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Original paper

Effects of prebiotics on acid and bile resistance of *Bifidobacterium lactis* and *Lactobacillus acidophilus* probiotic bacteria

AYLA MUMCU*[,1], AYHAN TEMİZ[2]

[1]Olive Research Institute, Izmir, Turkey
[2]Hacettepe University, Faculty of Engineering, Department of Food Engineering, Ankara, Turkey

Abstract

The effects of prebiotics on acid and bile resistance of probiotics were evaluated. Two commercial strains, *Bifidobacterium lactis* BB-12 and *Lactobacillus acidophilus* LA-5 had higher acid and bile resistance characteristics than the ATCC strains. *B. lactis* BB-12 was the most resistant to acid and bile and showed higher survival capacity. The counts of *B. lactis* BB-12 viable cells in pH 2 medium decreased by 2.7 log unit while the viability of other strains decreased by 4.5-7 log units. The counts of *B. lactis* BB-12 viable cells in the bile medium after 6 and 24 hours of incubation were determined as 6.1 and 2.8 log CFU/ml, respectively, while no viable bacteria could be determined for the other strains after 24 hours of incubation. The acid and bile resistance of probiotic bacteria remarkably varied with the type of prebiotics. Acid and bile resistance properties of *L. acidophilus* LA-5 and *L. acidophilus* ATCC 4356 were found higher when INU was present in the growth medium. Acid and bile resistance properties of *B. bifidum* ATCC 15969 was higher when the medium contained GOS whereas acid and bile resistance of *B. lactis* BB-12 was found to be higher when medium contained XOS.

Keywords Probiotic, prebiotic, acid resistance, bile resistance

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*Corresponding author: Ayla Mumcu, ayla.mumcu@tarimorman.gov.tr

Introduction

The effects of probiotics and prebiotics on human health are of great interest to both consumers and food manufacturers and always up to date. Probiotics, derived from the Greek words meaning "for life", are living microorganisms which actively enhance health of consumers by improving the balance of microbiota in the gut when ingested in sufficient numbers (FULLER [1]). 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 of diarrhea, reduction of serum cholesterol and lactose intolerance, enhancement of immune system, pre-digestion of proteins, improved absorption, enhancement of bowel motility and maintenance of mucosal integrity (COLLINS and GIBSON [2]; ZIEMER and GIBSON [3]; HOLZAPHEL and SCHILLINGER [4]). The most commonly used probiotics in many functional foods and nutritional supplements are *Bifidobacterium* and *Lactobacillus*, the members of the normal colonic bacterial flora. *Bifidobacterium longum*, *B. bifidum*, *B. breve*, *B. infantis*, *Lactobacillus plantarum*, *L. acidophilus*, *L. helveticus*, *L. rhamnosus*, *L. reuteri* and *L. casei* are the most widely studied probiotic strains and have been shown to exert a wide number of health benefits (GIBSON and ROBERFROID [5]; GIBSON [6]; GISMONDO & al [7]; SHAH [8]; HOLZAPHEL and SCHILLINGER [4]).

Probiotics in the normal intestinal microbiota need some special carbohydrates called "prebiotics" for their survival and growth. Prebiotics, such as fructo-oligosaccharides, gluco-oligosaccharides galacto-oligosaccharides, xylo-oligosaccharides, isomalto-oligosaccharides, gentio-oligosaccharides, lactulose, lactitol, lactosucrose, polydextrose, pyrodextrin, raffinose, resistant starch and inulin are non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of probiotic bacteria (GIBSON and ROBERFROID [5]; GIBSON [6]; ZIEMER and GIBSON [3]; GIBSON & al [9]; HOLZAPHEL and SCHILLINGER [14; TUOHY & al [10]; SAMINATHAN & al [11]). Prebiotics are short-chain carbohydrates that pass into the large intestine without being digested in the stomach and small intestine. They support the growth and activities of probiotic bacteria such as *Lactobacillus* spp. and *Bifidobacterium* spp. in the large intestine. The synergic combinations of probiotics and prebiotics in nutritional supplements and foods are called synbiotics.

