A Study on Preparation of Probiotic Cheese and its Antagonistic Effect against Pathogenic Microorganisms

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The present study aims to isolate Lactobacillus sp which fulfills the basic criterion expected from probiotic strains, that is, capable of surviving at low pH and in the presence of bile salts. This isolated species were used for the preparation of probiotic cheese. Within the framework of the performed investigations, the author evaluated the physical, chemical, microbiological properties and antagonistic activity of probiotic cheese, antagonism towards selected pathogens. The performed experiments revealed that the isolated Lactobacillus sp were found to exhibit high antagonistic effect against Escherichia coli, followed by Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Proteus vulgaris, Clostridium difficile, Salmonella typhimurium, Vibrio cholerae and Campylobacter jejuni.

Key words: Lactobacillus sp, Probiotic, Bile tolerance, Antagonistic activity.

Let food be thy medicine and medicine be thy food" the age old quote by Hippocrates is certainly the tenet of today. As a result the market for functional foods or foods that promote health beyond providing nutrition, is flourishing. Within the functional foods, is the small but rapidly expanding arena of probiotics-live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance (Suvarna and Boby, 2005).

Probiotic bacteria have a long history of association with dairy products. A probiotic may be made out of a single bacterial strain or it may be consortium as well (Gilliland et. al., 1977).

Probiotics can be in powder form, liquid form, gel paste, granules or available in the form of capsules, sachets, etc.

Probiotic microorganisms exhibit some inhibitory activity against pathogenic bacteria by producing organic acids such as acetic, lactic and butyric acid, hydrogen peroxide and medium range antibiotics-like compounds designated as bacteriocins. The present study was conducted to prepare probiotic cheese and to assess the antagonistic activity of probiotic cheese against pathogenic microbes.

MATERIAL AND METHODS

Isolation & Identification of Lactobacillus sp

MRS agar media were used to isolate the Lactobacillus sp. from the milk samples. The isolated Lactobacillus sp was identified using gram staining, motility test and biochemical tests such as catalase, oxidase, starch hydrolysis,

Bile resistance
The ability of strains to grow in the presence of bile was determined according to the method of Gilliland (1997) with some modifications. The strain was inoculated (2% v/v) into MRS broth with 0.3% (v/v) of bile. Then the culture was incubated at 37°C and after 24 hours optical density was measured at 560 nm and it is compared to control culture (without bile salts).

Preparation of probiotic cheese
The milk was pasteurized at 70 to 76°C for 20-25 minutes. The probiotic Lactobacillus suspensions in 0.9% NaCl was added into the pasteurized milk. CaCl2 (25 g per 100 g) was added and coagulation occurs within 35 minutes at 35°C. The coagulated product was cooked to 39°C. The acidity of curd was pH of 6.4-6.5 at the end of the procedure. After cooking, the curd was placed into the mold to drain off the whey. Cheese was removed from the mold, salted in 18% brine at 9°C. The extraction from the brine broth allowed drying for 2 days and the cheese was stored in refrigerator.

(Dinakar and Mistry., 1994).

Physical analysis of cheese
Color, appearance, odour, flavor, body and texture of probiotic cheese were noted based on Gupta et al., (2002)

Chemical analysis

Determination of acidity in probiotic cheese
10 g of probiotic cheese was weighed, along with 25 ml of distilled water was added, homogenized and heated to near boiling. 0.4 ml of potassium oxalate and 1 ml phenolphthalein was added and titrated against 0.1 N NaOH. The end point is appearance of pink color. 2 ml of formaldehyde was added and pink color turns into white color. Again titrate with 0.1 N NaOH. The end point is appearance of pink color. The experiment was repeated to get concordant value. Percentage of titrable protein was obtained by using the following formula (Gupta et al., 2002)

\[ \% \text{ of titrable protein} = \frac{V 	imes 1.7}{V_2} \]

Where,
\( V \) = titrate value

Microbiological analysis
Coliform count was tested using MacConkey agar, Yeast and Mould count were enumerated using Potato Dextrose Agar U.S. Food and Drug Administration BAM (2001).

