

Molasses as Bifidus Promoter on Bifidobacteria and Lactic acid Bacteria growing in skim milk

R Reyed, A El-Diwany

Citation

R Reyed, A El-Diwany. *Molasses as Bifidus Promoter on Bifidobacteria and Lactic acid Bacteria growing in skim milk*. The Internet Journal of Microbiology. 2007 Volume 5 Number 1.

Abstract

Two potential probiotics strains were cultivated in skim milk containing different food additive. The total numbers of Bifidobacteria were stable throughout the study period in both subjects, but lactobacillus numbers were less constant and stable. Samples were examined for (a) viability, and (b) level of organic acids biosynthesis (lactic and acetic acids). Bifidobacteria were evaluated for their potential use in skim milk. Strains were grouped according to their growth rate in reconstituted skim milk. Molasses supported and enhanced growth of Bifidobacteria and Lactobacillus more than other dietary carbohydrates. Organic acids biosynthesis were enhanced when Bifidobacteria were grown in the presence of molasses.

Presented at a First international Biological and Environmental Sciences held in Hurgada Egypt 13-16 March 2008.

INTRODUCTION

Probiotics have a very long history of use in humans and animals, with the first recorded intakes dating back to several hundred years ago. Metchnikof (1908) in his fascinating treatise 'The Prolongation of Life' propounded that the longevity of Bulgarians was in part due to their consumption of large quantities of fermented milks containing lactic acid bacteria. He also launched the theory that the intestinal microflora exerts important influences on health and longevity. Although controversial, his theory was responsible for much subsequent scientific research regarding the role of fermented and culture-containing dairy products in health. This observation has led to burgeoning activity in the elucidation of the role of lactic acid cultures and cultured milk products in alleviation of human and animal gastrointestinal disorders. Recent advances in our knowledge of the biosynthetic activities of lactic bacteria and their antagonistic action against pathogenic, toxigenic and putrefactive

organisms have added a new dimension to the importance of fermented milks in human nutrition and health. The emergence of antibiotic-resistant bacteria and natural ways of suppressing the growth of pathogens has contributed to the concept of 'probiotics'. Probiotic bacteria not only compete and suppress 'unhealthy fermentation' in human

intestine, but also produce a number of beneficial health effects of their own. Fuller 1989 have defined probiotics as 'a live microbial feed supplement, which has beneficial effects on the host by improving its intestinal microbial balance'. Probiotics can influence the structure and functions of the gastrointestinal tract, there are opportunities for using diet as a 'management tool' to affect the resident microbiota. Fermentable milk increase the densities of beneficial bacteria and stimulate growth and functions of the healthy intestine, therefore Probiotic bacteria could be applied to balance disturbed intestinal microflora and related dysfunction of the gastrointestinal tract (Fuller 1989). Although yet to be verified decisively, it is understood that probiotic organisms fight and hold back the growth of undesirable microorganisms in the colon and small intestine, and thus help to make digestive system stable. Other effects include prevention of intestinal infections, expression of antitumor activities, and improvement of lactose utilization in the human gut (Kirjavainen and Gibson, 1999; Glodin 1998) Probiotics are traditionally regarded as safe for human use. When ingested in sufficient numbers, probiotics are believed to play an important role in the control of host intestinal microbiota and maintenance of its normal state. Microbes that are frequently isolated from human and animal intestines and selected as probiotics, include species of the genera Bifidobacterium, Research performed with germfree animals and the introduction of improved anaerobic culture techniques have been particularly useful in clarifying the significance of the interrelationships of diet