The human gastrointestinal tract 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 microbiota in the gut (GIBSON and ROBERFROID [5]; ZIEMER and GIBSON [3]; HOLZAPHEL and SCHILLINGER [4]; TUOHY & al [10]). Prebiotic compounds are consumed by probiotics as a carbon or energy source in the colon. Increasing in the probiotics count in the gut helps the reduction of gut pathogens. The beneficial effects of probiotics in the gut depend on their viability and metabolic activity. To provide health benefits, probiotics must reach to the large intestine in sufficient numbers. It is recommended to consume about $10^6 - 10^9$ viable probiotic cells per day (LEE and SALMINEN [12]). Therefore, the concentration of probiotic bacteria in a functional food product is suggested to be 10^8 cfu (colony forming unit)/g or over (SHORTT [13]). For sustaining positive effects in humans, the probiotic carrier foods must contain a minimum viable microorganism count of at the time of consumption. However, during the processing, transportation, storage, and marketing of probiotic products, probiotics are harmfully affected by external adverse factors. Furthermore, to fulfill their crucial role, probiotics must survive in the acidic conditions of the stomach and be delivered to the intestines in high numbers. After stomach passage, the probiotic bacteria reach the intestinal tract and meet with bile which also reduce their survival. For this reason, it is extremely important to select the appropriate carrier foods and prebiotics that will increase the resistance of the probiotics and help them to pass adequately into the gastrointestinal tract. Effects of prebiotics on growth and acidifying activity of probiotic bacteria were determined as stated in our previous study (MUMCU and TEMIZ [14]). The results of our previous study indicated that an appropriate prebiotic substance should be selected for each probiotic bacterial strain for its good growth and acidifying performance. In general, as the concentration of the prebiotics increases, the growth and acidifying activity performance of the probiotic strains increases.

Considering the potential influence of prebiotics on the resistance of probiotics to the gastrointestinal conditions, the aim of this study was to investigate the effects of the certain prebiotics on the resistance and viability of probiotic bacteria *in vitro* acid and bile environment (as simulated gastrointestinal conditions).

Materials and methods

Probiotic cultures

Lactobacillus acidophilus ATCC 4356, *L. acidophilus* LA-5 (Chr. Hansen, Denmark), *Bifidobacterium bifidum* ATCC 15969 and *B. animalis* subsp. *lactis* BB-12 (*B. lactis* BB-12; Chr. Hansen, Denmark), purchased in lyophilized form, were used as probiotic test bacteria. *L. acidophilus* cultures were activated in MRS broth (de Man, Rogosa and Sharpe, Merck) at 37 °C for 24 hours. *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 hours.

Prebiotics

As prebiotics, commercial preparations of fructooligosaccharide (FOS; Dora/Orafti, Turkey), inulin (INU; Dora/Orafti, Turkey), galactooligosaccharide (GOS; Oligomate55, Yakult, Japan), soybean oligosaccharide (SOS; Calpis, Japan), and xylo-oligosaccharide (XOS; Suntory, Japan) were used. Stock solutions of 10% prebiotic substances were prepared in distilled water and filter-sterilized by using 0.45 µm pore size membrane filters (Millipore). Considering the results of our previous study (MUMCU and TEMIZ [14]), the prebiotics FOS and INU for *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5, GOS and SOS for *Bifidobacterium bifidum* ATCC 15969, XOS and SOS for *B. lactis* BB-12 were used in the trials at 2% final concentration.

Basic growth media

MRS broth and RCM without glucose were used as basic growth medium for the growth of *L. acidophilus* LA-5 and *B. lactis* BB-12, respectively.

Saline solution with pH 1, 2 and 3 (similar to gastric juice)

Saline solution (5 g/L NaCl) was adjusted to pH 1, 2 and 3 with 1 N HCl and then sterilized at 121 °C for 15 min.

Bile solution (similar to intestine)

Bile solution containing 0.5% bile (Ox bile, Merck) was prepared in distilled water and then sterilized at 121 °C for 15 min.

Effects of prebiotics to acid tolerance (*in vitro* assay)

For acid tolerance assay, the sterilized saline solutions with pH 1, 2 and 3 were used as gastric juice media. Firstly, the stock solution of the test prebiotic was transferred into the basic growth medium (MRS broth or RCM without glucose) with the final concentration of 2% (w/v). The basic growth medium was used as negative control and glucose (Merck) was used instead of the test prebiotic for comparison. Activated bacterial culture was added into the prebiotic containing media with the final concentration of 1% (v/v) and incubated at 37 °C for 24 h. After the incubation, bacterial cultures were centrifuged at 3000 rpm for 10 min and precipitates were suspended in 4 ml of sterile distilled water. Inoculations of 2% (v/v) were made from the obtained cell suspensions to saline solutions with pH values of 1, 2 and 3. The same amount of cell suspension was transferred directly to 0.5% NaCl solution (pH 5.35) for control. These inoculated solutions were incubated at 37 °C and viable cell counts were assessed after 0, 1/2, 1, 2 and 3 hours of incubation. The effects of prebiotics on the acid-resistance property of probiotic bacteria were evaluated based on the changes in viable cell number levels.

Effects of prebiotics to bile tolerance (*in vitro* assay)

For bile tolerance assay, the sterilized bile solution containing 0.5% bile (similar to intestine) was used. Firstly, the stock solution of the test prebiotic was transferred into the basic growth medium (MRS broth or RCM without glucose) with the final concentration of 2% (w/v).The basic growth medium was used as negative control and glucose (Merck) was used instead of the test prebiotic for comparison. Activated bacterial culture was added into the prebiotic containing media with the final concentration of 1% (v/v) and incubated at 37 °C for 24 h. After the incubation, the cultures were centrifuged at 3000 rpm for 10 min and precipitates were suspended in 4 ml of sterile distilled water. Inoculations of 2% (v/v) were made from the obtained cell suspensions to 25 ml of sterile bile solution. Same amount of cell suspension was transferred directly to 25 ml of sterile distilled water for control. These inoculated solutions were incubated at 37 °C and viable cell counts were assessed after 0, 3, 6 and 24 hours of incubation. The effects of prebiotics on the bile resistance property of probiotic bacteria were evaluated based on the changes in viable cell number levels in bile-containing media.

