FORMULATION OF PROBIOTICS FROM RICE WASH FERMENTATION

Project Reference No.: *48S_MSC_0271*

College : Maharani Lakshmi Ammanni College for Women, Autonomous, Bengaluru
Branch : Biotechnology
Guide(s) : Dr. Jolitha A B
Dr. Babitha B
Student(s) : Ms. S Afreen Banu
Ms. Rakshitha N
Ms. Sushma N

Keywords:

Probiotics, Rice Wash, Fermentation, Gut Health, Probiotic Formulation.

Introduction:

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. Traditionally, probiotics have been derived from dairy products and fermented foods, but there is growing interest in alternative, non-dairy sources due to lactose intolerance and dietary preferences. Rice wash, a byproduct of rice rinsing, contains a diverse microbial population that could potentially serve as a novel source of probiotics.

Rice fermentation is a traditional practice in many cultures, often resulting in the production of beneficial microorganisms such as lactic acid bacteria (LAB). These microorganisms are known for their gut health benefits, including enhancing digestion, modulating the immune system, and preventing pathogen colonization. However, the potential of rice wash fermentation as a probiotic source remains underexplored.

This research aims to isolate, characterize, and evaluate probiotic microorganisms from fermented rice wash and develop a stable probiotic formulation. Additionally, the study will use Wistar rats as an animal model to investigate the efficacy and safety of the formulated probiotics, providing valuable insights into their potential applications in functional foods and therapeutics.

Objectives:

1. To isolate and identify potential probiotic microorganisms from fermented rice wash.
2. To formulate a probiotic preparation using the isolated microorganisms and evaluate its stability and viability under storage conditions.
3. To study the biochemical and microbiological properties of the probiotic formulation.
4. To assess the efficacy of the formulated probiotics in improving gut health and other physiological parameters using Wistar rats as an animal model.
5. To analyze the safety profile of the isolated probiotic strains by conducting toxicity and histopathological studies.

Methodology

Materials Required:

1. Rice (various varieties), sterile containers
2. Incubator (25–30°C), MRS agar and broth
3. Gram staining kit, catalase test reagents
4. 16S rRNA sequencing kit and PCR reagents
5. Centrifuge, freeze dryer, encapsulation materials
6. Acid and bile salt solutions
7. Pathogens: E. coli, Salmonella spp.
8. Intestinal epithelial cell lines, microplate reader
9. Wistar rats (6–8 weeks, 180–200 g), standard diet
10. Biochemical assay kits: SCFAs, LDH, ALP, lipid profile
11. Antioxidant enzyme assay kits (SOD, catalase)
12. Histopathology reagents, microscope

Experimental Steps:

1. **Isolation of Probiotics:**

   Collect rice wash and ferment at 25–30°C for 24–48 hours.

   Streak on MRS agar to isolate LAB.

Perform Gram staining, catalase tests, and 16S rRNA sequencing.

2. **Formulation**:

   Grow isolates in optimized broth.

   Freeze-dry or encapsulate for stability testing under various storage conditions.

3. **In Vitro Characterization:**

   Test acid and bile tolerance.

   Assess antimicrobial activity against E. coli and Salmonella spp.

   Evaluate adhesion to intestinal cells.

4. **Animal Studies:**

Groups / Rice type	Control group	Positive control group	Low dose group	High dose group
1.Raw rice	1R1	1R2	1R3	1R4
2.Ration rice	2R1	2R2	2R3	2R4
3.Boiled rice	3R1	3R2	3R3	3R4

5. **Biochemical Analysis:**

   Measure SCFAs using gas chromatography.

   Perform LDH and ALP assays in serum and tissues.

   Analyze lipid profile and antioxidant enzyme activity.

6. **Safety Evaluation:**

   Conduct acute/sub-chronic toxicity studies.

   Perform histopathological analysis of liver, kidney, and intestine.

Result and Conclusion:

The final result will be summarised after the completion of the study.

