GIDA (2014) 39 (2): 71-77
doi: 10.5505/gida.66375

Research / Araştırma

EFFECTS of PREBIOTICS on GROWTH and ACIDIFYING ACTIVITY of PROBIOTIC BACTERIA

Ayla Şener Mumcu[1], Ayhan Temiz[2]*

[1]Republic of Turkey Ministry of Food, Agriculture and Livestock, Bodrum District Directorate, Muğla Provincial Control Directorate, Muğla, Turkey
[2]Hacettepe University, Faculty of Engineering, Department of Food Engineering, Ankara, Turkey

Received / Geliş tarihi: 13.01.2014
Accepted / Kabul tarihi: 17.01.2014

Abstract

In this study, in vitro effects of six commercial prebiotics on growth and acidifying activity of two strains of *Lactobacillus acidophilus* and two strains of *Bifidobacterium* spp. were investigated. Fructo-oligosaccharide, inulin, galacto-oligosaccharide, soybean oligosaccharide, xylo-oligosaccharide and lactulose were used as prebiotics. The prebiotics were tested at three different concentrations. Growth and acidifying activity of the probiotic bacteria were variable depending on the type and concentration of the prebiotics. In general, as the concentration of the prebiotics increases, the growth and acidifying activity performance of the probiotic strains increases. The difference in the strains of *L. acidophilus* was not significant in terms of both growth performance and acidifying activity. However, the difference in the species of *Bifidobacterium* was found to be significant ($P<0.05$). The results of this study indicated that an appropriate prebiotic substance should be selected for each probiotic bacterial strain for its good growth and acidifying performance.

Keywords: Prebiotics, probiotic bacteria, *Lactobacillus acidophilus, Bifidobacterium* spp., growth, acidifying activity.

PREBİYOTİKLERİN PROBİYOTİK BAKTERİLERİN GELİŞMESİ ve ASİTLEŞTİRME AKTİVİTELERİ ÜZERİNE ETKİLERİ

Özet

Bu çalışmada altı adet ticari prebiyotik maddenin in vitro koşullarda iki adet *Lactobacillus acidophilus* suşu ve iki adet *Bifidobacterium* spp. suşunun gelişme ve asitleştirme aktivitesi üzerindeki etkileri araştırılmıştır. Prebiyotik olarak frukto-oligosakkarit, inulin, galakto-oligosakkarit, soya fasulyesi oligosakkariti, ksilo-oligosakkarit ve laktuloz kullanılmıştır. Prebiyotikler üç farklı konsantrasyonda denenmiştir. Probiyotik bakterilerin gelişme ve asitleştirme aktivitesi üzerindeki etkileri prebiyotik çeşidine ve konsantrasyonuna bağlı olarak değişim göstermiştir. Genel olarak, prebiyotik konsantrasyonu arttıkça, probiyotik bakteri suşlarının gelişme performansı ve asitleştirme aktivitesinde artışlar meydana gelmiştir. *L. acidophilus* türündeki suş farklılığı, suşların gelişme performansı ve asitleştirme aktivitesi üzerinde önemli bir etki yaratmamıştır. Buna karşılık *Bifidobacterium* cinsindeki tür farklılığının bu yönlerdeki etkisi önemli bulunmuştur ($P<0.05$). Araştırma sonuçları, bir probiyotik bakteri suşunun iyi bir gelişme ve asitleştirme performansı gösterebilmesi için ona uygun bir prebiyotik madde seçilmesinin gerekliliğine işaret etmektedir.

