

## Production of synbiotic corn extract: application against diarrhea causing microorganisms

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### ABSTRACT

Production of functional foods containing health-promoting components has experienced rapid growth in recent years. In this study, a prebiotic containing fructo-oligosaccharide was incorporated to corn extract to stimulate the growth of probiotics strains and development of a synbiotic corn product. The antimicrobial activity of probiotic corn extract using different strains of probiotics *Lactobacillus kefirifaciens*, *Candida kefir* and *Saccharomyces boulardii* was determined. Antimicrobial activities of synbiotic extract could differ in their antagonistic activities against diarrhoeal causing organism which could be due to the metabolite secreted by the lactic acid bacteriocin especially type of organic acids and added inulin as a prebiotic and for food preservation.

**Keywords:** corn extract, diarrhea, microorganisms, food industry, nutritional value, consumers.

### 1. INTRODUCTION

Nowadays the common concern of the food industry and food scientists is the nutritional value of foods that would make them more acceptable for consumers. However, there is a trend towards the development of foods containing components with an additional biological function, the so-called functional foods [1], [2].

Incorporation of probiotics in several foods such as cornflakes [3], pomegranate juice [4], Doogh [5], cheese [6], fermented drink [7], yogurt [8-11], fermented milk [12] is reported. Probiotics possess many health beneficial intrinsic properties for their host. Also, they can produce many useful metabolites during their growth and metabolism like the production of bioactive compounds [13], conjugated linolenic acid [14], and propionic acid [15]. Recently, reduction of oxidative stress and inflammatory factors [16, 17], removal of toxins and heavy metals [18] are stated for these amazing microorganisms. To stimulate probiotic growth prebiotics are administered [19-21].

Diarrhoea is an intestinal disorder's common symptom and one of the global threats to human health [22]. It is a reason for mortality over 4 million deaths annually especially in children under 5 years old. It is the second children killer disease in the developing countries. Diarrhoea caused by *Escherichia coli* is common in the world [23, 24].

As stated in the definitions, a synbiotic is a supplement that contains both a prebiotic and a probiotic that works together to improve the friendly flora of the human intestine [25]. 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 [26]. The concept of functional foods has gained universal acceptance as a preventive and therapeutic approach to combat many diseases that decrease the work productivity due to poor health [27]. The objectives of the study were to isolate and identify the beneficial bacteria [probiotics] from fermented milk sample such as yogurt, kefir, butter, cheese, and koumiss [28]. 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 Corn Extract and allowed for fermentation. Administration of prebiotics, the non-digestible 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 colon thus improving host health offers an attractive alternative [29]. Among prebiotics, non-digestible 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 Corn Extract in the presence of inulin for synbiotic fruit extract preparation [30]. The Extract contains potassium, phosphorus, magnesium, calcium, iron, sodium, zinc, copper, manganese, 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 [22, 31]. In the present study, was to determine the antagonistic action of synbiotic fruit extract against diarrhoeal causing organism.

### 2. EXPERIMENTAL SECTION

**2.1. Sample Source and Enrichment Technique.** The fermented milk sample was collected from the 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 80 ml MRS

(demanrogosa and sharpe) broth medium in 150 ml conical flask. The enriched samples were incubated under static conditions [29]. The high volume of the media provided suitable conditions for the facultative anaerobic microorganisms and made it unnecessary to incubate the samples anaerobically. The enrichment process was conducted in triplicate and repeated on a weekly basis for one month period.

**2.2. 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°C, the isolated bacterial cultures were characterized and identified using colony morphology, biochemical test and in selective medium carbohydrate fermentation. Five species of probiotics were isolated to identify best combinational approachment group of probiotics to confirmed and inoculated into the fruit Extract [32, 33]. The isolated bacterial cultures were characterized using colony morphology, biochemical test and in selective medium, carbohydrate fermentation (Table 1).