and intestinal microbiota in health and disease (Kirjavainen and Gibson, 1999; Friedrich, 2000.; Lu 2001). In spite of a rather large list of literature, the therapeutic effects of the preparations of Bifidobacteria as well as their culture-containing dairy products on human gastrointestinal disorders remain obscure or controversial. Some strains of microorganisms have been used for a long time in animal feeding to improve their zootechnical performances. Over the last few decades, some strains of lactic acid bacteria belonging to Bifidobacteria and Lactobacilli have been introduced in food products for human consumption, with the aim to improve human health (Guarner and Schaasama 1998). Dairy products have been the preferred medium to reintroduce viable populations of Lactic acid bacteria and Bifidobacteria into the GI tract of both children and adults. More than 70 products that contain bifidobacteria, mostly of dairy origin, including sour cream, buttermilk, yogurt, powdered milk, cookies, and frozen desserts, are produced worldwide (Kim 1988; Rasic and Kmann. 1983) Products fermented with the bifidobacteria culture have a mild, acidic flavor that is similar to that of yogurt (Gibson et al., 1995) In Japan, foodstuffs that contain bifidobacteria are very well-known and are sold by most of the large dairy companies. Total yogurt sales in Japan have practically doubled in the past 10 yr. foodstuffs that have bifidobacteria report for more than one-third of the total (Hughes and Hoover. 1991) A parallel large growth in yogurt sales has happened in France. currently, 11% of all yogurt put up for sale in France holds supplementary bifidobacteria (Hughes and Hoover. 1991). Yoplait (Paris, France) and Dannon (Paris, France) have also recently introduced products that contain bifidobacteria into this rapidly growing European market (Hughes and Hoover. 1991). Bifidobacteria must remain viable in large numbers in the carrier food to be used with confidence as a dietary adjunct. However, maintaining the viability of bifidobacteria during processing and refrigerated storage has been a challenge to dairy processors. Another approach to increasing the numbers of Bifidobacteria in the GI tract is the incorporation of prebiotics in the diet. A prebiotic is a non-digestible dietary supplement that modifies the balance of the intestinal microflora stimulating the growth and/or activity of the beneficial organisms and suppressing potentially deleterious bacteria. Molasses is used to Food additive some dessert dishes and jam-like preserves. It is also used as a snack dip and dessert, in combination with tahini and pita bread. Egyptian black strap molasses (50.0% sugars) was suitable as carbon source in the fermentation medium (Abou-Zeid et al., 1978). Molasses

are by-products of the sugar cane industry; they have been widely used as a cereal substitute in livestock feeds (FAO 1992). Molasses referred specifically to the final effluent obtained in the preparation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugar cane and from sugar beets (Fermin et al 1994) Molasses Known in Egypt as 'black honey', Cane (blackstrap) molasses is extracted from sugar cane, which grows well in Egypt's hot summers. Traditionally kept for relatively long periods in earthenware jars known as ballas. Blackstrap Molasses- A leftover sludge of the sugar making process. Teeming with minerals and vitamins. Ounce per ounce it contains more calcium than milk, more iron than eggs, and more potassium than any other food. blackstrap molasses is that the body seems to assimilate all the minerals quite readily. It's great for anemia problems. Cane molasses are by-products of the sugar industry. There are different kinds of cane molasses depending on the process utilized to obtain sugar. Among them, blackstrap molasses is considered the one which is no longer used to recover sugar, and is normally used for industrial fermentation. The overall composition of the various molasses differs according to specific geographical areas of production. In Egypt, molasses are the cheapest carbon sources in addition to being a good substrate which is rich in fermentable sugars and mineral. In Vietnam, two types of molasses are produced; "C" molasses from industrial sugar production and "A" molasses from the artisan method. Van and Men (1990) described the production of "A" molasses and showed that pig growth was similar to that on rice bran with a slight tendency for better feed conversion on the molasses. Under these economic conditions, the use of "A" molasses will only be profitable if its price is about 50% lower than that of rice bran. Growth and viability of bifidobacteria in fermented milk can be improved significantly by the integration of molasses in milk prior to fermentation. All of the sweetness provided by the sugar may be substituted with sugar replacement ingredients by sweetening agents like molasses. Natural product of sugar cane refining is usually one of the cheapest sources of energy available. Rich in sugars, it is also high in dry matter; helps bind feeds, eliminates dust and reduces waste. Suitable for all classes of livestock, it assists the breakdown of fiber and non-protein nitrogen by stimulating rumen bacteria. Molasses (sugar cane) utilized to provide sweetening products known to the art as sugar replacements or substitutes. Such products normally contain a nutritive or non-nutritive binder as a major ingredient (e.g., sugars in combination with an

artificial sweetening agent (usually a noncaloric sweetener).

