

## Executive summary of Final Report of Work Done of UGC-Major Research Project

1. **Title of the Project:** "Microbial Screening and Molecular characterization of potential probiotic strains from Traditional fermented foods/products used in Karnataka, India."
2. **Name and Address of the Principal Investigator:** Dr. M Y Sreenivasa  
Assistant Professor  
Department of Studies in  
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Mysore, Manasagangotri,  
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3. **Name and Address of The Institution :** Department of Studies in Microbiology  
University of Mysore, Manasagangotri,  
Mysore-06, Karnataka, India
4. **UGC Approval Letter No. and Date:** No.F. 40-134/2011 (SR) dated 24-06-2011
5. **Date of Implementation:** 01/07/2011
6. **Tenure of the project:** 3 years (2011-2014)
7. **Total Grant Allocated:** Rs. 9,44,080/-
8. **Total Grant Received:** Rs. 8, 65,352/-
9. **Final Expenditure:** Rs. 8,61,100/-
10. **Objectives of the project:**
  - (a) Screening of traditional fermented food/products for potential probiotic strains used in Karnataka
  - (b) Evaluation of selected probiotic strains for their *in vitro* probiotic properties isolated from traditional fermented food/products
  - (c) Biochemical characterization of the selected probiotic strains isolated from traditional fermented food/products
  - (d) Molecular characterization of the selected probiotic strains isolated from traditional fermented food/products
11. **Whether Objectives were achieved:** YES, All the objectives have been successfully completed and the results have been published.
12. **Achievements from the project**

A total of 15 Traditional fermented products such as Sorghum based traditional fermented food, Sannas, Fermented wine samples such as Nell wine, a wine prepared from the natural fermentation of paddy, Apple wine, finger millet wine, fermented bamboo, fermented soybean etc., have been screened, characterized and documented. Totally 82 Lactic acid bacteria strains have been isolated and characterized. A total of 22 Sequences of Lactic acid bacteria isolates have been deposited at NCBI, GenBank, Maryland, USA and accession numbers are obtained. A novel compound has been isolated which has shown a significant antifungal activity against *Fusarium* species and *Aspergillus* species occurring on animal and poultry feeds. Eight promising *Lactobacillus* isolates have shown significant probiotic attributes have been isolated and maintained in the laboratory. Three potential Lactic acid bacteria strains has been isolated and shown a good biopreservative efficacy of poultry feeds.

### **13. Summary of the findings: (IN 500 WORDS)**

A total of 15 traditional fermented foods such as Sorghum based traditional fermented food, Sannas, Fermented wine samples such as Nell wine, a wine prepared from the natural fermentation of paddy, Apple wine, finger millet wine, fermented bamboo, fermented soybean etc., which have been collected from different parts of Karnataka, India have been screened for the potential probiotic lactic acid bacteria (LAB) strains. The LAB strains were characterized for growth at different temperature, effect of pH, bile salt concentrations, NaCl concentrations, Antibiotic susceptibility test, haemolytic test using 5% human blood, tolerance to artificial duodenum juice, Microbial adherence to hydrocarbon assay. Biochemical characterization such as sugar fermentation tests, catalase test, Arginine hydrolysis has been carried out. Species identification was done by 16s rRNA sequence analysis and sequences were deposited in NCBI Gen Bank and accession numbers were obtained. Further the LAB isolates were tested for the additional health benefits like hypocholesteromic activity, Antibiofilm assay, antioxidant assay and Antifungal activity.

Totally 82 potential LAB strains were isolated from the 15 unexploited traditional fermented foods of Karnataka. The morphological, biochemical and molecular methods revealed the different species of LAB strains viz., *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Enterococcus faecium*, and *Enterococcus durans*. Twenty LAB strains have been sequenced and the sequences were deposited in Gen Bank NCBI and accession numbers were obtained and dendrogram was derived. The strains showed growth at different temperatures (10, 37 and 45°C) and showed tolerance to low pH (2) for 1h. The LAB strains also showed tolerance to 0.3% bilesalt, 6% NaCl and resistant to methicillin, kanamycin, vancomycin and norfloxacin, sensitive to penicillin, amoxicillin and erythromycin. The neutralized Cell free supernatant of eight LAB strains showed good antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Enterococcus faecalis*. All the strains showed negative for haemolytic test. The strains showed varied degree of cell surface hydrophobicity. The results of the present study suggest that the *Lactobacillus* strains isolated and characterized from Traditional fermented product may be used as probiotic strains for therapeutic applications.

