

Study on the Fermented Boiled Srilankan Red Rice- Scientific and Traditional View

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Abstract Rice is one of the major cereals and essential food for Srilankan population. It provides dietary energy and nutrition. But it is found that even after cooking, rice may not be completely digestible by the human body. Many of the micronutrients present in the grain may not be bio-available for absorption. People who mainly depend on freshly cooked rice may suffer from deficiency of micronutrients. The presence of phytic acid in rice and other food grains decreases the bio-availability of micronutrients such as phosphorous, magnesium, iron and zinc. It also inhibits the enzymes pepsin, amylase and trypsin. Due to the nutrient inhibiting property phytate is considered an anti-nutrient. The fermented rice which was consumed by most of the ancient people has several health benefits. The present study involves the isolation and identification of bacteria in fermented rice which break-down the anti-nutritional factors and improves the bio-availability of micro-nutrients. In this study, the presence of lactic acid bacteria was confirmed in the fermented rice by gram staining and some biochemical tests.

Keywords *Phytic acid; fermented rice; bio-availability*

1. Introduction

Among the 100 trillions of cells that make up the human body, only 10% what we would call human cells. Other cells that make up our body, 90% are bacteria. The microbial ecosystem that envelopes human body is important to maintain the wellbeing of an individual. The modern medicine focuses on fighting against the pathogens through the antibiotics. Undoubtedly, they produce miraculous improvement in infectious diseases. But it should be thought how the world community could control the pathogens before the invention of the antibiotic. So the forces of evolution are more powerful than anything medical science can come up with. Therefore the disease can be controlled or cured by improving the public nutrition which strengthens the human body to fight off potential infection on their own. The human gut has thousands of different species of bacteria that are part of the human symbiote live in the gut, mainly in the large intestine. Many foods, carbohydrates can only be digested if gut flora is present where the probiotic bacteria plays major role in the digestion. The symbiote is in

facilitating absorption of essential nutrients and mineral from all manner of foods. The symbiote bacteria make some of the essential vitamin that the human body needs. It K, Vit .B 12, & B5 are made up in the gut and absorb through the lining of the gut. The human gut is the front line where the body's immune system end and our symbiote bacteria begins, 90% of bacteria present in the gut. Keeping the bacteria ecosystem in the gut in a stable symbiotic balance occupies most of the resource of the human immune system.

The one day water soaked boiled rice is known as 'Palam Sooru' (fermented rice) among folklore people. Significance of 'Palam Sooru' has been explained in relationship to every sphere of human life including social culture and religion. The review of traditional siddha medical literature indicate the medicinal properties of 'Palam Sooru' such as reduce body heat, cure peptic ulcer, abdominal pain and constipation. Fermented rice water known as Palamsootru kanchi or Nisineer also used as healthy water in natural medicine in folklore practice in ancient times. It provides energy and helps with stomach issues like bloating, constipation and diarrhea, thus prevent dehydration act as effective electrolyte solution. In addition regulate the body temperature and also protect skin from the sun due to cooling effect and cures acne and red blisters of the face. Due to optimal PH of fermented rice water, it keeps hair shiny, long hair, improving skin elasticity, reducing surface friction and preventing grey hair. In spite of its great importance in day to day human life, scientific approach with respect to microbial and chemical studies are not much expedited. Therefore the present generation is reluctant to take these types of traditional foods nowadays. Folklore usage of this food would act against pathogens which would have enhanced the healthy life of ancient people. Therefore there is a constant and urgent need to develop new strategy to introduce this type of traditional food among the young generation of urban and town life where the scientific findings of medicinal value should be expedited. The present study was done to see whether the boiled fermented rice has useful micro flora which can enhance intestinal flora and increase the digestibility.

2. Materials and Methods

Rice was cooked and excess water was drained. It was allowed to cool at room temp. It was soaked fully in water and stored in a container. It was covered and left overnight at room temp. A loopful of inoculums was taken from the surface of the rice and streaked on a nutrient agar medium and incubated at 37 °C for 24hrs.

Preservation and storage of isolates

After purification of isolates, they should be maintained in the laboratory. Preservation and storage of isolates are important in microbial studies. 2-8g nutrient agar powder was dissolved in 100ml of distilled water. Then this medium was shaken in a hot plate with magnetic stirrer. After shaking a small quantity of medium (about 4ml) was poured individually in each small sized Mac-Cartney bottles. Then these bottles were autoclaved at 121 °C for 15mins. After sterilization, bottles were kept in slant position to solidify. A loopful of fresh isolates was streaked individually on nutrient agar slants. After that these slants were incubated at 37 °C for 24hrs in an incubator. The following tests were done in the stored isolates.