Therefore, the objective of this research was to:

1. Determine the effect of molasses on growth and activity of bifidobacteria in milk in comparison to conventional sugars.
2. Compare the growth-promoting and effects of molasses on population of bifidobacteria to that of commercially available Lactose and Fructose .
3. Determine the level of acetic acid and lactic acid produced by these organisms when grown in the presence of food additive like molasses, fructose and lactose.

MATERIAL AND METHODS

Strains and Cultivation: The species of *Lactobacillus platarum* No. 8P-A3 and *Bifidobacterium bifidum* No.1, 791 “Russian strains” used in this study were cultivated weekly in the MRS broth (Difco Laboratories, Detroit, MI) supplemented with 0.05% (w/v) L cysteine- HC1 (Sigma Co., St. Louis, MO), 0.075% (wt/v) Bacto agar (Difco), 0.02% (w/ v) Na₂CO₃, and 0.01% (w/v) CaCl₂.2H₂O. Active cultures were incubated aerobically for 72 h at 37°C without agitation.

Treatment of Molasses (Sugar Cane Honey). Molasses was kindly supplied by the Sugar and Integrated Industries Company, SIIC, Egypt. Molasses were prepared by dilution with water. The diluted molasses (one part of molasses to 3 parts water) was repeatedly centrifuged (3 times) at 4000 rpm for 20 min each. The muddy precipitate was then discarded.

Enumeration of Microorganisms. For each skim milk sample, the dilutions 10⁻², 10⁻³, and 10⁻⁴ were prepared according to the IDF standard (Anonymous. 1996.) Inoculation, incubation, and enumeration of the samples were performed according to the IDF standard (Anonymous. 1991) One milliliter of the dilution was, in duplicate, transferred to Petri dishes, and 12 to 15 mL plate count agar containing 5% reconstituted nonfat dried milk produced according to the IDF standard (Anonymous. 1991) were poured into each Petri dish. The inoculum and the medium were carefully mixed and allowed to solidify. The Petri dishes were inverted and incubated at 30 ± 1°C for 72 ± 3 h. After incubation, the colonies on the Petri dishes were counted, and this count was divided by the dilution factor to

obtain the CFU/mL.

Growth of Bifidobacteria and Lactobacteria in Milk. The fermentation characteristics of each strain were determined in sterile reconstituted skim milk. Skim Milk powder (Difco Laboratories, Detroit, MI) was reconstituted to 12% solids with distilled water and sterilized by autoclaving 10 min at 121 ° C. Flasks containing 100 ml of sterile reconstituted skim milk were inoculated with 3 ml of a supplemented MRS broth culture for Lactobacteria and for Bifidobacteria MRS broth supplemented with cysteine- HC1 (Sigma Co., St. Louis, MO), .075% (w/ v) incubated at 37 ° C for 24 h.

Bifidobacterial and Lactic acid bacteria Activity. Sample preparation: To determine culture activity, Skim milk containing 5 percent (w/v) Molasses ,Fructose, or Lactose and Fermented with the two strains of Bifidobacteria and Lactobacteria were prepared for High Performance Liquid Chromatography (HPLC Agilent HP 1100) analysis using the method described according to a modification of the method of Bevilacqua and Califano (1989). One hundred µL sample was added to 40 ml of buffer-acetonitrile mobile phase (0.5% (wt/vol) (NH₄)₂HPO₄ (0.038 M) - 0.4% (v/v) acetonitrile (0.049 M), at pH 2.24 with H₃PO₄), extracted for 1 h, The samples were centrifuged at 5000 x g for 10 minutes. The supernatants were filtered using (NY 0.45 µm membrane filter, Chemiton, Barcelona, Spain) and eluted through a reverse phase Superclean tube and stored in HPLC Agilent vials at -20°C until the HPLC analysis. Culture activity was determined by measuring the end products of fermentation (lactic acid and acetic acid) using HPLC. HPLC analysis: HPLC System (HP1100) can include; G1329A Thermostatted Autosampler G1316A, PEEK Sample Loops Analytical Injection Valves PEEK 9725i They have a 20 µl loop, Column reversed phase, Compartment, G1310A Isocratic Pump, G1365A Multi-wavelength Detector. Solvent /Degasser Nylon Filter Membranes 47 mm, pore size 0.45 µm (Reyed et al. 2006).