### **14. Contribution to the society**

The Potential LAB strains of the present study could be considered as probiotic organisms since they showed good probiotic attributes, as there is a need for a natural therapeutic agent with probiotic benefits. The isolated strain *L. plantarum* of the present study showed good results for the hypocholesteromic ability and after the randomized *in vivo* studies, the strain could be as cholesterol lowering agent. Serum bad cholesterol is one of the major problems to target in the form of natural therapeutic agents. The isolated strains *Enterococcus durans* and *Lactobacillus plantarum* the two potential isolates have showed the reduction of pathogenic biofilm of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. After the *in vivo* validation of these strains for the antibiofilm activities, the same could be used for eradication

of pathogenic biofilms. Pathogenic biofilm is an emerging link to the disease pathogenesis and these are considered as reservoirs of the pathogens. Hence the antibiofilm efficacies of the LAB isolates have been tested and shown promising results and can be used as antibiofilm agents. The isolated strain *Lactobacillus plantarum* exhibited good antioxidant ability indicating the use of the strain during the treatment of oxidative stress associated diseases, however the placebo *in vivo* studies are needed to confirm the use of the strain as natural antioxidant. Oxidative stress and associated diseases are of critical issues and thus the probiotic strain could be useful to be used as an antioxidative agent. Traditional fermented foods documented in the present study explored a good number of probiotic agents that have therapeutic applications. The unexploited traditional fermented foods have been documented and the importance of these have been highlighted. Overall, the potential probiotic agents isolated and characterized in the present study given some novel avenues in the field of probiotic research.

#### **Poster Presentations**

1. Poornachandra Rao K, H. Nagaraja, M.Y. Sreenivasa 2012. Isolation and Preliminary Characterization of Potential Probiotic strains from Traditional Sorghum based fermented food products used in Karnataka. First Annual Conference of Probiotic Association of India and International Symposium on Probiotics for Human Health: New innovations and emerging trends on 27th & 28th August 2012, Gulmohar hall, India habitat Center, New Delhi, India.
2. Poornachandra Rao K, H. Nagaraja, V. Thamankar, M.Y. Sreenivasa 2012. Isolation and Characterization of Bacteriocin Producing Lactic acid bacteria from Srikanth- an Indigenous fermented milk product. International Conference on Advances In Biological Sciences. Department of Biotechnology and Microbiology Inter University Centre for Biosciences, Kannur University, Kannur, Kerala, India.
3. Poornachandra Rao K and M Y Sreenivasa 2013. Potential probiotic LAB strains from Sannas - A traditional fermented food used in Karnataka, India. 54th Annual Conference of Association of Microbiologists of India (AMI-2013) & International Symposium on 'Frontier Discoveries and innovations in Microbiology and its interdisciplinary Relevance' (FDMIR-2013). (November 17-20, 2013). Maharshi Dayanand University, Rohtak, Haryana, India.
4. Poornachandra Rao K, Hemanth Kumar N K, Sreenivasa M Y. 2015. Antioxidant potential of Lactic acid bacteria isolated from traditional fermented foods of Karnataka. Indian Science Congress Association. Jan 3-7, 2015. University of Mumbai. Mumbai, India.

#### **Oral Presentation**

1. Poornachandra Rao K and Sreenivasa M Y. 2015. Probiotic potential of *Lactobacillus plantarum* MYS14 isolated from a traditional fermented food. UGC sponsored national symposium on "Microbes and Human Welfare" 23<sup>rd</sup> and 24<sup>th</sup> March 2015. JSS College of Arts, Commerce and Science, Ooty road, Mysore, Karnataka, India.

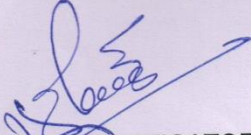
16. Whether any Ph.D. Enrolled/Produced out of the project:

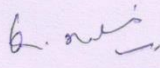
1. Mr. Poornachandra Rao K- UGC-MRP, project fellow has been registered for Ph. D  
(Ph. D reg no: DOR.9.9/Ph.D/PCRK/202/2012-13)


2. No. of publications out of the project:

1. One Research article has been published in the spinger journal, Probiotics and Antimicrobial proteins, Volume 7, Issue 2 Pages 146-156.
2. One Research article has completed the review process with Food bioscience
3. Still another three potential Research manuscripts are under preparation.
4. A total of four posters have been presented in International Conferences
5. One oral presentation in National Symposium with regard to the present work.

(Copies of the same have been enclosed)

  
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