**Table 1.** Characteristic Feature of Probiotics.

Characteristics	<i>L. kefirano faciens</i>	<i>L. mesenteroides</i>	<i>L. bulgaricus</i>	<i>Candida kefir</i>	<i>S. boluradii</i>
Cellwall	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 15-20°C, 20-30°C, 30-40°C, 40-50°C	+	+	+	+	+
pH 3.5, 4.5, 6.5, 8.5	+	+	+	+	+
Salt 6.5 10%	+	+	+	+	+
Carbo hydrate fermentation					
Arabinose	+	+	+	+	+
Cellobiose	+	+	+	+	W
Esculin	+	+	+	+	+
Fructose	+	W	+	+	+
Galactose	+	+	W	+	+
Gluconicacid	+	+	+	-	-
Lactose	+	+	+	+	+
Maltose	+	+	+	-	+
Mannitol	+	+	+	-	+
Mannose	+	+	+	-	+
Mellibiose	+	+	+	-	+
Raffinose	-	+	+	-	+
Rhamnose	-	+	+	-	+
Ribose	+	+	+	-	+
Salicin	+	+	+	-	+
Sorbitol	+	+	+	-	-
Sucrose	+	+	+	-	-
Xylose	-	+	+	-	+

(++)-Luxurious growth  
(+) -growth  
(W) -Weak Growth  
(-) -No growth

PPM – Phosphopentidomannan, PLM – Phospholipomannan  
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 - Nonspore

**3. RESULTS SECTION**

**3.1. 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 organisms were further identified to effective against diarrhoeal causing organisms, the best combinational approachment of probiotic bacteria and yeast confirmed as a probiotic by using acid tolerance test, bile tolerance and cell adhesion test from which three probiotic species were distinguished namely, *Lactobacillus kefiranofaciens*, *Candidakefir* and *Saccharomyces boluradii*. These probiotics are grown well in

**2.3. Production synbiotic corn extract.**

Corn was purchased from a local market. The extract was prepared from homogenized skinless slices and was filtered it properly, 100ml of Corn Extract were inoculated with 2ml of MRS broth containing probiotic yeast and bacteria (*L. kefiranofaciens*, *saccharomyces boluradii*, *Candida kefir*) they were allowed for fermentation. After fermentation, Extract was separated in two different containers. One of that container inulin could be added. This was an *in vitro* study on the antibacterial activity of synbiotic corn Extract against five diarrhoeal causing organisms.

**2.4. Microbiological methods.**

**2.4.1. Test organisms.** The bacteria used as test organisms were *Staphylococcus aureus*, *Enterotoxigenic E.coli*, *Vibrio Cholerae*, *Salmonella Paratyphi A*, and *Shigella Dysenteriae*. All of these microorganisms were obtained from the Microbial Type Culture Collection (MTCC), Iran.

**2.4.2. Preparation of inoculum.** The inoculum was prepared by adding one loopful of test pathogen in 50ml of BHI broth and then incubated at 37°C for 24 h.

**2.4.3. Determining the antagonistic activity of synbiotic corn extract using *in vitro* test**. The anti-bacterial activity of symbiotic Extract was done by Agar well diffusion method [34], [35]. Sterilised MHA medium was poured into sterile Petri plates and allowed to solidify. After solidification of the medium, the inoculums was vortexed and 100 ml of the inoculum was spread evenly over the surface of the agar plates using a glass spreader. A well of 8mm diameter was made on each plate with a sterile cup borer. The symbiotic Extract (100ml) was introduced and into the separate plate and in a single well. (Fresh Extract, fermented Extract with inulin). The fruit Extract extract was allowed to diffuse at room temperature for 24hrs. The plates were then incubated at 37°C for 48hrs. The antagonistic test was performed in triplicate. The efficiency was determined by measuring the diameter of the zone of inhibition exhibited by the synbiotic Extract 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 Extract 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.

temperature range of 20 - 30° C and characterized by 18 type of carbohydrate fermentation. In some cases, week and negative sugar fermentation were reported.

**3.2. *In vitro* antagonistic activity of synbiotic corn extract against diarrhoeal causing organism.** On the basis of the result obtained, there was a significant in antagonistic effect against the pathogens between fruit Extract, fermented Extract, fermented Extract with inulin. The antagonistic test was performed in triplicate and their efficiency was determined by measuring the

diameter of zone of inhibition around the well. In triplicate assay mean value was taken for analysis (Table 2 and Figure 1).

