Antidiarrhoeal effect of feeding probiotic fermented indigenous food blend in ampicillin treated mice

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Abstract

The study was undertaken to study the efficacy of a developed probiotic fermented food blend in the prophylaxis of ampicillin-induced diarrhoea. Mice were used as experimental animals. An indigenous food mixture was developed by mixing rice flour, milk co precipitate, sprouted green gram paste and tomato pulp in the ratio of 2:1:1:1 (w/w) and coded as RCGT. This mixture was sequentially fermented with S. boulardii and L. casei. The final lyophilized product had a cell count of 9.25 and 9.54 cfu/g (yeast and lactobacilli), respectively. To study the therapeutic effect, two groups of mice were treated with ampicillin which was added at the rate of 75mg/Kg of their diet. Control group received unfermented while experimental group received fermented food mixture. It was observed that simultaneous feeding of fermented food mixture with ampicillin successfully prevented the moisture, ash and nitrogen from rising in faeces of majority of mice. It also prevented the sharp decline of faecal lactobacilli as was observed in the control group.

Keywords: Antibiotic induced diarrhoea, L. casei, probiotics, S. boulardii

1. Introduction

Diarrhoea is a common side effect of antibiotics. It may prolong hospital stay, increase the risk of other infections, develop into more serious forms of disease (colitis, toxic mega colon), and lead to premature discontinuation of the needed antibiotic. Antibiotic associated diarrhoea may develop in 5-30% of patients, with the rates increasing as the antibiotic spectrum gets broader (McFarland et al., 2007). Diarrhoea associated with antibiotic use may result from the disruption of the barrier of normally protective colonic microflora that are inadvertent targets of the inciting antibiotic. In 20-30% of these cases, an opportunistic pathogen, Clostridium difficile, takes advantage of this opening, colonises the intestine, and produces toxins, resulting in diarrhoea or colitis. A strategy to re-establish this microbial barrier is through the use of probiotics (McFarland, 2000).

The term “probiotic” was first used (Fuller, 1991) to describe “a live microbial supplement, which beneficially affects the host by improving its microbial balance.” Since then, research has looked at possible clinical uses for these agents and in 1995, when a greater understanding of their properties had developed, the term "bio therapeutic agents" was proposed (McFarland and Elmer, 1995) to describe micro-organisms with specific therapeutic properties that also inhibit the growth of pathogenic bacteria. The probiotic species used in clinical practice include Lactobacillus spp., Saccharomyces spp., Bacillus subtilis, Bifidobacterium spp. and many others. Probiotics have been advocated for the prevention and treatment of a wide range of diseases, and there is strong evidence for their efficacy in some clinical scenarios. Probiotics are now widely used in many countries by consumers and in clinical practice. Probiotics are becoming increasingly available as capsules and dairy based food supplements sold in health food stores and some supermarkets. A multitude of probiotic products are available in the global marketplace. But very few are based on
cereals, legumes or their blends, which are the staple foods in the diets of a large section of world’s population. Keeping this in view, a rice based probiotic fermented mixture was developed. The present communication reports about the development and anti diarrhoeal effect of the mixture. Following work was carried out at the Deptt. of Food and Nutrition, COHS, CCSHAU Hisar and the research was approved by the appropriate committee of the institution.

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 h. and kept in incubator (30°C, 12 h) 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. bouardi 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 rice 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 RCGT. 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. bouardi (12 h old culture) and incubated at 25°C for 24 h. 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 h. 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. The food mixture was also analysed for moisture, crude protein, fat and ash, dietary fibre constituents (AOAC, 1990), total soluble sugars (Cerning and Guilhot, 1973), reducing sugars (Somogyi, 1945; Yemm and Willis, 1954), starch (Clegg, 1956), phytic acid (Haug and Lantzsch, 1983), polyphenols (Swain and hill, 1959; Singh and Jambunathan, 1981) and minerals (Peterson et al., 1943; Lindsey et al., 1969).

2.5 Test animals

Twenty young (weanling) Swiss mice weighing 12± 2 g were obtained from the disease free small animal house, CCSHAU Hisar. The mice were housed individually in metabolic cages kept in air conditioned room with 12 h light and dark cycle.

2.6 Feeding trials

Mice were divided into two groups, each group comprising of ten mice. Mice in the control group received unfermented food mixture while those in experimental group received fermented food mixture. Prior to feeding, the lyophilized food mixture were rehydrated (1:4, w/v) with boiled and cooled water. Antibiotic ampicillin was added to both the diets at the rate of 75mg/kg of the rehydrated feed.

Feeding trial was carried out for one week. Food and water were given ad lib. Actual amount of food eaten daily by mice was recorded. It was observed that on an average, each mouse ate 10.2 ±1.8 g of the food mixture daily.

