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ISOLATION AND CHARACTERIZATION OF PROBIOTIC BACTERIA FROM FERMENTED KANJIKA SODA ON THE BASIS OF AMYLASE PRODUCTION

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ABSTRACT

Fermented beverages made from fruits and vegetables are the greatest source of probiotic bacteria and are been consumed from ages. The fermented beverages can be consumed in place of milk based probiotic foods to avoid the problems like lactose digestion, regertation. The present study was to prepare vegetable Kanji soda (Kanjika) drink considering it to be beneficial to human health. Different samples were prepared and various isolates were obtained. Acceptibity of the fermented soda drink was done by sensory evaluation test. Various biochemical tests, gram staining, morphological analysis was done to select the potential bacteria. Starch hydrolysis test was performed for the analysis of amylase production by the potential bacteria. Out of 14 isolates, 8 isolated showed this probiotic potential. Overall the fermented kanji soda drink was found a health promoting drink exhibiting probiotic properties.

Keywords: Fermented Beverage, Gram Positive, Health Promoting, Probiotic Bacterial Isolates, Starch Test

I INTRODUCTION

The term 'Probiotic' is well explained by the FAO and WHO expert team that a large number of microorganism exists in nature which includes probiotic bacteria that offer health benefits on the host body when administered in adequate amounts [1]. LAB are a large group of bacteria which are gram positive, non-spore forming, catalase negative cocci or rods which produces lactic acid as major end product from fermentation of carbohydrate. They are generally designated as safety (GRAS) microorganisms as they are heath promoting ones and had shown effective against many health problems and various disorders [2].Probiotics is like a boon to human health .Various health problems include disturbed gut flora, gastrointestinal syndrome, diarrhea, irritable bowel syndrome, weak immune system, allergy, immunomodulatory activity, antimutagenic/anti-carcinogenic activity, cholesterol problem, dental

problems ,bacterial vaginosis [3,4] Fermentation is one of the oldest and healthiest methods of food preservation around the world. Fermented vegetables are vegetables processed and preserved by the action of microorganisms. There are various categorization of fermented foods like cereal based (with / without pulses) fermented foods, cereal/pulses and buttermilk based fermented foods, milk based fermented foods, vegetable / unripe fruit based fermented foods , meat based fermented foods [5].

Kanjika or kanji is a lactic fermented rice product. It has been prescribed by various medical practitioners as a cure of many chronic diseases [6]. Carrot Kanji have high nutritional value as well as cooling and soothing properties [7]. Beetroot kanji is found beneficial for preventing infection and malignant disease [8].Bile tolerance and gastric juice tolerance is the main characteristic feature of probiotic bacteria ,as to maintain gut flora the bacteria has to resist high bile salt concentration , high pH and acid concentration [9,10].

II MATERIAL AND METHODS

2.1 Sample collection

Beetroot and carrot was bought from the Gwalior sabji mandi and edible sodium carbonate, sodium bicarbonate and sugar was bought from confectionary shops.

2.2 Measurements of the components for the preparation of novel fermented beverage

Vegetables were chopped longitudinally and sodium carbonate, sodium bicarbonate and sugar were measured accordingly. All the samples were fermented for 7 days and offered good taste without spoilage .The fermentation process was not extending for more days to avoid spoilage.

2.3 Preparation of Test samples

All the components along with the chopped vegetables were added to the glass bottles containing hot water . The hot water helped in extracting the liquid from the vegetables .The bottles were capped so that the soda formed may not escape out of the bottles. The samples were kept in room temperature .The presence of effervesces showed the presence of soda in the samples. The samples were mixed well each day without opening to avoid the release of soda. Due to the presence of beetroot, the sample turned pink.

2.4 Sensory Evaluation

Out of 10 panelists, 6 liked the taste , 3 found to be sour and 2 found it average in taste but all of them found the taste of soda .When maximum accepted the taste of the fermented beverage ,the beverage was studied for the strain isolation and studing its probiotic characteristics

2.5 Strain Isolation

- 1ml from each sample was pipetted out and added to MRS broth and mixed well which then incubated at 37 C for 24-48hrs.
- 1ml of sample was taken and mixed well with 9ml of distilled water .From this suspension, 1.0 ml solution was again pipetted and transferred into second test tube containing 9.0 ml of distilled water and mixed well.
- This series of dilutions were made up to 10^{-6} dilutions .MRS agar was prepared and 100 µL from 10-4 and 10-6 dilutions was spreaded evenly on plates by the help of spreader .
- The petriplates were incubated an-aerobically at 37^{o}C for 24 hours.
- After incubation several white coloured colonies were isolated and sub-streaked again on MRS agar plates to obtain pure colonies.
- These colonies were further characterized by observing their colonial morphology and some physiological tests [11].

