African Journal of Microbiology Research Vol. 5(32), pp. 5877-5881, 30 December, 2011
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2011 Academic Journals
DOI: 10.5897/AJMR11.801

Full Length Research Paper

The effects of *bifidobacterium lactis* and galactooligosaccharide (GOS) on ileum and distal colon motility: *In vitro* study

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Accepted 13 September, 2011

Twenty one male Wistar albino rats each weighing approximately 280 g were used in this study. Animals were divided into three groups. The first group (n = 7) consisted of sham controls, in the second (n = 7), rats were administrated 0.1 g/1 ml/galactooligosaccharide by by oral gavage for 4 weeks. In the third group (n = 7), rats were administrated 10^9 CFU/1ml/day *Bifidobacterium lactis* by oral gavage for 4 weeks. After 4 weeks, rats were sacrified; ileum and proximal colon segments were removed. The spontaneous contractions of ileum and proximal colon were evaluated by using organ bath. It has been detected that both prebiotics and probiotics increased intestinal motility. While probiotics have effects on both ileum and proximal colon, prebiotics seem to be effective in colon. All data are expressed as mean ± SEM (standard error of mean). Statistical comparisons between groups were performed using general linear models of analysis of variance (ANOVA) followed by the Turkey test.

Key words: *Bifidobacterium lactis,* galactooligosaccharide, ileum, rat, distal colon, *in vitro.*

INTRODUCTION

Epidemiological studies indicate that diet has a major impact on human health: a diet low in fat and high in fruit and vegetables has been correlated with a decreased incidence of so-called Western diseases such as coronary heart disease and colon cancer (Trock et al., 1990). Such a diet contains not only nutrients that are readily absorbed in the small intestine but also coponents that escape digestion by pancreatic and small bowel enzymes. The latter are the principal substrates of the bacteria resident in the human intestinal tract. Since a number of nutritional health effects are mediated by the intestinal microflora, diet is key in influencing their composition and activity. It has been increasingly recognized that the bacterial community in the intestine influences human health and well-being (Cummings and Macfarlane, 1997). Consequently, nutrition may be considered as a tool for influencing the intestinal microbiota in such a way that harmful bacteria are suppressed and beneficial bacteria are stimulated. Dietary strategies that serve to support health-promoting effects of the intestinal microflora include the ingestion of probiotics (Goldin, 1998) and or prebiotics (Gibson and Roberfroid, 1995), as well as a diet rich in fiber (Salminen et al., 1998).

Three approaches exist to increase the number of health-promoting organisms in the gastrointestinal tract. The first is the oral administration of live beneficial microorganisms. At present, these microorganisms, called probiotics, have been selected mostly from lactic acid bacteria and bifidobacteria that form a part of the

Abbreviations: GOS, galactooligosaccharide; **KBS,** Krebsbicarbonate solution; **CFU,** colony forming units; **NDO,** non-digestible oligosaccharides; **NaH_2PO_4,** sodium phosphate; **NaCl,** sodium chloride; **KCl,** potassium chloride; **$CaCl_2$,** calcium chloride; **$MgCl_2$,** magnesium chloride; **$NaHCO_3$,** sodium bicarbonate; **SEM,** standart error of mean; **ANOVA,** analysis of variance.

normal intestinal microflora of humans, these organisms are also indigenous to the colon. These bacteria have been suggested to be useful in the treatment of diarrhea (Rota virus, traveler's diarrhea, and *Clostridium difficile*), constipation, irritable bowel syndrome, and inflammatory bowel disease. They also have putative effects on enhancing the immune system and decreasing lactose intolerance (Bhutto and Morley, 2008).

The mechanisms of probiotic action appear to be multifactorial. Probiotic bacteria can promote fermentation processes that metabolize varying quantities of lactic, acetic, and formic acids; synthesis of vitamins; and the production of antimicrobial bacteriocidins and fatty acids (Bourlioux et al., 2002). Probiotics can also affect innate intestinal host defenses, including strengthening intestinal tight junctions, increasing mucous secretion, enhancing motility, and producing metabolic products (amino acids such as arginine and glutamine and short-chain fatty acids) that secondarily function as protective nutrients. They contribute to microflora diversity, thus helping to establish a normal commensal flora that protect against potential microbial pathogens (Neu and Caicedo, 2005).