The Bifidogenic factors activity. The assay for measuring the bifidogenic factors activity in various samples was done. It was determined by serially diluting 2-fold with sterilized distilled water for the sample containing cell-free filtrate. Every 100 µL of appropriately diluted sample subcultured twice in medium different kind of sweetener anaerobic incubation at 37°C for 72 h. After 72 h, the growth of B. bifidum and lactic acid bacteria was assessed from the absorbance at 580 nm Uv/ Vis spectrometer (Lambda EZ 201 Perkin Elmer).

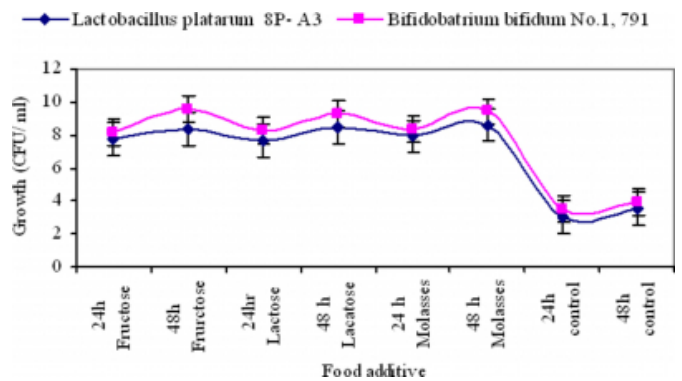
Titrate acidity (TA). It was measured for all the treatments at 24 and 48h. 10 ml of each culture broth was titrated with 0.1 NaOH required to neutralize to an end point of pH 8.2. The results were expressed in mmol of NaOH from which the value at 0 had been subtracted (Marshall, et al 1982.).

RESULTS AND DISCUSSION

Strains of *Lactobacillus platarum* No. 8P-A3 and *Bifidobatrium bifidum* No.1, 791 grew in skim milk (Control) (Fig. 1). Growth patterns were quite variable when skim milk was used as the culture medium with Food additive (Fig. 2). After approximately 48 h, growth in skim milk reached the stationary phase for *Lactobacillus platarum* No. 8P-A3 and *Bifidobatrium bifidum* No.1, 791 use of skim milk as influenced by sweetener added to a growth medium increased growth density and reach to the stationary phase after 24 h for *Lactobacillus platarum* No. 8P-A3 and *Bifidobatrium bifidum* No.1, 791 if it compared with the uses of skim milk as a growth medium without any additive (control). Numerous growth factors have been proposed over the years for optimization of bifidobacterial growth, such as amino sugars, bovine casein digest. (Rasic and Kurmann. 1983.) Following 2 days of incubation *Lactobacillus platarum* No. 8P-A3 and *Bifidobatrium bifidum* No.1, 791 attained higher population and acid production in skim milk. *Lactobacillus platarum* No. 8P-A3 and *Bifidobatrium bifidum* No.1, 791 had the highest growth rate in molasses 13 % whereas the growth of these bacteria showed poor growth in skim milk supplemented with other sweeteners type. In case of *Bifidobatrium bifidum* No.1, 791, high cell number were obtained when this organism was grown in the presence on molasses, Cultural viability under these conditions was much better than expected.

Figure 1

Figure 1: Growth promotion of No. 8P-A3 and No.1, 791 culture in skim milk as influenced by Food additive (Cfu/ ml 10).



