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Effect of Prebiotics on Growth of the Selected Lactobacilli Culture Isolated from Dairy Products

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Abstract

Prebiotics beneficially affects the host by selectively stimulating the growth or activity of one or limited number of bacteria such as bifidobacteria, lactobacilli in the colon that can improve the host health. Inulin and gum acacia are the most commonly available prebiotics. The ability of lactobacilli cultures (i.e. Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus casei) to utilize inulin, gum acacia, honey and fructo-oligosaccharide @ 2% for different time intervals (6, 12 and 24 h) were tested. The growth rate of Lactobacillus acidophilus was found be higher in 2% honey than other isolates as well as prebiotics taken. Potential probiotic Lactobacillus acidophilus isolated from curd were allowed to grow in selected prebiotics, i.e., honey at different concentrations (2.0, 3.0 and 4.0%) for a time period of 0–24 h. Lactobacillus acidophilus, could utilize all the concentrations of honey (2.0, 3.0, and 4.0%) with control (glucose, 1%) and exhibited maximum growth 4.5×10^7 *at 6 h interval,* 5.4×10^8 *at 12 h time period and* 6.3×10^9 *after 24 h of incubation. At 2% honey concentration the growth rate is nearly similar to that of 3% honey. Whereas, at 4% honey the growth of probiotic bacteria decrease in comparison to that of 2 and 3%. When combining both lactobacilli cultures (potential probiotics) and a prebiotic in a single food product, the expected benefits are an improved survival during the passage of probiotic bacteria through the upper intestinal tract.*

Keywords: *prebiotics, lactobacilli, host health*

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INTRODUCTION

Once isolated, the probiotic cultures can be used for the functional food development. To enhance the growth and activity of these probiotic organisms certain substances can be used and these are called as prebiotics. The prebiotics are defined as non-digestible substances (dietary fibers) that exert some biological effect on humans by selective stimulation of the growth or bioactivity of beneficial microorganisms in the intestine [1, 2]. Actually, prebiotics undergo fermentation by beneficial micro-flora in the large intestine (contrary to the dietary fibers). Inulin and resistant starch are the most common molecules to comprise the prebiotics group [3]. Prebiotics are selective in their action; therefore, for using a prebiotic with a specific probiotic organism their mutual synergism must be studied [4–7] so as to obtain the maximum benefit when used in combination as food supplement [8–10].

Prebiotics: The Food for Probiotics

Two separate approaches exist to increase the number of health promoting organisms in the gastrointestinal tract. The first is the oral administration of live beneficial microorganisms. These microorganisms, called probiotics, have been selected mostly from lactic acid bacteria and bifidobacteria that form a part of the normal intestinal micro-flora of humans, since these organisms are indigenous to the colon. Another strategy for increasing their number is to supply those already present in the intestine with selective carbon and energy source that provides them with competitive advantage over other bacteria in this ecosystem, thus selectively modifying the composition of the micro-flora using dietary supplements. These selective dietary components were named 'prebiotics'. They are specific, naturally occurring carbohydrates, mainly of plant origin, and fructo-oligosaccharides are mostly known representatives. A prebiotic is defined as 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health [11]. Prebiotics have been added to probiotic yoghurt as a food source for improving the ability of beneficial bacteria colonizing the gut. At present, most prebiotics are directed towards the growth of lactic acid bacteria due to their purported health promoting properties. The prebiotics identified as no digestible carbohydrates include lactulose, inulin, resistant starch and a range of oligosaccharides that supply a source of fermentable carbohydrate for probiotic bacteria in the colon [12]. Much of the interest is aimed at no digestible oligosaccharides (fructo-oligosaccharides, trans-galactooligosaccharides, iso-maltooligosaccharides, xylo-oligosaccharides, gluco-oligosaccharids and lactosucrose). In addition to providing substrate for probiotic organisms in the colon, prebiotics have their own specific health benefits. Inulin and fructo-oligosaccharides have been found to selectively stimulate the growth of *Bifidobacterium* in mixed batch and chemostat study [13].

