Original Research Article

Antimicrobial activity of probiotic fermented barley based food products

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Abstract

This study was conducted to determine the presence of antimicrobial activities in the probiotic fermented barley based indigenously developed food products against common microbial pathogens namely E. coli, Shigella dysenteriae and Salmonella typhimurium. An indigenous food mixture was developed by mixing barley flour, milk co precipitate, sprouted green gram paste and tomato pulp in the ratio of 2:1:1:1(w/w) and coded as BCYT. This mixture was fermented with single cultures of L. plantarum, L.casei and S. boulardii. Sequential fermentation was also carried out with S.boulardii + L.casei as well as S.boulardii + L.plantarum. Cell viability was studied before and after lyophilization. To study the antimicrobial effect, cell free filtrates of fermented food mixtures were used. Cup well essay method was used to study the inhibition zones formed against the pathogens. The developed blend was able to maintain sufficient viability of strains in both single as well as sequential culture fermentations. The pH ranged from 4.13 - 4.55 while titratable acidity ranged from 2.63-2.92 g lactic acid/100ml of fermented food products. Methanol-acetone cell free extracts of all fermented mixtures formed inhibition zones against all three pathogens ranging from 13-18 mm diameter. Results showed the presence of antimicrobial activity in all developed food blends. The spectrum of their antimicrobial activity varied. Considering that it was a blend of staple foods, the products have the potential of providing nutrition along with its therapeutic effects.

Keywords: L. casei, L. plantarum, S. boulardii, E. coli, Shigella dysenteriae, Salmonella typhimurium, probiotics, cell viability, antimicrobial activity

1. Introduction

Probiotics are living, health promoting microorganisms that are incorporated into various kinds of foods. The ability of probiotics to withstand the normal acidic conditions of the gastric juices and the bactericidal activity of the bile salts, as well as the production of lactic acid that inhibits the growth of other microorganisms, allow them to be established in the intestinal tract (Lee and Salminen, 1995; Saarela et al., 2000).

The reported health benefits include: boosting of the immune system, inhibition of the growth of pathogenic organisms, prevention of diarrhea from various causes, prevention of cancer, reduction of the risk of inflammatory bowel movements, improvement of digestion of proteins and fats, synthesis of vitamins, and detoxification and protection from toxins (Andersson et al., 2001; Salminen, 2001; Kullisaar et al., 2003).

The concept of probiotics progressed around 1900, when Elie Metchnikoff hypothesized that the long and healthy lives of Bulgarian peasants were the outcome of their consumption of fermented milk and milk products. Members of the genera Lactobacillus, Bifidobacterium and
Streptococcus are the most common probiotics used in commercial fermented and non-fermented dairy products today. One of the most important properties of probiotics is protection against pathogens in the intestinal tract of the host. The role of antimicrobial compounds produced by probiotic strains as prophylactic agents against enteric infections is crucial and well documented (8–10) Also the probiotic strain should withstand the manufacturing process without loss of viability or negative effect on the sensory properties of the food product. (Sanders and Huis in’ t Veld, 1999; Saarela et al., 2000). Therefore, it is of great importance to control the stability of probiotic numbers and properties in developed products.

This study was conducted to determine the presence of antimicrobial activities among the probiotics incorporated into barley based indigenously developed food product against common microbial pathogens. Substantiating the antimicrobial activities of probiotics will affirm their use in the development of functional foods for the betterment of the health of the consuming public.

2. Materials and Methods

2.1 Materials

Skimmed milk for co precipitate was procured from the Deptt. of Animal products Technology, CCSHAU Hisar. Green gram (whole), rice and fresh tomatoes were purchased from the local market in a single lot.

Rice was cleaned thoroughly and ground in an electric grinder (cemotec1090, m/s Tecator, Hoganas, Sweden) using 1.5 mm sieve size. After cleaning, green gram was soaked in distilled water for 6 hr. and kept in incubator (30°C, 12 hr) for sprouting. The sprouted green gram was ground to paste in a homogeniser. Tomato pulp was obtained by mashing and sieving the blanched tomato in a thick strainer. Co precipitate of medium calcium was prepared from skimmed buffalo milk (Mann and Mulay 1987).

2.2 Microbial cultures

L.casei (NCDC-19) was collected from the National Collection of Microorganisms Unit, NDRI, Karnal, India. S.boulardii was obtained from Department of Food Science and Human Nutrition, Univ. of New South Wales, Sydney, Australia.

