

# Isolation and In-Vitro Characterization of Lactobacillus SP, From Traditionally Fermented Black Rice (Karupu Kavuni Arisi) and Brown Rice (Kai Kuthal Arisi)

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**Abstract:-** Probiotics are live and beneficial organisms that helps to maintain the natural balance of gut microbiome. Traditionally fermented Indian foods act as a rich source for isolation of probiotic bacteria. Rice based fermented foods are nourished with micro and macro nutrients. The consumption of Black rice (karupu kavuni arisi) is believed to increase the longevity of the life. The Brown rice (kai kuthal arisi) is excessively packed with wholesome nutrients and vitamins. Black rice and Brown rice have huge advantage over white rice when it comes to nutrient content. The present study focus on Isolation and screening of probiotic organisms from traditionally fermented black rice and brown rice. Different Strains were identified according to morphological, biochemical and physiological criteria. The potential probiotic features was determined by invitro characterization test. The efficient strain was selected and confirmed in accordance with Bergey's Manual of Determinative Bacteriology which can be used as an ideal probiotic substitute for medical application and also to fulfill the economic needs.

**Keywords:-** Probiotics, Fermented Rice, Isolation, Characterization, Identification, Lactobacillus Sp,

## I. INTRODUCTION

Probiotics are viable, non pathogenic organism intended to provide health benefits. The beneficial probiotics regulates the function of intestinal layer to act as barrier against pathogenic organisms and chemicals. The search for new probiotic strains has increased in recent years due to emerging health hazards and economical needs. Probiotic therapy is unique because it recreates the normal flora in a beneficial way without any adverse effects even at low cost. The probiotics contain dominant groups of bacteria known as lactic acid bacteria (LAB). *Lactobacillus* and *Lactococcus* are grouped under (GRAS) Generally Recognized as Safe due its non pathogenic nature (1).

The important aspect of probiotics is to regulate immune response which stimulates the healthy reactions against infectious organisms. The potential role of probiotics is to promote intestinal lactose digestion which has positive influence on intestinal and urogenital microbiome in antibiotics and radiation induced colitis, yeast infections and vaginitis women. It also aids in prevention and reduction of intestinal tract infections like *Candida enteritis*, *Helicobacter pylori* and leads to the improvement of immune system by providing antagonistic environment for pathogens by blocking its adhesion sites (2).

The main source of probiotics is edible foods which has hidden medicinal properties. Fermented foods are designated as "naturally fortified functional food" build with physiologically active ingredients that aids beyond basic nutrition (3).

Traditionally fermented Indian foods are loaded with friendly bacteria which makes the food highly nutritious. In India, rice has attained its unique status since ancient times because of its nutritional and medicinal values. In comparison with dairy based products rice is one of the most promising alternative foods that naturally promotes the growth of probiotic bacteria. Black rice (karupu kavuni arisi) is highly packed with rich vitamin and mineral content. Black rice is an alternative food for healthy and disease free life as it contains antioxidative agent, such as proline and anthocyanin (4). Brown rice (kai kuthal arisi) has been consumed by our ancestors because of its promising health benefits. Brown rice contains beneficial nutrients and vitamins which can be consumed as an replacement of white rice. These indian foods will naturally leads to the discovery of new drugs. The aim of the present research work was to isolate and screen the probiotic microbes from fermented black rice and brown rice. The traditional materials and methods were adopted for the fermentation process. Probiotic strains was identified by morphological, biochemical and invitro characterization test. The phenotypic characterization was done according to Bergey's Manual of Determinative Bacteriology.

## II. MATERIAL AND METHODS

### A. Sample Preparation (5)

Black rice (karupu kavuni arisi) and Brown rice (kai kuthal arisi) belong to family *Poaceae* were procured from local market, Coimbatore. The raw black and brown rice were cooked with water for 30min to obtain soft consistency. Fermentation was carried out by soaking cooked rice (200g) in water (rice:water 1:3) for 8 to 10 hours in earthened pots at ambient temperature(27°C).

### B. Identification and biochemical characterization of isolated strain

Fermented rice steep (10ml) from both cooked black and brown rice were collected separately after mixing. Dilution method was adopted by using saline (0.85%) at different concentrations. Aliquots (100µl) of the prepared dilution was inoculated on to different media using spread plate technique and the plates were incubated at 37°C for 24 hrs. The plates with typical colonies were randomly selected and tested by standard methods (6). The isolates were grown at different temperatures (10,25,37,45 and 55°C) in MRS broth and MRS agar plate. Gram positive and catalase neagative strains were selected and checked for fermentation ability of different sugars (Glucose, Lactose, Fructose and Mannitol) according to (7). The tubes containing different sugars were inoculated with the test strains and noted for the colour change and gas production.

