

Preparation of Bifidus Milk using Different Substrates and its Antagonistic Effect on Pathogens

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Human infant faecal samples were taken as a source of *Bifidobacterium sp.* The bacterial species were isolated with the aid of Transgalacto oligosaccharide Propionate (TP) medium and it can be identified based on their morphological carbohydrate fermenting ability and unique enzymatic property (bifidus shunt). The selected strain was used as a inoculum for the preparation of various types of fermented milk. Based on the sensory evaluation soy based bifidus milk was selected for further analysis which includes crude protein analysis and antagonistic property.

Key words: *Bifidobacterium*, Bifidus shunt, Transgalacto oligosaccharide propionate.

Bifidobacterium species are Gram positive anaerobic bacteria that inhabit the intestinal tracts of humans and animals. These bacteria were found to be a predominant component of intestinal flora in breast fed infants (Bezkorovainy and Miller-catch-pole, 1989 and Mitsuoka, 1990). *Bifidobacterium* was first described by Tissier in 1899 as they often exist in the Y – shaped obbifid form but it requires special nutrient supplements.

Bifidobacteria are differentiated from *Lactobacillus spp.* By their metabolic activity to utilize carbohydrates. Bifids use fructose 6 – phosphate pathway for hexose fermentation while

Lactobacillus uses glucose 6-phosphate shunt (De Vries *et al.*, 1990).

Establishment of high numbers of *Bifidobacterium* is reported to form barriers against the proliferation of exogenous pathogens. (Gibson and Wang, 1994). The purpose of this work was to screen the *Bifidobacterium* from the infant faecal (Predominant source) and used for the production of fermented milk and to determine the affectivity against pathogens.

MATERIAL AND METHODS

Collection of Samples

Thirty five Faecal samples were collected from breast fed infants (age 2 – 4 months). They were carefully transferred to the laboratory by using saline water containing sterilized screw capped bottles.

Isolation of strains

One gram of fresh feces was transferred

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in to the flask containing 9 ml of 0.2 % cysteine-HCl and the suspension was homogenized for 2 minutes. Serial dilutions were made and using spread plate technique, the suspension was inoculated in MRSc agar and TP agar. Then the plates were incubated at 37°C for 48 hours in the anaerobic chamber. Colony with distinct morphology was selected for further analysis. Morphological (Gram staining) and their metabolic properties were analysed with selected colonies.

F-6-PPK Test

According to Scardovi (1986) method, cells were grown in 5 ml of MRSc broth at 37°C for 48 hrs under anaerobic condition and the cells were harvested by centrifugation at 5000g for 10 minutes. The pellet was twice washed with 5 ml of 0.5 g/l phosphate cysteine buffer. After centrifugation, the pellet was collected in 1 ml buffer and disrupted by the addition of 0.4 ml of cetridium bromide (0.4 mg CTAB in 1 ml of distilled water). Carbohydrate fermentation test

The carbohydrates fermentation was determined on TPY containing bromocresol purple (0.04g/l) as a pH indicator and supplemented with 1% of the following carbohydrates: lactose, glycerol, adonitol, fructose, maltose, raffinose, xylitol, tagatose and gluconate. To ensure the anaerobic condition, each tube was supplemented with two drops of sterile liquid paraffin after inoculation (Samelis *et al.*, 1994 and Saidi *et al.*, 2002).

Production of fermented bifidus milk products

Preparation of milk:

100 ml of milk from cow (a1), buffalo (a2) and goat (a3) were collected in respective container. 100 grams of clean, dry soy (b1), wheat(b2) and cotton seed(b3) were taken and washed with water, then soaked for 6 -16 hrs. Then soaked materials rinsed again with water, grounded. Using cheese cloth, filtrate was collected. Milk samples were pasteurized.

Fermentation

The culture was inoculated (1%) in a respective containers and incubated at 37°C for 24 hrs under anaerobic condition

Measurement of pH, titratable acidity and Estimation of crude protein

The pH values were determined using a digital pH meter. Two drops of 1% phenolphthalein was added with 5 ml of fermented milk, mixed well

and titrated with 0.1N NaOH until the appearance of pink colour. Total acidity was calculated using formula. $\text{Acidity}(\%) = \frac{\text{volume of titrate (ml)} \times \text{Normality of NaOH} \times 64 \times 100}{\text{volume of sample} \times 1000}$ Quantity of Crude protein was estimated by determination of the total nitrogen following the kjeldahl method (AOAC, 1970) with conversion factor of 6.25.

Sensory evaluation (hedonic scale experiment)

Sensory attributes such as appearance, aroma, colour, taste and mouth feel and overall accessibility was tested in a hedonic scale experiment. A testing panel of 50 panelists was assembled for this study. Panelists were asked to evaluate the above criteria and overall accessibility of the sample.