The current status includes

- Preliminary studies conducted in our laboratory.

- The study showed that all the three types of rice washes had lactobacillus sp.,

Project outcome and Industry relevance

Expected Project Outcome:

- Potential probiotic strains will be isolated from fermented rice wash.
- A stable and viable probiotic formulation will be developed.
- The isolated strains will demonstrate acid and bile tolerance.
- Antimicrobial activity will be observed against gut pathogens like E. coli and Salmonella.
- The organism isolated will be tested in wistar model.
- Increased SCFA production and antioxidant enzyme activity will be noted.
- Safety studies will confirm the absence of toxicity or adverse effects.

Industry Relevance:

- The project will offer a non-dairy probiotic alternative for vegan and lactose-intolerant consumers.
- It will enable value addition to rice byproducts, encouraging sustainable practices.
- Findings will support the development of functional foods and nutraceuticals.
- It will align with the growing demand for plant-based, eco-friendly health solutions.
- Products derived from this research will have potential in probiotic drinks and supplements.
- The project will promote cost-effective and scalable probiotic production using local resources.

Working model vs simulation/study

Working Model (Lab-based Experimental Study):

- This is Laboratory based pilot study to determine efficacy of plant based Probiotic.

- We are isolating probiotic strains from rice wash, formulating a product, and testing its properties — this involves hands-on lab work.
- 2nd stage of the study would be animal study to try and compare the isolated organism with existing commercial one.
- Animal trials using Wistar rats are conducted to evaluate physiological effects — this is part of an actual working biological model.
- Biochemical and histological analyses give measurable outcomes, simulating how the product would work in real-life systems.

Simulation:

Not applicable in the traditional computational sense — no computer-based or mathematical model is used to simulate outcomes.

Theoretical Study:

- Literature survey suggested that the current market rate of Probiotics are high and because of its production cost
- Answer also those Probiotics are dairy based and shelf life are shorter.
- From the studies it is seen that plant based Probiotics, especially lactobacillus strains., can be isolated at relatively low cost and its formulation can have a greater shelf life.
- The method can be very simple that it can be produced indigenously at households.
- The study also suggest that Indians were consuming concentrated starch liquid from cooked rice(Ganji) which had Probiotic and soothing effect on intestines.
- Based on that, idea of finding Probiotic organism in rice wash is hypothesised.

Project outcomes and learning

- Hands-on experience in isolation and identification of probiotic microorganisms.
- Familiarity with biochemical testing and molecular techniques such as 16S rRNA sequencing.
- Understanding of probiotic formulation and evaluation of stability and viability.

- Practical skills in assessing acid and bile tolerance and antimicrobial activity.
- Knowledge of designing and executing animal studies using Wistar rats.
- Experience in conducting biochemical assays for gut health, enzyme activity, and lipid profile.
- Competence in performing toxicity studies and histopathological analysis.
- Ability to analyze experimental data and interpret results effectively.
- Improved skills in scientific documentation, report preparation, and presentation.
- Awareness of the industrial potential and relevance of non-dairy probiotic formulations.

Future scope

- The future scope of this project includes
- Large-scale production and commercialization of rice-based probiotic supplements.
- Development of non-dairy probiotic beverages targeting lactose-intolerant and vegan consumers.
- Integration of probiotic strains into functional foods such as health drinks, yogurts, or snack bars.
- Genetic characterization and enhancement of probiotic strains for improved functionality.
- Clinical trials to evaluate efficacy in humans for gut health, immunity, and metabolic regulation.
- Exploration of other traditional fermented rice products for novel probiotic sources.
- Potential for patenting unique strains or formulations derived from rice wash.
- Scope for collaboration with the food, nutraceutical, and pharmaceutical industries.
- Use of AI or biosensor-based tools for monitoring probiotic growth and product quality.
- Expansion into prebiotic-probiotic (synbiotic) combinations using rice-based substrates.