The Bioscan 12(1): 85-87, 2017
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

ACTION OF BIFIDOBACTERIA ON WHEY PROTEINS

RITA NARAYANAN*, B. SURESH SUBRAMONIAN AND DOROTHY PUSHPARANI
Department of Dairy Science,
Madras Veterinary College, TANUVAS Chennai - 600 007, INDIA
e-mail: ritanarayanan@yahoo.com

KEYWORDS
Whey proteins
Bifidobacteria
Chromatogram

Received on :
02.12.2016

Accepted on :
25.01.2017

***Corresponding author**

ABSTRACT
Bifidobacteria species are known to possess probiotic property. Millets like finger millet and whey proteins present in bovine milk act as prebiotics and hence their use in the preparation of probiotic foods would enhance the functionality of the product. Finger millet contains fractions which is known to contribute to fermentation. In the present study the *Bifidobacterium longum* was isolated from infant faeces and cultured in samples containing whey and whey with malted ragi extract. A HPLC chromatogram conducted showed the degradation of the proteins by the bifidobacterium. The chromatogram revealed a gradual decrease in the peak of whey protein fractions like alpha-lactalbumin and beta- lactoglobulin due to the culture activity of *B.longum* during fermentation. The results of the HPLC analysis shows that the degradation of both lactalbumin and lactoglobulin content due to action of *B. longum* was higher in whey + ragi + probiotic (77.5% and 75.5%) than in whey sample + probiotic (42.2% 37.7%) which may be due to the enhanced growth pattern of *B.longum* in the presence of ragi. Hence it can be concluded that the isolate under study had shown enhanced viability with the prebiotics.

INTRODUCTION

Colonic foods, which encourage the growth of favourable bacteria, are referred as prebiotics. Oligosaccharides such as lactulose galacto oligosaccharides, inulin, fructo oligosaccharides (FOS) and other food carbohydrates are some of the examples of prebiotics. Breast milk contains prebiotic oligosaccharides that are fermented by colonic *Lactobacilli* and *Bifidobacteria* stimulating their growth and activity. This helps to develop and mature the intestinal immune function. (Allan Walker, 2006). This group of oligosaccharides are of interest because, they are neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract, and may beneficially affect the growth or activity of desirable bacteria in the colon (Cummings *et al*., 2001).

Consumer awareness and interest in nutritious healthy foods have driven much of the research into the beneficial effects of whey and whey fractions. Whey, a byproduct obtained from cheese manufacture was often disposed off as waste in the past. Whey is a biological source of most valuable proteins and is rich in minerals and vitamins especially Vitamin B2. It is an important source of lactose, serum proteins and soluble vitamins, which makes this product to be considered as a functional food (Ha and Zemel , 2003). Fennema (1965) reported that alpha-lactalbumin represents 28 per cent of the total whey protein content in bovine milk. Seventy per cent of protein in human milk is whey protein and 41 per cent of that protein is alpha - lactalbumin. Addition of bovine alpha lactalbumin is strongly advocated to humanize infant formulas and create other products for people with limited or restricted protein intakes. Finger Millet (Ragi, *Eleusine coracana*) is an important food in many parts of India. It is rich in protein, iron, calcium, phosphorous, fibre and vitamin content. By virtue of its nutritive value, finger millet has industrial potential in the manufacture of baby and sick person's food formulations. Traditionally ragi is processed either by malting or fermentation. Malting and fermentation of finger millet improves its digestibility, sensory and nutritional quality as well as pronounced effect in lowering the antinutritional factors. The stimulatory effect of malted ragi milk was due to arabinoxylan, the fraction contributing prebiotic action / prebiotic precursor. (Pokharia, 2008)

In the present study an attempt was made to study the action of *B.longum* on the breakdown of whey proteins in whey and in whey based ragi malt for easy assimilation

MATERIALS AND METHODS

Bifidobacterium longum was isolated and identified from infant faeces by using phenotypic and molecular characterization. The stock culture was grown and propagated in Yoshika broth. Five percent of *B.longum* culture was inoculated in whey and incubated for 4 hours. Another treatment consisted of inoculating five percent *B.longum* in whey containing 9 per cent ragi malt extract and incubated The inoculated and incubated samples were then analysed for degradation of whey proteins using HPLC.

Degradation of whey protein by *B. longum* using HPLC

WPC standards were quantified by means of reverse phase high performance liquid chromatography (Ruprichova, 2012).