Determination of anti-pathogenic effect of probiotic cheese
Cross Streak Method
Muller Hinton agar plates were prepared and inoculated with the Lactobacillus as a streak across the surface of the agar plate and incubated at 37°C for 24-48 hours. Next day the sensitive / indicator cultures (Salmonella typhi, Vibrio cholera, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Campylobacter jejuni, Clostridium difficile and Listeria monocytogenes) were collected from Meenakshi Mission Hospital and Research Centre, Madurai were cross streaked perpendicular to the Lactobacillus culture and incubated again for overnight. Next day plates were observed for inhibition of growth at each side of cultures.

RESULTS AND DISCUSSION

Isolation and identification of Bacterial species
Lactobacillus sp present in the milk sample was enumerated as 22 × 10^6 CFU/ml. The occurrence of Lactobacillus sp in milk and milk products mostly in butter milk ranges from 1 million to 1 billion colonies per ml. This is due to high nutritive substrates of milk.

The bacterial isolates from MRS agar plates were identified as Lactobacillus sp. was
carried out by Bergy’s manual of bacteriology (1994) based on their colony morphology (white, round colonies), gram staining (positive), motility (non-motile) along with biochemical tests viz., catalase (negative), oxidase (negative), and starch hydrolysis (positive).

**Bile resistance**

The presence of bile salts was more inhibitory to lactic acid starter bacteria than other probiotic organisms. *Lactobacillus* sp proved to be quite resistant to bile salt and showed an OD value of 0.25 at 560 nm. This result showed the ability of *Lactobacillus* growth in the presence of bile. Similar result were observed by Hofmann and Mysels, 1992, Gilliland and Speck, 1977.

**Probiotic Cheese**

**Physical analysis**

The organoleptic evaluation of probiotic cheese attributes such as color (Yellowish), appearance (Smooth, glossy surface), odor (Pleasant smell), flavor (Salty in taste), body and texture (Soft and firm) were determined. According to the results, probiotic cheese with *Lactobacillus* sp shows changes in flavor i.e., slight souring during the storage period. Gupta et al., (2002) reported that wide variation occurred in the sensory characteristics like flavour, body, texture, colour and appearance of market misti curd.

### Table 1. Determination of acidity in probiotic cheese

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>Acidity (in percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>1</td>
<td>Cheese</td>
<td>0.65</td>
</tr>
</tbody>
</table>

### Table 2. Determination of protein in probiotic cheese

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>Protein content (in percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>1</td>
<td>Cheese</td>
<td>4.6</td>
</tr>
</tbody>
</table>

### Table 3. Determination of coliform count in probiotic cheese

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>Coliform (CFU/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>1</td>
<td>Cheese</td>
<td>5.4 ×10&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Table 4. Determination of yeast and mould count in probiotic cheese

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>Yeast and Mould count (CFU/gm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
</tr>
<tr>
<td>1</td>
<td>Cheese</td>
<td>2.7×10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Chemical analysis

Determination of acidity in probiotic cheese

Acidic content of probiotic cheese is listed in Table 1. Similar results were observed by Gupta et al., (2002) reported that the lactic acid bacteria produced maximum acidity in the absence of sugar and increase in sugar concentration, decreased the acid production.

Determination of protein in probiotic cheese

The protein percentage of sample is listed in Table 2. Similar results were obtained by Gupta et al., (2002)

Microbiological analysis

Determination of coliform count in probiotic cheese

The result of coliform count of probiotic cheese is given in Table 3. Similar results were obtained by Gupta et al., (2002) were it increases the shelf life of the products.

Determination of yeast and mould probiotic cheese

The yeast and mould count of probiotic cheese under different storage period are given in Table 4. Similar results were obtained by Gupta et al., 2002, Sakakibara et al., (2008). Similar kind of results was reported by the workers Suvarna and Boby, 2005, Sue et al., 1989. Vincent et al., (1959) reported that the antibacterial effect has been ascribed to the production of antibiotics or antibiotic like substances such as acidophilin and lactocidin produced by Lactobacillus acidophilus.

REFERENCES

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