In this study, we found that Molasses are growth promoters for *Lactobacillus platarum* No. 8P-A3 and *Bifidobatrium bifidum* No.1, 791. the fermentation process was monitored by measuring growth rate, pH, TA, and organic acid, both of two strains demonstrated a relation between growth rate (CFU/ml) and acid production that was measured as Titratable Acidity (TA) in 48 h of incubation. Fast growing bacteria generally produced more acetic and lactic acids after 48 h in 13 % molasses than supplementary sweeteners type that shown the slow growing strains (Fig.2). Skim milk had increased lactic acid (D+L) throughout the 2 d of fermentation, and in agreement with the decrease in the pH values (Table I), at 48 h the highest values were found in skim milk with *Lactobacillus platarum* No. 8P-A3 and *Bifidobatrium bifidum* No.1, 791 added, at the end of fermentation the organic acid concentrations in case of bifidobacteria were reached to 15%. Kosikowska (1978) reported that strains of *Lactobacillus bifidus* (now *B. bifidum*) differed in their ability to acidify milk. That report showed TA that ranged from 0.60 to 1.40% LA after 48 h of incubation for different strains of *L. bifidus*. Brown and Townsley (1970); Collins and Hall (1984) and Marshall et al. (1982) made similar observations. Desjardins et al., (1990). have reported that a great part of the total lactic and acetic acids is produced by bifidobacteria after growth, especially during the stationary phase. Using lactic acid bacterial culture, the pH of skim milk dropped as expected following 48 h incubation, thus indicating that molasses support acid production in a similar manner to other sweeteners and were not inhibitory. Molasses enhanced *Bifidobatrium bifidum* No.1, 791 to produce organic acids more than lactose, fructose and control. Therefore, further studies were undertaken to determine the influence of these sweeteners on acid production by lactic acid bacteria and

bifidobacteria (Table II). Bifidobacteria produce acetic and lactic acids in a molar ratio of 3:2 from 2 mol of glucose in an ideal synthetic medium Scardovi (1965). Lactic acid is in the L-(+) form, which is more easily metabolized by infants than is the D-(-) form. (Rowland 1997). Production of acetic and lactic acids varied among species and among formulae within species. Percentage of acetic acid produced at maximal rates ranged from 9 % to 15 % in skim milk but Mistxy et al (1985). reported that during fermentation of ultra filtrated skim milk, growth of bifidobacteria were uncoupled from organic acid production 55 to 75% of Lactic acid and Acetic acid were produced during the stationary phase of growth shows the level of lactic acid produced when bifidobacteria were grown in the presence of Fructose, Lactose, Molasses and in control. Bifidobacteria were influenced by sweetener type. Bifidobacteria are known to be a fastidious organism. Numerous researchers have reported that Bifidobacteria grow poorly in milk. (Biavati. et al., 1992; Klaver, et al., 1993) .Enhanced organic acid production by Bifidobacteria, in the presence of molasses was unexpected. Molasses is considered Bifidogenic factor as like all oligosaccharides have been shown to increase growth and activity. Fructooligosaccharides (FOS) and Galactooligosaccharides (GOS) having lower degree of polymerization (DP), were best in supporting growth of Bifidobacteria. These low substrates with low DP oligosaccharides may be the favored substrates for Bifidobacterial support, thereby enhancing lactic acid production as observed in the present study. In contrast, carbohydrates with high DP were poor Bifidobacteria substrates. Very little is known about the mechanism of carbohydrate uptake by Bifidobacteria (Kleessen et al ., 1997).

