

Acid producing bacteria Isolated from Brown Rice Vinegar

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Abstract- Organic brown rice was fermented under anaerobic condition to produce rice vinegar. Lactic acid bacteria which were grown in fermented rice vinegar (pH 4, total acidity 6%) were isolated. Totally six bacterial isolates were identified as *Lactobacillus* spp. by some biochemical characteristics. All isolates gave clear zone which were cultured on MRS agar supplemented with 0.3% CaCO₃. The cell concentration of the strain BRV 4 was higher acid producing than any other probiotic tested when they were grown in MRS medium. Colonial morphologies of all isolates were convex and white-creamy. All isolates were gram positive and shape of rod. Among these isolates, BRV 4 gave catalase positive and the rest were negative and BRV2 gives methyl red negative. As for motility test, all strains showed negative results. Moreover, the carbohydrate fermentation test (glucose, sucrose, dextrose and lactose) were carried out, it has been found that, all isolates can ferment all carbohydrates.

Index Terms- Brown rice vinegar, Anaerobic Condition, Lactic Acid Bacteria, *Lactobacillus* spp.

I. INTRODUCTION

Rice vinegar is made from fermented rice. Brown rice vinegar tastes better than other vinegar from the store, add the flavor to get more tasteful. BRV can also use for herbs and spices. Vinegar is a the fermented food or cleanser by bacteria. The BRV gives its tangy flavor and also the ingredient that makes useful for household cleaning.

Definition of probiotic

The microbes in BRV that protects its host and prevents disease. The best-known probiotic in BRV is *Lactobacillus acidophilus* which is found in yogurt acidophilus milk and supplement. Probiotic counter the decimation of helpful industrial bacteria by antibiotic. Probiotic given in combination with antibiotic. It can promote the intestinal tract... Among the probiotic in BRV *L. acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, and *Lactobacillus reuteri*. *Bifidobacterium* strains include *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium infantis*. This probiotics provides dairy products specifically, yogurt like products that is probiotic products. The consequences of this quality with respect to consumer perception are that

- Fermented foods already have promoted as being healthful.
- Consumers are familiar with fermented products contain viable microorganisms.
- Probiotics organisms combine the positive images of both fermented food and fermentation organisms.
- The fermented food like products as healthful foods that is recommend for consumption of probiotics.

In addition, there is the important technologic reason for the use of fermented products as carriers of probiotics: many of these products have already been optimized to some extent for survival of the fermentation organisms. Thus, the existing technology can be relatively easily adapted to guarantee sufficient survival of the added probiotic bacteria. However, it must be pointed out that other fermented products (eg, raw sausages and sauerkraut) can serve as carriers of probiotic organisms, but few such products are already on the market.

Test	Result
Spore forming test	Negative
Catalase test	Negative
Methyl Red test	Positive
Voges Proskauer test	Negative
Citrate test	Negative
Starch hydrolysis	Negative
Glucose fermentation test	Positive(Acid and gas)
Tentative identification	<i>Lactobacillus</i> spp
Lactose fermentation test	Positive

Objectives

- To study the traditional method of rice fermentation
- To isolate acid producing bacteria from fermented food.
- To assume the isolated bacteria are safe to consume.

The chemical composition of the BRV are acidic, available carbohydrate content, nitrogen sources, mineral content, water activity, and oxygen content, and possible interactions of the probiotics with the starter cultures (eg, bacteriocin production, antagonism, and synergism).

II. MATERIAL AND METHODS

Material

Brown rice, brown sugar and salt were collected from Ocean Super Market, Mandalay All glass wear, chemical and lab facilities were used in Department of Biotechnology, Technological University (Kyaukse).

Methods

Traditional fermentation methods, weight brown rice, brown sugar and salt were used to add together into distilled water. And released gas and shaken very well every day. After five days, brown rice vinegar was collected by sieving. Bacteria were incubated by plate culture methods on De Man, Rogosa and Sharpe agar (MRS). It is selective culture media for *Lactobacilli*. The Gram stain technique is a differential stain, a procedure that distinguishes between various microorganisms according to their ability to retain certain dyes. The Gram stain depends on the ability of some bacterial cells to resist decolorization longer than others. After another water rinse, you have created a differential stain with purple gram-positive bacteria and colorless gram-negatives. To see the gram-negative cells, it is helpful to add the pink counter stain, safranin.