2.6 Morphological identification and Gram staining

The colonies obtained were gram positive round, rod shaped shiny white colonies, convex elevated and smooth rough textured.

TABLE 1: Morphological and Gram staining Characterization of Isolates

S. No.	Isolates from fermented beverage	Gram staining	Shape
1.	Isolate 1	+	Rod shape
2.	Isolate 2	+	Rod shape
3.	Isolate 3	+	Rod & cocci shape
4.	Isolate 4	+	Rod & cocci shape
5.	Isolate 5	+	Rod shape
6.	Isolate 6	+	Rod shape
7.	Isolate 7	+	Rod shape
8.	Isolate 8	+	Rod shape
9.	Isolate 9	+	Rod shape
10	Isolate 10	+	Rod & cocci shape
11	Isolate 11	+	Rod & cocci shape
12.	Isolate 12	+	Rod shape
13.	Isolate 13	+	Rod shape
14.	Isolate 14	+	Rod shape

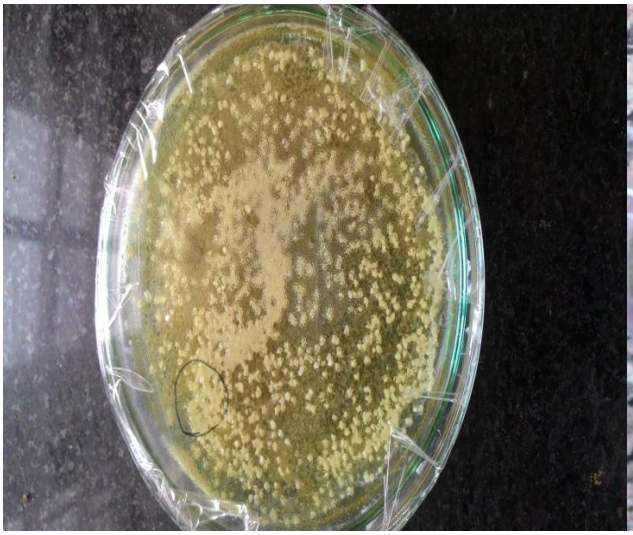

Fig 1 : Spreaded probiotic colony on MRS agar

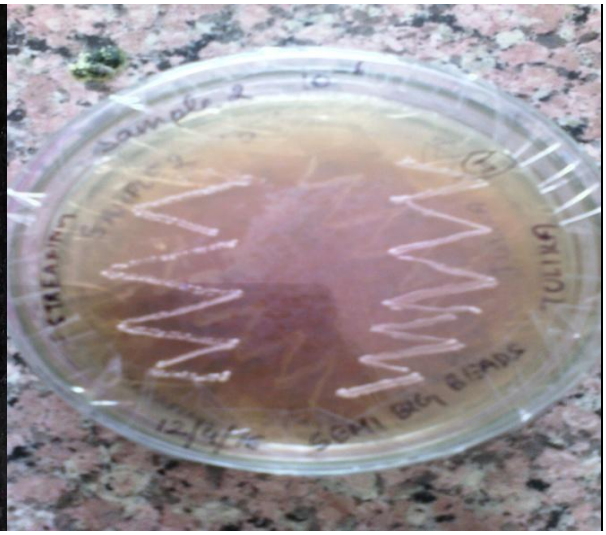

Fig 2: Streaked probiotic white colony on MRS agar plate

2.7 Biochemical Test

Various biochemical tests were applied on bacterial isolates for evaluation of probiotic potentiality. Catalase, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, indole test and triple sugar iron were conducted to characterize probiotic organisms present in the Fermented vegetable beverage.

