Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(6): 279-282
Received: 07-09-2021
Accepted: 18-10-2021

Prabha R
Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bangalore, Karnataka, India

Ramachandra B
Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bangalore, Karnataka, India

PA Shankar
Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bangalore, Karnataka, India

Corresponding Author:
Prabha R
Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Bangalore, Karnataka, India

Effect of vegetable extract as bifidogenic agents on the growth of wild strains of bifidobacteria

Prabha R, Ramachandra B and PA Shankar

Abstract
Bifidobacteria are considered as good bacteria residing in the intestinal tracts of human beings, predominated in breast fed infants extending the therapeutic benefits. They are the indictors of healthy gut. A bifidus factor or Bifidogenic factors are compounds that specifically enhance the growth of bifidobacteria in the intestines of humans. The bifidogenic factors include natural food ingredients like oligosaccharides (Fructo, Xylo, Galacto), resistant starch, pantathine etc. Vegetables contain growth stimulants for bifidobacteria in the form of dietary fibres. In this article an attempt has been made to find the good vegetable that stimulate bifidobacteria through incorporating their cold extract into MRS broth. Among 33 commonly available vegetables, ash gourd, carrot, chow-chow and tomato consistently stimulated the wild strains of bifidobacteria such as *B. longum* F51(isolated from infant faeces) & PF1(isolated form probiotic feed); *B. adolescentis* F35 (infant faecal isolate) and *B. bifidum* S26 (skin isolate of lactating mother) with direct microscopic counts ranging from 8.14 to 8.60 $\log_{10}$/ml. In sterile skim milk, the vegetable extracts led to early setting and improved in titratable acidity. The skim milk took 48 to 96 hours to curdle but incorporation of cold extracts of ash gourd (*Benincasa hispida*), carrot (*Daucus carota*) and tomato (*Solanum lycopersicum*) reduced the curdling time and made the milk to set at 20 to 22 hours at 37 ^{0}C with DMC 8.05 to 8.22 $\log_{10}$/ml and 0.33 to 0.48% lactic acid. This indicated that some of the vegetables have the ability to stimulate the growth of bifidobacteria.

Keywords: Bifidogenic factor, oligosaccharides, cold extract, Incorporation

Introduction
The beneficial effects of Bifidobacterium as an intestinal microbe are well known. Breast fed infants are characterized by a predominance of Bifidobacterium in the intestine of up to 10^{10} cells/g [1] and are protected from enteric infection by these intestinal microflora. The relative abundance of bifidobacteria has been shown to decrease following weaning, and from adolescence into adulthood, with a further decline when their hosts become elderly [2]. In many recent studies the health benefits resulting from the proliferation of bifidobacteria in the intestine have been observed not only in infants but also in adults. A detailed understanding of carbohydrate metabolism of a particular bifidobacterial species and/or strains may offer opportunities to increase its abundance in the adult gut by dietary means. One way to positively modulate the gut microbiota is by the supplementation of so-called prebiotics, where a prebiotic is defined as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' [3]. Prebiotics that specifically stimulate bifidobacterial growth are termed 'bifidogenic' [4]. Knowledge on which plant carbohydrates can be metabolized by a bifidobacterial species may therefore offer an opportunity to increase the abundance of bifidobacteria in the adult gut. For instance, *Bifidbacterium longum* subsp. *infantis* is associated with the infant gut, and is specialized in HMO metabolism, whilst *B. longum* subsp. *longum*, associated with both the infant and adult gut, can metabolize plant-derived oligosaccharide [5].

In light of the health benefits of bifidobacteria to promote the proliferation of indigenous bifidobacteria in the intestine, vegetables which are the normal portion of the human daily diet may have the bifidogenic factors in the fibre portion thus stimulate the growth. The plant cell wall consists of a matrix comprised of cellulose fibrils, hemicellulose, pectin and lignin [6]. Dietary fibers represent polymeric carbohydrates, including lignin, consisting of ten or more monomeric subunits that cannot be hydrolysed by enzymes found in the upper part of the human gastrointestinal tract (such as lactases, amylases and sucrases) [7]. Glycan is a much broader term that refers to a wide variety of carbohydrates (polymers and oligosaccharides). Glycans of dietary origin are generally indigestible to the human host, yet may be metabolized by the gut microbiota, and may include carbohydrates with less than 10 monomeric units that have been generated by the gut microbiota following dietary fibre degradation [8].