### C. Invitro Characterization of Potential Probiotic Strain

#### ➤ Ph Tolerance

The selected strains was tested for pH tolerance according to (8). MRS broth tubes was adjusted to pH (2.0,3.0,4.0,5.0,6.0 and 8.0) using HCL and NaOH. The one ml of test strains were inoculated and incubated at 37°C for 24 hrs. OD was measured at 600 nm after 24 hrs of incubation.

#### ➤ Bile Tolerance

Bile tolerance for selected strains was performed according to (9). About one ml of test strains were inoculated in to MRS broth tubes supplemented with (0.05, 0.1, 0.20,0.30 and 0.50%) of bile and incubated at 37°C. Absorbance was read at 600 nm at after 24 hrs of incubation.

#### ➤ Nacl Tolerance

NaCl tolerance for selected strain was performed according to (10). About one ml of test strains were inoculated in MRS broth tubes with varying NaCl concentration ( 1,3,5 and 7%). The tubes were incubated at 37°C for 24 hrs and the final OD was measured at 600nm after 24 hrs of incubation.

#### ➤ Phenotypic Characterization

The identification of selected strain was done based on their morphological, biochemical and physiological characters by comparing it with the Bergey's Manual of Determinative Bacteriology (11).

## III. RESULTS AND DISCUSSION

### A. Identification and Biochemical Characterization of Isolated Strain

A total of twelve strains were selected based on the colony morphology and growth in selective MRS agar plate. Ten strains showed gram positive rods, some was short rods, 2 strains were found to be cocci and all the bacterial isolates were non spore formers and found to be non motile. Catalase test showed negative result. All the strains grown well at 25°C, 37°C and 45°C but no growth were obtained at 15°C. Based on the gram nature, morphology, growth in MRS agar and catalase test four strains were selected for sugar fermentation analysis and the results were reported in table 1. Similarly (12) reported that strains are gram positive, catalase negative, non spore forming irregular short rods which grows well at 45°C. Four strains were found to ferment glucose, lactose, fructose and mannitol. Similarly (13) reported that the strain isolated from idli batter ferments glucose, lactose, fructose and mannitol respectively.

Test	ST1	ST2	ST3	ST4
Gram staining	gram +ve rods	gram +ve rods	gram +ve rods	gram +ve rods
Endospore staining	non spore former	non spore former	non spore former	non spore former
Motility test	non motile	non motile	non motile	non motile
Catalase	-ve	-ve	-ve	-ve
<b>Sugar fermentation</b>				
Glucose	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve
Fructose	+ve	+ve	+ve	+ve
Mannitol	+ve	+ve	+ve	+ve

Table 1:- Morphological and biochemical characteristics of selected strains (ST1-Strain1, ST2-Strain 2, ST3- Strain 3, ST4-Strain 4)

➤ Legend: -ve - negative result, +ve – positive for sugar fermentation.

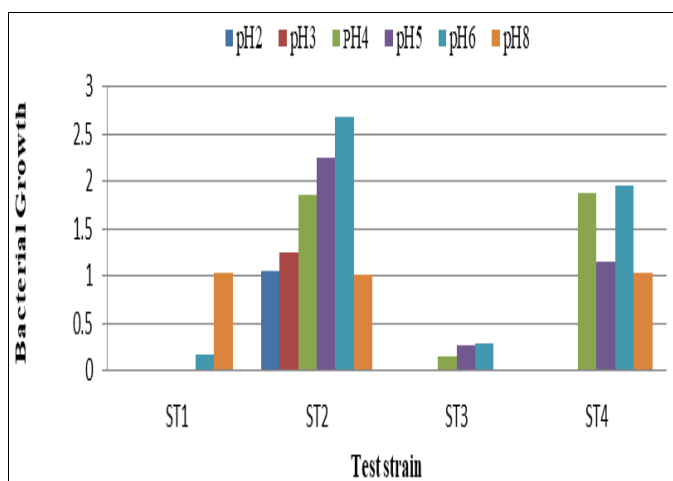
### B. Invitro Characterization of Potential Probiotic Strain

The positive strains are assayed for Bile tolerance, salt tolerance test along with pH.

#### ➤ pH Tolerance

Lactic acid bacteria has to pass through stressful conditions to reach the small intestine. pH tolerance test was performed to identify the ability of survival rate of (ST1,ST2,ST3and ST4) at different pH levels. The isolates that showed growth at different pH (2,3,4,5,6 and 8) was shown in graph1 (ST2) tolerated and grown at different pH levels. The strain also had ability to grow even at lower pH.

