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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 monocytogens, Proteus vulgaris, Clostridium difficile, Salmonella typhimurium, Vibrio cholerae* and *Camphylobacter 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 asses 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,

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glucose fermentation based on Bergy's manual of bacteriology., 1994.

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 37^0 C and after 24hours optical density was measured at 560nm and it is compared to control culture (without bile salts).

Preparation of probiotic cheese

The milk was pasteurized at 70 to 76^0C for 20-25 minutes. The probiotic *Lactobacillus* suspensions in 0.9% NaCl was added into the pasteurized milk. $CaCl_2$ (25g per 100g) was added and coagulation occurs within 35 minutes at 35^0C. The coagulated product was cooked to 39^0 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 9^0 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

10g of probiotic cheese was weighed, along with 25 ml of distilled water was added, homogenized and heated to near boiling. 1ml of phenolphthalein indicator was added and titrated against 0.1N NaOH. The end point is appearance of pink color. The titrate value (V1) was noted and repeated the experiment to get concordant value. Percentage of titrable acidity was obtained by using the following formula Gupta *et al*., (2002).

$$\% \text{ of titrable acidity} = \frac{9 \times V1 \times N1}{V2}$$

Where,

V1= titrate value

V2= weight of sample

N1= normality of NaOH

Determination of protein in probiotic cheese

10g of probiotic cheese was weighed, along with 25 ml of distilled water was added, homogenized and heated to near boiling. 0.4ml of potassium oxalate and 1ml phenolphthalein was added and titrated against 0.1 NaOH. The end point is appearance of pink color. 2ml of formaldehyde was added and pink color turns into white color. Again titrate with 0.1N 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)

$$\% \text{ of titrable protein} = V \times 1.7$$

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 37^0c for 24-48 hours. Next day the sensitive / indicator cultures (*Salmonella typhimurium, Vibrio cholera, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Camphylobacter jejuni, Clostridium difficile* and *Listeria monocytogens)* 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 22×10^5 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. Similiar 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

S. No	Sample	Acidity (in percentage)			
		1st day	2nd day	3rd day	4th day
1	Cheese	0.65	0.77	0.87	0.95

Table 2. Determination of protein in probiotic cheese

S. No	Sample	Protein content (in percentage)			
		1st day	2nd day	3rd day	4th day
1	Cheese	4.6	4.6	4.4	4.8

Table 3. Determination of coliform count in probiotic cheese

S. No	Sample	Coliform (CFU/gm)			
		1st day	2nd day	3rd day	4th day
1	Cheese	5.4×10^1	4.5×10^1	2.7×10^1	1.8×10^1

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

S. No	Sample	Yeast and Mould count (CFU/gm)			
		1st day	2nd day	3rd day	4th day
1	Cheese	2.7×10^2	5.5×10^2	6.3×10^2	8.1×10^2

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).

Determination of antipathogenic effect of probiotic cheese

The probiotic cheese was able to inhibit the growth of pathogens such as *Salmonella typhimurium, Vibrio cholerae, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Camphylobacter jejuni, Clostridium difficile, and Listeria monocytogens.* The results are expressed as the diameter of zone of inhibition i.e., 12mm, 10mm, 9mm, 8mm, 5mm, 5mm, 4mm, 4mm, 3mm and 3mm respectively to the pathogens such as *E. coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogens, Proteus vulgaris, Clostridium difficile, Salmonella typhimurium, Vibrio cholerae* and *C. jejuni.* 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*.

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