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Prebiotic effect of honey on the growth of *bifidobacterium longum* bb-46 in synbiotic whey drink

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Abstract

A study was conducted to assess the prebiotic potential of honey towards *Bifidobacterial species* in the preparation of synbiotic whey. Both the cheese whey and paneer were inoculated with *Bifidobacterium longum* bb-46 at 2 per cent level and prebiotic substance-Honey at 1.5, 2.0 and 2.5 per cent levels (designated as SWD_{1.5}, SWD₂ and SWD_{2.5}) and incubated at 37°C for 8 hrs and analysed for the bifidobacterial count at every 2 hours. The prebiotic effect of honey on the growth of *Bifidobacterium longum* was high in SWD₂ at 4 hours with a colony count of 8.93 log₁₀ cfu/ml paneer whey compared to cheese whey with a maximum count of 8.83 log₁₀ cfu/ml. the maximum count of 8.93 log₁₀ cfu/ml was reached in 4 hr of incubation in SWD₂.

Keywords: Whey, *Bifidobacterium longum*, prebiotics, honey

Introduction

In India, nearly 50 per cent of the milk produced is utilized for the preparation of dairy products. During this process, several by-products viz., whey, skim milk, and buttermilk are obtained. Among this, whey is the major by-product obtained during the preparation of cheese, paneer, Channa, shrikhand and casein. Whey is a watery liquid by-product of dairy industry during the preparation of channa, paneer, cheese and casein. World whey production is 180 to 190 tonnes/year with an annual increment of 1-2% and only 50% of whey is utilized/processed. (Roman *et al.* 2012) [7]. Whey contains 45-50% total milk solids, 70% lactose, 20% milk proteins and 70-90% milk minerals and almost all the water soluble vitamins originally present in milk (Horton, 1995) [4]. Whey is a serious pollutant as it imposes a very high BOD of 30,000- 50,000 mg/lit and chemical oxygen demand of 60,000-80,000 mg/lit. Discarding of whey also constitutes a significant loss of potential nutrients and energy and has been looked upon seriously by the environmentalists and technologists due to its potent polluting strength. So, Whey disposal becomes a serious environmental pollutant being loaded with high amount of organic matter. Whey possesses preventive and curative elements responsible for treatment of ailments such as arthritis, anemia and liver complaints. (Cruz *et al.* 2009) [2] Since whey has a number of nutritional aspects and to increase the nutritional value of fermented as well as non fermented whey drinks have emerged in the market. To make it more dietetic, probiotic cultures are being used for fermentation and prebiotic substances like honey was incorporated in the preparation. Addition of probiotics imparts many potential health benefits like alleviation of lactose intolerance, hypocholesterolemic effect, immunostimulatory effect and anti-carcinogenic effect. Addition of probiotics imparts many potential health benefits like alleviation of lactose intolerance, hypocholesterolemic effect, immunostimulatory effect and anti-carcinogenic effect. In recent years, *Bifidobacterium bifidum* has been considered as an important bacteria for human health, the main probiotic effects attributed to these bacteria include, improvement in lactose utilization, prevention of diarrhoea, colon cancer, hypercholesterolemia, improvement of vitamin synthesis and calcium absorption, development of longer villi and significantly deeper crypts in the ileal region of the gut and production of substances of low molecular mass with antimicrobial activity (Vinderola *et al.*, 2000) [10].

It has been revealed that honey had a beneficial impact over bifidobacteria attributing to its enhanced growth and due to the carbohydrate and complex mixture of oligosaccharides present in honey (Ustunol and Gandhi, 2001) [9].

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In this regard, a study was conducted to prepare synbiotic containing *Bifidobacterium longum* bb-46 and honey (as a prebiotic source) whey drink and the results of prebiotic potential of honey is given and discussed in this article.

Materials and Methods

Fresh cow milk obtained from University Research Farm was used to paneer and cheese whey at the model Dairy Plant, Department of Livestock Products Technology (Dairy Science), Madras Veterinary College. Citric acid was used for the preparation of paneer whey and lactic acid and Fromase (Gist-brocades, France) were used for the preparation of cheese whey. Agmark Grade A honey from the finest apiaries of India, marketed by Dabur India Ltd., (West Bengal) was used in the study. *Bifidobacterium longum* Bb – 46, DVS culture was procured from Chr. Hansens Lab and the culture was directly added to the sterile whey. Propagation of bifid culture were carried out using yoshioka media (Suresh Subramonian, 2001)

Preparation of paneer whey

Paneer whey was prepared as per the procedure described by Bhattacharya (2001) [1].

Preparation of cheese whey

Cheese whey was prepared as per the procedure adopted by Nareshkumar (1993) [5].

