Antimicrobial Activity of Probiotic Microorganisms from Probioticated Carrot Juice against Selective Pathogenic Strains

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Abstract- Probiotics are defined as live microbial feed supplement that beneficially affects the host by improving its intestinal balance. The objectives of the present study were to study the fermentation of carrot juice with probiotic microorganisms and to determine the effects of probioticated carrot juice on inactivation of selected pathogenic microorganisms such as *E. coli, E. faecilis, K. pneumonia, S. dysentrae, S.epidermidis* and *S. aureus* by using agar well diffusion method. Carrot juice was inoculated with probiotic culture and incubated at 37° C for 24 h. The results of the agar well diffusion method showed that probioticated carrot juice were able to inhibit the growth of most of the selected pathogens and show significant increase in anti microbial activity against five pathogenic microorganisms.

Index Terms- Probiotics, Antimicrobial Activity, Pathogenic microorganisms, Probiotic foods, Carrot Juice, Fermentation, Zone Of Inhibition.

1 Introduction

PROBIOTICS are defined as live microbial feed supplement that beneficially affects the host by improving its intestinal balance and are added into appropriate food vehicles, usually fermented milks "[1]". These bacteria affect the gut micro flora positively and decrease the microbial toxic activity "[2], [3]". Several genera of bacteria and yeast have been proposed as probiotic cultures such as *Lactobacillus, Bifidobactreium, Streptococcus, Ruminococcus, Enterococcus, Lactococcus, Leuconostoc, Pediococcus* and *Saccharomyces* "[4], [5]". These bacteria are gram positive, rod-shaped, nonspore- forming, and non-aerobic as well as acid tolerant, aero tolerant, and fastidious, with lactic acid as the major end product of sugar fermentation "[6]". The microorganisms primarily associated with this balance are *Lactobacilli* and *Bifidobacteria*. It is generally recognized that an optimum 'microbial population balance' in our digestive tract is associated with good nutrition and health "[7]".

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Research has shown that addition of probiotics to food provides several health benefits including reduction in the level of serum cholesterol, improved gastrointestinal function, enhanced immune system, and lower risk of colon cancer "[8], [9], [10], [11]". Lactic acid bacteria are commercially used as starter cultures for the manufacture of dairy-based probiotic foods "[12]".

Traditionally, probiotics have been added to yogurt and other fermented dairy products, but lactose intolerance and the cholesterol content are two drawbacks related to their consumption "[13], [14], [15], [16], [17], [18]". Nowadays there is an increasing consumer demand for non-dairy-based probiotic products and these organisms are being incorporated into drinks as well as marketed as supplements in the form of tablets, capsules and freeze dried preparations (e.g. Multibionta, Enterogermina, Reuterina, UltraLevure, Florastor) "[19], [20]". They are safe for people with lactose intolerance or allergy to dairy products and for children suffering from galactosemia "[21]".

Fruits and vegetables are rich in functional food components such as minerals, vitamins, dietary fibers, and antioxidants (phytochemicals). Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population "[22]". Adding probiotic cultures to fresh juice is a novel concept and provides a research opportunity for food professionals "[23]". The Carrot (*Daucus carota L.*) is a widely available

and commonly used vegetable that is rich in functional food components such as vitamins (A, D, B, E, C, and K) and minerals (calcium, potassium, phosphorus, sodium, and iron). Beta carotene content of carrot is 2–10 mg per 100 g of carrot. Carotenoids and other antioxidants present in carrot juice and it is useful in the inhibition of oxidation processes, as well as in counterbalancing free radical activities "[21], [24], [25]". The changes in microbial population and some components in carrot juice during the fermentation with *Bifidobacterium* and *Lactobacillus* species has been seen by Kun et al., 2008. They also tested fermentation of carrot juice with The objectives of the present study were to study the fermentation of carrot juice with probiotic microorganisms, to determine the effects of probioticated carrot juice on inactivation of selected pathogenic microorganisms and comparing it with fresh carrot juice.