Brown rice vinegar was measured pH by using pH paper star paper. Cn) Detecting of the acid content was made by titration methods. The present of catalase enzyme in the isolate is detected using hydrogen peroxide. If the bacteria possess catalase, when the amount of bacteria isolate is added to hydrogen peroxide, bubble of oxygen are observed. It is catalase positive. Bubble are not observed, negative. Methyl red broth is used for MR test. Inoculate tube containing Methyl red broth with a pure culture of the microorganism under investigation. Incubate at 35°C for up to 4 days. After that add about of the methyl red indicator solution to the tubes. If the color of the

medium change to red, it is positive reaction. If the color change yellow negative medium.

III. RESULT

The brown rice were fermented by traditional method for rice vinegar. The air were released in the fermented bottle because the culture of anaerobic probiotic bacteria were needed. After five days, rice vinegar was measured pH-4, acid content were concerned by titration method (Table 4.1). So it was assume that bacteria in it acid producing bacteria. Probiotic bacteria were culture by plate culture method by using MRS media under anaerobic condition (Plate 4.1 and Plate 4.2). All six isolated samples are gram positive (Plate 4.3) bacteria and five samples are catalase negative. Five samples are methyl red positive. Therefore, it was assumed that sample BRV₁, BRV₃, BRV₅ and BRV₆ may be *Lactobacillus spp.* (Table 4.2)

Table 4.1 Physico chemical properties of FRV

Sample	pH	Alcohol	Acid
Sample1	4	–	0.023
Sample2	4	–	0.03
Sample3	4	–	0.03

Table 4.2 Morphology and Biochemical Properties of Isolated Bacteria

Sub culture	Cony –Mor	Gram	Shape	Catalase	MR
BRV1	White-Creamy Convex	+	rod	–	+
BRV2	White-Creamy Convex	+	rod	–	–
BRV3	White-Creamy Convex	+	rod	–	+
BRV4	White-Creamy Convex	+	rod	–	+
BRV5	White-Creamy Convex	+	rod	+	+
BRV6	White-Creamy Convex	+	rod	–	+

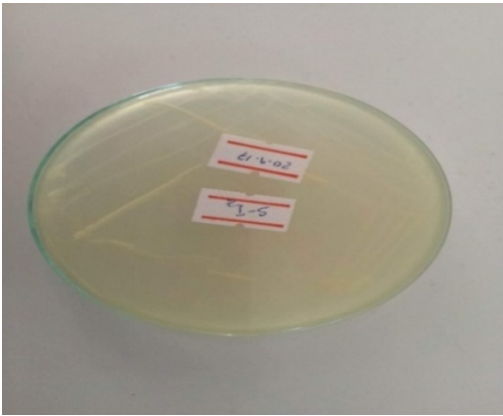


Plate 4.1

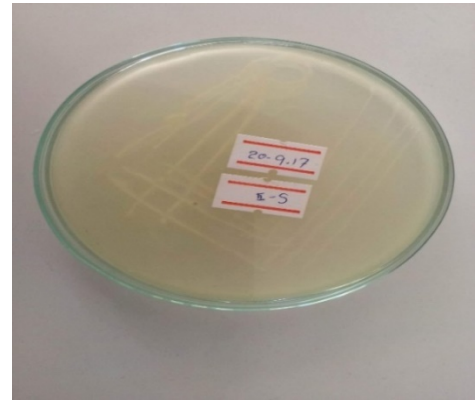


Plate 4.2



Plate 4.3

IV. DISCUSSION AND CONCLUSION

Six acid producing bacteria were isolated from three sample bottle in traditional rice vinegar. It can be examined that culture on MRS media, titration for acid content, measure pH, alcohol and specific gravity. The isolated bacteria samples were isolated by gram staining by gram and test by biochemical reaction. According to result data, SI₁, SII₁, SIII₁ and SIII₂ are assumed as the Lactic acid producing bacteria but SI₂, SII₂ are not. Because SII₂ is catalase positive and SI₂ is Methyl red negative. According to literature Lactic acid producing bacteria are catalase negative.

BRV₁, BRV₃, BRV₅ and BRV₆ may not confirmed, Lactic acid producing bacteria. It was needed to test acid in rice vinegar that was lactic acid and biochemical reaction for confirmation. Other probiotic bacteria will collect and study for further study. And BRV will be measured its nutritive value for production and food safety.

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