Statistical analyses

SPSS 15.0 statistical software program was used in the evaluation of the results. The differences in the treatments were established by using the analysis of variance (ANOVA) test at 5% significant level.

Results and Dicussions

Probiotic bacteria should survive during the passage through the stomach and the small intestine and reach enough amount to be effective in the gastrointestinal tract of the host. However, acid secretion in the stomach and bile

secretion in the intestinal tract could significantly affect the probiotic bacterial growth, survival and activities in the gut. On the other hand, it is known that prebiotics can support the growth and activities of probiotic bacteria in the gut. The results of our previous study (MUMCU and TEMIZ [14]) indicated that an appropriate prebiotic substance should be selected for each probiotic bacteria for its maximum growth and acidifying activity. In general, as the concentration of the prebiotics increases, the growth and acidifying activity of the probiotic strains also increases. *Lactobacillus acidophilus* ATCC 4356 and *L. acidophilus* LA-5 exhibited the best growth with FOS and INU, *Bifidobacterium bifidum* ATCC 15696 with GOS and SOS, *B. lactis* BB-12 with XOS and SOS. Considering these results, prebiotics FOS and INU for *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5, GOS and SOS for *Bifidobacterium bifidum* ATCC 15969, XOS and SOS for *B. lactis* BB-12 were used at the highest concentration (2%) which stimulated the growth of probiotics at maximum level in the trials of the present study. The survival capability of tested bacteria strains varied in terms of prebiotics present in the growth medium. HERNANDEZ & al [15] reported that resistance to gastrointestinal conditions in *Lactobacillus* strains is dependent on the type of carbon source and strain.

Effects of the prebiotics on acid resistance of the probiotic bacteria

The effects of FOS and INU on the acid resistance of *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5 were evaluated according to the changes in the viable bacterial cell numbers during 3 hours of incubation at pH 1, pH 2 and pH 3 (Figure 1 and Figure 2). Both *L. acidophilus* strains were found to be sensitive to pH 1 medium. No viable bacterial cell was determined at pH 1 after 30 min of incubation. Since the results obtained by pH 3 trials were very close to each other, the effects of FOS and INU on the acid resistance property of both *L. acidophilus* strains were evaluated according to the results obtained at pH 2 trials. As it can be seen in Figure 1 and 2, there were important decreases in the levels of viable bacterial cell especially after the first hour of incubation at pH 2 medium. Both *L. acidophilus* strains showed more acid-resistance when INU was included in the growth medium. The initial viable bacterial cell levels of both *L. acidophilus* strains were approximately 7 log CFU/ml. The initial viable cell numbers of *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5 decreased to 4 log and 4.9 log CFU/ml with INU, 2.9 log and 3.5 log CFU/ml with FOS, and 2.4 log and 2.3 log CFU/ml with GLU, respectively after 3 hours of incubation at pH 2. Compared to GLU, it was observed that FOS also increased the acid resistance property of these bacterial strains. Although *L. acidophilus* ATCC 4356 displayed the best growth with FOS (MUMCU and TEMIZ [14]), it is noteworthy that INU caused more acid resistance in this bacterial strain than FOS. It was found that the differences between the prebiotic substances and the incubation period were significant in terms of the acid resistance of both *L. acidophilus* strains ($p<0.05$). According to the results of KOCER and UNAL's study [16], it was also found that supplementation with INU may increase the viability of *L. acidophilus* La-5 during the simulation of GI conditions. At pH 3, the decreasing in the level of live bacteria was much less than in pH 2. The changes in the bacterial levels of *L. acidophilus* strains, grown in environments with INU and FOS, during the incubation period in pH 3 were found to be statistically insignificant ($p<0.05$), the changes were very low decrease and almost close to the control. When the results are evaluated in general, it is possible to say that the acid resistance property of *L. acidophilus* LA-5 is higher than that of *L. acidophilus* ATCC 4356.