MATERIALS AVD METHODS

Materials and Equipment: Carrots were purchased from local market, probiotic capsule from the medical store (Darolac brand, each containing *Lactobacillus acidophilus, Lactobacillus rhamnous, Bifidobacterium longum, Saccharomyces boulardii*), Pathogenic microorganisms including *E. coli, E. faecilis, K. pneumonia, S. dysentrae*, *S.epidermidis* and *S. aureus* From IMTECH, Chandigarh, Mann Rogosa Sharpe (MRS) broth, agar agar, yeast extract, bacteriological peptone, tryptone, Scale for zone of inhibition and beef extract were purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India), NaCl (Merck ltd), Glucose and Mannitol (Rankem), Grinder (Lumix), Incubator (Thermotech), Digital Camera (Canon/Sony), Laminar Flow (Scientech), Micropipette (Nichiryo, Japan), Microwave (LG), Autoclave (Jyoti Scientific Instrument), Refrigerator (LG), Weighing Balance (Citizen).

Methods:

Bacterial strains media preparation: Stock cultures of all pathogenic microorganisms were maintained as shows in Table 1. Inocula for all experiments were prepared by transferring a loop full of stock cultures to the flask containing appropriate medium and incubating at 37° C for 18-24 h.

Table 1: Bacterial strains and there growth medium.

S. No.	Pathogenic bacterial strains	Growth medium
1.	*Escherichia coli* 25922	Luria Bertani (LB) broth medium
2.	*Staphylococcus aureus* 23923	MSA medium
3.	*Entrococcus faecilis*	Nutrient broth medium
4.	*Klebsiella pneumonia*	TSB medium
5.	*shigella dysentrae*	MacConky medium
6.	*S.epidermidis*	TSB medium

Carrot juice preparation: Carrot was washed, peeled, chopped, and blanched for 10 minutes. The pulp was ground by a blender before filtration to obtain carrot juice. The fresh juice was filtered through a muslin cloth and collect in to air tight steel mug "[26]".

Fermentation of Carrot Juice: Fermentation experiments were conducted in air tight steel mug, each containing 40 mL of carrot juice and inoculated with probiotic culture (>10^8 CFU/mL). Both mug (carrot juice & carrot juice with probiotic culture) have tightly packed and incubated at 37° C for 24 h.

Antimicrobial activity test by agar well diffusion method and analysis of Zone of inhibition (ZOI): The agar well diffusion method was used to determine the antimicrobial property of the probiotic culture, carrot juice and probioticated carrot juice. A 24 hr culture of the pathogenic strains were grown in appropriate medium as shown in Table 1 and incubated at 37° C. A lawn of the indicator strain was made by spreading the cell suspension over the surface of Muller hilton agar plates with a sterile spreader. The plates were allowed to dry and a sterile well borer of diameter (5 mm) was used to cut uniform wells in the agar. Each well was filled with 100 µl probiotic culture, carrot juice and probioticated carrot juice. After incubation at 37° C for 24 hrs, the plates were observed for a zone of inhibition (ZOI) around the well. Results were considered positive if the diameter (mm) of the ZOI was greater than 1mm "[27]".

RESULTS AND DISCUSSION

Probiotic microorganisms were found capable of growing well on carrot juice without nutrient supplementation. They grew rapidly on carrot juice and reached nearly 2 x 10^8 CFU/mL after 24 h of fermentation at 37° C.

The results of the agar well diffusion method showed that probioticated carrot juice were able to inhibit the growth of most of the, if not all of the selected pathogens. The spectrum of their antibacterial effects varied. Inhibition of pathogenic bacteria by probiotic culture, carrot juice and probioticated carrot juice are shown in Table 2. Probioticated carrot juice showed the highest antibacterial activity.

