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In vitro Study of Anti-diarrhoeal Activity of Synbiotic Cabbage Juice against Dysentery Causing Organisms

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This study evaluated that the effect of prebiotic food containing fructo oligosaccharide to enhance the growth and activity of probiotics strains to select a suitable prebiotics for the development of synbiotic fruit juice. The anti microbial activity of cabbage juice probioticated using different strains of *Lactobacillus kefiranofaciens, candida kefir* and *Saccharomyces boluradii.* Anti microbical activites of synbiotic juice could differ in their antagonistic activities against diarrhoeal causing organism which could be due to the metabolite secreted by the lactic acid bacteriocin special type of organic acids and added inulin as a prebiotic and for food preservation.

Key words: Probiotics, Inulin, Cabbage, Diarrhoea, Antimicrobial activity.

A synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the friendly flora of the human intestine. A synbiotic product should be considered a functional food rather than some obscure chemistry formulation. In the synbiotic present scenario, food is no longer consumed for satisfaction of hunger alone but for promoting nutrition and health. The concept of functional foods has gained universal acceptance as a preventive and therapeutic approach to combat many disease that decrease the work productivity due to poor health. The objectives of the study were to isolate and identify the beneficial bacteria [probiotics] from fermented milk sample such as yoghurt, kefir, butter, cheese, and koumiss. Five species of probiotics isolated, and its combinational approachment to treat against diarrhoeal causing organisms. Effective combinational group of organisms is identified, and inoculated with cabbage juice and allowed for fermentation. Administration of prebiotics, the non-digestable food ingredients that beneficially affect the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the column thus improving host health offers an attractive alternative. Among prebiotics, non-digestable carbohydrate like inulin and oligofructose have received much attention. The keeping above facts in view, present investigation was undertaken to evaluate prebiotics strains for their compatibility with cabbage juice in the presence of inulin for synbiotic fruit juice preparation.

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One half cup of cabbage, cooked, boiled, drained with no added salt has 0.95 grams protein, 17 calories and 1.4 grams of dietary fiber and it also contain potassium, phosphorus, magnesium, calcium, iron, sodium, zinc, copper, managanese, selenium, vitamin C, niacin, vitamin B1, vitamin B2, vitamin B6, folate, pantothenic acid, vitamin A,K,E, and also contain some other vitamins in small amounts. Chemical analysis of juice performed by gas chromatography and mass spectrometry showed the presence of fatty acids.

In the present study, was to determine antagonistic action of synbiotic fruit juice against diarrohoeal causing organism.

Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) analyses

The identification of volatile constituents from juice was performed using a gc clarus 500 perkin elmer equipped with a mass detector turbo mass gold perkin elmer, turbomans5.2 injector temperature 250c.The ge-ms electron ionization system was set at 70e. A sample of the oil was methylated and solubilized in ethyl acetate for analyses the quantification of the components was performed on the basis of their gas chromatography / flame ionization detector peaks on the hp-5column.

MATERIAL AND METHODS

Sample source and enrichment technique

Fermented milk sample was collected from market was used for isolating probiotic bacteria and yeast. The milk sample was inoculated and allowed to ferment at room temperature for a week spontaneously without any additives through the milk endogenous microorganisms. The enrichment process of the identified collected organisms inoculated same was carried out as follows, low volume of inoculated milk was added to 80ml MRS [Deman rogosa and sharpe] broth medium in 150/ ml conical flask. The enriched samples were incubated under static conditions. The high volume of the media provided suitable conditions for the facultative anaerobic micro organisms and made it unnecessary to incubate the samples anaerobically. The enrichment process was conducted in triplicate and repeated on weekly basis for one month period.

Isolation characterization and identification of probiotic bacteria and yeast

The isolation process was carried out by streaking the enriched samples on MRS agar media and the isolated bacteria were incubated at 37^0C, the isolated bacterial cultures were characterized and identified using colony morphology, bio-chemical test and in selective medium carbohydrate fermentation. Five species of probiotics were isolated. To identified best combinational approachment group of probiotics to confirmed and inoculated in to the fruit juice.

