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Research Article

STUDIES ON SYNBIOTIC BARLEY GRAIN EXTRACT AGAINST SOME HUMAN PATHOGENS

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ABSTRACT
This study evaluated that effect of prebiotic food containing oligosaccharide to enhance the growth and activity of probiotic strains. Barley grains probioticated using different strains of probiotics are *Lactobacillus kefiranofaciens, Candida kefir*,and *saccharomyces boluradii*. To select a suitable prebiotics like inulin for the development of Synbiotic barley and tested for antibacterial activity against diarrhoea causing pathogen such as *Esherichia coli, Staphylococcus aureus, Salmonella paratyphi A, Shigella dysenteriae, Vibrio cholerae*. Analysis of identified compound from synbiotic barley grain using GC-MS.

Key words: Antibacterial activity, Barley, GC-MS analysis, Probiotics, Synbiotics.

INTRODUCTION

Diarrhoea is a common symptom of intestinal disorders and it is a global threat to human health. It is a leading cause of morbidity and mortality, with over 1000 million episodes and over 4 million deaths annually in children under 5 years of age. Diarrhoeal infection is a second killer disease of children in the developing countries. Diarrhoea caused by *Escherichia coli* is common in India with occasional outbreaks (Kahali et al., 2004). Where as *Escherichia coli* (58.4%) *Salmonella* sp (20%) and *Shigella* sp (20%) were found to be extremely uncommon agents of childhood diarrhea making only 1.6 per cent of the positive culture in Yeman (Banajeh et al., 2001). Synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the friendly flora of the human intestine.Reasearch and development of synbiotic products have been increasingly focusing on evidence of functional benefits including resistance to infection, antibacterial activity, and improved immune status (Gibson and Roberfroid, 1995). 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. Effective probiotic organisms identified, and inoculated with barley grain extract 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. Inulin consists of 2-60 fructose units linked by a β-(2-1) glycosidic linkage often with a terminal glucose unit. Many researches proved that consumption of prebiotics, such as inulin, could stimulate intestinal peristalsis by means of increasing fecal bulk and moisture (Gibson et al., 1995). The keeping above facts in view present investigation was undertaken to evaluate prebiotics strains for their compatibility with barley in the presence of insulin for synbiotic barley preparation.

Barley grain contains several vitamins and minerals including niacin (vitamin B3), thiamine (vitamin B1), selenium, iron, magnesium, zinc, phosphorus and copper. Barley contains antioxidants, which are also important for maintaining good health and also it contains phytochemicals, which are natural plant-based chemicals that may decrease the risk for certain diseases such as heart disease, diabetes and cancer. In addition to that fermented barley grain with inulin might be a good source of prebiotic and also nutritional components even after 2 weeks storage at 4°C so inulin acts as food preservative.

MATERIALS AND METHODS

Preparation of synbiotic barley grain extract

Barley grains were purchased from a local market. Extract was prepared for soaking the grain with water in over night, homogenized and was filtered it properly and 100ml of barley grain extract was inoculated with 2ml of MRS broth containing probiotic bacteria and yeast. (*Lactobacillus kefiranofaciens Candida kefir*,and *Saccharomyces boluradii*) they were allowed for fermentation (Yoonky et al., 2006) After fermentation synbiotic barley grain extract was used for antibacterial analyses.

Test organisms

The bacterial test organisms were *Esherichia coli, Staphylococcus aureus, Salmonella paratyphi A, Shigella dysenteriae, Vibrio cholerae* were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The organisms were maintained on agar slant and were subsequently subcultured into newly prepared nutrient agar media. All the chemicals and medium used in this study were supplied by Himedia Pvt. Ltd., Mumbai, India.

