular pectinase by *Bacillus subtilis* (To be communicated)
7. Achamma Thomas and Mohan S. (2017) Metagenomic study of soil bacterial diversity from a site near Thattekad Bird Sanctuary in the Western Ghats (To be communicated).

ii **Manpower trained on the project**

Research Scientists or Research Associates NIL

No. of Ph. D produced 1 Research scholar – Thesis to be submitted.

Other Technical Personnel trained 12 M Sc students

iii. Innovations/Technology developed Rare isolates were obtained. Multi enzyme producing soil bacteria with were identified from Western Ghats sites. Metagenomic analysis revealed the presence of unculturable organisms.

iv. Patents taken, if any NIL

v. **Application potential :** The bacteria and fungi isolated and identified in this study were found to be secrete degrading enzymes. Hence they may prove to be useful in the degradation of house hold wastes and industrial efluents. The cellulase and pectinase producers may find applications in food and cattle feed industries. However further studies in this regard are essential.

11. **Summary of the work done (not more than 500 words) highlighting the outcome separately.**

Analysis of soil bacterial diversity of five different samples including three Western Ghats sites were carried out in the study. A total of 110 bacteria were isolated and characterized using biochemical and molecular biological tools. Selected bacteria from the first sample and all the bacteria from the third sample were tested for enzyme production and found that many bacteria from the Western Ghats are multienzyme producers. Some bacteria produce large quantities of

enzymes and may be utilized for industrial applications after further characterization. These studies only identified the culturable bacteria. Detailed metagenomic studies are going on for the identification of highly potent and novel molecules of economic, industrial and medicinal importance.

Bacteria and fungi were isolated and identified from inland and sea shore soil of Amini island of Lakshadweep**.** A total of twenty bacteria and six fungi were identified**.** The total bacterial count in surface sample is higher in both the sites compared to 15 and 30 cm depth samples. Total number of *Azotobacter* count in Ashby's medium and total number of *Rhizobium* and *Agrobacterium* count of soil in CR-YEMA medium was found to be highest in soil taken from 15 cm depth when compared to surface soil and soil taken from 30 cm depth. In GPA plate in case of inland sample soil taken from 15 cm depth and in case of seashore sample soil taken from 30 cm depth show highest number of mould count.

Lipase producing bacteria was isolated from the soil in the premises of a dairy. A bacterial consortium comprising *Bacillus coagulans, Bacillus simplex* and *Trichococcus* sp. was prepared and its efficiency in the degradation of lipid rich dairy effluent was studied. When the dairy effluent was treated with the bacteria individually and as a consortium, there was a reduction in Biological oxygen demand (BOD) and lipid content. The secretion of lipase was higher by the consortium than when the organisms were added individually. There was a positive correlation between the lipase secretion and the reduction in lipid content. The decrease in the lipid content may have contributed to the reduction in the BOD.

In another study thirteen pectin degrading bacteria were isolated by serial dilution plate technique and identified by microbiological techniques and 16S rRNA gene sequencing. Further studies on the optimization of culture media and the cultural conditions and pectinase production using immobilized cells were carried out using *Bacillus subtilis* whose halo zone was greater than the other isolates. Maximum pectinase production by Bacillus subtilis was at 72 hours of incubation with pectin concentration 1%. The natural substrates like orange peel and rice bran at were found to be better substrates than pure pectin. Optimum temperature was 30ºC, p^H -6. Additional carbon sources - carbon sources were starch and glucose increased pectinase production, whereas additional nitrogen sources decreased pectinase production. Pectinase production by immobilized cells was higher than that of free cells. Three percent sodium alginate was better than agar agar for the production of pectinase. Both agar-agar entrapped cells and cells entrapped in sodium alginate could be used for five cycles.

Based on the different characteristics like antagonistic activity, enzyme production, 10 potent bacterial strains were used for the study of waste degradation. Two sets of microbial consortium was used in two formulations one as broth and another as husk previously adsorbed by microbial consortia. Composting study was conducted in earthen pots, by the application of consortium as broth or husk. Cow dung was used as a natural starter. Different parameters were studied during the course of composting, at intervals of 2nd, 7th, 14th and 21st day. pH and temperature range were 6-8.5, and 25°C to 35°C, respectively, degradation time was 21 days resulting in compost with dark brown color, even textured and having an earthy aroma. Results of enzyme activity during composting process showed that the two sets of compost were active when compared to control. Of the 2 sets studied set II showed more enzyme activity On the basis of parameters studied, among the 2 sets of microbial consortia, 2nd gave promising result. Among the broth and husk, more stable form was husk formulation

11. Financial Details:

No.	**Financial Position/Budget Head**	**Funds Sanctioned INR**	**Expenditure INR**	**% of Total cost**
1	Equipment	19,84,173.00	19,81,579.00	99.86%
2	Consumables	4,75,000.00	4,75,554.00	100%
3	Contingency	50,000.00	50,000.00	100%

12. Procurement/Usage of Equipment:

a)

Sl.No	**Name of equipment**	Make/Model	Cost (/Rs.)	Date of Installation	Utilization Rate (%)	Remarks regarding maintenance/breakdown
1	**Major**					
2	-80^0 C Deep freezer (vertical)	Eppendorf New Brunswick U410 230v 50 Hz	5,49,999.00	07 07 2014	100%	Developed technical snag once. Corrected by the company
3	CO_2 Incubator	Symbiogen Biotechnologies Necternova EP 165 A	3,99,997.00	27 11 2015	5% Installed in a separate room for cell culture and standardized	
4	Inverted Microscope	EVOS XL Core Imaging	6,18,987.00	03 02 2017		

		system – THERMO FISCHER SCIENTIFIC Invitrogen Bioservices			and work started.	
	Bio Safety Cabinet (Class 100)	LABLINE – B S C 4	1,29,843.00			
	Minor					
5	Refrigerator	LG 365 GL – B252V MGY 2014	18320.00	29 3 2014	100%	
6	Rotary Shaker for bacterial culture	ROTEK - RRS06	28511.00	25 04 2014	100%	
7	Water bath with thermostat (2 nos)	ROTEK - RSN 04	34808.00	17 06 2014	100%	
8	Vortex mixer	REMI - CM 101	4694.50	29 03 2014	100%	
9	Micropipetter (2 Sets)	Thermoscientific – F2 variable volume	59,724.00	20 03 2014	100%	
10	Magnetic mixer (2L and5 L)	REMI 2ML - 5 ML	5267.00 6641.00	29 03 2014	100%	
11	Balance(2 Nos)	INFRA – IN 200	38930.00	26 03 2014	100%	
12	Electric pipette aid	TARSONS –Cat 2020	20,839.00		100%	
13	pH meter	ELICO – L1 120/L1 610	10019.00	17 06 2014	100%	
14	Incubator	LABPLUS – LP1M - 3	21766.00	19 06 2014	100%	
15	Autoclave	LABPLUS –LAC -2	33233.00	19 06 2014	100%	

b) **Plans for utilizing the equipment facilities in future**

- 80 ° C freezer will be used for the storage of bacterial cells and cell lines. Carbon dioxide incubator, Biosafety cabinet and inverted microscope will be used for animal cell culture experiments by research scholars. All the minor equipments will be used for routine laboratory experiments.

Head of the Department
Name & Signature with Date

Co- ordinator
Name and Signature with Date

Head of the Institution
Name & Signature with date