Cheese and paneer whey were obtained from whole milk. The whey was partially deproteinised at 78°C for 30 min and filtered through a muslin cloth. The whey were inoculated with *Bifidobacteria species* at 2 per cent level and prebiotic substance-Honey at 1.5, 2.0 and 2.5 per cent levels (designated as SWD_{1.5}, SWD₂ and SWD_{2.5}) and incubated at 37°C for 8 hrs and analysed for the bifidobacterial count at every 2 hours.

Results

The number of colonies formed by different dilutions of bifidus cultured in paneer and cheese whey with added honey at 1.5 per cent, 2 per cent and 2.5 per cent (SWD_{1.5}, SWD₂, and SWD_{2.5}) at 0, 2, 4, 6, 8 h of incubation were counted. The mean values of six trials were tabulated in Table 4 as log₁₀ colony forming units per ml (log₁₀ cfu/ml). The log₁₀ cfu/ml were paneer whey (8.65, 8.68, 8.71, 8.59, 8.46) for control, (8.81, 8.81, 8.83, 8.81, 8.75) for SWD_{1.5}, (8.81, 8.84, 8.93, 8.85, 8.83) for SWD₂ and (8.81, 8.74, 8.80, 8.74, 8.69) for SWD_{2.5}. The log₁₀ cfu/ml cheese whey were (8.54, 8.66, 8.69, 8.65, 8.51) for control, (8.72, 8.76, 8.74, 8.82, 8.65) for SWD_{1.5}, and (8.72, 8.83, 8.83, 8.77, 8.72) for SWD₂ and (8.72, 8.68, 8.71, 8.65, 8.54) for SWD_{2.5}. Statistical analysis of the data revealed significant difference at ($p \leq 0.01$) between the treatments and hours.

Discussion

The bifidobacterial growth in paneer and cheese whey with honey at 1.5 per cent, 2 per cent and 2.5 per cent (SWD_{1.5}, SWD₂ and SWD_{2.5}) after 0, 2, 4, 6, 8 hr of incubation were assessed. The colonies formed were measured as log₁₀ cfu and it revealed a maximum of 8.93 log cfu after 4 hr of incubation in paneer whey. There was a slight reduction in the colony count after 4 hr. This could be due to the accumulation of metabolic end products (Dubey and Mistry, 1996) [3].

The probiotic load of 10⁸ cfu/ml gives better health benefits when consumed and the maximum count of 8.93 log₁₀ cfu/ml was reached after 4 hrs of incubation in SWD₂. The low count

in the control and higher count after honey addition could be due to the presence of fructo-oligosaccharides (Ustunol, 1996) [8].

As preparing of synbiotic whey drink is done preferably at the log phase at a lower acidity and higher pH, the significant difference in prebiotic effect of honey SWD₂ at 4 hr over the control was considered beneficial.

Statistical analysis revealed significant difference at $p \leq 0.01$ between the prebiotic effect of honey on the growth of *Bifidobacterium longum* at 4 and 8 hr. in SWD_{1.5}, SWD₂ and SWD_{2.5} from their initial colonies of 8.81, 8.81, 8.81 as log₁₀ cfu/ml.

The prebiotic effect of honey in SWD with 2.5 per cent honey revealed a decline in the colony count. This could be related to the bacteriostatic effect of higher level of "Inhibine" present in honey and also due to other enzymatic by products like hydrogen peroxide (NHB, 1996) [6].

Table 1: Prebiotic effect of honey on the growth of *Bifidobacterium longum* in whey drink

Hours	Colonies as log ₁₀ CFU/ml							
	Paneer whey				Cheese whey			
	Control	SWD _{1.5}	SWD ₂	SWD _{2.5}	Control	SWD _{1.5}	SWD ₂	SWD _{2.5}
0	8.65	8.81	8.81	8.81	8.54	8.72	8.72	8.72
2	8.68	8.81	8.84	8.74	8.66	8.76	8.72	8.68
4	8.71	8.83	8.93	8.80	8.69	8.74	8.83	8.71
6	8.59	8.81	8.85	8.74	8.65	8.82	8.77	8.65
8	8.46	8.75	8.83	8.69	8.51	8.65	8.72	8.54
F – Value	30.50**				28.11**			
S.E.	0.021				0.017			
C.D.	0.079				0.065			

Percentages (mean ± SE) Average of six trials. Mean values bearing different superscripts in a column differs significantly ** ($P < 0.01$).

Control - Synbiotic whey drink using paneer and cheese whey with *Bifidobacterium longum*

SWD_{1.5} - 1.5 per cent honey added SWD using paneer and cheese whey with *Bifidobacterium longum*

SWD₂ - 2 per cent honey added SWD using paneer and cheese whey with *Bifidobacterium longum*

SWD_{2.5} - 2.5 per cent honey added SWD using paneer and cheese whey with *Bifidobacterium longum*

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