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Application of Probiotics in Fruit Juices - Challenge and New Approaches

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Abstract:-Fruit juices could be served as the suitable media for cultivating probiotic bacteria when consumed in enough amounts exert their health benefits. Therefore the present study was carried out to evaluate the synergistic activity of probiotic bacteria with the fruit juices against clinical bacterial pathogens. Lactobacillus sp. was isolated from the curd sample and used in combination with three types of fruit juices (orange, pineapple and pomegranate) and mixture of these juices against the bacterial pathogens (Escherichia coli, Salmonella typhi and Klebsiella spp.). It was observed that orange and pineapple juices have antimicrobial effect on selected bacterial pathogens. Antimicrobial activity of pomegranate juice was observed only against Salmonella typhi and Klebsiella spp. The juices have also shown the antimicrobial effect in combination with Lactobacillus sp. The mixture of three juices has shown good antimicrobial activity as well as synergistic activity with Lactobacillus sp. against tested bacterial pathogens.

Keywords:-Lactobacillus SP., Fruit Juices, Bacterial Pathogens

I. INTRODUCTION

Probiotics are defined as a live microbial supplement, which beneficially affect the host by improving its intestinal microbial balance. Recent research has underlined the importance of a vital and healthy microbial population of the gastro intestinal tract. Increased research efforts during the last three decades, have confirmed the beneficial association of lactic acid bacteria with the human host [1].

Dairy products have been traditionally considered as the best carriers for probiotics; but, it may be also limited by allergies, cholesterol diseases, dyslipidemia, vegetarianism and lactose intolerance; therefore, several raw materials have been extensively investigated [2]. The development of fruit-juice based functional beverages, with the probiotic ingredients can be advantageous in this context. The fruit juices have been suggested as an ideal medium for the functional health ingredients because they contain beneficial nutrients, they have taste profiles that are pleasing to all the age groups [3]. Fruits are considered good matrices and can provide ideal substrates for probiotics, since they contain minerals, vitamins, antioxidants and fibers [4]. Therefore the present study was carried out to study the synergistic activity of probiotic bacteria with the fruit juices against clinical bacterial pathogens.

II. MATERIALS AND METHODS

A. Collection of Curd Samples

Curd sample was collected from the local market of Nagpur region, Maharashtra. The sample was collected in sterile polythene bag and brought to the Microbiology laboratory for isolation of *Lactobacillus* sp. from it.

B. Collection of Fruit Juices

Three types of fruit juices such as orange juice, pineapple juice and pomegranate juice were collected in sterile polythene bags from local fruit market in Nagpur region, Maharashtra and brought to the Microbiology laboratory for further processing.

C. Selection of Clinical Bacterial Pathogens

The three types of clinical bacterial pathogens such as *Escherichia coli, Salmonella typhi* and *Klebsiella* spp. were selected for the studies. Two strains each of these organisms were collected from the pathology laboratory in Nagpur region, Maharashtra.

D. Isolation of Lactobacillus sp. from Curd

- *Serial Dilution*

  The stock culture of curd was prepared (1 ml curd+9 ml sterile distilled water). The prepared samples were serially diluted up to 10^{-5}.

- *Isolation and Identification of Lactobacillus sp.*

  A loopful of curd sample was inoculated on de Man, Rogossa and Sharpe (MRS) agar plate. Inoculated petriplate was incubated at 37^0C for 24 hrs. The isolated bacterium was identified on the basis of morphological, cultural and biochemical characteristics [5].

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E. Synergistic Activity of Lactobacillus sp. with Fruit Juices against

a). Clinical Bacterial Pathogens

- *Addition of Lactobacillus sp. into Each Fruit Juices:* Equal proportion of each fruit juice in combination with *Lactobacillus* sp. was prepared in sterile tubes. In this way three sets of two tubes of each were prepared for three fruit juices such as Orange, Pineapple and Pomegranate Juice.

- *Antimicrobial Efficacy of Fruit JuicesSynergistically with Lactobacillus sp.* The MRS agar plates were lawned with the selected bacterial pathogen and the wells of 6mm diameter were prepared with the help of gel puncture. In two wells, 20 µl each of the specific fruit juice and Ampicillin solution was added separately while in remaining one well, the mixture of specific fruit juice and Lactobacillus culture (10µl each) was added. In this way the procedure was followed for all three types of juices against six strains of clinical bacterial pathogens for one type of set of experiment. In another set of experiment, instead of single fruit juice, a mixture of equal proportion of three types of fruit juices was added in one well, in the remaining wells the above mentioned addition was followed. The plates were incubated at 37^0C for 24 hours for zone of inhibition [6].

III. RESULTS AND DISCUSSION

A curd sample was collected from the local market in Nagpur city. The sample was processed and the Lactobacillus sp. was isolated from the curd sample. It was observed that orange and pineapple juices have antimicrobial effect on selected bacterial pathogens (Table 1). Antimicrobial activity of pomegranate juice was only against Salmonella typhi and Klebsiella sp. The juices have also shown the antimicrobial effect in combination with *Lactobacillus* sp. The mixture of three juices has shown good antimicrobial activity as well as synergistic activity with Lactobacillus sp. against tested bacterial pathogens.

Recent studies reported that fruit juices could serve as suitable media for cultivating probiotic bacteria [7]. The results of the present study agreed with the previous research work [8] [9] in which it was reported that antimicrobial substances produced by Lactobacillus sp. have a great potential for inhibiting the growth of pathogenic microorganisms.

However, mixture of three juices has also shown antimicrobial activity against tested bacterial pathogens. Fruit juices is a good medium for the growth of Lactobacillus sp. Fruits are reported to contain a wide variety of antioxidant components, including phytochemicals [10]. Juices can represent a suitable carrier for probiotics, as they can combine the appearance of healthy and fresh foods, designed for a wide range of consumers, and the healthy benefits from probiotics.

Bacterial pathogens	OJ	OJ+L	PiJ	PiJ+L	PoJ	PoJ+L	MxJ	MxJ+L	A
E. coli 1	17mm	22mm	17mm	19mm	R	R	22mm	24mm	16mm
E. coli 2	16mm	24mm	11mm	18mm	R	R	19mm	25mm	16mm
S. typhi 1	R	R	16mm	24mm	R	R	19mm	24mm	15mm
S. typhi 2	20mm	25mm	13mm	25mm	15mm	19mm	23mm	26mm	13mm
Klebsiella sp.1	13mm	17mm	21mm	23mm	17mm	26mm	23mm	27mm	16mm
Klebsiella sp. 2	10mm	15mm	R	R	16mm	17mm	18mm	18mm	12mm

Table 1 : Probiotic Action of Fruit Juices and Lactobacillus SP.

Where, R- Resistant, OJ- Orange Juice, PoJ- Pomegranate Juice, PiJ- Pineapple Juice, MxJ- Mixture of 3 Juices, L- *Lactobacillus* sp., A- Ampicillin

IV. CONCLUSION

Juices can represent a suitable carrier for probiotics, as they can combine the appearance of healthy and fresh foods, designed for a wide range of consumers, and the healthy benefits from probiotics. Orange and Pineapple fruit juices have shown antimicrobial activity against selected bacterial pathogens while Pomegranate juice showed antimicrobial activity against only against Salmonella typhi and Klebsiella spp. The probioticated juices could differ in their antagonistic activities against the pathogens. Results obtained in this study will be helpful for developing an appropriate probiotic juice with more health benefits which could be served as a health beverage for consumers who are allergic to dairy products.

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