A similar result was obtained by [14] who found a significant increase in the counts of *Bifidobacterium* in 10 senile adults that were fed 20-40g of inulin for 20 d. In another study, fructo-oligosaccharides were found to increase intestinal Riga of mice, thus improving the body's defense against invaders [15]. There are several types of oligosaccharides that have been shown to be prebiotics using *in vitro* models, animal models and human trials. Oligosaccharides that have been studied include lactulose, fructo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides and lactosucrose, isomalto-oligosaccharides, gluco-oligosaccharides, and xylo-oligosaccharides [16].

The Sources of Prebiotics

A number of plants worldwide contain fructo-oligosaccharides; some sources of inulin are onion (2–6%), garlic (9–16%), banana (0.3–0.7%), asparagus (10–15%), chicory (13–20%) and even wheat (1–4%). Yet the levels are too low for a significant gastrointestinal tract effect. Consumption of more than 4 g of fructo-oligosaccharides is needed daily to induce changes in lactic acid bacteria levels. Prebiotics are increasingly used in development of new food product, e.g., drinks, yogurts, biscuits and spreads. The positive effects of prebiotic consumption are: improvement of bowel habit, reduction of diarrhoea and constipation, modulation of lipid metabolism by normalizing cholesterol levels, reduction of osteoporosis by improved mineral absorption, reduction of allergy risks through immune system modulation, reduction of colon cancer risk [17]. However, many of the above mentioned health claims still require further research in this direction. [11] Introduced the term prebiotic, who exchanged "pro" for "pre," which means "before or for". They defined prebiotics as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon." This selectivity was shown for bifidobacteria that may be promoted by the ingestion of substances like fructo-oligosaccharides and inulin [18], transgalactosylated oligosaccharides and soybean oligosaccharides [19]. To be an effective prebiotic it must:

a) Neither be hydrolyzed or absorbed in the upper part of the gastrointestinal tract,
b) Have a selective fermentation such that the composition of the large intestinal micro-biota is altered towards a healthier one, and
c) Induce luminal or systemic effects that are beneficial to the host. In small intestine the prebiotics are reported to have desirable influence on sugar digestion and absorption, glucose and lipid metabolism and protection against known risk factors of cardiovascular disease. Due to the fermentation of prebiotics in the colon short chain fatty acids are produced and this is considered a major beneficial feature related to the primary prevention of colorectal cancer [20]. Another important role prebiotics can play, is in the prevention of intestinal permeability or leaky gut

syndrome', which could lead to chronic bowel inflammation caused by mucosa's exposure to foreign bodies. Moreover, there is a high level of evidence of positive effects of some prebiotics to alleviate constipation and treat hepatic encephalopathy. Already proven beneficial effects with reference to prebiotics include: non-digestible and low energy value, increase and modulation of stool volume by stimulation of beneficial bacteria (*Bifidobacterium*, *Lactobacillus* and *Eubacterium* spp.) and inhibition of "undesirable" bacteria (*Clostridium* and *Bacteroides*) [21].

Different Types of Prebiotics

Inulin

Inulin has been defined as a poly-disperse carbohydrate material consisting mainly, if not exclusively, of β-(2-1) fructosyl-fructose links. Inulin-producing plant species are found in several monocotyledonous and dicotyledonous families, including liliaceae, amaryllidaceae, gramineae, and compositae. However, only one inulin-containing plant species (chicory, *Cichorium intybus*) is used to produce inulin industrially. Inulin-type fructans are used as sugar substitutes, as fat replacers (inulin only), and as a means of providing texture, stabilizing foam, or improving mouth feel in miscellaneous products such as fermented dairy products; desserts such as jellies and ice creams; bakery products such as cookies, breads, and pastries; spreads; and infant formulas. Moreover, inulin is also found to improve calcium absorption [22].

