Progress Report for Minor Research Project entitled 'Development of appropriate technology for production of wines from locally grown tropical fruits' Project No. 23-84/2000(WRO)

Project Investigator- Dr. Carolina F. E. Fernandes
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Annual Report- Progress report for the year 2000-2001

Status of the Project (Phase I)

The project was sanctioned on 13th Nov. 2000 and was received by the College along with D.D. of Rs. 22,000/- (Rupees twenty two thousand only) on 20th Nov. 2000. After receiving the sanction letter, the work commenced immediately.

1. A field survey and sampling of appropriate seasonal tropical fruits was carried out.
2. Standard isolation media such as Saboraud's agar, Malt extract agar, Wickerham's agar were used for isolation and selection of wild type yeasts from locally grown tropical fruits.
3. Identification of the fermenting natural wild type of yeasts was carried out by referring to the relevant literature.
4. A preliminary study of wine-making process from locally grown tropical fruits using the axenic wild yeast strains was undertaken.

Statement of expenses towards fieldwork and travel for the first year for the Project No. 23-84/2000(WRO).

Date	Particulars	Amount
20.12.2000	Voucher-Taxi	500/-
29.12.2000	Voucher-Rickshaw	35/-
27.1.2001	Voucher-Taxi	500/-
1.2.2001	Voucher-Taxi	500/-
15.6.2001	Voucher-Taxi	500/-
23.10.2001	Voucher-Taxi	465/-

Final Report of the Minor Research Project submitted to the University Grants Commission (UGC)

Significant findings of the study:

- The survey conducted in various places in Goa, indicated the variability in availability of tropical fruits as per seasons. Twenty-three morphotypes of wild yeasts on the surface of various fruits viz. papaya, pineapple, cashew apple, mango, jamun, kokam, banana and guava, were isolated. Studies indicated that on an average, six yeast microflora are associated as a consortium for each fruit thus demonstrating diversity. The pool or origin of wild yeasts is in soil, which are transmitted by pollinators, arboreal. It was observed that some of the wild yeasts show fruit specificity. These twenty-three morphotypes of yeasts were further evaluated for their fermentation potential, out of which ten possessed this trait. Morphological, cultural and biochemical characterization of these morphotypes indicated to be members of the genus *Saccharomyces*.
- Standardization of wine making was carried out by considering two parameters viz. effect of concentration of sucrose and yeast inoculum. A matrix of patterns of 80 combinations were employed in the study keeping control Baker's yeast for each.
- Different wine samples were analyzed using gas chromatography. The conditions for analysis were as follows: 0.5 µl of wine sample was injected into a packed SE-30 column. The initial column temperature was 60°C, the prog rate was set at 6°C/min, the FID detector and injector temperature were set at 290°C with nitrogen being the carrier gas and hydrogen and air being the other gases used for maintaining the FID detector. The results from the gas chromatographic profiles indicated wines from cashew apple, kokam, mango and jamun showed a good balance between fusel oil, ester and soluble solids compared to wines from guava, banana, pineapple and papaya.

The study showed that the various axenic wild yeasts could be used to produce wines from a number of tropical fruits, despite of different origin of these yeasts. Such yeast collection would thus contribute to preserving the genetic resources in order to develop yeast strains with improved functional traits.

Statement of expenses towards fieldwork and travel for the second year for the Project No. 23-84/2000(WRO).

Date	Particulars	Amount
5.12.2001	Voucher-Taxi	500/-
10.12.2001	Voucher-Taxi	500/-
7.1.2002	Voucher-Taxi	500/-
12.2.2002	Voucher-Taxi	500/-
16.7.2002	Voucher-Taxi	500/-

Purpose of fieldwork and travel was for reference work and to survey and collect fruit samples from various places in Goa.

P.I. has deposited the equipments purchased under this project in the Department of Microbiology of the College. Books have been accessed and submitted to the Library of the College.

Part of this work was presented as a poster paper at the National Seminar on 'Advances in Life Sciences' organized by the Department of Botany, St. Xavier's College, Mapusa-Goa on 7th and 8th December, 2015.

Dr. Carolina F. E. Fernandes
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Progress report for Minor Research Project entitled 'Exopolysaccharides from hypersaline habitats ' Project No. 47-953/09(WRO)

Project Investigator- Dr. Carolina F. E. Fernandes
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Farmagudi, Ponda, Goa

Annual Report- Progress report for the year 2009-2010

Status of the Project (Phase I)

Sanction of Project dated: 22.09.2009
Letter of Grant-in-aid dated: 23.12.2009
D.D./Cheque dated: 18.12.2009
Amount of D.D./Cheque: Rs. 1,60,000/-
Received by College on: 4.01.2010

1. Brine and sediment samples were collected from various salt pans of Goa at Assolna, Arpora, Arambol, Batim, Ribandar, located in three Talukas of Goa namely, Pernem, Bardez and Tiswadi.
2. The samples were plated on nutrient rich halophilic media namely, Tryptone Yeast Extract containing 25% crude solar salt (NTYE), *Halobacterium sodomense* (HS), Kustr, *Natronobacterium pharaonis(NP)* and Synthetic Medium containing 20% NaCl and 0.2% of glucose (NGSM); after carrying out necessary dilutions, for isolation of microflora.
3. The isolates were then studied for their morphological and cultural characteristics.
4. The isolates were screened for Exopolysaccharides(EPS) production, based on light microscopy (alcian blue adsorption assay).
5. The physico-chemical properties and preliminary studies on the role of EPS in environmental pollution control was undertaken.