Many scientists have tried adding various natural food items like green leafy vegetables, tomato, carrot and other vegetable in the form for the stimulation purpose of bifidobacteria [9, 10, 11].

Materials and Methods

Collection of vegetables: commonly available fresh vegetables in the commercial markets were collected in 100 g capacity and brought to the laboratory for extraction.

Preparation of vegetable extracts: one part of vegetable with 5 parts of water was sterilized at 121 ºC for 15 min, macerated in blender and filter sterilized. Cold extract was prepared by 1:4 ratio. Grounded and muslin cloth filteration and membrane filtered for sterility

Preparation of MRS broth: Hot extract of vegetable was used as base for the preparation of MRS broth (Harrigan, 1998). To the prepared sterile MRS broth containing the vegetable extract, was inoculated with four cultures of bifidobacteria such as *B. longum* F51 (isolated from infant faeces) & PF1 (isolated form probiotic feed); *B. adolescentis* F35(infant faecal isolate) and *B. bifidum* S26(skin isolate of lactating mother).

Addition of vegetable extracts to milk: Both hot and cold extracts of selected vegetables were neutralized to milk pH and separately added to sterile double strength reconstituted skim milk(18% TS) in the ratio of 1:1 and inoculated with four cultures of bifidobacteria such as *B. longum* F51 & PF1; *B. adolescentis* F35 and *B. bifidum* S26. Incubation was done at 37^{0}C till curdling and later titratable acidity (% Lactic acid), DMC were determined.

Addition of different levels of tomato, carrot and ash gourd to skim milk: Cold extracts of tomato(*Solanum lycopersicum*), carrot (*Daucus carota*) and ash gourd (*Benincasa hispida*), were individually added to double strength skim milk at 1:1, 1:0.8, 1:0.4 and 1:0 and sterilized, inoculated with above mentioned 4 cultures of bifidobacteria individually.

Results and Discussion

Effect of incorporation of vegetable extracts into MRS broth on the growth of selected cultures of bifidobacteria:
The growth stimulatory property of different vegetable extracts was initially tested using MRS broth. It may be seen in the Table 1A & Table 1B that the growth of culture *B. longum* F51 was stimulated by the extracts of ash gourd, carrot, ridge gourd and tomatoes DMCs were 8.44, 8.30, 8.33 and 8.35 $\log_{10}$/ml respectively compared to 8.20 in control (only MRS broth). Similarly the growth of *B. longum* PF1 was stimulated by ash gourd, raddish and tomato. In respect of *B. adolescentis* F35, growth was stimulated by ash gourd, chow-chow and tomato and the DMC was 8.51, 8.69, 8.66 and 8.60 $\log_{10}$/ml respectively. The growth of *B. bifidum* S26 was stimulated by the extracts of carrot, ash gourd, snake gourd and tomato with DMC of 8.66, 8.61, 8.56 and 8.55 $\log_{10}$/ml respectively compared to 8.39 in control. It could be seen that ash gourd, tomato and carrot consistently stimulated the growth of all the four cultures of bifidobacteria.