Gum Acacia

Gum acacia is an exudate gum from the trees of various *Acacia* species, particularly *Acacia senegal*, consists mainly of a highly complex polysaccharide of a branched β-(1, 3)-linked galactose backbone with branches through the 1,6 positions, with arabinose, rhamnose and uronic acids in ramified side-chains [23]. This gum is useful for its functional properties (stabilizing and emulsifying) in several applications in the food and pharmaceutical industries and in painting [7, 24]. For use in the probiotic food industry, it has added advantages of providing the health benefits associated with dietary fiber, showing antibacterial activity against periodontal pathogens [25] and causing a rapid change in faecal flora when used as a part of the human diet [26].

Polydextrose

Polydextrose is a glucose polymer, which is resistant to hydrolysis by human digestive enzymes. It is fermented slowly in the colon where it stimulates the growth of lactobacilli and bifidobacteria. Slowly fermentable prebiotics such as polydextrose are able to sustain fermentation from the proximal through to the distal end of the large intestine and hence its prebiotic benefits are experienced along the whole length of the colon [27]. It also has a well-documented glucose attenuating effect. This results in a reduction of gas produced and no lactic acid accumulation, thus reducing gastrointestinal stress. Polydextrose has been shown to express prebiotic effects; it decreased fecal pH, increased the residual concentration of short-chain fatty acids, and increased numbers of bifidobacteria in feces [28].

Fructo-Oligosaccharide

The bifidogenic nature of fructo-oligosaccharides. Administration of fructo-oligosaccharides (15g/day) significantly increased the bifidobacterial numbers, concomitantly decreasing the numbers of bacteriodes, fusobacteria and clostridia in human [18]. In a human volunteer trial, [28] it was demonstrated that administration of fructo-oligosaccharides increased the beneficial lactobacillus and bifidobacteria and suppress the numbers of *Bacteriodes* spp. and *Candia*. Inhibition of growth of some human enteropathogens such as salmonellae has been reported in presence of fructo-oligosaccharides [29] whilst other reported bifidobacterial antagonistic activity against Gram-negative species, such as *Salmonella*, *Campylobacter* and *Escherichia coli* [30]. Therefore, administration of probiotics and prebiotics can modulate the gut flora balance towards the health benefit direction by increasing bifidobacteria and *Lactobacillus* thus suppression of other harmful micro-flora [31].

Honey

Honey is a natural and healthy food additive containing primarily fructose (38.5%), glucose (31.3%), maltose (7.2%), sucrose (1.5%) and various oligosaccharides (4.2%). The oligosaccharides in honey result from the action of honeybee α-D-glucosidase. Honey also contains a variety of organic acids such as acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic and succinic acids (0.17 to 1.17%), which give the product an average pH of 3.9. Inhibitory properties of honey against pathogens such as *E. coli*, *Salmonella typhi*, *Listeria monocytogenes*, etc. have been demonstrated. Microbial inhibition of honey has been attributed to its low pH, low water activity, presence of enzymes such as glucose oxidase, catalase and lysozyme, H_2O_2, flavonoids and phenolic antioxidants [32]. Despite its antimicrobial properties, honey did not inhibit the growth of these organisms at 5% level and appears suitable as sweetener in fermented milk products. In case of bifidobacteria, high cells numbers and enhanced lactic acid production were observed. The oligosaccharides with low DP have been suggested to favour the growth and activity of bifidobacterial strains [33].In view of above facts in consideration, the present research has been carried out to study the effect of prebiotics on growth of the selected lactobacilli culture isolated from dairy products.

MATERIALS AND METHODS

Lactobacilli Cultures

Lactobacilli cultures (i.e. *Lactobacillus acidophilus*, *Lactobacillus plantarum and Lactobacillus casei*) used in this study were previously isolated and identified [34].