For alpha lactalbumin and beta lactoglobulin determination, an analytical HPLC unit (Waters ,USA), Rheodyne injector with 20 μl loop with PDA detector and C18 (Phenominex), 150x 5 um was used. Column oven temperature for whey protein detection was 40^{o}C .

Mobile phase A included 0.1 per cent trifluoroacetic acid (TFA) and 5 per cent acetonitrile in water and mobile phase B

included 0.08 per cent trifluoroacetic acid (TFA) and 90 per cent acetonitrile in water. Gradient elution and flow rate of 1.0ml per minute was applied. The detection was performed at 214nm. The samples under study were filtered through a nylon membrane filter (0.22μm) into vials and used for analysis. The sample analysis lasted for 20 minutes.

RESULTS AND DISCUSSION

Degradation of Whey proteins by *B. longum*

Figure 1 illustrates the chromatogram of whey protein present in both the samples . The chromatogram revealed the presence of lactalbumin and lactoglobulin in whey and whey with ragi milk and *B.longum* after 4 hours of incubation. The gradual decrease in the peak area of the alpha lactalbumin and beta lactoglobulin in the whey and ragi samples with *B.longum* indicates the effect of the isolate *B.longum* on whey proteins during fermentation.

Degradation of Whey proteins by *B. longum*

Figure 1revealed the breakdown of whey proteins like alpha-lactalbumin and beta- lactoglobulin in whey and whey based ragi malt with *B.longum* after 4 hours of incubation.

The peak area within samples indicated a gradual decrease which may be due to the culture activity of *B.longum*

during fermentation. As given in Fig. 2 & 3,in comparison to whey used as standard the lactalbumin content on analysis exhibited a 42.2 % and 77.5 % of degradation in whey sample + probiotic and whey + ragi + probiotic respectively due to action of *B.longum*. In comparison to standard the lactoglobulin content on analysis exhibited a 37.7% and 75.5 % of degradation in whey sample + probiotic and whey + ragi + probiotic respectively due to action of *B.longum*. This is concordant with the study conducted by Belkaaloul *et al.* (2010) who evaluated the growth, acidification and proteolytic activity of *B.longum, L.plantarum, S.thermophilus* and reported that the associations (*L.plantarum* + *B.longum*) give the best protein degradation.

The decrease in concentration of lactalbumin and lactoglobulin after incubation may be due to hydrolysis of soluble proteins into peptides by *B.longum*. This is concordant with the study of Mullally *et al*. (1997) and Belem *et al*. (1999).

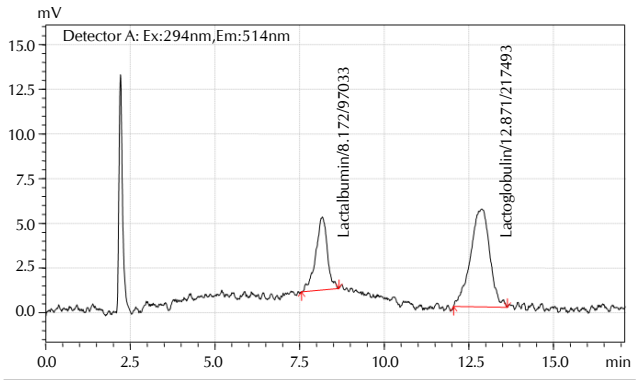
The composite mix contained many simple sugars and complex carbohydrates which provided a source of energy for maintaining the growth and viability of *Bifidobacteria*.

Table 1: Action of Bifidobacteria on Lactalbumin

Sample	Whey protein	Retention time	Area	Action of Bifidobacteria on lactalbumin (expressed as % degradation based on HPLC chromatogram area)
Whey	Lactalbumin	8.172	97033	
Whey sample + Probiotic	Lactalbumin	7.882	56110	42.20%
Whey + ragi + probiotic	Lactalbumin	8.138	21795	77.50%

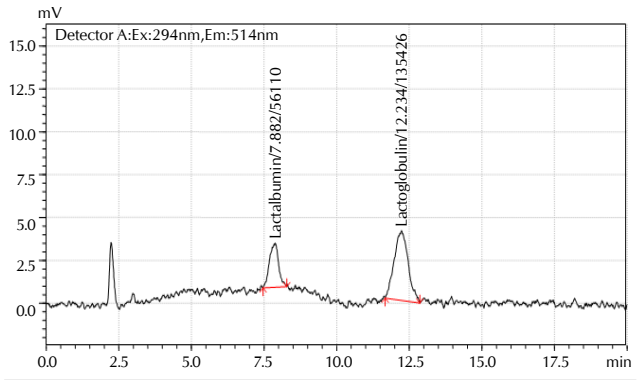
Table 2: Action of Bifidobacteria on Lactoglobulin