Anahtar Kelimeler: Prebiyotikler, probiyotik bakteriler, *Lactobacillus acidophilus, Bifidobacterium* spp., gelişme, asitleştirme aktivitesi

* *Corresponding author* / Yazışmalardan sorumlu yazar;
temiz@hacettepe.edu.tr, (+90) 312 297 7101, (+90) 312 299 2123

INTRODUCTION

In recent years, the effects of probiotics and prebiotics on human health are of great interest to both consumers and food manufacturers. Many efforts have been made to develop novel functional foods or preparations containing probiotics and prebiotics. The combinations of probiotics and prebiotics in nutritional supplements in a form of synergism are called synbiotics. The human gastrointestinal tract (GIT) is a kinetic micro-ecosystem that enables normal physiological functions of host organism unless harmful and potentially pathogenic bacteria dominate it. It is stated that systematic supplementation of the diet with probiotics, prebiotics or synbiotics may ensure maintaining a proper equilibrium of the microflora in the GIT (1-4).

Probiotics, derived from the Greek words meaning "for life", are living microorganisms which actively enhance health of consumers by improving the balance of microflora in the gut when ingested in sufficient numbers (5, 6). The health benefits attributed to probiotic bacteria can be summarized as nutritional benefits, enhancing bio-availability of some minerals, synthesis of vitamins, increase in natural resistance to infectious diseases of the intestinal tract, prevention diarrhea, reduction of serum cholesterol, reduction of lactose intolerance, enhancement of immune system, pre-digestion of proteins, improved absorption, enhancement of bowel motility and maintenance of mucosal integrity (2, 3, 7). Traditionally, probiotics have been added to yoghurt and other fermented foods. Recently, they have also been incorporated into drinks, as well as marketed as supplements in the form of tablets, capsules, and freeze-dried preparations (3, 8).

Prebiotics have become an exciting and challenging concept in nutrition. A prebiotic is "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 the host health" (1). This definition was revised in 2004 and prebiotics are now defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (9). Any dietary ingredient that can reach the colon has the potential of being a prebiotic. However, there are some criteria which allow the classification of a food ingredient as a prebiotic. These include (10);

- It must be neither hydrolyzed, nor absorbed in the upper part of the gastro-intestinal tract.

- Selective fermentation by potentially beneficial bacteria in the colon.

- Alteration in the composition of the colonic microbiota towards a healthier composition.

- Preferably, induce effects which are beneficial to the host health.

The certain carbohydrates in the form of oligo- and polysaccharides, meeting the criteria of prebiotics, have been isolated from different natural sources at large scale by using different technologies and have become commercially available. There are many prebiotic oligosaccharides in the markets including fructo-oligosaccharides, inulin, galacto-oligosaccharides, soybean oligosaccharides, xylo-oligosaccharides, lactulose, gentio-oligosaccharides, raftiloses, raftiline, isomalto-oligosaccharides and mannan-oligosaccharides (1-4, 11-13).

Bifidobacterium and *Lactobacillus*, the members of the normal colonic bacterial flora, are the most commonly used probiotics in many functional foods and nutritional supplements. These are the most widely studied probiotic strains of the lactic acid bacteria and have been shown to exert a wide number of health benefits (1, 3, 8, 11, 14, 15). The beneficial effects of probiotics in the GIT depend on their viability and metabolic activity. To provide health benefits, probiotics must reach the large intestine in sufficient numbers. It is recommended to consume about 10^6-10^9 viable cells per day. Hence, the concentration of probiotic bacteria in a functional food product is suggested to be 10^8 CFU (colony forming unit)/g or over (16). The previous studies showed that the growth and activity of probiotics is greatly stimulated by the prebiotics. There are various in vitro and in vivo experiments on the effect of prebiotics on the growth and activity of probiotic bacteria (13, 17-25). In each study, only the limited numbers of prebiotics or saccharides having prebiotic importance were used to investigate their effects on the limited numbers of probiotics

or human gut microflora. On the other hand, it was stated that the greatest scientific interest was focused on the nutritional and health benefits of oligofructose and inulin (17).

Because of the considerable effects of prebiotics on the viability and growth of probiotics, it is crucial to select suitable prebiotic substances to produce functional foods containing a combination of prebiotics and probiotics. As indicated by Goderska et al. (18), it seems feasible to undertake in vitro research concerning the growth and activity of potential probiotic bacteria in the media supplemented with different prebiotics, and to study differences between bacterial strains. Therefore, the aim of this study was in vitro investigation of the effects of the commercially available prebiotics on the growth and acidifying activity of the potential probiotic bacteria, two strains of *Lactobacillus acidophilus* and two strains of *Bifidobacterium* spp.