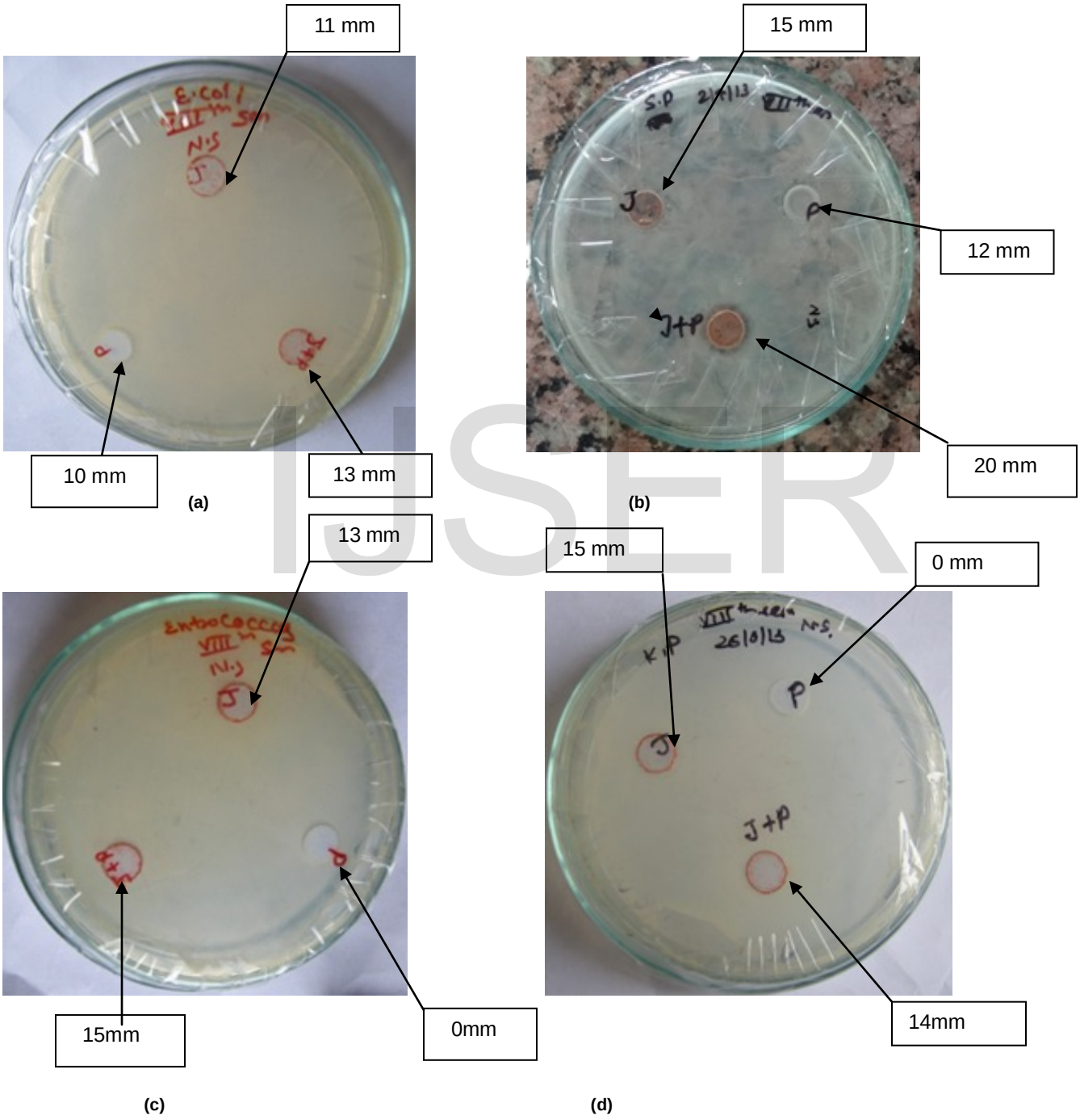
Table 2: Antibacterial activity of probiotic culture, carrot juice and probioticated carrot juice:

S. No.	Test organisms	Sample ZOI(mm)		
		Probiotic culture	Carrot juice	Probioticated Carrot juice
1.	*Escherichia coli*	10	11	13
2.	*S.epidermidis*	12	15	20
3.	*Entrococcus faecilis*	0	13	15
4.	*Klebsiella pneumonia*	0	15	14
5.	*Shigella dysentrae*	0	0	14

6.	*Staphylococcus aureus*	No	No	No

[Note: No = No Inhibition, ZOI = Zone Of Inhibition]

Comparison of the sensitivity of the tested pathogenic microorganisms towards probioticated juice showed that *E. coli, E. faecilis, K. pneumonia, S. dysentrae* and *S.epidermidis* were the most sensitive, while S. aureus was the most resistant, as showed in Table 2 and Figure 1.