Figure 2

Table 1: The pH of skim milk fermented with No. 8P-A3 and No.1, 791 influenced by food additive

Culture	Fructose		Lactose		Molasses		Control	
	24h	48 h	24h	48h	24h	48h	24h	48h
<i>Lactobacillus platarum</i> 8P-A3	5.83 ± 0.09	3.82 ± 0.03	5.81 ± 0.09	3.92 ± 0.06	5.82 ± 0.06	3.79 ± 0.03	5.86 ± 0.07	3.94 ± 0.12
<i>Bifidobacterium Bifidum</i> No.1, 791	6.0 ± 0.04	5.1 ± 0.14	6.03 ± 0.08	5.01 ± 0.22	6.03 ± 0.07	4.55 ± 0.09	6.09 ± 0.07	5.06 ± 0.05

N = 3 for all treatment
Result were expressed by mean SD., for triplicate determination.

Figure 3

Figure 2: Titratable acidity (TA) of skim milk fermented with No. 8P-A3 and No.1, 791 influenced by food additive

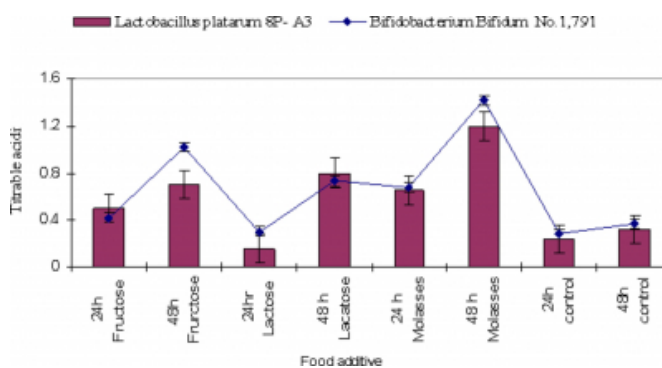


Figure 4

Table 2: Organic acid production in skim milk fermented with as influenced by Food additive

Organic acid	Fructose		Lactose		Molasses		Control	
	24h	48 h	24h	48h	24h	48h	24h	48h
Acetic acid (mmol)	0.95 ± 0.06	1.33 ± 0.17	1.9 ± 0.11	2.2 ± 0.20	2.7 ± 0.26	3.9 ± 0.38	0.4 ± 0.03	1.8 ± 0.06
Lactic acid (mmol)	0.06 ± 0.05	1.19 ± 0.07	1.1 ± 0.07	1.5 ± 0.12	2.2 ± 0.08	2.9 ± 0.07	0.2 ± 0.02	1.4 ± 0.08

The acetic acid and lactic acid in fermented broth were determined by HPLC.
Results were expressed by Mean ±S.D. for triplicate determination.

Growth and acid production were quite different among the Bifidobacterium and lactobacillus species tested in this study. The fast growing Bifidobacteria in skim milk were mainly of infant origin, these results suggest a relationship between the preparation of freshly autoclaved media for all samples, followed by incubation or holding in the anaerobic and nutritional conditions, may have contributed to greater viability of the intestinal origin of Bifidobacteria, Lactobacillus strains and increased their growth performance in skim milk. The more obligatory anaerobic species might have grown poorly because the oxidation- reduction potential of milk was not adequate. The growth of anaerobic organisms might be stimulated by the addition of L-cysteine to decrease the oxidation-reduction potential of milk. The inoculum was grown in supplemented MRS that contained yeast extract and L cysteine. However, the dilution into milk might have been too great for the strains to use these growth factor. Growth conditions could affect the metabolism and explain the differences among growth of bifidobacteria and lactic acid bacteria. In practical terms, the in vitro properties of new prebiotics will probably relate reasonably well to their physiologic function and analytic results, and these can