Preparation of Inoculum

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

Isolation and Characterization of Probiotics

Fermented milk samples such as yoghurt, kefir, cheese, and koumiss were collected from market. The milk samples were enriched and inoculated into the MRS broth (Man rogosa sharpe) The enriched samples were incubated under static

conditions for a week. Probiotic isolation was carried out by streaking the enriched milk sample on MRS agar media and incubated at 37°C. Isolated bacterial cultures were characterized using colony morphology, bio-chemical test and in selective medium, carbohydrate fermentation. (Table 1)

Agar well diffusion assay

The antibacterial activity of synbiotic barley was evaluated by agar well diffusion method. (Chung et al., 1990) Muller Hinton agar medium was prepared and poured into the petriplates and allowed to solidify. Then it was inoculated with a swab of culture and spread through out the medium uniformly with a sterile cotton swab. Using sterile cork borer (10mm diameter) wells were made in the agar medium. The test compound (Synbiotic grain extract) was introduced into the separate well in a single plate. All the plates were incubated at 37°C for 24h. The antagonistic test was performed intriplicate 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).

GC-MS analysis

The volatile constituents from synbiotic barley extract was analysed using GC-MS (GC Clarus 500 Perkin Elmer) with Elite-1 column and a mass detector, which was operated in EI mode at 70eV. Injector and detector temperatures were set at 250^0C (Al-Delaimy and Ali, 1970). Barley extract (1µl) was injected and analysed with a column held initially at 110°C for 2min and then increased by 5^0C per min up to 280^0C. Helium was used as carrier gas (1ml/min). The relative amount of individual components of the total extract expressed as percentage peak area relative to total peak area. Quantitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds, or by retention indices (RI) and mass spectra.

RESULT AND DISCUSSION

As Table 1, shows isolation characteristics of probiotic bacteria and yeast. From these observation all the three strains characterized by morphology, biochemical test, different growth condition of pH, temperature and salt concentrations. Probiotics such as *Lactobacillus kefiranofaciens, Candida kefir, Saccharomyces boluradii* grown well temperature range of 20-30°C at 6.5 salt concentration and mainly characterized by 18 type of carbohydrate fermentation. In some cases week and negative sugar fermentation was observed.

From Table 2 it is very clear that fermented barley grain extract with inulin showed growth inhibition activities of five diarrhoeal causing test pathogens and the higher antibacterial activity (Fig 1). Similar type of work was to evaluated the influence of prebiotic additives on gluten-free breads, and to assess the effectiveness of selected prebiotics inulin (Grzelak, 2006). Barley exhibit antifungal activity on *candida albicans* by TLPs (Thaumatin like protein) (Hejgaard et al., 1991).

To further study the nature of components present in the grain extract GC-MS was performed (Fig 2). The major compounds of GC-MS analysis and their retention time were listed in Table 3, these include n-Hexadecanoic acid (26.52%), and 9,12-Octadecadienoic acid (73.48%). These observations when correlated with earlier studies (Mc Murrough, 1983) reported phenolic acids in barley grain include benzoic acid and cinnamic acid derivatives and simple flavanoids accounts for 58-68% of total phenolics.

Probiotic approach through barley grain extract increases residence bacteria which are beneficial to human health. The inhibitory action of probiotic bacteria and yeast is mainly due to accumulation of main primary metabolites such as lactic acid , acetic acid, ethanol and carbondioxide. It is earlier reported as lactic acid bacteria were also able to control the growth of gram negative pathogens including food borne pathogens by the production of organic acids and hydrogen peroxide (Lu and Walker, 2001 and Ito et al., 2003). Similar types of incidence have been reported as Production levels and the proportions among these compounds depend on the strain, medium compounds and physical parametrs (Tannock, 2004). The chromatographic analysis of compounds obtained from these synbiotic barley grain extract can be used as natural “anti bacterial activity” for developing plant derived anti microbial drugs.

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Table 1: Isolation characteristics of Probiotics

Characteristics	*Lactobacillus kefiranofaciens*	*Candida kefir*	*Saccharomyces boluradii*
Cellwall	G+ve	Chitin mannose PPM, PLM	Chitin mannose PPM, PLM
Morphology	Rod	Yeast like pseudohyphae	Pseudohyphae
Motility	NM	-	-
Spore forming	NS		
Selectivemedium	MLR	YMA	SGA
Growth at 15^0C-20^0C 20^0C -30^0C 30^0 C-40^0C 40^0 C-50^0C	+	+	+
pH 3.5 4.5 6.5 8.5	+	+	+
Salt 6.5 10%	+	+	+
Carbohydrate fermentation			
Arabinose	+	+	+
Cellobiose	+	+	W
Esuculin		+	-
Fructose	+	+	+
Galactose	+	+	-
Gluconicacid	+	-	+
Lactorose	+	+	+
Maltose	+	-	+
Mannitol	+	-	+
Mannose	+	-	+
Mellibiose	+	+	+
Raffinose	-	-	+
Rhamnose	-	-	+
Ribose	+	-	+
Salicin	+	-	+
Sorbitol	+	-	-
Sucrose	+	-	+
Xylose	-	-	+