Maintenance and Propagation of Cultures

For their further assessment of probiotic attribute and other analysis all the isolated lactobacilli cultures were maintained in chalk litmus milk at refrigeration temperature after their growth at 37°C for overnight. The cultures were sub-cultured at regular intervals in chalk litmus milk and stored under refrigeration conditions. Before use the cultures were activated in MRS broth. All the isolates were also maintained at -70°C in glycerol stock in triplicates for use in experiment at different stages.

Growth of Lactobacilli Cultures in Presence of Prebiotics

Five different prebiotics (i.e., inulin/honey/gum acacia/fructo-oligosaccharide/poly-dextrose) were taken in liquid minimal medium in order to assess the utilization ability of selected lactobacilli culture and compare [35] the utilization pattern of each prebiotic. For this, liquid minimal media containing prebiotics (2%), meat peptone (1%), buffering salts and indispensable ions of Garches medium [36] was prepared. Active lactobacilli cultures were inoculated to give an initial cell count to about 7 log cfu/ml and incubated at 37°C. At the end of 6, 12 and 24 h viable cells were enumerated using MRS agar at 37°C for 48 h for interpretation of effects.

RESULTS AND DISCUSSIONS

A prebiotic is a non–digestible food ingredient that beneficially affects the host by selectively stimulating the growth or activity of one or limited number of bacteria in the colon that can improve the host health. Fructo-oligosaccharides, trans galacto-oligosaccharides and several of the experimental prebiotics, have shown benefits in favorably modulating the microbial ecology of the gut, protecting against various intestinal pathogens and in some instances, boosting gastrointestinal immunity [37]. *In vitro* fermentability of inulin by faecal bacteria to that of rye, wheat and oat bran were compared [38]. Inulin was the most rapidly fermented of the test substrates giving most butyrate production and the largest decrease in pH but also the highest and fastest acid production. *Lactobacillus* has complex nutritional requirements such as nucleic acid derivatives, carbohydrates, amino acids, salts and vitamins, which vary markedly from species to species. Incorporation of prebiotics has been proved to improve the viability of probiotics by selectively stimulating their growth, though they differ in the degree of polymerization and the type of oligomers [39].

Prebiotic utilization efficiency of lactobacilli was studied by determining the growth of selected lactobacilli spp. in presence of different prebiotics (inulin, honey, gum acacia, fructo-oligosaccharide and polydextrose @ 2% at various time intervals (6, 12 and 24 h). The growth rate of selected probiotics in presence of different prebiotic reflects the ability of a given substrate to support the growth of a probiotic. Therefore, for a substance to be a good prebiotic, it should be selectively metabolized by the probiotic bacteria.

The growth of *Lactobacillus acidophilus* after 6 h duration was found to be more in media containing honey ($2.1x10^7$) as compared to other prebiotics (Table 1 A, B and C). Similar results were obtained with *Lactobacillus casei* and *Lactobacillus plantarum*. Hence, during the first 6 h of incubation honey was found to fasten the growth rate of all the three cultures compared to that of other prebiotics taken in consideration. Our results are in close agreement with that of [40] who have reported the *Lactobacillus* spp. utilize effectively honey during first 6h of incubation.

It can be concluded from the 6 h results that all the three cultures got well adapted in the honey containing medium in contrast to that of media containing rest of the prebiotics. After 12 h of incubation, there was 2 log cycle increase in growth rate of *Lactobacillus acidophilus* in comparison to *Lactobacillus casei* and *Lactobacillus plantarum* showing only one log cfu/ml (10^8) increment (initial growth count 10^7 cfu/ml) in growth in media containing honey as prebiotic. In the other media supplemented with inulin, gum acacia and fructo-oligosaccharide as sole carbon source growth rate was same. The results are in similar fashion of that of 6 h. The study was continued for 12 more hours. After the 24 h of growth period, *Lactobacillus acidophilus* reached up to 10^{10} cfu/ml count indicating 3 log cycle increase, whereas in *Lactobacillus planatrum* and *Lactobacillus casei* log count reached upto 10^9 cfu/ml. All the log counts are indicated in Table 1 (A, B and C).