Effect of incorporation of vegetable extracts into skim milk on the growth of selected cultures of bifidobacteria:
All the four cultures of bifidobacteria grew slowly in plain skim milk and took between 48 and 96 hours to curdle the milk. In contrast it may be seen in Table 2 that *B. longum* F51 & PF1 took 18 hour to curdle while *B. adolescentis* F35 and *B. bifidum* S26 took 20 hours to curdle skim milk containing cold extract of tomato with TA values ranging from 0.36 to 0.43% and DMC ranging from 8.11 to 8.32 $\log_{10}$/ml. However in the presence of hot extract of tomato the curdling time was marginally increased as it ranged from 20 hours to 22 hours in all the cultures tested with TA values ranging from 0.33 to 0.43% and DMC ranging from 8.05 – 8.21 $\log_{10}$/ml. Similarly addition of cold carrot juice resulted in curdling times ranging from 18 to 20 hours with TA value ranging from 0.41 to 0.43% and DMC ranging from 8.13 – 8.21 $\log_{10}$/ml. while hot carrot extract when added to milk, the curdling times ranged from 20 to24 hours with TA value ranging from 0.36 to 0.48% and DMC ranging from 8.07 – 8.15 $\log_{10}$/ml. Cold extracts of ash gourd, curdled the milk within 20 hours by all the four cultures of bifidobacteria with TA of 0.37 to 0.41% TA and DMC of 8.17 to 8.22 $\log_{10}$/ml.
The screening of vegetable extracts including green leafy vegetables revealed their bifidogenic nature. Among that hot and cold extracts of tomato, carrot and ash gourd exerted appreciable stimulatory activity on the growth and acid production by bifidobacteria in milk suggesting that the active principles present in these samples are heat resistant. Zbiskowski and Zikjka (1986) [11] have found heat resistant growth factors in carrot extract. Similarly potato extract [9] and maize extract [12] have shown to cause stimulation of bifidobacteria. Heat stability of these stimulatory factors reported for carrot extract as well for many vegetable extracts [8, 10].

Table 1A: Growth of bifidobacteria in MRS broth containing different vegetable extracts at 37^{0}C for 24 hours

Control & Name of the vegetable extract (botanical nomenclature)	Species of *Bifidobacterium*							
	***B.longum* F51 (faecal isolate)**		***B.longum* PF1 (Feed isolate)**		***B.bifium* S26 (faecal isolate)**		***B. adolescentis* F35 (faecal isolate)**	
	pH	**DMC ($\log_{10}$/ml)**	**pH**	**DMC ($\log_{10}$/ml)**	**pH**	**DMC ($\log_{10}$/ml)**	**pH**	**DMC ($\log_{10}$/ml)**
MRS as control	4.10	8.20	3.72	8.20	3.78	8.39	3.78	8.27
Green Leafy Vegetables								
Amaranthus (Dantu) -A*maranthus dubius*	4.24	7.95	3.94	8.09	4.18	8.27	4.18	8.11
Amaranthus (Harivae) *Amaranthus viridis*	4.33	8.04	3.99	8.10	4.20	8.23	4.20	8.14
Cabbage *Brassica oleracea*	4.20	7.90	3.63	8.09	3.73	8.25	3.73	8.47
Coriander leaves *Coriandrum sativum*	4.17	7.69	3.79	7.87	3.80	8.00	4.07	7.60
Curry leaves *Murraya koenigii*	4.23	8.04	3.90	8.02	3.90	7.84	4.18	7.82
Fenugreek leaves *Trigonella foenum-graecum*	4.29	8.14	3.94	7.97	3.95	8.51	4.14	8.27
Mint leaves *Mentha piperita*	4.25	7.69	3.85	7.81	3.91	8.02	4.19	7.95
Shepu *Anethum graveolens*	4.18	7.77	3.90	7.87	3.80	8.40	4.09	8.09
Spinach *Spinacia oleracea*	4.23	8.17	3.97	8.32	3.86	8.47	4.10	8.35

Gourd family vegetables								
Ash gourd *Benincasa hispida*	4.14	8.44	3.79	8.33	3.95	8.61	3.95	8.51
Bitter gourd *Momordica charantia*	4.15	8.11	3.76	8.13	3.93	8.38	3.93	8.02
Bottle gourd *Lagenaria siceraria*	4.11	8.06	3.78	8.09	3.91	8.49	3.91	8.00
Cucumber *Cucumis sativus*	4.17	8.13	3.88	8.00	3.83	8.51	4.08	8.27
Gharkin *Cucumis sativus*	4.08	8.30	3.79	8.29	3.77	8.54	3.99	8.34
Ridge gourd *Luffa acutangula*	4.12	8.33	3.86	8.26	3.79	8.41	3.95	8.27
Snake gourd *Trichosanthes cucumerina*	4.13	8.31	3.82	8.23	3.7	8.56	3.99	8.32