2.3 Development of food mixture

The food mixture was developed by mixing barley flour, milk co precipitate sprouted green gram paste and tomato pulp in the ratio of 2:1:1:1 (w/w) and was coded as BCGT. The developed food mixture (100 g) was mixed with water (500 ml) and stirred sufficiently to obtain homogenous slurry which was autoclaved at 1.5 kg/cm² for 15 min. It was then cooled and inoculated with 2% (v/v) liquid culture of S. boulardii (12 h old culture) and incubated at 25°C for 24 hr. This was followed by inoculation with 2% (v/v) liquid culture of L. casei (6 h old culture) and incubated at 37°C for another 24 hr. Both the cultures contained 10⁶ cells/ml broth. The fermented product was subjected to lyophilisation and the lyophilized product had a cell count of 9.25 and 9.54 cfu/g (yeast and lactobacilli), respectively. The unfermented autoclaved and lyophilized slurry served as control.

2.4 Chemical analysis

Titratable acidity was determined as lactic acid per 100 ml (Amerine et al 1967). The pH was measured by a pH meter against a standard buffer of 4.0 pH.

2.5 Determination of antibacterial activity

Antibacterial activity of probiotic fermented food mixtures was determined by Agar Well assay Technique as recommended by the British Standard Method (BS4020, 1974)

3. Results and Discussion

3.1 Survival of the Strain

For single as well as sequential culture fermentations, autoclaved food mixture slurries were inoculated each with 2 per cent of the inoculum containing pure culture of L. casei, L. plantarum or S. boulardii at a level of 10⁶ cells/ml of inoculum. At the end of fermentation periods, the cell counts of Lactobacilli as well as yeast increased in all the fermented food slurries.
In single culture fermented BCGT food mixture, the one fermented with L. casei had the highest cell count (9.99 cfu/g) (Table 1). In food mixture fermented sequentially with S. boulardii + L. casei and S. boulardii+ L. plantarum, the respective cell counts of lactobacilli (8.99 and 8.90 log cfu/g) were higher as compared to those of yeast (7.70 and 7.63 log cfu/g). After lyophilization, the Lactobacilli counts in single fermented food mixture with L. casei and L. plantarum were 10.21 and 10.32 log cfu/g respectively which indicates sufficient viability of these probiotic organisms in lyophilized food mixtures. The yeast count after lyophilization was 9.03 and 8.91 log cfu/g in sequential fermentations with L. casei and L. plantarum respectively.

3.2 pH and titratable acidity

With the decline in pH, there was simultaneous increase in titratable acidity (Table 1). The pH of unfermented BCGT food mixture was 6.00 and after fermentation with different probiotic organisms, pH of food mixture fermented sequentially with S. boulardii + L. casei, was significantly (P<0.05) lower than that obtained after fermentations with other single (L. casei; L. plantarum) and sequential (S. Boulardii+ L. plantarum) culture fermentations (Table 1). Sequential culture fermentation of BCGT food mixture with S. boulardii+ L. casei increased the titratable acidity from 1.78 to 2.95 g lactic acid per 100 ml which was significantly higher than those of single culture fermented mixtures. Hence, sequential culture fermentations were significantly (P<0.05) more effective in lowering the pH and simultaneously increasing the titrable acidity as compared to any of the single culture fermentations.

3.3 Antibacterial activity

The cell free filtrates of BCGT food mixture fermented with different probiotic organisms showed inhibition towards different pathogenic microorganisms namely, Shigella dysenteriae, Salmonella typhemurium and E. coli.

Against E. coli, the inhibition zone diameter was the largest (18mm) with cell free extract prepared from L. casei fermented food mixture. (Table 2). Against Shigella dysenteriae, maximum inhibition zone diameter (18mm) was obtained when cell free extract of S. boulardii + L. casei was used. The cell free extracts of both the single cultured food mixtures had similar inhibition zones (16mm) against Shigella. Against Salmonella typhemurium, both the sequentially fermented mixtures showed inhibition diameters of 16 mm each while single culture fermentations with L. casei and L. plantarum showed inhibition zones of 14 and 13 mm respectively.

4. Discussion

As the optimal temperatures for growth of probiotic organisms were used, it appears that the food mixtures containing cereal, legume, milk byproducts and tomato pulp supported the growth of both yeast and Lactobacilli well. Similar findings have been reported earlier by Arora et al., (2011) who developed pearl millet based food blends containing raw and germinated pearl millet flour and fermented with 5 percent Lactobacillus acidophilus. The blend containing germinated pearl millet flour exhibited better cell viability (8.64 cfu g⁻¹) as compared to non-germinated food blend.

During fermentation, probiotic organisms convert glucose to lactic acid which is responsible for the decline in pH of the food product. A rapid drop in pH with corresponding increase in titratable acidity has also been reported in cereals and legumes (Arora et al., 2010) cabbage (Yoon et al., 2006) and soy (Cavallini et al., 2010).