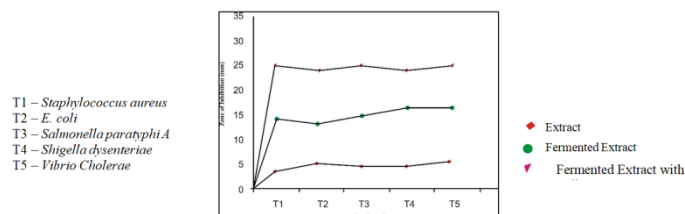
**Table 2.** Inhibitory activity of synbiotic extract against test pathogens.

Pathogens	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>
<i>Staphylococcus aureus</i>	+	++	+++
<i>Escherichia coli</i>	+	++	+++
<i>Salmonella paratyphi A</i>	+	++	+++
<i>Shigella dysenteriae</i>	+	++	+++
<i>Vibrio cholerae</i>	+	++	+++

S<sub>1</sub>-Corn Extract

S<sub>2</sub>-Extract probicated with organism

S<sub>3</sub>-Fermented Extract with Inulin



**Fig. 1.** Effect on synbiotic corn extract against pathogens.

Among the three samples (S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>) demonstrated, the higher antagonistic activity was shown by S<sub>3</sub> sample. Inhibition of bacterial growth compared with that of S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> were more effective. The preceding studies have shown protective and therapeutic effects of synbiotic extract vs diarrhogenic organisms our study showed that, the antibacterial activity of synbiotic extract exists at effective against *Staphylococcus aureus* shigella *dysenteriae* and *vibrio cholerae* compared with other two pathogenic organisms. Similar work was carried out to evaluate

#### 4. CONCLUSIONS

The present study has represented the use synbiotic corn extract against diarrhea causing microorganisms. The result of our study demonstrated that probiotic combination *Lactobacillus kefiranofaciens*, *Candida kefir* and *Saccharomyces boluradii* were excellent probiotic approachment for all the five test diarrhoea causing pathogen. The proceeding studies have shown the protective and therapeutic effect of synbiotic corn extract to diarrhoeal causing organisms. The probiotics used for fermentation showed inhibitory toward Gram negative bacteria.

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the influence of prebiotic additives on gluten-free bread, and also to assess the effectiveness of selected prebiotics inulin [36, 37].

A Synbiotic nature in the gut should be maintained which is a mixture of a probiotic and a prebiotic, and this combination is used to stimulate the growth of probiotic in the gut thereby increasing its effectiveness [38, 39]. Thus prebiotic approach though diet increases residence bacteria which are beneficial to human health [40-43]. The inhibitory action of probiotic bacteria and yeast is mainly due to the accumulation of main primary metabolites such as lactic acid, acetic acids, ethanol and carbon dioxide [44, 45]. Additionally, they it also capable of producing antimicrobial compounds such as formic and benzoic acids hydrogen peroxide, diacetylaceton, and bacteriocin [46-49]. The production levels and the proportions among these compounds depend on the strain, medium compounds and physical parameters probiotics has shown to process inhibitory activities mostly towards pathogens and closely selected bacteria due to the bactericidal effect of protease sensitive bacteriocins [50-53] still LAB were also able to control the growth of Gram negative pathogens including food borne pathogens by the production of organic acids and H<sub>2</sub>O<sub>2</sub> [54-56]. Corn extract fermented with *L. kefiranofaciens* (kefir & s. bolud and inulin might be a good source of probiotic lactobacilli and also nutritional components even after 2 weeks storage at 4°C [57, 58]. In addition, it would be completely functional when ingested [59, 60]. On the whole, the results impact positively towards a broader utilization of Corn extract suitable on the basis of a complex functional production with higher added values [61, 62].

The probiotics of *lactobacillus sp*, *bifidobacteria* as well as probiotic *saccharomyces boulardii* have been investigated with medical use, either in mixed cultures or as single strains. The antibacterial and inhibitory activity of synbiotic corn extract against test pathogens was evaluated and the activity of the extract with this organism was higher than the corn extract and also among the three samples, the highest antagonistic activity was shown by the fermented corn extract with Inulin.

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