Table 1B: Growth of bifidobacteria in MRS broth containing different vegetable extracts at 37^0C for 24 hours

Control & Name of the vegetable extract	**Species of *Bifidobacterium***							
	***B. longum* F51 (faecal isolate)**		***B. longum* PF1 (Feed isolate)**		***B. bifium* S26 (faecal isolate)**		***B. adolescentis* F35 (faecal isolate)**	
	pH	**DMC (log_{10}/ml)**	**pH**	**DMC (log_{10}/ml)**	**pH**	**DMC (log_{10}/ml)**	**pH**	**DMC (log_{10}/ml)**
MRS as control	4.10	8.20	3.72	8.20	3.78	8.39	3.78	8.27
Roots & Tubers								
Beet root *Beta vulgaris*	4.11	7.90	3.98	8.32	4.04	8.46	4.04	8.50
Carrot *Daucus carota*	4.20	8.30	3.75	8.33	3.73	8.66	3.90	8.69
Knol – khol *Brassica oleracea*	4.20	8.27	3.71	8.16	3.82	8.47	3.90	8.53
Onions *Allium cepa*	4.14	8.30	3.84	8.16	3.86	8.47	3.98	8.51
Potato *Solanum tuberosum*	4.16	8.28	3.89	8.13	3.88	8.45	3.95	8.49
Pumpkin *Cucurbita pepo*	4.10	8.24	3.79	7.97	3.77	8.43	3.97	8.14
Radish *Raphanus raphanistrum ssp. sativus*	4.13	8.09	3.74	8.30	3.90	8.38	3.67	8.54
Other Vegetables								
Beans *Phaseolus vulgaris*,	4.15	8.09	3.69	8.20	3.79	8.53	3.79	8.24
Brinjal *Solanum melongena*	4.20	7.95	3.88	8.06	4.07	8.29	4.07	8.39
Capsicum *Capsicum annuum*	4.15	8.08	3.84	8.30	3.99	8.43	3.99	8.38
Cauliflower *Brassica oleracea var. botrytis*	4.22	8.17	3.82	8.20	3.84	8.38	3.98	8.57
Chilli *Capsicum frutescens*	4.21	8.04	3.88	8.14	3.80	8.34	3.99	8.35
Chow-chow *Sechium edule*	4.15	8.14	3.75	8.24	3.68	8.39	3.83	8.22
Cluster beans *Cyamopsis tetragonoloba*	4.17	8.20	3.80	8.22	3.80	8.53	3.99	8.54
Drumstick Moringa oleifera	4.20	8.00	3.96	8.24	3.97	8.09	4.18	8.24
Ladies Finger *Abelmoschus esculentus*	4.18	8.06	3.85	8.09	3.96	8.41	3.96	8.22
Tomato *Solanum lycopersicum*	4.15	8.35	3.80	8.59	3.82	8.55	3.93	8.60

Table 2: Performance of bifidobacteria in skim milk incorporated with different levels of selected vegetable extracts at 37^0C