Sample	Whey protein	Retention time	Area	Action of Bifidobacteria on Lactoglobulin (expressed as % degradation based on HPLC chromatogram area)
Whey	Lactoglobulin	12. 872	217493	
Whey sample + Probiotic	Lactoglobulin	12.234	135426	37.70%
Whey + ragi + probiotic	Lactoglobulin	12.851	53365	75.50%



Name	Ret. Time	Area
Lactalbumin	8.172	97033
Lactoglobulin	12.871	217493

Treatment 1: Chromatogram on the effect of *B. longum* in fermented whey using HPLC



Name	Ret. Time	Area
Lactalbumin	7.882	56110
Lactoglobulin	12.234	135426

Treatment 2: Chromatogram on the effect of *B. longum* in fermented whey and malted ragi milk using HPLC

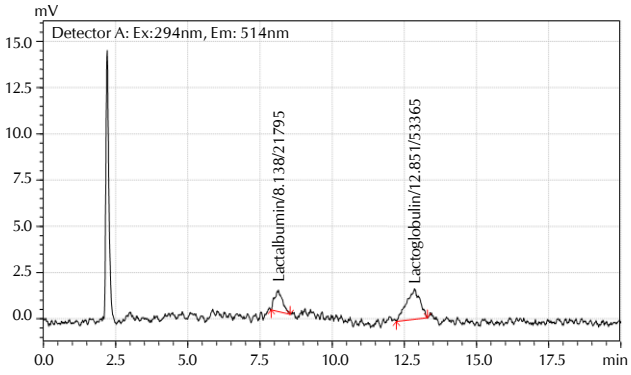


Figure 1: Chromatogram on the effect of *B. longum* in fermented whey and whey based malted ragi using HPLC standard

The results of the HPLC analysis shows that the degradation of both lactalbumin and lactoglobulin content due to action of *B.longum* was higher in whey + ragi + probiotic (77.5% and 75.5%) than in whey sample + probiotic (42.2% 37.7%) which may be due to the enhanced growth pattern of *B.longum* in the presence of ragi. The enhanced growth patterns with malted finger millet may be due to the crude xylo-oligosaccharides present in ragi as suggested by Chithra and Muralikrishna (2011) who established the prebiotic nature of the crude xylo-oligosaccharides on *Bifidobacterium* and *Lactobacillus* sp. This study thus confirmed the isolate under study had shown enhanced viability with the prebiotics used in this study.

REFERENCES

Allan Walker, W. 2006. Prebiotic oligosaccharides induce optimal development of the intestinal immune system in newborn and infants. Active Food Scientific Monitor, *ORAFTI* Newsletter Nr.14 Spring.

Belem, M. A. F., Gibbs, B. F. and Lee, B. H.1999. Proposing sequences for peptides derived from whey fermentation with potential bioactive sites. *J. Dairy Sci.* **88:** 486-493.

Chithra Manisseri and Muralikrishna Gudipati 2011. Prebiotic Activity of Purified Xylobiose obtained from ragi (*Eleusine coracana*, Indaf-15) bran. *Indian J. Microbiol.* pp. 1-7.

Cummings, J. H., Macfarlane, Y. T. and Englysl, H. N. 2001. Prebiotic digestion and fermentation. *Am. J. Chem. Nutr.* **73(2):** 415S-420S.

Fennema, O. 1965. Composition of Milk Proteins. Food chemistry 2nd edition Marcel. *Dekker Inc.,* New York. NYP. p. 796.

Ha, E. and Zemel, M. B. 2003. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. *J. Nutr. Biochem.* **14**: 251-258.

Mullally, M. H. Meisel and Fitzgerald, R. 1997. Angiotensin- enzyme inhibitory activities of gastric and pancreatic proteinase digests of whey proteins. *Int. Dairy J.* **7:** 299-303.

Pokharia, A. K. 2008. Paleoethnobotanical record of cultivated crops and associated weeds and wild taxa from Neolithic site. Tokwa, Uttar Pradhesh, India. *Curr. Sci.* **94:** 248-255.

Ruprichova, Lenka, Michaela Drackova, Ivana Borkovcova and Lenka Vorlova 2012. Determination of proteins in yoghurt. *J. Microbiol. Biotechnol and Food Sci.***1**(4): 644-650.