The effects of GOS and SOS on the acid resistance property of *B. bifidum* ATCC 15696, and XOS and SOS on the acid resistance property of *B. lactis* BB-12 were evaluated

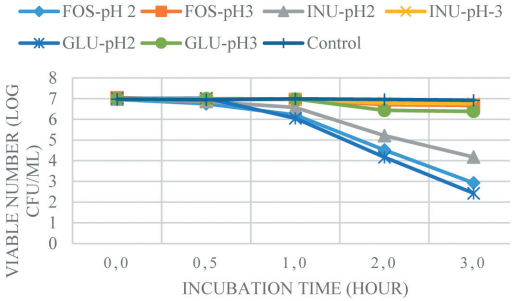

Figure 1. Effects of prebiotics on acid resistance of *L. acidophilus* ATCC 4356 (viable cell count as log CFU/mL at pH 1, 2 and 3)

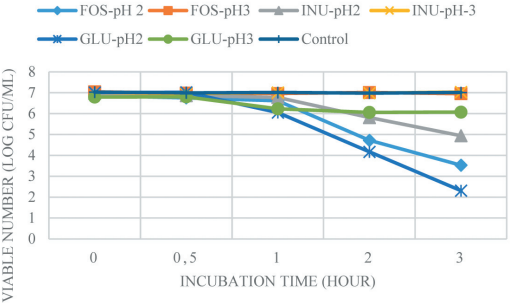

Figure 2. Effects of prebiotics on acid resistance of *L. acidophilus* LA-5 (viable cell count as log CFU/mL at pH 1, 2 and 3)

according to the changes in the viable bacterial cell numbers during 3 h of incubation at pH 1, pH 2 and pH 3 (Figure 3 and Figure 4). Both *Bifidobacterium* spp. strains were found to be sensitive to pH 1 medium, as similarly *L. acidophilus* strains. No viable bacterial cell was determined at pH 1 after 30 min of incubation. *B. bifidum* ATCC 15696 strain showed much more acid sensitivity than *B. lactis* BB-12. No viable *B. bifidum* ATCC 15696 cell was determined at pH 2 after the first hour of incubation (Figure 3). In addition, the viable cell count results of *B. bifidum* ATCC 15696 obtained by pH 2 trials were very close to each other and the difference was statistically insignificant. For this reason, the effects of GOS and SOS on the acid resistance properties of *B. bifidum* ATCC 15696 were compared according to the results obtained by pH 3 trials. It was found that the differences between the prebiotic substances and the incubation period were significant in terms of the the acid resistance of *B. bifidum* ATCC 15696 strain ($p<0.05$). The initial viable cell level (approximatelly 7 log (CFU/ml) of *B. bifidum* ATCC 15696 decreased to 5.1, 3.8 and 4.5 log (CFU/ml) with GOS, SOS and GLU, respectively after 3 hours of incubation at pH 3. The acid resistance property of *B. bifidum*

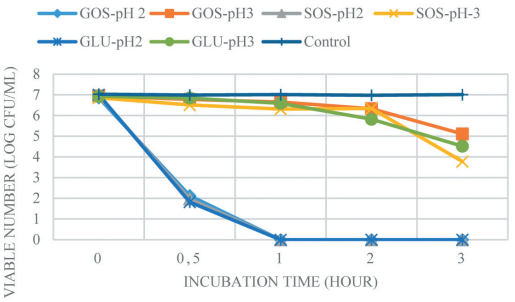

Figure 3. Effects of prebiotics on acid resistance of *B.bifidum* ATCC 15696 (viable cell count as log CFU/mL at pH 1, 2 and 3)

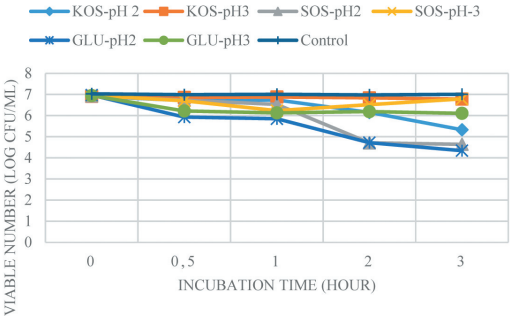

Figure 4. Effects of prebiotics on acid resistance of *B.animalis* subsp. *lactis* BB-12 (viable cell count as log CFU/mL at pH 1, 2 and 3)

ATCC 15696 was higher at pH 3 when GOS was included in the growth medium. The fact that the number of viable bacteria could not be determined at the end of 30 minutes of incubation in pH 1 and after the first hour of incubation in pH 2, it can be said that *B. bifidum* ATCC 15696 was highly sensitive to pH 1 and 2 and the test prebiotics could not be effective on the acid resistance property of this strain at those pH values.

Contrary to the results from *B. bifidum* ATCC 15696 trials, the viable cell count results of *B. lactis* BB-12 obtained by pH 3 trials were very close to each other and the difference was statistically insignificant. For this reason, the effects of XOS and SOS on the acid resistance properties of *B. lactis* BB-12 were compared according to the results obtained by pH 2 trials. It was found that the differences between the prebiotic substances and the incubation period were significant in terms of the the acid resistance of *B. animalis* subsp. *lactis* BB-12 strain ($p<0.05$). The initial viable cell level (approximatelly 7 log (CFU/ml) of this strain decreased to 5.3, 4.5 and 4.3 log (CFU/ml) with XOS, SOS and GLU, respectively after 3 hours of incubation at pH 2. XOS was more effective on the acid resistance property of this strain than SOS. The common prebiotic examined for both *Bifidobacterium* spp. strains was SOS. According to the results obtained by SOS trials, it can be said that *B. lactis* BB-12 is more acid resistant strain than *B. bifidum* ATCC 15696.