Control & Name of the vegetable extract	**Levels of incorporation**	**Species of *Bifidobacterium***											
		***B. longum* F51 (faecal isolate)**			***B. longum* PF1 (Feed isolate)**			***B. bifium* S26 (faecal isolate)**			***B. adolescentis* F35 (faecal isolate)**		
		Curdling time (h)	**TA (%)**	**DMC (log_{10}/ml)**	**Curdling time (h)**	**TA (%)**	**DMC (log_{10}/ml)**	**Curdling time (h)**	**TA (%)**	**DMC (log_{10}/ml)**	**Curdling time (h)**	**TA (%)**	**DMC (log_{10}/ml)**
Plain Skim milk as control		48	0.49	8.08	84	0.37	8.04	96	0.49	8.00	84	0.43	8.03
Tomato	1:1	18	0.40	8.23	18	0.37	8.32	20	0.43	8.19	20	0.43	8.19
	1:0.8	18	0.40	8.20	18	0.37	8.30	20	0.43	8.19	20	0.43	8.19
	1:0.4	40	0.43	8.04	48	0.38	8.25	72	0.41	8.10	48	0.41	8.10
Carrot	1:1	18	0.43	8.15	18	0.43	8.34	20	0.43	8.20	18	0.43	8.20
	1:0.8	18	0.43	8.13	18	0.43	8.32	20	0.43	8.19	18	0.43	8.19
	1:0.4	40	0.43	8.04	48	0.37	8.27	72	0.41	8.10	72	0.41	8.10
Ash gourd	1:1	20	0.41	8.21	20	0.40	8.22	20	0.38	8.17	20	0.38	8.17
	1:0.8	40	0.34	8.18	20	0.41	8.20	20	0.38	8.15	20	0.38	8.15
	1:0.4	48	0.34	8.16	48	0.41	8.10	84	0.36	8.10	72	0.36	8.10

References

1. Nagpal R, Kurakawa T, Hirokazu Tsuji H, Takahashi T, Kawashima K, Nagata S, *et al*. Evolution of gut *Bifidobacterium* population in healthy Japanese infants over the first three years of life: A quantitative assessment. Scientific Report-Nature Research. 2017;7:10097. Doi: 10.1038/s41598-017-10711-5
2. Odamaki T, Kato K, Sugahara H, Hashikura N, Takahashi S, Xiao JZ, *et al*. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. BMC Microbiol. 2016;16:90. Doi:10.1186/s12866-016-0708-5.
3. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, *et al*. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat. Rev. Gastroenterol. Hepatol. 2017;14:491. Doi:10.1038/nrgastro.2017.75.
4. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125:1401-1412. Doi:10.1093/jn/125.6.1401.
5. Odamaki T, Bottacini F, Kato K, Mitsuyama E, Yoshida K, Horigome A, *et al*. Genomic diversity and distribution of *Bifidobacterium longum* subsp. *Longum* across the human lifespan. Sci. Rep. 2018;8:85. Doi: 10.1038/s41598-017-18391-x

6. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microbes 2012;3:289-306. Doi: 10.4161/gmic.19897.
7. Alimentarius C. Guidelines on Nutrition Labelling CAC/GL 2-1985 as Last Amended. Joint FAO/WHO Food Standards Programme, Secretariat of the Codex Alimentarius Commission. Rome: FAO, 2010.
8. Yoshika M, Yoshika S, Tamura Z, Ohta K. Growth responses of *B. bifidum* coenzyme A, its precursors and carrot extract. Jap. J. Microbiol. 1968c;12:395-402.
9. Klaassen MT, Trindade LM. RG-I galactan side-chains are involved in the regulation of the water-binding capacity of potato cell walls. *Carbohydr*. Polym. 2020;227:115353. Doi: 10.1016/j.carbpol.2019.115353.
10. Jonker D, Fowler P, Albers R, Tzoumaki MV, van Het Hof KH, Aparicio-Vergara M. Safety assessment of rhamnogalacturonan-enriched carrot pectin fraction: 90-Day oral toxicity study in rats and *in vitro* genotoxicity studies. Food Chem. Toxicol. 2020;139:111243. Doi:10.1016/j.fct.2020.111243.
11. Zbiskowski Z, Zikjka S. Technology of obtaining humanized infant formula with enhanced bifidogenic properties by adding carrot juice Indian J Dairy Science. 1986;39:69-75.
12. Semenikina, Sundukova. Use of bifidobacteria in manufacture of cultural milk products for babies. Molo. Prom. 1980;3:33-44.