Effect of Bile Salts on the bacterial isolates from Fermented Kanji Soda

Tulika Mukherjee
Department of Biotechnology
Madhav Institute of Technology and Science,Gwalior, India

Abstract- **Fermentation is a primitive technique which is been used since ages .Fermentation process creates an anaerobic environment which supports the growth of anaerobic bacteria which leads to the formation of lactic acid as an end product .The present study was conducted to prepare a fermented kanjika soda beverage which was examined for its resistibility against high bile salts concentration and its capability to inhibit diarrheal microorganisms. Various samples were prepared and out of 14 isolates, 7 isolates showed the resistance against 0.3% and 5 isolates showed resistance for 0.5% bile salts concentration. One of the main characteristic feature of probiotic bacteria is to resist high bile salts concentration and survive in that surroundings. Acceptibity of the fermented kanjika soda beverage was done by sensory evaluation test. Overall the fermented kanji soda drink was found a health promoting drink exhibiting probiotic nature and helpful against diarrhea causing agents.**

***Index Terms*— Fermentation, Fermented kanjika soda drink, bile salts concentration, probiotic isolates.**

I. INTRODUCTION

Probiotics is the topic upon the information was limited earlier but now a large number of clinical investigation is been done which gave positive results and proved that they are health promoting and a complement to human health. Today yeast (*Saccharomyces cerevisiae*) has gained popularity and used intercontinentally in many foods and bacteria is used as probiotics, including Lactic acid bacteria of same genera like Lactobacillus, Streptococcus, Enterococcus, Bifidobacterium, Propionibacterium, Bacillus. Some strains of *Escherichia coli* can also be added in this category. Microbes either intrinsic or native or they occurs momentary have impact on the immune system and is directly affect on maintaining gut microflora and immune equilibrium and evenness [1].

The gut acts as a highly eclectic barrier and information delivering organ between the gut bacterial habitat and the host. The miscarriage of this companionship leads to the mucosal restraint dysfunction which gives chances to opportunistic health depleting pathogens to get through the mucosal barrier and disturb the gut micro flora and opens the gateway for various inflammation based disorders [2]. Other than the inflammation disorders ,some critical illness like multiple organ failure ,abdominal surgery ,the mutual deal between equilibrium of the normally symbiotic alliance between the intestinal microbiota, epithelium and immune system are disturbed and obstructed due to the reason of nutrient imbalance , high doses of broad spectrum antibiotics ,pH, psychological imbalance, mood swings etc [3] . Probiotic strains and their various blends have a three way inhibitory actions either by restricting the colonization by secreting antimicrobial bacteriocins and blocking toxin mediated responses [4] or by competitive growth by not allowing the pathogens to utilize the nutrients and uphold [5] or by not allowing them to bind to the receptors and restricting their activity ,occupancy and survival rate [6,7,8] .Some probiotics are known to possess anti-apoptotic properties [9,10] and prevent disruption of tight junctions and joints [11,12]. Most common mechanism of probiotic bacteria is their nature of immunomodulation which is capable of releasing anti-inflammatory cytokines such as interleukin-10 [13] and modulating the highly complex part of human body i.e. human dendritic cell function [14]. According to various review papers and surveys the survivality rate is also a factor which states that probiotics must outlast both gastric acid and bile juice to reach the small intestine and colon where they can evolve healthy micro flora.

Bile- Bile is a dark green to yellow coloured liquid substance whose concoction and storage occurs in liver and gall bladder and finally transferred in duodenum after food consumption .The constituents of bile includes phospholipids, bile acids, cholesterol, pigment biliverdin [15,16] which are in different proportionality and mostly importantly water. Bile functions as a lipid solubilizing agent which is involved in fat digestion procedure. It inhibits the growth of pathogens by puncturing and dissolving their outer membranes [17].Bile acids are absorbed by passive diffusion along the entire gut and by active transport in the terminal ileum [15] whereas reabsorbed bile acids is taken up by hepatocytes and resecreted into bile. Bile salts are the bactericides developed in host body that suppress the activity of unhealthy responsible for disease causing.

Fermented Kanjika Soda- Fermented Kanji (commonly known as Kanjika) an Indian fermented drink whose end product is lactic acid which is prepared from vegetables and suitable for those who finds it difficult to digest milk products and is prescribed by Aryurvedic acharyas as it is helpful in chronic diseases [18]. Kanjika can be served with Urad Dal Vadas which is named as kanji –vada. Carrot Kanji alleviates nutritional value, cooling and calming properties [19] whereas Beetroot kanji is documented for exhibiting protection against various infection and tumour [20].