Effects of the prebiotics on bile resistance of the probiotic bacteria

The effects of FOS and INU on the bile resistance property of *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5 were evaluated according to the changes in the viable bacterial cell numbers during 24 hours of incubation at 0.5% bile solution (Figure 5 and Figure 6). The initial viable bacterial cell levels of both *L. acidophilus* strains were approximately 7 log CFU/ml. The effects of the test prebiotics on the bile resistance property of both *L. acidophilus* strains can be easily observed with the results obtained at the third hour of incubation. The viable cell counts of *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5 detected at the third hour of incubation were 6.3 and 6.9 log CFU/ml with INU, 5.5 and 5.5 log CFU/ml with FOS, and 4.7 and 5.3 log CFU/ml with GLU containing media, respectively. At the sixth hour of the incubation, the viable cell levels of *L. acidophilus* ATCC 4356 were found to be very close to each other, and at the end of the 24-hour incubation, the viable cells could not be determined in any of the samples. In contrast, the viable cell levels of *L. acidophilus* LA-5 at the third and sixth hour of the incubation was significantly higher when

the growth medium was included INU. It was found that the differences between the prebiotic substances and the incubation period were significant in terms of the the bile resistance of *L. acidophilus* strains ($p<0.05$). In the control samples, sharp decreases in the number of viable bacteria were observed in the transition from the sixth hour of incubation to the 24-hour incubation of both test strains. The viable cell level of *L. acidophilus* LA-5 was 4.1 log CFU/ml at the end of the 24-hour incubation period in the medium containing INU whereas no viable *L. acidophilus* ATCC 4356 cell was determined at the end of 24-hour incubation. According to these results, it is possible to say that bile resistance of *L. acidophilus* LA-5 is much more higher than *L. acidophilus* ATCC 4356. The prebiotic INU increased the bile resistance properties of both *L. acidophilus* strains. However, INU had a significantly more positive effect on the bile resistance of *L. acidophilus* LA-5 during the incubation period compared to *L. acidophilus* ATCC 4356.

The prebiotics GOS and SOS were used for the evaluation of the bile resistance of *B. bifidum* ATCC 15696, whereas XOS and SOS for *B. lactis* BB-12 (Figure 7 and Figure 8). *B. bifidum* ATCC 15696 exhibited the highest bile resistance property when the growth medium containing GOS. It was found that the differences between prebiotic substances and the incubation period were important in terms of their effects on the bile resistance ($p<0.05$). *B. bifidum* ATCC 15696 was found to be seriously sensitive to 0.5% bile culture medium. No viable bacterial cell was detected at the third hour of incubation of the bile culture medium containing SOS, and at the sixth hour of incubation when GOS and GLU were included in the bile culture media. After three hours of the incubation, the viable cell counts of *B. bifidum* ATCC 15696 decreased to 4.2 and 3.1 log CFU/ml with GOS and GLU containing media, respectively. At the end of the 24 hour incubation, the presence of viable cells could not be detected in the control sample either. *B. lactis* BB-12 was found to be greatly resistance to 0.5% bile culture medium. In all prebiotic trials, there was very little reduction in the number of viable bacteria during the incubation period. After 24 hours of incubation in bile solutions, viable bacterial cell levels of *B. lactis* BB-12 were determined as 4.8, 4.1 and 2.8 log CFU/ml with XOS, SOS and GLU containing bile culture media, respectively. When the results obtained are evaluated comparatively, it is possible to say that the bile resistance of *B. lactis* BB-12 is much higher than that of *B. bifidum* ATCC 15696. While the prebiotic XOS significantly increased the bile resistance of the *B. lactis* BB-12 strain during the 24-hour incubation period, GOS was able to increase the bile resistance of *B. bifidum* ATCC 15696 strain only to a certain level until the third hour of incubation (Figure 7 and Figure 8).

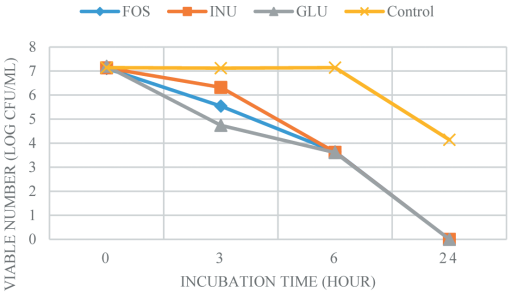

Figure 5. Effects of prebiotics on bile resistance of *L. acidophilus* ATCC 4356 (viable cell count as log CFU/mL)