Characterization and identification of probiotics

A number of bacterial and yeast species were isolated from the fermented milk sample, and were identified as probiotics. The five isolated organism were further identified to effective against diarrhoeal causing organisms, the best combinational approachment of probiotic bacteria &yeast confirmed as a probiotic by using acid tolerance test, bile tolerance & cell adhesion test etc. From which three probiotic species were distinguished namely, *Lactobacillus kefiranofaciens, Candidakefir and Saccharomyces boluradii.*

Probiotication of synbiotic cabbage beverage

Cabbage was purchased from a local market. Juice was prepared from homogenized skin less slices and was filtered it properly and100 ml of cabbage juice were inoculated with 2 ml of MRS broth containing probiotic yeast and bacteria. *(ie L. kefiranofaciens,Saccharomyces boluradii,Candida kefir*) they were allowed for fermentation. After fermentation, juice were separated in to two different containers. One of that containers inulin could be added. This was an invitro study on the antibacterial activity of synbiotic cabbage beverage against five diarrhoeal causing organisms.

Test organisms

The bacteria used as test organisms were *Staphylococcus aureus, Enterotoxigenic Escherichia coli, Vibiro cholerae, Salmonella paratyphi A,* and *Shigella dysenteriae*. These were procured from MTCC (Microbical Type Culture Collection) IMTECH Chandigarh india.

Preparation of inoculum

Inoculum was prepared by adding one loopful of test pathogen in 50ml of BHI broth and then incubated at 37^0C for 24hrs.

Agar well diffusion method

The anti-bacterial activity of symbiotic beverage was done by Agar well diffusion method. Sterilised MHA medium was poured in to sterile petriplates and allowed to solidify. After solidification of the medium, the inoculums was

Table 1. Characteristic feature of Probiotics

Characteriistics	*L.kefirano faciens*	*L.mesenteroides*	*L.bulgaricus*	*Candida kefir*	*S.boluradii*
Cell wall	G+ve	G+ve	G+ve	Chitin mannose PPM, PLM	Chitin mannose PPM, PLM
Morphology	Rod	Cocci	Rod	Yeast like pseudohyphae	Pseudohyphae
Motility	NM	NM	NM	-	-
Spore forming	NS	-	NS		
Selective medium	MLR	TJA	LBB	YMA	SGA
Growth at					
15^{0}C-20^{0}C					
20^{0}C -30^{0}C	+			+	+
30^0 C-40^{0}C		+	+		
40^0 C-50^{0}C					
PH3.5				+	+
4.5	+	+			
6.5			+		
8.5					
Salt					
6.5	+	+	+	+	+
10%					
Carbo hydrate fermentation					
Arabinose	+	+	+	+	+
Cellobiose	+	+	+	+	W
Esuculin		-	+	+	-
Fructose	+	W	+	+	+
Galactose	+	+	W	+	-
Gluconicacid	+	+	+	-	+
Lactose	+	+	-	+	+
Maltose	+	+	+	-	+
Mannitol	+	+	+	-	+
Mannose	+	+	+	-	+
Mellibiose	+	+	+	+	+
Raffinose	-	+	+	-	+
Rhamnose	-	+	+	-	+
RiboseSalicin	++	++	++	--	++
Sorbitol	+	+	+	-	-
Sucrose	+	+	+	-	+
Xylose	-	+	+	-	+

(+) - growth
(W) - Weak Growth
(-) - No growth
PPM - Phophopetidomannan, PLM - Phospholipomannam
MLR - Modified Lactobacillus Agar medium TJA - Tomato Juice Agar medium
LBB - Lacto bacillus bulgaricus agar medium YMA - Yeast morphology agar medium
SGA - Sabrouds glucose medium NM - Nonmotile
NS - Nospore

Table 2. Combinational approachment of probiotics against diarrhoeal causing organisms