MATERIALS and METHODS

Bacterial Strains

Lactobacillus acidophilus ATCC 4356, *L. acidophilus* LA-5 (Chr. Hansen), *Bifidobacterium bifidum* ATCC 15969 and *B. animalis* subsp. *lactis* BB-12 (Chr. Hansen), purchased in lyophilized form, were used as probiotic test bacteria. *Lactobacillus acidophilus* cultures were activated in MRS broth (de Man, Rogosa and Sharpe, Merck) at 37 °C for 24 h. *Bifidobacterium* spp. cultures were activated in RCM broth (Reinforced Clostridial Medium, Fluka) under anaerobic incubation conditions by using anaerobic test kits (GENbox anaer, Biomérieux) at 37 °C for 24 h.

Prebiotic Substances

As prebiotics, commercial preparations of fructo-oligosaccharide (FOS; Dora/Orafti, Turkey), inulin (INU; Dora/Orafti, Turkey), galacto-oligosaccharide (GOS; Oligomate55, Yakult, Japan), soybean oligosaccharide (SOS; Calpis, Japan), xylo-oligosaccharide (XOS; Suntory, Japan) and lactulose (LAC; Sigma) were used. The prebiotics were tested at three different concentrations of 0.5%, 1% and 2% (w/v). Stock solutions of 10% prebiotic substances were prepared in distilled water and filter-sterilized by using 0.45 µm pore size membrane filters (Millipore).

Effects of the Prebiotics on Growth and Acidifying Activity of the Probiotic Bacteria

Carbohydrate-free MRS broth and carbohydrate-free RCM broth was used as basal growth medium for *Lactobacillus acidophilus* cultures and *Bifidobacterium* spp. cultures, respectively. Sterile prebiotic solutions were added into the basal MRS and RCM broth to obtain final prebiotic concentrations of 0.5%, 1% or 2%. Activated bacterial culture was transferred (1%) into the basal growth media supplemented with prebiotics. The basal growth medium with glucose at 2% concentration was used as positive control and the basal growth medium was used as negative control. Initial viable cell numbers of the inoculated growth media were determined by pour plate method by using MRS agar and RCM agar for *Lactobacillus acidophilus* and *Bifidobacterium* spp., respectively. Inoculated MRS agar and RCM agar were incubated at 37 °C for 24 h under aerobic and anaerobic incubation conditions, respectively. After the incubation with prebiotics, viable cell numbers in the culture media were determined by pour plate method by using MRS agar or RCM agar. The effects of prebiotics on the growth performance of the probiotic bacteria were evaluated according to the difference between the viable cell number at the end of the incubation and the initial viable cell number in the inoculated basal medium. Acidifying activity of the cultures was determined by measuring pH with a pH meter (Mettler-Toledo, Seven Multi). The study was carried out by three replicates for each prebiotic.

Statistical Analyses

Statistical analyses were performed by using SPSS software (SPSS Inc., version 15.0). The differences in the treatments were established by using the analysis of variance (ANOVA) test at 5% significant level.

RESULTS and DISCUSSION

The effects of the prebiotic substances on the growth and acidifying activity of the tested probiotic bacterial strains are shown in Table 1. The results showed that the growth performance of probiotic bacterial strains was variable depending on the type and concentration of prebiotics used in the basal media.