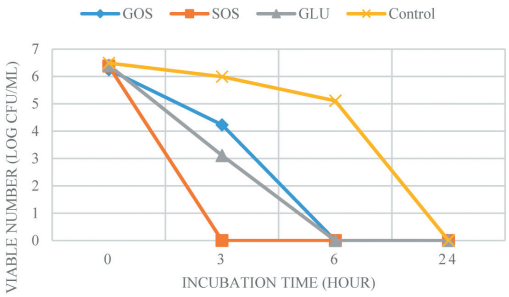

Figure 7. Effects of prebiotics on bile resistance of *B.bifidum* ATCC 15696 (viable cell count as log CFU/mL)

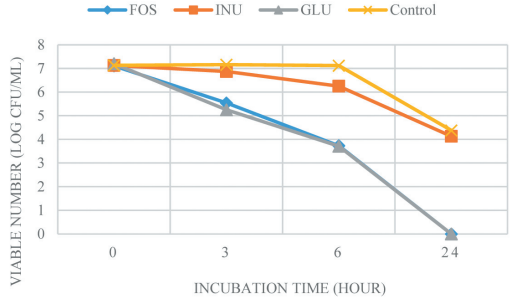

Figure 6. Effects of prebiotics on bile resistance of *L. acidophilus* LA-5 (viable cell count as log CFU/mL)

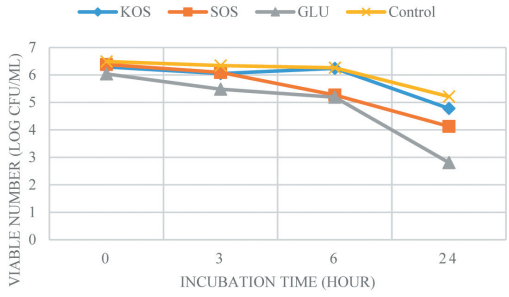

Figure 8. Effects of prebiotics on bile resistance of *B.animalis* subsp. *lactis* BB-12 (viable cell count as log CFU/mL)

The overall results of the present study indicated that commercial probiotic strains, *L. acidophilus* LA-5 and *B. lactis* BB-12 exhibited higher acid and bile resistance characteristics than the other two test probiotic strains. It is possible to compare the acid and bile resistance property of the tested probiotic bacterial strains by looking at the viable cell count results of the strains grown in the medium containing GLU (Figure 1-8). In this sense, it was found that *B. lactis* BB-12 was the most resistance strain to acid and bile. *B. lactis* BB-12 showed higher survival rates compared to other tested bacteria strains. While the viability of the other probiotic strains in the acid medium decreased 4.5-7 log units, the viable bacterial cell level of *B. lactis* BB-12 decreased around 2.7 log unit only. After 24 hours of incubation, the viable cell level of *B. lactis* BB-12 in the bile medium was determined as 2.8 log CFU/ml, whereas the presence of viable cells could not be determined in the other probiotic strains. Although *B. lactis* BB-12 showed more resistance to acid and bile than the other strains, in fact a noteworthy decrease in the viable cell level occurred. KOCER and UNAL [16] studied the effects of inulin, polydextrose and resistant starch (Hi-maize) on viability of *L. acidophilus* La-5, *B. animalis* subsp. *lactis* BB-12, and *Streptococcus thermophilus* under simulated gastrointestinal conditions. They found that *B. animalis* subsp. *lactis* BB--12 presented higher survival rates under gastrointestinal stress than *L. acidophilus* La-5. The higher survivability of *B. animalis* subsp. *lactis* BB-12 compared to that of *L. acidophilus* La-5 during *in vitro* simulated gastrointestinal conditions has also been reported by some other researchers (BEDANI & al [17]; BEDANI & al [18]; CASAROTTI & al [19]; CASAROTTI and PENNA [20]). CRITTENDEN & al [21] demonstrated that B. *animalis* subsp. *lactis* BB-12 was both acid and protease tolerant among commercial strains. In a study of AMBALAM & al [22], the high resistance property of *B. animalis* subsp. *lactis* strains to the bile environment was also verified. VERNAZZA & al [23] found that most bifidobacteria were poorly resistant to strongly acidic conditions with the exception of *B. lactis* BB-12. It was stated that bile tolerances of five *Bifidobacterium* strains were widely variable. *B. lactis* BB-12 and *B. infantis* 20088 were able to grow in the bile-containing medium as demonstrated by the high viable counts. MADUREIRA & al [24] pointed out that resistance property of a *B. animalis* strain to the simulated gastrointestinal conditions (acid and bile environment) was higher than those of *L. casei* and *L. acidophilus* strains.

Another essential result in the present study was the positive effects of the tested prebiotics on improving acid and bile resistance properties of the tested probiotic bacteria. Certain prebiotics added to the growth media enhanced the acid and bile resistance properties of the test probiotic strains. Acid and bile resistant characteristics of *L. acidophilus* ATCC 4356 and *L. acidophilus* LA-5 strains were higher when INU was included in the growth medium. Acid and bile resistance characteristics of *B. bifidum* ATCC 15969 and *B. lactis* BB-12 were higher with GOS and XOS, respectively. As a result, INU in the growth medium increased the acid and bile resistance properties of LA-5 to a higher level whereas acid and bile resistance properties of B. *lactis* BB-12 were further enhanced when XOS was included in the growth medium. These results emphasize the importance of prebiotic selections to be added to the growth medium for increasing the acid and bile resistance of the probiotic strains.