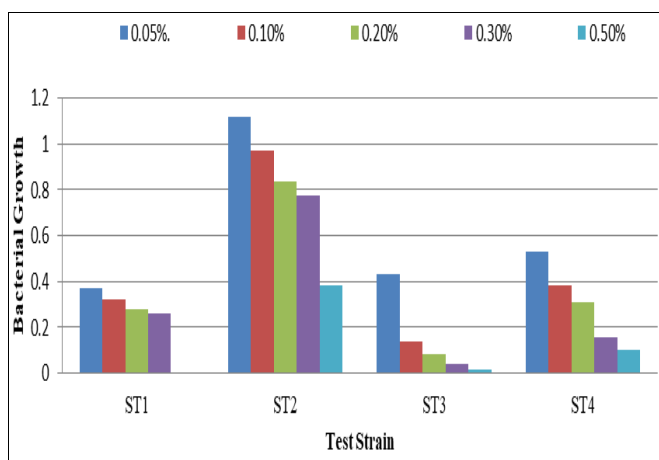
LAB strains showed survival at lower pH (2,3,4 and 7) isolated from south Indian fermented foods (12).



Graph 1:- Analysis of pH tolerance for selected strains (ST1-Strain1,ST2-Strain 2,ST3- Strain 3.ST4-Strain 4).

➤ **Bile Tolerance**

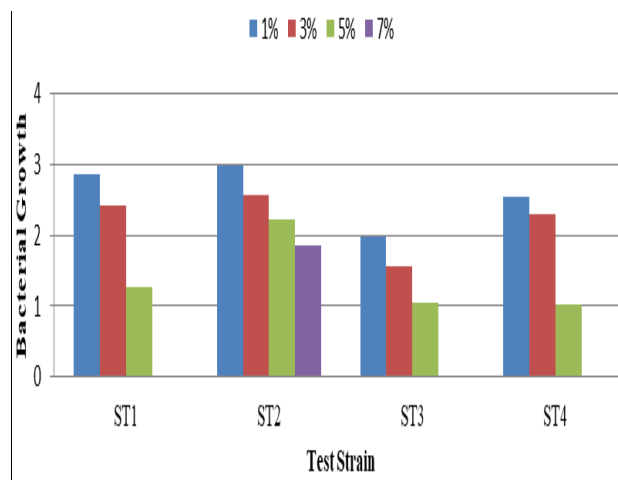
The bile concentration of small intestine is 0.3% which is an essential factor for the growth of lactic acid bacteria. Graph 2 shows the growth of bacteria at different bile concentrations (0.05, 0.1, 0.20,0.30 and 0.50%) among all the strains (ST2) showed better tolerance. The LAB isolates from idli batter also showed good resistance to 2% bile salt even after exposure for 48 hrs (13).



Graph 2:- Analysis of Bile tolerance for selected strains (ST1-Strain1,ST2-Strain 2,ST3- Strain 3.ST4-Strain 4).

➤ **NaCl Tolerance**

NaCl concentrations (1,3,5 and 7%) was selected to check the ability of (ST1,ST2,ST3 and ST4) was shown in graph 3. All the strains were able to grown at 1,3 and 5% of NaCl concentrations but (ST2) alone showed growth at 7% of salt concentration. Correspondingly LAB isolates from south Indian fermented foods tolerated to grow at 4 to 8% of salt concentration (12)



Graph 3:- Analysis of NaCl tolerance for selected strains (ST1-Strain1,ST2-Strain 2,ST3- Strain 3.ST4-Strain 4).

➤ **Phenotypic Characterization of (ST2)**

The selected strain was found to tolerate bile, NaCl and pH effectively. The (ST2) was confirmed to be the *Lactobacillus* sp., by morphological, biochemical and physiological characteristics according to Bergey’s Manual of Systematic Bacteriology (7) Similar results were reported by (14) from various food samples.

**IV. CONCLUSION**

*Lactobacillus* sp., isolated from fermented rice was able to tolerate high bile concentration, low pH level and high salt concentration. The in vitro characterization test also notified that the isolated strain can reach the intestinal tract in good numbers. This potential strain can be recognised as safe which makes a way for wide application. The industrial utilization of strain will lead to the discovery of new drugs. The positive perspective of the strain can be applied as probiotics for the production of functional foods. However, The bacterial isolate has to be further identified by gene sequencing and in vivo experiments to reveal the possible health benefits.

**ACKNOWLEDGMENT**

We would like to thank Sri Ramakrishna College of Arts & Science for Women, Coimbatore-641004 for their technical support.

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