be used to screen potential prebiotics. These analytic results should be taken in hand because the nature of the carbohydrate determines its fermentability is a question that has barely been addressed and include 1) a detailed description of the chemical structure, 2) measurement of resistance to gastric juice, 3) measurement of resistance to pancreatic enzymes, and possibly 4) measurement of resistance to brush border enzymes. Results from this study has shown that Molasses contains highly active growth promoters for *B. bifidum*, much of the activity of Blackstrap Molasses has been attributed to blackstrap Molasses is rich in nutrients such as copper, calcium, iron, potassium, magnesium, chromium, manganese, molybdenum, zinc, phosphorous, pantothenic acid, vitamin E and Inositol, also contains high concentrations of C6 sugars and other fermentable carbohydrates as well as significant concentrations of B vitamins, especially biotin, which enhance fermentation rates. Molasses were not inhibitory to lactic acid and bifidobacteria when added to skim milk at a level with 5 %. Furthermore, understanding the substrate preference of bifidobacteria will facilitate development of probiotics, perbiotics, and synbiotics (Beerens, et al . 1980.; Poch, and Bezkorovainy. 1989; Bezkorovainy and Topouzian. 1981) In conclusion, we have shown that molasses can be used effectively as a prebiotic because of the increase in Bifidobacteria and lactic acid bacteria numbers with the subsequent increase in acetic acid and lactic acid concentrations, giving an added possible health benefit consequently inhibit the growth of pathogenic bacteria, through the production of short-chain fatty acids, lowering of colonic pH, production of antimicrobial compounds, and/or competition for growth substrates and adhesion sites (Benno, et al., 1984; Gibson, and Wang. 1994, Hudault, et al.1997).

CORRESPONDENCE TO

REYED M. REYED M.Sc. PhD Bioprocess Department, Genetic Eng. & Biotechnology Research Institute, Mubarak City for Scientific Research and Applied Technology e.mail. reyed@colon.la; riad@ncbs.res.in; rmrkater@mail.ru

References

1. Metchnikof E. (1908). *The Prolongation of Life*, 1st ed. New York: SP Putmans Sons.
2. Fuller R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol.* 66:365-378.
3. Kirjavainen PV, Gibson GR. (1999). Healthy gut microflora and allergy: factors influencing development of the microbiota. *Ann. Med.* 31, 288-292.
4. Glodin B R. (1998). Health benefits of probiotics. *Br. J. Nutr.*; 80 (Suppl. 2): S203-7.
5. Friedrich M J. (2000). A bit of culture for children: probiotics may improve health and fight diseases. *J. Am. Med. Assoc.* 284, 1365-1366
6. Lu L, Walker WA. (2001). Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium. *Am. J. Clin. Nutr.* 73 (6), 1124S-1130S.
7. Guarner F, Schaafsma G J. (1998). Probiotics. *Int. J. Food Microbiol.* 39: 237-238.
8. Kim H S. (1988). Characterization of lactobacilli and bifidobacteria as applied to dietary adjuncts. *Cult Dairy Prod. J.* 23; 37
9. Rasic, J L, and J A Kurmann. (1983). *Bifidobacteria and Their Role*. Birkhauser Verlag, Basel, Switzerland.
10. Gibson GR, Beatty EH, Wang X, Cummings JH. (1995). Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology.* 106: 975-82.
11. Hughes D B, Hoover DG. (1991). Bifidobacteria: their potential for use in American dairy products. *Food Technol.* 45(4):74.
12. Abou-Zeid A A, Fouad M, Yassein M.: (1978). Microbiological production of acetone-butanol by *Clostridium acetobutylicum*. *Zentralbl Bakteriol Naturwiss.* 133(2):125-34
13. Fermin D, Fermin R, Pina A, Mena A. (1984). Sugar cane juice as a substitute for maize and molasses in diets for fattening pigs. *Tropical Animal Production* 9:271-274
14. Van B H, Men L T. (1990). "A" molasses in diets for growing pigs. *Livestock Research for Rural Development*, Volume 2, Number 3:76-79.
15. Anonymous. IDF Standard 122C. (1996). Milk and milk products. Preparation of samples and dilutions for microbiological examination. *Int. Dairy Fed., Brussels, Belgium.*
16. Anonymous. IDF Standard 100B. (1991). Milk and milk products. Enumeration of microorganisms. *Int. Dairy Fed., Brussels, Belgium.*
17. Bevilacqua A E, Califano A N. (1989). Determination of organic acids in dairy products by high-performance liquid chromatography. *J. Food Sci.* 54, 1076-1079.
18. Reyed Reyed M, Sahar Zaki A, Desouky Abd-El-Haleem, Elsayed AM M. (2006). Biochemical Characterization of Spent Culture Supernatant associated Polysaccharides in *Bacillus* Isolate", 3 th Conference the Role of Biochemistry in Environment and Agriculture, Cairo University, pp. 58- 67.
19. Marshall V M, Cole W M, Mabbitt L A. (1982). Fermentation of specially formulated milk with single strains of bifidobacteria. *J. Soc. Dairy Technol.* 35:143.
20. Kosikowska M. (1978). Biochemical activity of bifidobacterium. Page 549 in *Proc. 20th Int. Dairy Congr. Vol. E. Int. Dairy Fed.. Paris, France.*
21. Brown C D, Townsley PM. (1970). Fermentation of milk by *Lactobacillus bifidus*. *Can. Inst. Food Technol. J.* 3(4):121.
22. Collins E B, Hall B J. (1984). Growth of bifidobacteria in milk and preparation of *Bifidobacterium infantis* for a dietary adjunct. *J. Dairy Sci.* 67: 1376.
23. Desjardins M L, Roy D. (1990). Uncoupling of growth and acid production in *Bifidobacterium* sp. *J. Dairy Sci.* 73:1478.
24. Scardovi V, Trovatelli L. D. (1965). The fructose-6-phosphate shunt as peculiar pattern of hexose degradation in the genus *Bifidobacterium*. *Ann. Microbiol.* 15:19.
25. Rowland I R. (1997). Influence of non-digestible oligosaccharides on gut functions related to colon cancer. In: Hartemink R, ed. *Nondigestible oligosaccharides: healthy food for the colon? Proceedings of the International Symposium, Wageningen Graduate School VLAG. Wageningen, Netherlands: Wageningen Graduate School*