Table 1 A: *Growth of Selected Lactobacilli in Presence of Different Prebiotics (2%) and Incubation Time.*

Cultures	**Growth pattern in presence**							
	Inulin				**Honey**			
	0 h	**6 h**	**12 h**	**24 h**	**0 h**	**6 h**	**12 h**	**24 h**
Lactobacillus acidophilus	$1.1x10^7$	$3.4x10^7$	$4.9x10^8$	$5.3x10^9$	$1.3x10^7$	$2.1x10^7$	$5.7x10^8$	$6.7x10^9$
Lactobacillus casei	$8.9x10^6$	$1.8x10^7$	$6.7x10^8$	$1.5x10^9$	$1.4x10^7$	$2.1x10^7$	$4.2x10^8$	$2.4x10^9$
Lactobacillus plantarum	$1.7x10^7$	$4.3x10^7$	$1.7x10^8$	$2.7x10^9$	$0.9x10^7$	$3.2x10^7$	$3.7x10^8$	$1.4x10^9$

Table 1 B: *Growth of Selected Lactobacilli in Presence of Different Prebiotics (2%) and Incubation Time.*

Cultures	**Growth pattern in presence**							
	Gum acacia				**Fructo-oligosaccaride**			
	0 h	**6 h**	**12 h**	**24 h**	**0 h**	**6 h**	**12 h**	**24 h**
Lactobacillus acidophilus	$2.1x10^7$	$4.9x10^7$	$3.7x10^8$	$4.3x10^9$	$1.1x10^7$	$2.4x10^7$	$2.7x10^8$	$3.6x10^9$
Lactobacillus casei	$1.2x10^7$	$7.1x10^7$	$4.3x10^8$	$3.1x10^9$	$3.9x10^7$	$8.1x10^7$	$3.4x10^8$	$3.2x10^9$
Lactobacillus plantarum	$2.5x10^7$	$7.4x10^7$	$3.1x10^8$	$3.5x10^9$	$0.7x10^7$	$1.4x10^7$	$2.3x10^8$	$4.4x10^9$

Table 1 C: *Growth of Selected Lactobacilli Cultures in Presence of Prebiotics POLY-Dextrose (2%) and Incubation Time.*

Cultures	**Growth pattern in presence of Poly-dextrose**			
	0 h	**6 h**	**12 h**	**24 h**
Lactobacillus acidophilus	$1.8x10^7$	$1.9x10^7$	$7.7x10^7$	$7.3x10^8$
Lactobacillus casei	$3.0x10^7$	$4.1x10^7$	$6.3x10^7$	$6.2x10^8$
Lactobacillus plantarum	$2.4x10^7$	$3.4x10^7$	$8.1x10^7$	$5.4x10^8$

Effect of Selected Prebiotic (Honey) Concentrations on Counts of *Lactobacillus acidophilus*

During present investigation, potential probiotic lactobacilli isolated from curd were allowed to grow in selected prebiotics, i.e., honey at different concentrations (2.0, 3.0 and 4.0%) for a time period of 0-24 h. The Table 2 revealed that the isolate, *Lactobacillus acidophilus,* could utilize all the concentrations of honey (2.0, 3.0, and 4.0%) with control (glucose, 1%) and exhibited maximum growth 4.5 x 10^7 at 6 h interval, 5.4 x 10^8 at 12 h time period and 6.3x10^9 after 24 h of incubation. At 2% honey concentration the growth rate is nearly similar to that of 3% honey. Whereas, at 4% honey the growth of probiotic bacteria decrease in comparison to that of 2 and 3%. It is well revealed from Table 2 that though the bacteria could easily grow and multiply at all the concentrations but maximum growth was achieved at 2 and 3% of honey. [41] Suggested that the lowest dose of prebiotic that gives a demonstrable effect should be used because increasing the dose can lead to flatulence in the stomach. Buddington RK, et al. [42] has demonstrated that fructo-oligosaccharides are prebiotic at 4 g/d. Hence, 2% honey appears to be optimum for stimulating the growth of *Lactobacillus* species. Hence, at last we can conclude that *Lactobacillus acidophilus* was the most efficient among the three species recovered from dahi in utilizing honey as the sole energy source for their growth and exhibiting probiotic potential.