Gram staining

One drop of sterile water placed in the centre of the clean slide. A loopful of pure culture was transferred to sterile water. Then smear was prepared on a clean slide. The smear was air dried and rapidly passed over the flame for heat fixation. The slide was cooled at room temperature smear was covered with crystal violet for 1.5 minutes and washed off with distilled water. Then Gram's iodine

was added to cover the smear. After 1.5 minutes this was washed off with absolute alcohol and continuously washed off with distilled water. Then safranin was added and after 3 minutes slide was washed with distilled water and air dried. Then this was observed under the oil immersion objective of the microscope. Gram positive bacteria appear as purple color and gram negative bacteria appear as pink color in this test.

Motility test (hanging drop method)

A drop of water was placed at each corner of the clean cover slip. Then one drop of sterile water was placed on the centre of cover slip by sterile inoculating loop. A loopful of isolate pure culture was inoculated into sterile water with sterile inoculating loop. Then cavity slide was placed on it. After that, slide was turned in an inverted position quickly and carefully. The drop was examined under the high power of microscope for bacterial motility.

Catalase test

A clean slide was taken & one drop of H₂O₂ solution was placed on clean slide. Then a loopful of culture was inoculated into it. Bubble formation was tested under the high power of the microscope. Bubble formation indicates the presence of catalase enzyme. No bubble formation indicates the absence of catalase enzyme.

Oxidase test

Sterile filter paper was placed in a sterile petridish. Oxidizing agent TPDH was poured into it. Then isolated pure cultures were inoculated separately on the paper in the petri dishes by using tooth pick. If the filter paper turns into purple color this indicates the positive results for the formation of oxidase.

Sugar fermentation test

First, 0.1g of phenol red was dissolved in 50ml of distilled water. Then it was filtered through filter paper. This filtrate was named as solution (A) 1g of bacterial peptone and 0.5g of NaCl was dissolved in 50ml of distilled water. This mixture was named as solution (B). The solution (A) and solution (B) were mixed. After mixing, pH was adjusted to 7.4 with pH indicator. After that 1g of sugars (Glucose or mannitol or Galactose or Xylose) was added to the above mixture. Then 10ml of mixture was poured into tubes and immediately plugged with cotton wool. These tubes were autoclaved at 121 °C for 15 minutes. After sterilization the tubes were allowed to cool.

Inoculation

Isolated pure cultures were inoculated in the test tube containing andrade's peptone water with sugars like mannitol, glucose, xylose, arabinose and these were incubated at 37 °C for 48hours. One was kept as control. After incubation, these tubes were observed. In this test color change from red to yellow indicate the positive results. Initial color of control tube is red in color.

3. Results and Discussion

The simple staining and the biochemical tests gave the following results.

Biochemical test	A	B	C	D	E
Shape	Large rod	Small rod	Spherical	Curved rod	Large rod
Colony color	White	White	Yellow	White	White
Gram staining	Purple	Purple	Purple	Purple	Purple
Motility	+	-	-	-	+
Catalase	+	-	+	-	+
Endospore	+	-	-	-	+
Oxidase	-	-	-	-	-
Glucose(acid)	+	+	+	+	+
Growth in air	+	+	+	+	+
Anaerobic growth	-	+	+	+	-

A: *Bacillus* sp

B: *Leuconostoc* sp

C: *Staphylococcus* sp

D: *Lactobacillus* sp

E: *Bacillus* sp

In this study two lactic acid bacilli were isolated from the Srilankan fermented red rice, this conforms the observation made by researchers in the Assam Agricultural University (Robin Chandra Boro and Madhumitha Barooah, 2016). The plenty of Lactobacilli about one million per milliliter to one billion per milliliter indicate the media or food is highly nutritive because they use the nutrients in the substrate for their own metabolism and cell growth, the primary and secondary metabolites are produced during the fermentation process (Robinson, 1991). The medium that would support their growth must contain a fermentable carbohydrate and many growth factors (Lindquist, 1998). Therefore the excellent some of natural starch which will help friendly bacteria to grow in the digestive system. The Lactobacilli showed stronger antagonize property against gram positive bacteria like *Clostridium perfoegens* and *Staphylococcus aureus* and gram negative bacteria like *E.coli* and *Salmonella typhimurium* (Gilliland and Speck, 1977).

4. Conclusion

The Srilankan fermented red rice showed the presence of the lacto bacilli bacteria like *Leconostoc* sp and *lactrobacillus* sp which antimicrobial substance had led their potential as natural preservatives because it may be used to combat the growth of pathogens mechanism and also breakdown the anti-nutritional factors in rice that result in the improved bioavailability of micronutrients such as Phosphorus, Calcium and Iron.

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