Table 1. Effects of the prebiotic substances on the growth and acidifying activity of the tested probiotic bacteria strains.[a]

Prebiotics/ Concentration (%)		*Lactobacillus acidophilus* ATCC 4356		*L. acidophilus* LA-5		*Bifidobacterium bifidum* ATCC 15969		*B. animalis* subsp. *lactis* BB-12	
		Increase in viable cell number (log CFU/mL)	pH after incubation	Increase in viable cell number (log CFU/mL)	pH after incubation	Increase in viable cell number (log CFU/mL)	pH after incubation	Increase in viable cell number (log CFU/mL)	pH after incubation
Negative control[b]		0.1±0.1	5.6±0.2	0.2±0.3	5.9±0.1	0.1±0.2	5.7±0.1	0.5±0.4	5.6±0.2
Positive control[b]		1.5±0.3	3.9±0.1	1.6±0.2	4.1±0.3	2.2±0.3	4.3±0.2	1.8±0.3	4.1±0.2
FOS	0.5	1.2±0.3	4.3±0.2	1.8±0.2	4.6±0.1	1.8±0.1	5.3±0.1	1.8±0.1	4.7±0.2
	1	1.7±0.2	4.3±0.3	1.8±0.4	4.2±0.2	1.7±0.3	5.0±0.1	1.9±0.2	4.6±0.1
	2	2.1±0.3	4.1±0.1	2.2±0.3	4.2±0.1	2.1±0.1	4.7±0.2	2.0±0.5	4.4±0.3
INU	0.5	1.3±0.3	4.6±0.3	1.8±0.1	5.1±0.3	1.6±0.1	5.5±0.1	1.7±0.3	5.0±0.2
	1	1.4±0.2	4.2±0.2	1.9±0.2	4.7±0.1	1.8±0.4	5.4±0.2	1.7±0.4	4.8±0.1
	2	1.7±0.5	4.1±0.2	2.0±0.1	4.3±0.1	1.7±0.3	5.2±0.2	1.8±0.2	4.4±0.3
GOS	0.5	0.9±0.3	4.7±0.2	1.6±0.3	4.8±0.2	1.9±0.2	4.3±0.1	1.9±0.3	4.4±0.4
	1	1.2±0.2	4.5±0.1	1.9±0.2	4.4±0.2	2.2±0.4	4.2±0.2	2.0±0.2	4.2±0.2
	2	1.5±0.2	4.2±0.2	1.3±0.5	4.2±0.1	2.4±0.1	4.3±0.2	2.2±0.2	4.1±0.1
SOS	0.5	0.7±0.1	4.9±0.2	1.8±0.1	4.9±0.1	1.5±0.2	4.7±0.1	1.9±0.2	4.3±0.2
	1	0.8±0.2	4.4±0.3	1.8±0.2	4.6±0.1	1.7±0.2	4.5±0.2	2.0±0.4	4.2±0.2
	2	1.1±0.1	4.2±0.2	1.5±0.1	4.3±0.3	2.2±0.3	4.2±0.2	2.2±0.2	4.0±0.1
XOS	0.5	0.8±0.1	5.5±0.1	0.9±0.1	5.7±0.1	1.2±0.2	5.6±0.1	1.8±0.6	4.4±0.3
	1	0.7±0.1	5.4±0.2	1.1±0.3	5.6±0.2	1.8±0.5	5.5±0.3	2.0±0.5	4.2±0.1
	2	1.1±0.1	5.1±0.2	1.4±0.5	5.3±0.4	1.7±0.3	5.4±0.2	2.3±0.3	4.1±0.4
LAC	0.5	0.6±0.3	5.1±0.3	1.8±0.4	4.5±0.2	1.8±0.1	4.6±0.3	1.8±0.2	5.1±0.2
	1	1.3±0.2	4.5±0.2	2.0±0.3	4.3±0.1	1.9±0.1	4.5±0.1	1.8±0.5	5.0±0.1
	2	1.6±0.3	4.5±0.1	1.9±0.4	4.1±0.1	2.1±0.3	4.6±0.2	1.9±0.1	4.6±0.3

[a] Values are mean ± SD (n=3)

[b] Negative control, the basal growth medium; Positive control, the basal growth medium supplemented with 2% glucose

The effects of prebiotic type and the prebiotic concentration on the growth of *Lactobacillus acidophilus* ATCC 4356 were found to be significant (P<0.05). The initial viable cell numbers of this strain were between 6.3-6.7 log CFU/mL. After the incubation with prebiotics, viable cell numbers were found to be between 7.1-8.6 log CFU/mL. The values after the incubation were 6.7 and 7.9 log CFU/mL for negative and positive controls, respectively. The increase in the viable cell numbers with prebiotics varied within the range of 0.6-2.1 log CFU/mL.