Table 2: *Effect of Selected Prebiotic Concentration on Count of Lactobacillus acidophilus.*

Time in hours	**Growth at various concentration of honey (%)**		
	2.0%	**3.0%**	**4.0%**
0	1.2x10^7	1.2x10^7	1.1x10^7
6	4.3 x 10^7	4.5 x 10^7	2.3 x 10^7
12	1.9 x 10^8	5.4 x 10^8	1.0 x 10^8
24	5.6x10^9	6.3x10^9	8.1x10^8

CONCLUSION

A non–digestible food ingredient called prebiotics that beneficially affects the host by selectively stimulating the growth or activity of one or limited number of bacteria such as bifidobacteria, lactobacilli in the colon that can improve the host health. Inulin and gum acacia are the most commonly available prebiotics. The ability of lactobacilli cultures (i.e. *Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus casei*) to utilize inulin, gum acacia, honey and fructo-oligosaccharide @ 2% for different time intervals (6, 12 and 24 h) were tested. The growth rate of *Lactobacillus acidophilus* was found be higher in 2% honey than other isolates as well as prebiotics taken. Potential probiotic *Lactobacillus acidophilus* isolated from curd were allowed to grow in selected prebiotics i.e. honey at different concentrations (2.0, 3.0 and 4.0%) for a time period of 0–24 h. *Lactobacillus acidophilus,* could utilize all the concentrations of honey (2.0, 3.0, and 4.0%) with control (glucose, 1%) and exhibited maximum growth 4.5 x 10^7 at 6h interval, 5.4 x 10^8 at 12 h time period and 6.3x10^9 after 24 h of incubation. At 2% honey concentration the growth rate is nearly similar to that of 3% honey. Whereas, at 4% honey the growth of probiotic bacteria decrease in comparison to that of 2 and 3%. When combining both lactobacilli cultures (potential probiotics) and a prebiotic in a single food product, the expected benefits are an improved survival during the passage of probiotic bacteria through the upper intestinal tract. Probiotic and prebiotics may be used in synergistic combination are called synbiotics, that improve the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, either by stimulating growth or by metabolically activating the health promoting bacteria.

REFERENCES

1. Gibson GR, From Probiotics to Prebiotics and a Healthy Digestive System. *Journal of Food Science* 2004; 69: M141–M143.
2. Dhewa T, *Prebiotics: Potential Substrate for Probiotic Organism*, LAP Lambert Academic Publishing, Germany, 2011.
3. Tomasik PJ, Tomasik P, Probiotics and prebiotics. *Cereal Chemistry,* 2003; 80: 113–117p.