2.7.1 Starch hydrolysis

2.7.1.1 Principle

This test is performed to test the utilization of starch by bacteria by producing the enzyme Amylase. Amylase is an exoenzyme that hydrolysis starch by breaking a polysaccharide into monosaccharide and disaccharide such as glucose. Starch is a complex carbohydrate composed of glucose molecules that are linked together by α-1,4 and α-1,6 glycosidic bonds. To check the ability of a microorganism to degrade starch by producing amylase is done by starch hydrolysis test [12]

2.7.1.2 Procedure

- Starch agar media (Himedia) was prepared and poured in sterilized petriplates and allowed to solidify.
- Isolates were streaked on plates and incubated for 24hrs.
- After incubation Gram's iodine solution (Lugol solution) was spreaded and excess iodine was removed.
- The plates were observed for zone of clearance around the bacterial growth .If the clear zone was obtained it gave positive result and if the media turns black then it was a sign of negative result [12]

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Probiotics	Iso 1	Iso 2	Iso 3	Iso 4	Iso 5	Iso 6	Iso 7	Iso 8	Iso 9	Iso 10	Iso 11	Iso 12	Iso 13	Iso 14
Starch Agar Test	+	+	+	+	-	-	-	-	+	+	+	+	+	+

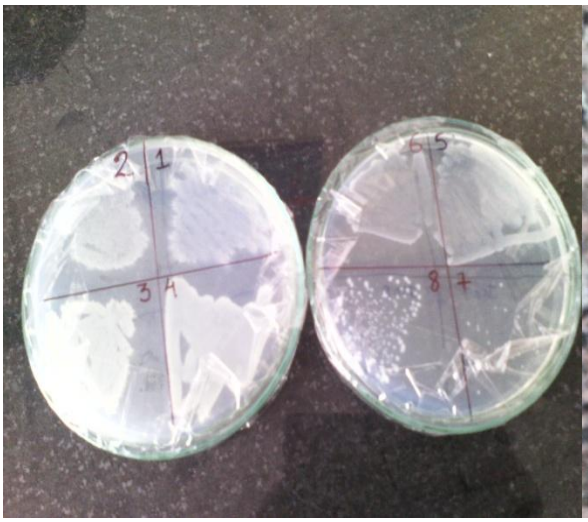

Fig 3 : bacterial growth on starch agar plate after incubation

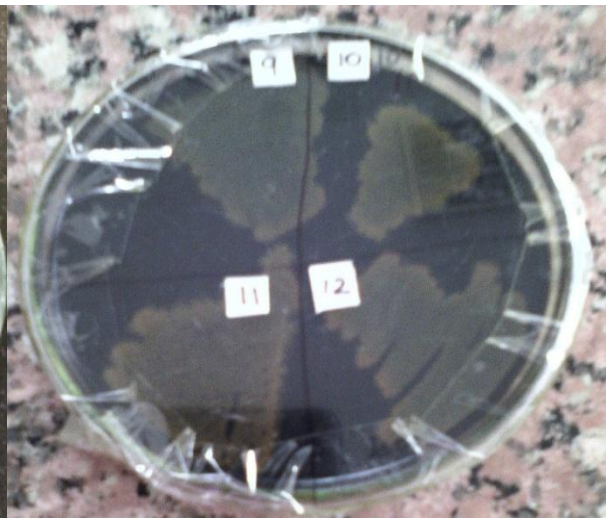

Fig 4: Clear zone on adding iodine solution showing positive test

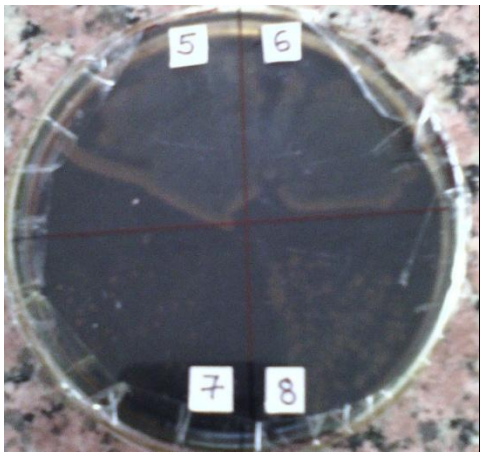

Fig 5: Black zone on adding iodine solution showing negative test for isolates 5,6,7,8

III CONCLUSION

Out of 14 isolates obtained from the vegetable soda beverage, all of them were Gram positive and exhibited rod and cocci shaped structure. Out of 14 isolates 10 isolates gave positive results for starch agar hydrolysis test which is the

biochemical test of the identification of probiotic bacteria. Apart from this encapsulation can be done to increase the shelf life and can be used in pharmaceuticals.

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