Statement of expenses towards fieldwork and travel for the first year for the Project No. 47-953/09 (WRO)

Date	Particulars	Amount
12.1.2010	Voucher-Taxi	500/-
20.3.2010	Voucher-Taxi	1100/-
10.4.2010	Voucher-Taxi	1000/-
14.4.2010	Voucher-Taxi	800/-
9.6.2010	Voucher-Taxi	850/-
4.5.2010	Voucher-Taxi	900/-

Total amount sanctioned was Rs. 5000/- where as Expenditure incurred was Rs. 5150/-

Final Report of the Minor Research Project submitted to the University Grants Commission (UGC)

Significant findings from the study:

- Out of 118 cultures isolated, only eighteen had the ability to produce slime(EPS).
- Growth of cultures on plates demonstrated slime which was thought to be extracellular. Negative staining revealed the nature of slime, to be attached as capsules. The slimy nature of the organisms was attributed to the presence of a capsule and not as a loose secretion. In this current study, it is believable that under most conditions, attached EPS probably represent an adaptation to protect cells from harsh microenvironments such as those prevalent in salt pans.
- The halophilic cultures were not fastidious in their requirements for growth at varied pH, temperature and NaCl concentration.
- All the cultures required a solid substratum for producing slime. Slime/EPS was not produced during growth at 0% salinity even on solid media. Liquid media supported only the growth of cultures devoid of slime production.
- Slime production by cultures was maximum on NTYE media containing 25% crude solar salt, than on NGSM, NP & Kustr media.
- Studies on the relative toxicity of the compounds for the cultures used could be put in the following order- $HgCl_2 > Pb(NO_3)_2 > NiSO_4 > FeSO_4 > CuSO_4 > MnSO_4 > MnCl_2$
- C_8 & C_{10} were most resistant to all the metals used. C_1, C_5 & C_6 appeared to be most sensitive. C_8 & C_{10} were tolerant to high concentrations of 5mM Mn^{+2} & Pb^{+2}, hence it would be possible to use C_8 & C_{10} as indicators of lead pollution.
- Exopolymer production was inhibited when the cultures were spread plated.
- All the cultures can be placed in the Family Halobacteriaceae. Some cultures lysed in distilled water, were resistant to penicillin & sensitive to bile salt, thus confirming their haloarchaeal nature. However, some of the halophilic cultures can be placed in a novel group of halophilic archaea based on variations in lysis in distilled water, resistance to Penicillin and sensitivity to bile salts.
- Pigment analysis of the isolates showed that the peaks represent some of the pigments dehydrosqualene for non-pigmented & dehydrosqualene & carotenoids for pigmented cultures, a characteristic of haloarchaea.
- The trait of lysis of the halophilic archaea with distilled water was made use of; in obtaining the EPS from the culture; although a number of chemicals and organic solvents were tried.
- Partially purified EPS from cultures showed a greater Alcian blue dye binding tendency than crude EPS.

- Crude EPS from C_{17} and C_{12} while partially purified EPS from C_{17} and C_{18} could sequester Mn ($KMnO_4$), hence may be used as potential candidates for cleaning-up of the Mn-contaminated environment, leading to metal recovery.
- Viscosity of crude EPS was found to be less than that of purified EPS estimated by Ostwald's viscometer.
- The flocculating activity of biopolymer C_{16} being unique, may have a great potential as flocculating agent to replace available synthetic flocculants.
- The biopolymers from all cultures could form gel, hence may find application in food and mining industry.
- The biopolymers possessed the ability to adhere to the hydrocarbon n-hexadecane, may find use in recovery / bioremediation in petroleum industry.
- EPS from all the cultures could emulsify lipids. Food industry may involve the use of such cultures as suspending agents and stabilizers.

  Thus, this work on biopolymers from halophilic archaea suggests great potential in exploiting them for human welfare.

Statement of expenses towards fieldwork and travel for the second year for the Project No. 47-953/09 (WRO)

Date	Particulars	Amount
4.8.2011	Voucher-Taxi	500/-
7.7.2011	Voucher-Taxi	1000/-
11.4.2011	Voucher-Taxi	900/-
5.3.2011	Voucher-Taxi	1000/-
9.2.2011	Voucher-Taxi	800/-
3.2.2011	Voucher-Taxi	900/-

Total amount sanctioned was Rs. 5000/- where as Expenditure incurred was Rs. 5100/-

Purpose of fieldwork and travel was for reference work and to survey and collect brine and sediment samples from various saltpans of Goa.

P.I. has deposited the equipments purchased under this project in the Department of Microbiology of the College. Books have been accessed and submitted to the Library of the College.

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