The inhibition of many pathogenic bacteria in fermented foods is believed to be the result of antimicrobial substances produced by LAB resulting in natural preservation through bacterial antagonism. Lactic acid is the major metabolite produced by lactic acid bacteria. Acetic acid is another organic acid produced. Their inhibitory effects have been extensively investigated. Low pH affects every aspect of cellular metabolism, with retardation of the growth of unwanted microbes in culture media. As evident from data, pH of the food mixtures was reduced substantially with fermentation. Undissociated lactic and acetic acids penetrate the cell membrane and disturb the transmembrane potential, resulting in inhibition of substrate transport (Maloney, 1990).
Hydrogen-peroxide produced by Lactobacilli during fermentation, also has its inhibitory action. Because Lactobacilli do not possess catalase, \( \text{H}_2\text{O}_2 \) accumulates in the surrounding medium, resulting in anaerobic conditions. The lethal effect \( \text{H}_2\text{O}_2 \) may be due to inactivation of essential biomolecules by the superoxide anion chain reaction. It may also function via the lactoperoxidase-thiocyanate system. The \( \text{H}_2\text{O}_2 \) oxidises the thiocyanate to release toxic oxidation products that are detrimental to foodborne pathogens (Fernandes et al., 1987).

Table 1. Effect of lyophilization on cell count (log cfu/g) of indigenously developed BCGT food mixture.

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>pH</th>
<th>Titratable acidity</th>
<th>Yeast (before lyophilization)</th>
<th>Lactobacilli (before lyophilization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.00 ± 0.01</td>
<td>1.78 ± 0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Autoclaved</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Single culture fermentation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>4.49 ± 0.01</td>
<td>2.63 ± 0.02</td>
<td>-</td>
<td>9.88 ± 0.01</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>4.55 ± 0.01</td>
<td>2.59 ± 0.03</td>
<td>-</td>
<td>9.11 ± 0.03</td>
</tr>
<tr>
<td><strong>Sequential culture fermentation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. boulardii + L. casei</em></td>
<td>4.13 ± 0.02</td>
<td>2.93 ± 0.03</td>
<td>7.70 ± 0.02</td>
<td>9.03 ± 0.02</td>
</tr>
<tr>
<td><em>S. boulardii + L. plantarum</em></td>
<td>4.20 ± 0.01</td>
<td>2.92 ± 0.04</td>
<td>7.63 ± 0.02</td>
<td>8.91 ± 0.01</td>
</tr>
<tr>
<td>S.E. (mean) ±</td>
<td>0.02</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>CD(P&lt;0.05)</td>
<td>0.06</td>
<td>0.12</td>
<td>0.9</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six independent determinations

Table 2. Antibacterial activity of different with different probiotic organisms in BCGT food mixture

<table>
<thead>
<tr>
<th>Food mixture</th>
<th>Methanol-acetone cell free extract inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><strong>Control</strong> (unfermented autoclaved mixture)</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Single culture fermentation</strong></td>
<td></td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>18</td>
</tr>
<tr>
<td><em>L. Plantarum</em></td>
<td>17</td>
</tr>
<tr>
<td><strong>Sequential culture fermentation</strong></td>
<td></td>
</tr>
<tr>
<td><em>S. boulardii + L. casei</em></td>
<td>17</td>
</tr>
<tr>
<td><em>S. boulardii + L. plantarum</em></td>
<td>17</td>
</tr>
</tbody>
</table>

\( \text{CO}_2 \) may exert its antimicrobial effect in several ways such as by rendering the environment more anaerobic, by inhibiting enzymatic decarboxylation and by disrupting the cell
membrane with the accumulation of the gaseous phase in the lipid bilayer (Dixon and Kell, 1989).

Some strains of Lactobacilli have been found to contribute to antimicrobial activity by the production of antibiotic or bacteriocins (Sanders and Klaenhammer, 2001). The bacteriocin Plantaricin A, Plantaricin 149 and Plantaricin SA 6 secreted by L. plantarum have been isolated. Bacteriocins produced by L. casei include Caseicin 80, Lactocin 705 have also been isolated (Klaenhammer, 1988).

Ali et al. (2013) studied the antibacterial activity of the selected 14 probiotic isolates against both S. aureus and E. coli using the agar well-diffusion method. The selected probiotic isolates in this test exhibited varying degrees of inhibitory activity against human pathogenic Staphylococcus aureus. It was concluded that the inhibitory activity of the isolates against E. coli was slightly less as compared to that obtained against S. aureus. Pathogen inhibition by probiotics has also been reported by several other workers. (Muhialdin and Hassan, 2011; Yesillik et al., 2011; Khanafari and Porgham, 2012)

References