Organism	T_1 (1)	T_2 (2)	T_3 (3)	T_4 (4)	T_5 (5)	T_6 (1+2)	T_7 (1+3)	T_8 (1+4)	T_9 (1+5)	T_{10} (2+3)	T_{11} (2+4)	T_{12} (2+5)	T_{13} (3+4)	T_{14} (3+5)	T_{15} (1+2+3)	T_{16} (1+2+4)	T_{17} (1+2+5)	T_{18} (1+3+4)	T_{19} (1+3+5)	T_{20} (1+4+5)	T_{21} (2+3+4)	T_{22} (2+3+5)	T_{23} (3+4+5)
S. auroeus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E.Coli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S.Paratyphi A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. Dysentriae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
V. Cholerae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\+ - > 1 to 10
++ - > 10 to 20
+++ - > 20 to 30

1 -*Lactobacillus kefirano faciens*
2 -Leuconostoc mesenteriodes
3 -Lactobacillus bulgaricus
4 -Candida kefir
5 -Sacharomyces boluradii

vortexed and 100 ml of the inoculum was spread evenly over the surface of the agar plates using a glass spreader. A well of 8 mm diameter was made in each plate with a sterile cup borer. The symbiotic beverage (100 ml) was introduced and into the separate plate and in a single well. (Fresh juice & fermented juice with inulin). The fruit juice extract were allowed to diffuse at room temperature for 24 h. the plates were then incubated at 37^0C for 48 h. The antagonistic test was performed intriplicate. The efficiency was determined by measuring the diameter of zone of inhibition exhibited by the synbiotic beverage against test pathogen.

After incubation, the diameter of the inhibition zone around the well was measured this clear zone was used an indication of the ability of symbiotic beverage antagonize to the tested pathogen. The diameter of the clear zone (mm) was determined by measuring the diameter between colonies and four different points of the clear zone surrounding the colonies and reporting the average.

RESULTS

Invitro antagonstic activity of synbiotic cabbage beverage against diarrhoeal causing organism

On the basis of result obtained, there was a significant in antagonistic effect against the pathogens between fruit juice, fermented juice, fermented juice with inulin, however, among three samples (s1, s2, & s3) demonstrated higher antagonistic activity was shown by s3 sample. Inhibition of bacterial growth compared with that of s1, and s2, the s3 was more effective. The precding studies have shown protective and therapeutic effects of synbiotic beverage vs diarrhogenic organisms our study showed that, anti bacterial activity of synbiotic beverage exists at effective against *Staphylococcusaureus, Shigella dysenteriae* and *Vibriocholrae* compared with other two pathogenic organism.

DISCUSSION

A synbiotic nature in the gut should be maintained which is a mixture of a proibiotic and a prebniotic, and the rationale for this combnination is that the prebiotic is used to stimulate the growth of probiotic in the gut there by increasing its

Table 3. Inhibitory activity of synbiotic beverage against test pathogens

Pathogens	S_1	S_2	S_3
Staphylococus aureus	+	++	+++
Escherichiacoli	+	++	+++
Salmonella paratyphi A	+	++	+++
Shigella dysenteriae	+	++	+++
Vibriocho lerae	+	++	+++

S_1 - Cabbage juice
S_2 - Juice probicated with organism
S_3 - Fermented beverage with Inulin

effectiveness. Thus prebiotic approach though diet increases residence bacteria which are beneficial to human health. The inhibitory action of probiotic bacteria and yeast is mainly due to the accumulation of main primary metabolites suchas lactic acid, aceticacids, ethanol and carbon dioxide. Additionally, they are also capable of producing anti microbial compounds such as formic and benzonic acids hydrogenperoxide, diacetylacetion, and bacteriocin. The production levels and the proportions among these compounds depend on the strain, medium compounds and physical parameters (Tannock 2004) probiotics has shown