Soda drinks are now a days consumed at a very high rate due to its taste but have no nutritional value .So the present study is to prepare a blend of Kanjika and soda i.e. Probiotic kanji soda taste health drink which will be capable of showing probiotic nature and inhibit the growth of pathogenic bacteria.
The present study is to isolate the bacterial colonies from fermented kanji soda and testing its resistibility by allowing the LAB to grow in selective media containing different concentrations of bile salts. The recommended concentration is between 0.15%-0.3% for human use and the LAB that can sustain the concentration can be used as probiotics [21].The tolerance to bile salts is due to bile salt hydrolase activity and the bacteria which proves to show this activity can be termed as LAB ,as it is main characteristic feature of probiotic bacteria [22,23]

II. MATERIALS AND METHODS

Sample collection:
Vegetables like Beetroot and carrot to prepare kanji was bought from nearby vegetable market and edible products usually required in making bakery foods like sodium carbonate, sodium bicarbonate, citric acid and sugar was bought from bakery shop.

Measurements of the components for the preparation of fermented beverage:
Vegetables were chopped and washed and sodium carbonate, sodium bicarbonate, citric acid and sugar were measured accordingly for each sample for preventing the drink to go off taste. All the samples were fermented for atleast 7 days and each day the samples were shaked in order to mix the ingredients properly .The fermentation process was not extending for more days to avoid spoilage in taste and smell.

Preparation of Kanji Test samples:
All the components along with the vegetables that were chopped were added to the glass bottles containing drinking mild hot water .The bottles were covered properly to prevent escaping of carbon dioxide. The presence of effervesces in the samples showed the presence of soda which was an important step. All the samples were turned pink due to the presence of beetroot. The sensory evaluation was performed so that the acceptibity and presence of soda can be checked.

Strain isolation:
Each of the sample for tested for the bacterial presence and 1ml of it was taken and added to MRS broth and properly mixed which then incubated at 37 C for 24-48hrs.This series of dilutions were made up to 10^{-6} dilutions.100 µL from 10-4 and 10-6 dilutions was spreaded evenly on MRS agar plates by the help of spreader .The plates were incubated at 37ºC for 24 hours. After incubation white colonies were isolated,some were morphologically different from one another which were then sub-streaked again on MRS agar plates to obtain pure colonies. Gram staining and biochemical test for the identification of colonies.

Biochemical Test
Various biochemical tests were implemented on bacterial isolates for judgment of probiotic potentiality among which Bile salt test is the main characteristic test for studing the resistibility of bacterial isolates and designated it as Probiotic.

Bile Test :
A series of Bile concentration was employed while altering its percentage each time. Two concentration was considered 0.3% and 0.5%,one within the range(0.15%-0.3%)for human use which LAB can sustain and other concentration was considered outside the range in which LAB has less chances of resistibility.

Procedure:
MRS broth was prepared and was divided into two parts , each containing bromocresol purple which will act as indicator .0.3% and 0.5% bile salts was added in previously divided MRS broths. Fourteen test tubes were prepared containing combination of MRS broth, bromocresol purple indicator and bile salt.100 µL of each bacterial isolates was inoculated in the test tubes and incubated at 37ºC for 2-4days to observe the color change.

Antimicrobial activity:
Antimicrobial activities of kanji samples were tested against *Klebsiella pneumoniae , Salmonella typhi , Shigella boydii ,Shigella flexneri and E.coli* and appreciable results were obtained as zone of inhibition .

III. RESULTS AND DISCUSSIONS

Out of 14 isolates obtained from fermented kanji soda ,isolate number 2,8,10,11,12,13,14 could resist the bile salt concentration of 0.3% whereas isolates 6,9,10,11,12,13,14 could resist 0.5% concentration of bile salt rest were not able to resist the either of the concentrations ,hence these isolates was further tested for antimicrobial activity against diarrhea causing pathogens through well diffusion method to study their zone of inhibition.

Table *BILE SALT TEST*

Probiotic isolates	*Bile salt conc. (0.3%)*	*Bile salt conc. (0.5%)*
Isolate 1	–	–
Isolate 2	+	–
Isolate 3	–	–
Isolate 4	–	–
Isolate 5	–	–
Isolate 6	–	+
Isolate 7	–	–
Isolate 8	+	–
Isolate 9	–	+
Isolate 10	+	+
Isolate 11	+	+
Isolate 12	+	+
Isolate 13	+	–
Isolate 14	+	–

III. CONCLUSION

This study showed that the bacterial isolates obtained from kanjika soda is capable to exhibit probiotic property as they can sustain in human gut and can participate in the maintaining and developing the healthy intestinal gut flora .These lactic acid bacteria also have the potential to suppress pathogenic bacteria which can disturb the pH and healthy gut flora. The kanjika soda thus formed showed the presence of soda even after 1 day of refrigeration without producing undesirable odour and taste.

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