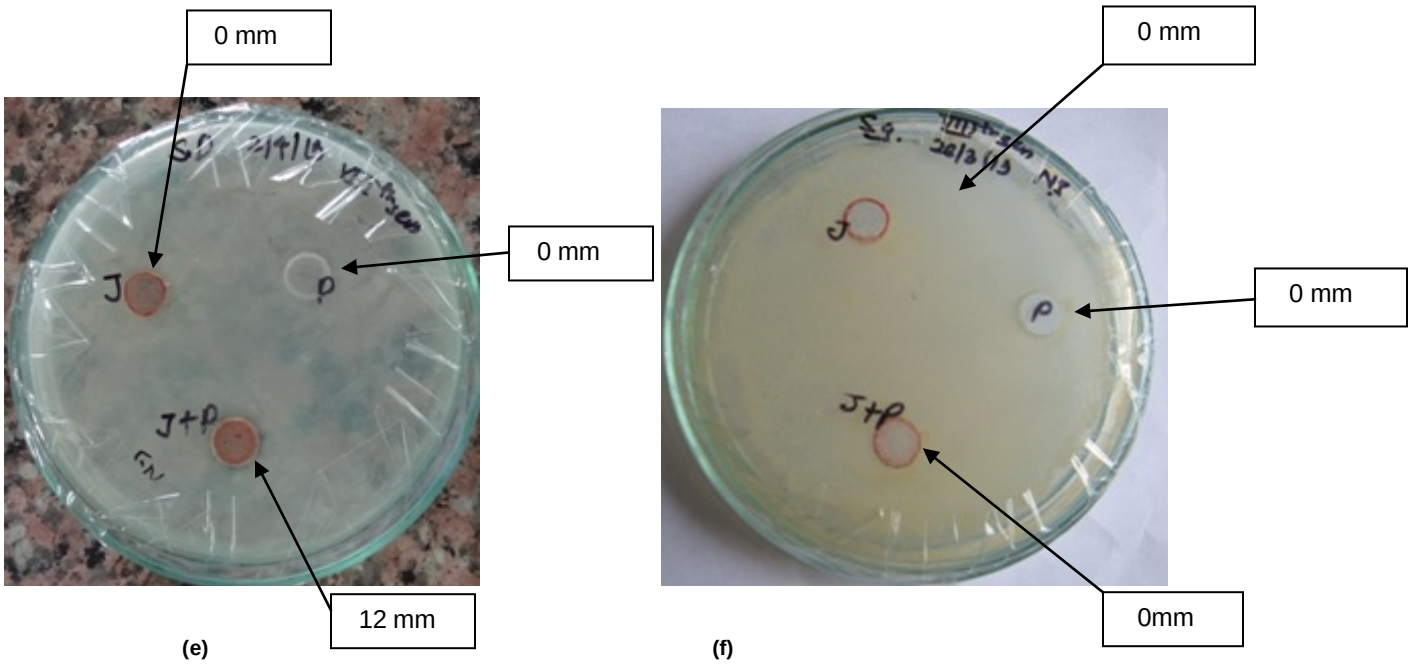


Figure 1:- Antimicrobial activity of probiocated carrot juice against tested strains (a) *E. coli* (b) *S. epidermidis* (c) *Entrococcus faecilis* (d) *K.pneumoina* (e) *Shigella dysentrae* (f) *S .auerus*.

CONCLUSION

Probiotic microorganisms were examined for their ability to utilize carrot juice for cell synthesis and lactic acid production without nutrient supplement. These lactic cultures grew well in carrot juice at 37° C, the viable cell counts reached nearly 2 x 10^8 CFU/mL after 24 h of fermentation at 37° C and show significant increase in anti microbial activity against five pathogenic microorganisms namely *E. coli, E. faecilis, K. pneumonia, S. dysentrae* and *S.epidermidis*.

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