Table 4. Compounds identified from the fermented cabbage juice

No.	RT	Name of the Compound	Pear Area %
1	12.09	Decanoic acid, decyl ester	16.67
2	16.39	n-Hexadecanoic acid	2.68
3	19.28	d-Mannitol, 1-decylsulfonyl	7.63
4	22.47	Hexanedioic acid, bis(29ehtylhexyl) ester	42.80
5	24.71	1,2 Benzenedicarboxylic acid, mono (2ethylhexyl) ester	30.23

to process inhibitory activities mostly towards g+ve pathogens and closely selected bacteria due to the bactericidial effect of protease sensitive bacteriocins (Jacketal 1995) still lactic acid bacteria were also able to control the growth of gram negative pathogens including food borne pathogens by the pdn of organic acids and H_2O_2. (Lu and Walker 2001 Itoetal 2003) Cabbage juice fermented with *L.kefiranofaciens,* kefier and Bolud and inulin might be a good source of probiotic

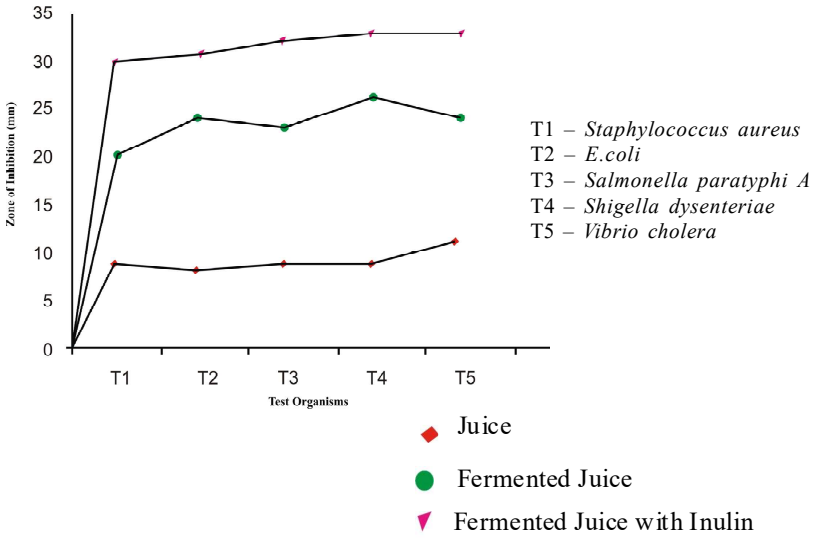

Fig. 1. Effect on synbiotic cabbage beverage against pathogens

Fig. 2. Cabbage

Fig. 3. Fermented Cabbage Juice

Fig. 4. Prebiotic Inulin

lactobacilli and also nutritional components even after 2 weeks storage at 4^0C. In addition it would be completely functional when ingested. On the whole, the results impact positively towards a broader utilization of cabbage juice suitable on the basis of a complex functional production with higher added values.

Cabbage juice were effective against diarrhoeal causing organisms. The chromatograms and compounds from fermented cabbage juice are prevented in Fig 1, Table 3. The chromoto graphic analysis of oils obtained from the juice of fatty acid, palmitic acid, stearic and the unsaturated acids, ollic besides metalinic acids (benzene

Fig. 5. Antibacterial Activity

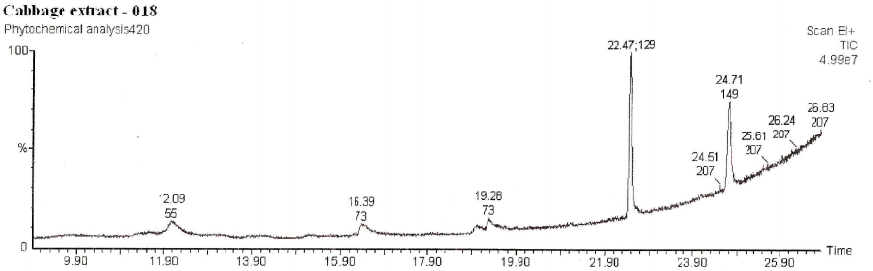

Fig. 6. Section of chromatogram from the fermented cabbage juice

dicarboxylic acid) fatty oils of fermented cabbage juice can be used as natural "anti bacterial potential activity" after further studies. It can be concluded that fatty oils of 'fermented cabbage juice' can be used for developing plant derived anti microbial drugs

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