4. Puniya M, Sangu KPS, Bhardwaj A, *et al.* Probiotic and Functional Attributes of *Lactobacillus* spp Isolated from Human Faeces. *Journal of Research in Antimicrobials*, 2012; 1: 032–042p.
5. Dhewa T, Pant S, Mishra V, Development of Freeze Dried Synbiotic Formulation using a Probiotic Strain of *Lactobacillus plantarum*. *Journal of Food Science and Technology*, 2011; 1–7. DOI 10.1007/s13197-011-0457-2.
6. Losada MA, Olleros T, Towards a Healthier Diet for the Colon: The Influence of Fructooligosaccharides and Lactobacilli on Intestinal Health. *Nutrition Research* 2002; 22: 71–84p.
7. Bertolini AC, Siani AC, Grosso CRF, Stability of Monoterpenes Encapsulated in Gum Arabic by Spray drying. *Journal of Agricultural and Food Chemistry* 2001; 49: 780–785p.
8. Mehra N, Majumdar RS, Dhewa T, Probiotics: Preventive and Clinical Applications. *Biotechnology*, 2012; 1: 015–020p.
9. Dhewa T, Screening, Production Purification and Potential Use of Bacteriocins from Lactic Acid Bacteria of Meat and Dairy Food Origin. International Conference on Nutrition and Food Sciences (ICNFS 2012), IACSIT Press, 2012.
10. Dhewa T, Bajpai V, Saxena RK, *et al.* Selection of Lactobacillus Strains as Potential Probiotics on basis of *in vitro* Attributes. *International Journal of Probiotics and Prebiotics* 2010; 5(1): 45–52p.
11. Gibson GR, Roberfroid MB, Dietary Modulation of the Human Colonic Microbiota. Introducing the Concept of Prebiotics. *Journal of Nutrition*, 1995; 125: 1401–1412p.
12. Crittenden R, *Probiotics: A Critical Review In: Tannoch* GW (Ed.) Wymondham, UK, Horizon Scientific Press, 1999, 141–156p.
13. Wang X, Gibson GR, Effects of the *in vitro* Fermentation of Oligofructose and Inulin by Bacteria Growing in the Large Intestine. *Journal of Applied Bacteriology*, 1993; 75: 373–380p.
14. Kleeson B, Sykura B, Zunft HJ, Effects of Inulin and Lactose on Fecal Microflora, Microbial Activity, and Bowel Habit in Elderly Constipated Persons. *The American Journal of Clinical Nutrition* 1997; 65: 1397–402p.
15. Nakamura Y, Nosaka S, Suzuki M, *et al.* Dietary Fructooligosaccharides Upregulate Immunoglobulin: A Response and Polymeric Immunoglobulin Receptor Expression in Intestines of Infant Mice. *Clinical and Experimental Immunology* 2004; 137: 52–58p.
16. Rastall RA, Gibson GR, *Probiotics and Prebiotics: Where are we going? (Caister Academic Press, Cambs, UK)*, 2002.
17. Conway PL, Prebiotics and Human Health: The State-of-the-Art and Future Perspectives. *Journal of Food and Nutrition Research* 2001; 45: 13–21p.
18. Gibson GR, Beatty ER, Wang X, *et al.* Selective Stimulation of Bifidibacteria in the Human Colon by Oligofructose and Inulin. *Gastrointrology* 1995; 108: 975–982p.
19. Saito Y, Tanaka T, Rowland IR, Effects of Soybean Oligosaccharides on the Human Gut Microflora *in vitro* Culture. *Microbial Ecology in Health and Disease* 1992; 5: 105–110p.
20. Scheppach W, Leuhers H, Menzel T, Beneficial Health Effects of Low-Digestible Carbohydrate Consumption. *British Journal of Nutrition* 2001; 85: S823–S830p.
21. Holzapfel WH, Schillinger U, Introduction to Pre- and Probiotics. *Food Research International* 2002; 35: 109–116p.
22. Linda MO, Improving the Gut Feeling. *Food Technology* 2002; 56: 67–70p.
23. Menzies AR, Osman ME, Malik AA, *et al.* A Comparison of the Physicochemical and Immunological Properties of the Plant Gum Exudates of *Acacia Senegal* (gum arabic) and *Acacia seyal* (gum tahla). *Food Additives and Contaminants* 1996; 13: 991–999p.
24. Fauconnier ML, Blecker C, Groyne J, *et al.* Characterization of Two Acacia Gums and their Fractions using a Langmuir Film Balance. *Journal of Agricultural and Food Chemistry* 2000; 48: 2709–2712p.
25. Clark DT, Gazi MI, Cox SW, *et al.* The Effects of Acacia Arabica Gum on the *in vitro* Growth and Protease Activities of Periodontopathic Bacteria. *Journal of*