The effect of the prebiotic preparations on the resistance property of the probiotic strains in the simulated gastrointestinal conditions has been investigated by some researchers. KOCER and UNAL [24] showed that the acid and bile resistance property of *L. acidophilus* La-5 was improved by INU among three prebiotics, while the use of Hi-maize resistant starch improved the acid and bile resistance property of *B. animalis* BB-12. Nevertheless, there are also some studies reported that the prebiotics, such as INU, had no effect on the survival of some commercial probiotics under gastrointestinal conditions. The study of BEDANI & al [17] showed that the addition of inulin and/or okara flour (a byproduct of the soymilk industry) into the fermented soy product (FSP) matrix did not affect the survival of *L. acidophilus* La-5 and *B. animalis* BB-12 under *in vitro* simulated gastrointestinal conditions. VERNAZZA & al [23] found that GOS and IMO (isomaltooligosaccharide) among the other substrates were generally well utilized by the tested bifidobacteria strains. It was demonstrated that the prebiotic GOS gave higher growth rates than XOS and was fermented by all of the tested bifidobacteria.

On the other hand, it was stated that probiotic survival in the simulated gastrointestinal conditions was dependent upon the type of matrix (CASAROTTI & al [19]). CASAROTTI & al [19] used MRS, milk and milk supplemented with INU as the matrix and demonstrated that milk and INU protected the probiotic strains from the deleterious conditions of the gastrointestinal conditions. These results suggest that it is critical to formulate the food matrix to be used as probiotic carrier. To exert its beneficial effects on the host, probiotic bacteria should be viable in the product upon consumption (SHAH [8]). Like prebiotics, the components of the food can have a protective effect on probiotics in the stomach and bile environment. WANG & al [25] demonstrated that the delivery of *L. casei* Zhang through fermented soymilk and bovine milk significantly enhanced the viability

of the strain in simulated gastric transit when compared to the pure culture suspended in sterile saline solution. For this reason, it can be said that the use of foods as a carrier in the intake of probiotics would be more appropriate. In addition, it was shown that the fermented milk with the fruit flours (apple, banana and grape) improved *L. acidophilus* tolerance to the simulated gastrointestinal conditions, specifically at days 14 and 28 of storage. Only banana flour had a protective effect on *B. animalis* subsp. *lactis* after 28 days of storage (CASAROTTI and PENNA [20]). The results of the present study indicate that the type of prebiotic exhibit a selective influence on the acid and bile resistance properties of probiotics under simulated gastrointestinal conditions.

Conclusions

The beneficial effects of probiotics depend on their viability and metabolic activity in the gastrointestinal environment. To provide health benefits, probiotics must reach to the large intestine in sufficient viable numbers. The resistance to low pH and bile salts are important for the growth and survival of probiotics in the gastrointestinal tract, thus the appropriate carrier foods and prebiotics should be selected for assuring their adequate passing into the gut. The *in vitro* analysis used in this study provided information about the survival rate of probiotic bacteria under gastrointestinal conditions and the effect of test prebiotics on improving the acid and bile resistance of the test probiotics. There were important differences in the resistance characteristics of probiotics to gastrointestinal conditions. As a result of this study, it is possible to say that *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 were the most resistant strains against acid and bile exposure than the other strains, indicating that these strains would have an increased chance of reaching to the large intestine in sufficient viable numbers. Prebiotics increased the resistance properties of probiotic organisms to gastrointestinal conditions. However, the ability of prebiotics to increase the acid and bile resistance properties of probiotics could vary depending on the prebiotic type in the growth environment. The results of this study indicated that the selection of appropriate probiotics and their appropriate prebiotics to be added to functional products is very important. The results suggest that it is critical to formulate the food matrix to be used as probiotic and prebiotic carrier. Therefore, choosing an appropriate prebiotic and the supplementation with prebiotics for the manufacture of functional products can contribute to maintain the viability of probiotic bacteria during the shelf-life of the product and in the gut. On the other hand, the *in vivo* conditions are difficult to simulate in the laboratory and the conditions of stomach and bile in the gastrointestinal system may vary by individual hosts. Thus, *in vivo* studies should be performed to confirm the *in vitro* studies.