Statistical analysis of experimental data

Statistical analysis was performed by using sensory evaluation subjected to ANOVA in two way classification.

Antibacterial activity

Antibacterial activity of various types fermented milk were tested by the well diffusion method using six bacterial strains, *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi*, *shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus*. Bacterial strains were spreaded over the Mueller Hinton agar; the wells were made with the aid of flamed cork borer on the surface of the agar plates. Approximately 0.1 ml of fermented product was transferred the well respectively (a1, a2, a3 and b1, b2, b3), incubated at 37 °C for 24 – 48 hrs.

RESULT AND DISCUSSION

Based on the cultural characteristics (creamy white colony, puncti form and 0.1-0.5 mm in diameter) morphology (Gram positive) the isolates were purified and presumed as lactic acid bacteria. All the isolates were catalase, oxidase and nitrate reduction negative. The presence of fructose 6 phosphate phosphoketolase enzyme was revealed by the appearance of reddish brown colour. This phenotypical characteristics and enzyme assay described by scardovi (1986) and Tamine *et al.*, (1995) resulted the identification of *Bifidobacterium*.

According the method of Vlkova *et al.*, (2002), modified F-6-PPK test was used for the

Table 1. Carbohydrate fermentation of the isolate

Carbohydrates	Characteristics of isolates
Lactose	-
Glycerol	-
Adonitol	-
Fructose	+
Mannitol	+
Maltose	+
Raffinose	-
Xylitol	-
Tagatose	+
Gluconate	-
Identified species	<i>Bifidobacterium bifidum</i>

identification of *Bifidobacterium*. Species level identification is accomplished based on the carbohydrate fermentation (Tab.1) (Miloud *et al.*, 2005). Titratable acidity and pH was determined at the interval of 12 hrs and 24 hours (Tab.2). Sensory evaluation of fermented milk values from 50 panels calculated based on the arithmetic median. A hedonic scale test showed the overall acceptances of milks were cow and soymilk (Table. 3 &4) that showed results which accept the previous work results of Vijith and Martin (2004). The antagonistic property of fermented products ((Fig.2) were evaluated against six pathogenic bacteria instead of four done by Misra and Kulia (1991).

Table 2. pH and titratable acidity of the fermented foods

Milk Substrates	Time interval (hours)			
	12		24	
	pH	Acidity	pH	Acidity
Cow	4.54	1.2	3.76	1.7
Buffolo	4.93	1.1	3.54	1.5
Goat	4.56	0.9	3.82	1.2
Soy	5.72	0.5	4.27	0.9
Wheat	5.91	0.4	4.32	0.6
Cotton seed	5.23	0.2	4.29	0.5

Table 3. Sensory evaluation of fermented milk

Characteristics	Types of Milk Substrates					
	Cow	Buffalo	Goat	Soy	Wheat	Cottonseed
Appearance	3.7	3.2	3.2	4.0	3.4	2.9
Aroma	3.7	3.2	3.3	3.6	3.4	3.0
Colour	3.8	3.1	3.4	3.6	3.1	3.0
Mouth Feel	3.6	3.2	3.2	3.7	3.1	3.0
Taste	4.1	3.6	3.2	3.8	3.3	3.1

0-1 Very Much Dislike, 1-2 Dislike, 2-3 Dislike Or Like, 3-4 Like, 4-5 Like Verymuch

Table 4. Statistical analysis

Source of variance	Computed value	At 1% level of significance	At 5% level of significance
Variance between the coloumn	4.0195	4.10	2.73
Variance between the row	4.9760	4.43	2.87

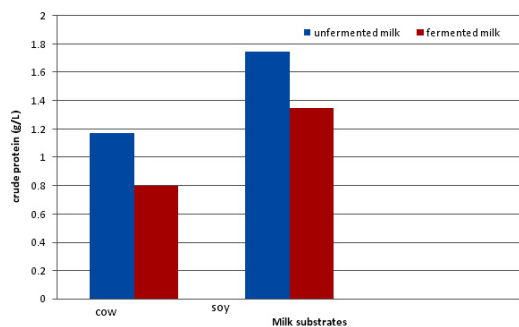


Fig. 1. Estimation of crude protein



Various types of milk substrates

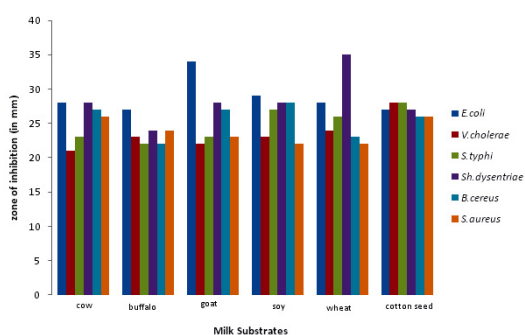


Fig. 2. Antagonistic activity of fermented milk



Fermented food products

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