2.7 Collection and analysis of faeces
Faecal samples of mice were collected before first feed (day 0) and then on day 2, day 4 and day 6 of the feeding trial. The collected samples of faeces were analysed for moisture, nitrogen and ash contents by employing standard methods of analysis (AOAC, 1990). Lactobacilli and yeast in fresh faecal samples were enumerated using MRS and YEPD mediums, respectively.

2.8 Statistical analysis

The data were analysed using suitable statistical measures (Snedecor and Cochran, 1991).

3. Results

3.1. Nutrient composition

The nutrient profile of the developed fermented food mixture is given in Table 1.

Table 1. Nutritional composition of the developed probiotic fermented food mixture (Dry matter basis).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count (after lyophilisation)</td>
<td></td>
</tr>
<tr>
<td>Yeast (log cfu/g)</td>
<td>9.25±0.03</td>
</tr>
<tr>
<td>Lactobacilli (log cfu/g)</td>
<td>9.54±0.02</td>
</tr>
<tr>
<td>pH</td>
<td>4.12±0.01</td>
</tr>
<tr>
<td>Titratable acidity (lactic acid/ml)</td>
<td>2.92±0.03</td>
</tr>
<tr>
<td>Proximate composition (g/100g)</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>1.75±0.06</td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.56±0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>2.57±0.04</td>
</tr>
<tr>
<td>Ash</td>
<td>2.26±0.02</td>
</tr>
<tr>
<td>Dietary fibre constituents (g/100g)</td>
<td></td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>0.66±0.17</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>1.98±0.14</td>
</tr>
<tr>
<td>Carbohydrate profile (g/100g)</td>
<td></td>
</tr>
<tr>
<td>Total soluble sugars</td>
<td>1.13±0.01</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>Non reducing sugars</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>Starch</td>
<td>16.11±0.38</td>
</tr>
<tr>
<td>Antinutrients (mg/100 g)</td>
<td></td>
</tr>
<tr>
<td>Phytic acid</td>
<td>Not detected</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>342.63±4.92</td>
</tr>
<tr>
<td>HCl- extractable minerals (mg/100g)</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>1.29±0.01</td>
</tr>
<tr>
<td>Calcium</td>
<td>243.65±0.14</td>
</tr>
<tr>
<td>Sodium</td>
<td>90.22±0.15</td>
</tr>
<tr>
<td>Potassium</td>
<td>133.48±0.12</td>
</tr>
</tbody>
</table>

Values are mean±SD of six independent determinations.

3.2. Faecal moisture content

Simultaneous feeding of unfermented RCGT food mixture and ampicillin to the control group mice increased their faecal moisture content (fig. 1). There were great individual variations as far as faecal moisture was concerned, which may be due to varying effect of antibiotic among the individuals. However, majority of mice (60 %) in control group had their faecal moisture per cent above 50 on day 2. In experimental group (fig. 1), none of the mice had faecal moisture content above 50 per cent during first two days. On the contrary it remained below 15 in 80 per cent of mice.

A significant rise (p<0.05) was observed in faecal moisture content of mice from control groups and it reached mean values of 41.47 and 42.35 per cent respectively on days 4 and 6 of the trial. The mean moisture per cent on corresponding days was 14.72 and 12.07 respectively in mice fed on fermented food mixture.

3.3. Faecal ash

The faecal ash content increased significantly (p ≤0.05) in 80 percent of mice from control group and only 50 per cent of mice from experimental group. On the whole, the mean faecal ash content of control group mice rose from 11.16 to 13.94 g/100g (dry matter basis) during the one week study period whereas in experimental group mice, the rise was from 10.89 to 11.14 g/100g (fig. 2).

3.4. Faecal nitrogen

Simultaneous feeding of unfermented RCGT food mixture and ampicillin to the control group mice increased their faecal nitrogen content (fig. 3)). On day 2, a significant (p<0.05) rise was observed in sixty percent of mice and the mean faecal nitrogen content raised from 1.17 on day 0 to 1.38 g/100g on day 2 whereas in experimental group, only twenty percent of mice experienced a significant rise of faecal nitrogen content on second day. Mean nitrogen content for control group was 1.43 and 1.40g/100g on days 4 and 5 respectively as against 1.18 and 1.17 for experimental group.

3.5. Faecal lactobacilli

Following the antibiotic treatment, a decline in lactobacilli count was observed in both the groups however the decline was more sharply focussed in the control group as compared to the experimental group (fig. 4). Initial counts of lactobacilli were
Fig. 1  Moisture (%) in faeces of mice treated with antibiotic ampicillin and simultaneously fed on unfermented (−) or fermented (+) developed food mixture

Fig. 2  Ash (%) in faeces of mice treated with antibiotic ampicillin and simultaneously fed on unfermented (−) or fermented (+) developed food mixture
Fig. 3 Nitrogen (%) in faeces of mice treated with antibiotic ampicillin and simultaneously fed on unfermented (−) or fermented (+) developed food mixture.