References

1. R. FULLER. History and development of probiotics. *In Probiotics: the Scientific Basis*, Fuller R. (ed), Chapman and Hall, London; pp.1-7 (1992).
2. M.D. COLLINS, G.R. GIBSON. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *American J Clin Nutr*.; 69 (5): 1052–1057 (1999).
3. C.J. ZIEMER, G.R. GIBSON. An Overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *International Dairy Journal*; 8: 473-479 (1998).
4. W.H. HOLZAPHEL, U. SCHILLINGER. Introduction to pre- and probiotics. *Food Research International*; 35: 109-116 (2002).
5. G.R. GIBSON, M.B. ROBERFROID. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutri*.; 125: 1401–1412 (1995).
6. G. R. GIBSON. Dietary modulation of the human gut microflora using prebiotics. *British J Nutr*.; 80 (2): S209-S212 (1998).
7. M.R. GISMONDO, L. DRAGO, A. LOMBARDI. Review of probiotics available to modify gastrointestinal flora. *Int J Antimicrob Ag*.; 12: 287-292 (1999).
8. N.P. SHAH. Functional foods from probiotics and prebiotics. *Food Technol*; 55 (11): 46-53 (2001).
9. G.R. GIBSON, R.A. RASTALL, M.B. ROBERFROID. *Prebiotics. In Colonic microbiota, nutrition and health*, Gibson GR, Roberfroid MB (ed.), Kluwer Academic Press. Dordrecht, The Netherlands, pp. 101-124 (1999).
10. K.M. TUOHY, G.C.M. ROUZAUD, W.M. BRÜCK, G.R. GIBSON. Modulation of the human gut microflora towards improved health using prebioticsAssessment of efficacy. *Curr Pharm Des*: 11: 75-90 (2005).
11. M. SAMINATHAN, C.C. SIEO, R. KALAVATHY, N. ABDULLAH, Y.W. HO. Effect of prebiotic oligosaccharides on growth of Lactobacillus strains used as a probiotic for chickens. *African J Microbiol Res*; 5 (1): 57-64 (2011).
12. Y. LEE, S. SALMINEN. The coming of age of probiotics. *Trends in Food Science & Technology*; 6 (7): 241-245 (1995).
13. C. SHORTT. The probiotic century: historical and current perspective. *Trends Food Sci Technol*; 10: 411-417 (1999).

14. A.S. MUMCU, A. TEMIZ. Effects of prebiotics on growth and acidifying activity of probiotic bacteria. *Gıda*; 39 (2): 71-77 (2014).
15. O. HERNANDEZ-HERNANDEZ, A. MUTHAIYAN, F.J. MORENO, A. MONTILLA, M.L. SANZ, S.C. RICKE. Effect of prebiotic carbohydrates on the growth and tolerance of *Lactobacillus*. *Food Microbiology*; 30 (2): 355-361 (2012).
16. E. KOCER, G. UNAL. Effects of different prebiotics on viability under *in vitro* gastrointestinal conditions and sensory properties of fermented milk. *Italian Journal of Food Science*; 30: 568-582 (2018).
17. R. BEDANI, E.A. ROSSI, S.M.I. SAAD. Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions. *Food Microbiology*; 34: 382-389 (2013).
18. R. BEDANI, M.M.S. CAMPO, I.A. CASTRO, E.A. ROSSI, S.M.I. SAAD. Incorporation of soybean by-product okara and inulin in a probiotic soy yoghurt: texture profile and sensory acceptance. *Journal of the Science of Food and Agriculture*; 94 (1): 119-125 (2014).
19. S.N. CASAROTTI, S.D. TODOROV, A.L.B. PENNA. Effect of different matrices on probiotic resistance to *in vitro* simulated gastrointestinal conditions. *International Journal of Dairy Technology*; 68(4): 595-601 (2015).
20. S.N. CASAROTTI, A.L.B. PENNA. Acidification profile, probiotic in vitro gastrointestinal tolerance and viability in fermented milk with fruit flours. *International Dairy Journal*; 41: 1-6 (2015).
21. R.G. CRITTENDEN, L.F. MORRIS, M.L. HARVEY, L.T. TRAN, H.L. MITCHELL, M.J. PLAYNE. Selection of a *Bifidobacterium* strain to complement resistant starch in a symbiotic yoghurt. *Jornal of Applied Microbiology*; 90: 268-278 (2001).
22. P. AMBALAM, K.K. KONDEPUDI, I. NILSON, T. WADSTRÖM, A. LJUNGH. Bile enhances cell surface hydrophobicity and Biofilm Formation of Bifidobacteria. *Applied Biochemistry and Biotechnology*; 172: 1970-1981 (2014).
23. C.L. VERNAZZA, G.R. GIBSON, R.A. RASTALL. Carbohydrate preference, acid tolerance and bile tolerance in five strains of *Bifidobacterium*. *Journal of Applied Microbiology*,; 100: 846-853 (2006).
24. A.R. MADUREIRA, C.I. PEREIRA, K. TRUSZKOWSKA, A.M. GOMES, M.E. PINTADO, F.X. MALCATA. Survival of probiotic bacteria in a whey cheese vector submitted to environmental conditions prevailing in the gastrointestinal tract. *International Dairy Journal*; 15 (6-9): 921-927 (2005).
25. J. WANG, Z. GUO, Q. ZHANG, L. YAN, W. CHEN, X-M. LIU, H-P. ZHANG. Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage. *Journal of Dairy Science*; 92 (6): 2468-2476 (2009).