Clinical Periodontology 1993; 20: 238–243p.
26. Wyatt GM, Bayliss CE, Holcroft JD, A Change in Human Faecal Flora in Response to Inclusion of Gum Arabic in the Diet. *British Journal of Nutrition* 1986; 55: 261– 266p.
27. Hollie MP, Apajalahti JHA, Rautonen N, *et al.* Polydextrose, Lactitol, and Fructo-Oligosaccharide Fermentation by Colonic Bacteria in a Three-Stage Continuous Culture System. *Applied and Environmental Microbiology* 2004; 70: 4505–4511p.
28. Jie Z, Bang-yao L, Ming-jie X, *et al.* Studies on the Effects of Polydextrose Intake on Physiologic Functions in Chinese People. *The American Journal of Clinical Nutrition* 2000; 72: 1503–1509p.
29. Ito, M., Kimura, M., Deguchi, *et al.* Effects of Transgalactosylated Disaccharides on the Human Intestinal Flora and their Metabolism. *Journal of Nutritional Science and Vitaminology* 1993; 39: 279–288p.
30. Gibson GR, Wang X, Bifidogenic Properties of Different Types of Fructooligosaccharides. *Food Microbiology* 1994; 11: 491–498p.
31. James L, Puniya AK, Singh K, Health Promoting Functional Synbiotics - A Mini Review. In: Proceedings of Summer Institute, ‘Advances in Applied Dairy and Food Microbiology’, Dairy Microbiology Division, National Dairy Research Institute, Karnal, 2002; 209p.
32. Molan PC, Russell KM, Non Peroxide and Bacterial Activity in Some New Zealand Honeys. *Journal of Apiculture Research* 1989; 27:62–67p.
33. Kurien A, Puniya AK, Singh K, Selection of Prebiotic and *Lactobacillus acidophilus* for Synbiotic Yoghurt Preparation. *Indian Journal of Microbiology* 2005; 45: 45–50p.
34. Bhardwaj Aarti, Puniya M, Sangu KPS, *et al.* Isolation and Biochemical Characterization of Lactobacillus Species Isolated from Dahi. *Research & Reviews: A Journal of Dairy Science and Technology* 2012; 1(2): 18–31p.
35. Bielecka M, Biedrzycka E, Majkowska A, *et al.* Effect of Nondigestible Oligosaccharides on Gut Microecosystem in Rats. *Food Research International* 2002; 35: 139–144p.
36. Rasic, J., Culture Media for Detection and Enumeration of the Bifidobacteria in Fermented Milk Products. *IDF Bulletin* 1990; 252: 24–31p.
37. Grizard D, Barthomeuf C, Non-Digestible Oligosaccharides used as Prebiotic Agents: Mode of Production and Beneficial Effects on Animal and Human Health. *Reproduction Nutrition Development* 1999; 39: 563–588p.
38. Karppinen S, Liukkonen K, Aura AM, *et al.* *In vitro* Fermentation of Polysaccharides of Rye, Wheat and Oat Brans and Inulin by Human Faecal Bacteria. *Journal of the Science of Food and Agriculture* 2000; 80: 1469–1476p.
39. Hull RR, Roberts AV, Mayes JJ, Survival of *Lactobacillus acidophilus* in Yogurt. *Australian Journal of Dairy Technology* 1984; 39: 164–166p.
40. Dhewa T, Goyal, Effect of Inulin, Honey and Gum Acacia on Growth of Human Faecal Potential Probiotic Lactobacilli. *The IUP Journal of Life Sciences* 2009; 3: 29–34p.
41. Ziemer CJ, Gibson GR, An Overview of Probiotics Prebiotics and Synbiotics in the Functional Food Concept: Perspectives and Future Strategies. *International Dairy Journal* 1998; 8: 473–479p.
42. Buddington RK, Williams CH, Chen SC, *et al.* Dietary Supplement of Neosugar Alters the Fecal Flora and Decreases Activities of Some Reductive Enzymes in Human Subjects. *The American Journal of Clinical Nutrition* 1996; 63:709–716p.