The growth of *L. acidophilus* ATCC 4356 was best supported by FOS at 2% concentration. INU and LAC at 2% concentration had relatively lower supporting growth effect on this strain than FOS had. SOS and XOS had low growth effect compared to other prebiotics. FOS and INU enhanced the growth of this strain much more when they were compared with 2% glucose (positive control). SOS and XOS at all tested concentrations had lower supporting effect on the growth of this strain than that of 2% glucose. The effects of prebiotic type and the prebiotic concentration on the growth of *Lactobacillus acidophilus* LA-5 were significant (P<0.05). The initial viable cell numbers of this strain were between 6.4-6.7 log CFU/mL. After the incubation with prebiotics, viable cell numbers increased to 7.5-8.6 log CFU/mL. The values after the incubation were 6.7 and 7.6 log CFU/mL for negative and positive controls, respectively. The increase in the viable cell numbers with prebiotics varied within the range of 0.9-2.2 log CFU/mL.

The growth of *L. acidophilus* LA-5 was best supported by FOS at 2% concentration. INU and LAC at 2% concentration had relatively lower effect on this strain when compared with FOS. In contrast, XOS had low growth effect compared to other prebiotics. All the tested prebiotic substances, except XOS, enhanced the growth of this strain much more when they were compared with 2% glucose (positive control).

In general, as the concentration of the prebiotics increases, the positive effect of the prebiotics on the acidifying activity of *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5 increases. The pH values of the culture media with prebiotics varied between 4.1 and 5.5 for *L. acidophilus* ATCC 4356 and 4.1 and 5.7 for *L. acidophilus* LA-5. The highest acidifying activity of *L. acidophilus* ATCC 4356 was obtained with positive control. Acidifying activity of *L. acidophilus* ATCC 4356 was best supported by the prebiotics of FOS and INU. The highest acidifying activity of *L. acidophilus* LA-5 was obtained with positive control and LAC. The prebiotic LAC had the highest effect on the acidifying activity of *L. acidophilus* LA-5. Except XOS, the prebiotics had almost similar effect on the acidifying activity of both *L. acidophilus* strains. XOS had the lowest effect on the acidifying activity of both *L. acidophilus* strains.

The effects of prebiotic type and the prebiotic concentration on the growth of *Bifidobacterium bifidum* ATCC 15696 were significant (P<0.05). The initial viable cell numbers of this strain were between 6.2-6.5 log CFU/mL. After the incubation with prebiotics, viable cell numbers increased to 7.7-8.7 log CFU/mL. The values after the incubation were 6.6 and 8.5 log CFU/mL for negative and positive controls, respectively. The increase in the viable cell numbers with prebiotics varied within the range of 1.2-2.4 log CFU/mL.

GOS at 2% concentration showed the best effect on the growth of *B. bifidum* ATCC 15696. SOS, LAC and FOS at 2% concentration had relatively lower supporting growth effect on this strain than GOS had. Only GOS at 2% concentration had higher supporting effect on the growth of this strain than that of 2% glucose. On the other hand, growth supporting effect of SOS at 2% concentration was identical with that of 2% glucose.

The effects of prebiotic type and the prebiotic concentration on the growth of *Bifidobacterium animalis* subsp. *lactis* BB-12 were significant (P<0.05). The initial viable cell numbers of this strain in the media were between 6.1-6.4 log CFU/mL. After the incubation with prebiotics, viable cell numbers increased to the levels of 8.0-8.6 log CFU/mL. The values after the incubation were 6.9 and 8.1 log CFU/mL for negative and positive controls, respectively. The increase in the viable cell numbers with prebiotics varied within the range of 1.7-2.3 log CFU/mL.