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

Table 2: Antibacterial activity of Barley grain extract

Pathogens	S_1	S_2
Staphylococus aureus	+	++
Escheriacoli	+	++
Salmonella paratyphi A	+	++
Shigella dysenteriae	+	++
Vibrio cholerae	+	++

S_1 - Extract probicated with organism
S_2 - Fermented extract with Inulin

Table 3: Major compounds identified from the fermented Barley extract

No	Retention time	Name of the compound	Molecular Formula	Molecular weight	Pear area%
1	16.38	n-Hexa decanoic acid	$C_{16}H_{32}O_2$	256	26.52%
2	18.93	9,12-OctaDecadienoic acid	$C_{18}H_{32}O_2$	280	73.48%

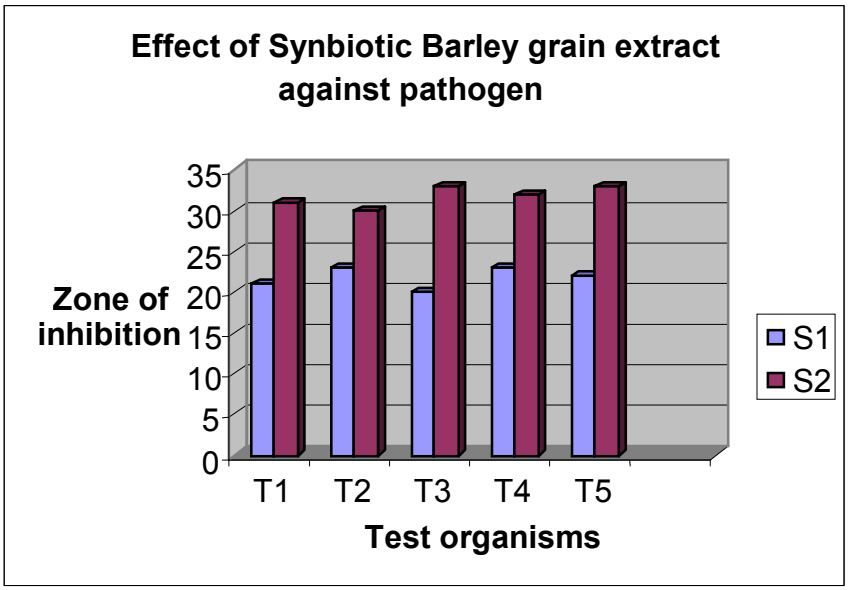

Fig 1

S_1-Extract probicated with organism, **S_2**-Fermented extract with Inulin, **T1** – *Staphylococcus aureus*, **T2** – *E.coli*, **T3** – *Salmonella Paratyphi A*, **T4** – *Shigella dysenteriae*, **T5** – *Vibrio Cholerae*

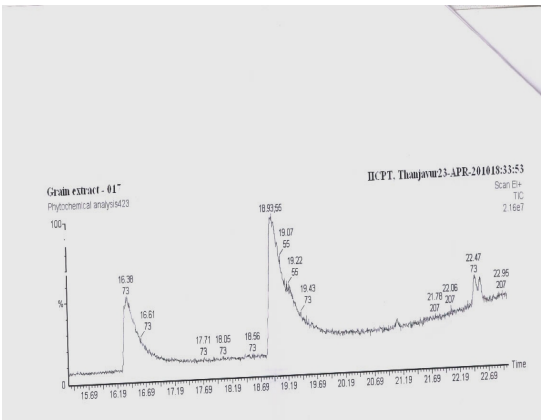

Fig 2: Gas Chromatography Mass Spectrum analysis of fermented plums juice

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