VLAG:100-5.

26. Mistxy V V, F V. (1985).Kosikowski. Fermentation of ultrafiltered skim milk retentates with mesophilic lactic cheese starters. *J. Dairy Sci.* 68: 161.
27. Biavati B, Sozzi T, P Mattarelli , Trovatelli L. D. (1992). Survival of bifidobacteria from human habitat in acidified milk. *Microbiologica* 15: 197.
28. Klaver A M, Kingma F, Weerkamp A H.(1993).Growth and survival of bifidobacteria in milk. *Neth. Milk Dairy J.* 47: 151-164.
29. Kleessen B, Sykura B, Zunft HJ, Blaut M (1997). Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. *Am J Clin Nutr.* 65:1397-402.
30. Beerens H, Romond C, Neut C. (1980). Influence of breast-feeding on the bifid flora of the newborn intestine. *Am J Clin Nutr.* 33(11 Suppl):2434-2439.
31. Poch M, Bezkorovainy A. (1989). Growth enhancing supplements for various species of the genus *Bifidobacterium*. *J. Dairy Sci.* 71:3214-3221.
32. Bezkorovainy A, Topouzian. N. (1981). *Bifidobacterium bifidus* var. pennsylvanicus growth promoting activity of human milk casein and its derivatives. *Int. J. Biochem.* 13: 585-590.
33. Benno Y, Sawada, K , Mitsuoka, T. (1984). The intestinal microflora of infants : composition of fecal flora in breast-fed and bottle-fed infants. *Microbiol Immunol* 28, 975-986.
34. Gibson G R, Wang X. (1994). Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J. Appl. Bacteriol.* 77:412-420.
35. Hudault S, Liévin V, Bernet Camard M F, Servin A L. (1997). Antagonistic activity exerted in vitro and in vivo by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection. *Appl. Environ. Microbiol.* 63: 513-518.

Author Information

Reyed M. Reyed, M.Sc., Ph.D.

Bioprocess Department, Genetic Eng. & Biotechnology Research Institute, Mubarak City for Scientific Research and Applied Technology

A.I. El-Diwany, M.Sc., Ph.D.

Chemistry of Natural and Microbial Products Dept, National Research Centre