The growth of *B. animalis* subsp. *lactis* BB-12 was best supported by XOS at 2% concentration. SOS and GOS at 2% concentration had relatively lower supporting growth effect on this strain than that of XOS. The prebiotics, except LAC and INU, at 2% concentration had higher supporting effect on the growth of this strain than that of 2% glucose.

In general, as the concentration of the prebiotics increases, the positive effect of the prebiotics on the acidifying activity of *B. bifidum* ATCC 15696 and *B. animalis* subsp. *lactis* BB-12 increases. The pH values of the culture media with prebiotics varied between 4.2 and 5.6 for *B. bifidum* ATCC 15696 and 4.0 and 5.1 for *B. animalis* subsp. *lactis* BB-12. The highest acidifying activity of *B. bifidum* ATCC 15696 was obtained with SOS at 2% concentration, followed by GOS and positive control. The highest acidifying activity of *B. animalis* subsp. *lactis* BB-12 was obtained with SOS at 2% concentration, followed by GOS, XOS and positive control with very similar acidity values. XOS and INU had the lowest effect on the acidifying activity of *B. bifidum* ATCC 15696. Also the increases in the viable cell number of this strain were lower with these prebiotics than those of other prebiotics. LAC had the lowest effect on the acidifying activity of *B. animalis* subsp. *lactis* BB-12 compared to the other prebiotics.

The results of this study indicated that type and concentration of prebiotics are important for the supporting effect of the prebiotics on the growth performance and acidifying activity of the probiotic bacterial strains. The results of this study about the supporting effect of the prebiotics on the growth performance of the probiotic bacterial strains are in good agreement with the results of several studies (13, 17, 18, 21, 26). In general, as the concentration of the prebiotics increases, positive effect of the prebiotics on the acidifying activity of the probiotic strains increases. Relatively higher acidifying activities were observed as the viable cell numbers of the probiotic strains increased. The difference in the strains of *Lactobacillus acidophilus* was not important in terms of both growth performance and acidifying

activity. However, species difference in the genus *Bifidobacterium* was found to be significant ($P<0.05$). The results of various studies also showed that ability of the probiotic bacteria to utilize prebiotics could be strain and/or substrate specific (13, 17-21, 24, 26).

In conclusion, an appropriate prebiotic substance should be selected for each probiotic bacterial strain for its viability and good growth and acidifying performance before the production of functional foods containing a combination of prebiotics and probiotics as synbiotic.

Acknowledgements

The authors would like to thank The Research Fund of Hacettepe University, Ankara, Turkey (Project Number: 0601602009). We are also grateful to Müge Kuyrukluyıldız in Peyma-Chr. Hansen's Cheese Enzyme Co. Inc., İstanbul, Turkey for supplying the Chr. Hansen's commercial probiotic bacterial cultures.

REFERENCES

1. Gibson GR, Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutri*, 125: 1401–1412.

2. Ziemer CJ, Gibson GR. 1998. An Overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *Int Dairy J*, 8: 473-479.

3. Holzaphel WH, Schillinger U. 2002. Introduction to pre- and probiotics. *Food Res Int*, 35: 109-116.

4. Tuohy KM, Rouzaud GCM, Brück WM, Gibson GR. 2005. Modulation of the human gut microflora towards improved health using prebiotics-Assessment of efficacy. *Curr Pharm Des*, 11: 75-90.

5. Fuller R. 1989. Probiotics in man and animals. *J Appl Bacteriol*, 66: 365-378.

6. Fuller R. 1992. History and development of probiotics. In *Probiotics: the Scientific Basis*, Fuller R. (ed), Chapman and Hall, London; pp.1-7.

7. Collins MD, Gibson GR. 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *American J Clin Nutr*, 69 (5); 1052–1057.