Fig. 4 Lactobacilli count (log cfu/g) in faeces of mice treated with antibiotic ampicillin and simultaneously fed on unfermented (−) or fermented (+) developed food mixture.
9.11 and 9.10 log cfu/g wet faeces in control and experimental groups, respectively. On day two, the counts had decreased to 6.57 and 6.85 log cfu/g faeces in the two respective groups. Throughout the study period, the experimental group which was being fed on fermented product, exhibited a higher viable count (6.63 and 6.53 log cfu/g on day 4 and 6 respectively) as compared to the control group (5.61 and 4.83 respectively on corresponding days). There was twenty percent mortality in the control group while it was nil in the experimental group.

4. Discussion

The results indicate that feeding of S. bouardii + L. casei fermented RCGT food mixtures along with antibiotic successfully prevented the ampicillin associated gastro-intestinal side effects in the mice.

Most scientists agree that probiotic strains shall be able to survive transit through the gastric acid environment as well as exposure to bile and pancreatic juice in the upper small intestine to be able to exert beneficial effects in the lower small intestine and the colon, although there are convincing data on beneficial immunological effects also from dead cells also (Mottet and Michetti, 2005). Best effect is achieved if the microorganisms colonise the intestinal surface mucus layer since they then can affect the intestinal immune system, displace enteric pathogens, provide antioxidants and antimutagens, and possibly other effects by cell signalling.

The large number of bacteria that normally inhabit the intestine act as an important host defence by preventing colonization by potential enteric pathogens. Persons with fewer intestinal bacteria, as in patients receiving antibiotics, are at significantly greater risk of developing infection with enteric pathogens. More than 99% of normal colonic flora is made up of anaerobic bacteria. The acidic pH and volatile fatty acids produced by these organisms appear to be critical elements in providing resistance to colonization conferred by the normal enteric flora (Dennis and Dori, 1998).

Antibiotic induced disturbance in the gut ecosystem include emergence of pathogens owing to a decreased barrier effect and decreased fermentation capacity. Petrino et al. (1977) reported that ampicillin therapy produced a decrease in number of strictly anaerobic bacteria with a remarkable overgrowth of enterobacter and translocation to liver. Antibiotic treatment may also impair mucosal integrity (Donohue and Salminen, 1996). When intestinal motility is impaired, the frequency of bacterial overgrowth and infection of small bowel with enteric pathogens is much increased. Lactobacilli are known to stabilize gut mucosal barrier (Majamma, 1995; Salminen et al., 1996).

Petrino et al. (1977) studied the influence of oral administration of different lactic acid bacteria including L. casei, on intestinal microflora and Ig-A secreting cells in mice treated with ampicillin. It was observed that oral administration of LAB improved the intestinal microflora avoiding bacterial translocation and increasing the number of Ig-A secreting cells. Since Igs are known to have a protective effect against pathogens so this can be one possible mechanism apart from stabilization of intestinal microflora and gut-mucosal barrier, of how lactobacilli can possibly be of use in antibiotic associated diarrhoea. Interestingly, S. bouardii also produces a protease which degrades both C. difficile toxin A and B, the main virulence factors in antibiotic-associated colitis (Castagliuolo et al., 1999).

Hickson et al. (2007) determined the efficacy of a probiotic drink containing Lactobacillus for the prevention of any diarrhoea associated with antibiotic use and that caused by Clostridium difficile. 135 hospital patients (mean age 74) taking antibiotics were given a 100 g (97 ml) drink containing Lactobacillus casei, L bulgaricus, and Streptococcus thermophilus twice a day during a course of antibiotics and for one week after the course finished. The placebo group received a long life sterile milkshake. 12% of the probiotic group developed diarrhoea associated with antibiotic use compared with 34% in the placebo group (P=0.007). No one in the probiotic group and 17% in the placebo group had diarrhoea caused by C. difficile (P=0.001). They concluded that consumption of a probiotic drink containing L casei, L bulgaricus, and S thermophilus can reduce the incidence of antibiotic associated diarrhoea and C. difficile associated diarrhoea.
A number of similar studies indicate safety and efficacy of probiotics including L. casei and S. boulardii against antibiotic associated diarrhoea (Cremonini et al., 2002; Boyle et al., 2006; McFarland, 2007; Monaghan et al., 2008). Most of them have used either commercial preparations of lactobacilli or dairy products as carrier vehicles for probiotics. Our study presents similar positive effects of probiotics using staple foods (rice and pulse) as the medium. Probiotic fermentation of such food ingredients has not only improved their nutritional attributes but has also added a therapeutic edge to it.

References