8. Shah NP. 2001. Functional foods from probiotics and prebiotics. *Food Technol*, 55 (11): 46-53.

9. Gibson GR, Probert HM, Loo VJ, Rastall RA, Roberfroid MB. 2004. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutr Res Rev*, 17: 259-275.

10. Fooks LJ, Fuller R, Gibson GR. 1999. Prebiotics, probiotics and human gut microbiology. *Int Dairy J*, 9: 53-61.

11. Gibson GR. 1998. Dietary modulation of the human gut microflora using prebiotics. *British J Nutr*, 80 (2): S209-S212

12. Gibson GR, Rastall RA, Roberfroid MB. 1999. Prebiotics. In *Colonic microbiota, nutrition and health*, Gibson GR, Roberfroid MB (ed.), Kluwer Academic Press. Dordrecht, The Netherlands; pp. 101-124.

13. Saminathan M, Sieo CC, Kalavathy R, Abdullah N, Ho YW. 2011. Effect of prebiotic oligosaccharides on growth of *Lactobacillus* strains used as a probiotic for chickens. *African J Microbiol Res*, 5 (1): 57-64.

14. Gismondo MR, Drago L, Lombardi A. 1999. Review of probiotics available to modify gastrointestinal flora. *Int J Antimicrob Ag*, 12: 287-292

15. Simmering R, Blaut M. 2001. Pro- and prebiotics - the tasty guardian angels? *Appl Microbiol Biotechnol*, 55 (1): 19-28.

16. Shortt C. 1999. The probiotic century: historical and current perspective. *Trends Food Sci Technol*, 10: 411-417.

17. Bielecka M, Biedrzycka E, Majkowska A. 2002. Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. *Food Res Int*, 35 (2-3): 125-131.

18. Goderska K, Nowak J, Czarnecki Z. 2008. Comparison of the growth of *Lactobacillus acidophilus* and *Bifidıbacterium bifidum* species in media supplemented with selected saccharides including prebiotics. *ACTA Sci Pol Technol Aliment*, 7 (2): 5-20.

19. Gopal PK, Sullivan PA, Smart JB. 2001. Utilisation of galacto-oligosaccharides as selective substrates for growth by lactic acid bacteria including *Bifidobacterium lactis* DR10 and *Lactobacillus rhamnosus* DR20. *Int Dairy J*, 11 (1-2): 19-25.

20. Hayakawa K, Mizutanit J, Wada K, Masai T, Yoshiharat I, Misuoka T. 1990. Effects of soybean oligosaccharides on human faecal microflora. *Microb Ecol Health Dis*, 3: 293–303.

21. Kneifel W, Rajal A, Kulbe KD. 2000. In vitro growth behavior of probiotic bacteria in culture media with carbohydrates of prebiotic importance. *Microb Ecol Health Dis*, 12: 27-34.

22. Mart nez-Villaluenga., Fr as J, Gómez R, Vidal-Valverde C. 2006. Influence of addition of raffinose family oligoaccharides on probiotic survival in fermented milk during refrigerated storage. *Int Dairy J*, 16 (7): 768-774.

23. Perin S, Warchol M, Gril JP, Schneider F. 2001. Fermentations of fructo oligosaccharides and their components by *Bifidobacterium infantis* ATCC 15697 on batch culture in semi-synthetic medium. *J Appl Microbiol*, 90: 859-865.

24. Saarela M, Hallamaa K, Mattila-Sandholm T, Mättö J. 2003. The effect of lactose derivatives lactulose, lactitol and lactobionic acid on the functional and technological properties of potentially probiotic *Lactobacillus* strains. *Int Dairy J*, 13 (4): 291-302.

25. Saito Y, Takano T, Rowland I. 1992. Effects of soybean oligosaccharides on the human gut microflora in in vitro culture. *Microb Ecol Health Dis*, 5: 105-110.

26. Pennacchia C, Vaughan EE, Villani F. 2006. Potential probiotic *Lactobacillus* strains from fermented sausages: Further investigations on their probiotic properties. *Meat Sci*, 73 (1): 90-101.