The second strategy for increasing their number is to supply those already present in the intestine with selective carbon and energy source that provides them with competitive advantage over other bacteria in this ecosystem, thus selectively modifying the composition of the microflora using dietary supplements. These selective dietary components were named "prebiotics".

A prebiotic has been defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995). Prebiotics are intended to modify the intestinal microbiota in such a way that bacterial activities advantageous to the host are stimulated and bacterial activities adverse to host health are suppressed. The concept of prebiotics arose from the observation that inulin and fructooligosaccharides selectively stimulate the growth of bifidobacteria (Potter et al., 1993; Cummings, 1994) which are considered to be beneficial for human health (Gibson and Roberfroid, 1995). Although most research has been done on inulin and fructooligosaccharides, other non-digestible oligosaccharides (NDO) including xylooligosaccharides, galactooligosaccharides and isomalto oligosaccharides have also been tested for their prebiotic effect (Fuchs et al., 1999). The majority of candidate prebiotics are oligosaccharides but also include polysaccharides.To serve as a bacterial substrate in the colon, a prebiotic may not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract.

And the last approach is a mixture of probiotic and prebiotic "synbiotic" has recently been proposed to characterise health-enhancing food and supplements used as functional food ingredients in human (Kontula et al., 1998).

Like probiotics, the prebiotics belong to a more general class of "colonic foods", that is foods entering the colon and serving as substrates for the endogenous colonic bacteria, thus indirectly providing the host with energy, metabolihc substrate and essential micronutrients (Gibson and Roberfroid, 1995).

Although it has been shown in clinical studies that pre and probiotics have positive effects on gastrointestinal motility, *in vitro* effects of pre and probiotics are not clear.

In this study we aimed to investigate and compare the effects of pre and probiotics on gastrointestinal motility in different segments of gastrointestinal track.

MATERIALS AND METHODS

Animal preparation twenty one male Wistar albino rats each weighing approximately 280 g were used in this study. The study was approved by. Animals were divided into three groups. The first group (n = 7) consisted of sham controls in which rats were administrated 1% ml 0.9 NaCl/ day by oral gavage for 4 weeks. In the second group (n = 7), rats were administrated 0.1 g/1 ml/day galactooligosaccharide (GOS) used as a prebiotic by oral gavage for 4 weeks. In the third group (n = 7), rats were administrated 10^9 CFU/1ml/day *Bifidobacterium lactis* used as a probiotic by oral gavage for 4 weeks. *B. lactis* were grown from frozen stocks (-80 ^{o}C) prepared for ingestion and counted as in Kamiya at al. (2006). At the end of the four weeks, rats were killed by cervical dislocation. The abdomen was opened with a midline incision. Ileum and proximal colon was removed and placed in previously aerated (95% O_2 and 5% CO_2) Krebs-bicarbonate solution (composition in mmol/L: NaCl, 120; KCl, 4.6; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 22; NaH_2PO_4 and glucose 11.5). Whole full-thickness segments of ileum and proximal colon were placed in circular direction in a 10 mL tissue baths, filled with pre-aerated Krebsbicarbonate solution (KBS) at 37°C. The upper end of the preparation was tied to an isometric transducer (Grass FT 03, Quincy, MA, USA) and preloaded with 1 to 1.5 g. Tissues were allowed to equilibrate for 30 min.

In vitro muscle contractility studies

Muscle segments from each group were contracted with 80 mmol/L KCl to ensure that they worked properly at the beginning and end of each experiment.

At the beginning of each experiment, 80 mmol/L KCl was added to the organ bath, and the contraction was considered as reference response. Subsequently, the amplitude of spontaneous contractions of the isolated Ileum and proximal colon muscle segments were calculated as a percentage of the contraction induced by KCl (80 mmol/L) from both control, prebiotic and probiotic groups. Changes in the frequency (number or min.) of spontaneous contractions were expressed as the number of contractions for 10 min intervals. Isometric tensions were recorded on a Grass model 79 E polygraph. All experiments were performed in duplicate.

Data analysis

All data are expressed as mean ± SEM (standard error of mean). Statistical comparisons between groups were performed using general linear models of analysis of variance (ANOVA) followed by the Turkey test and P-values of less than 0.05 were considered to be statistically significant.

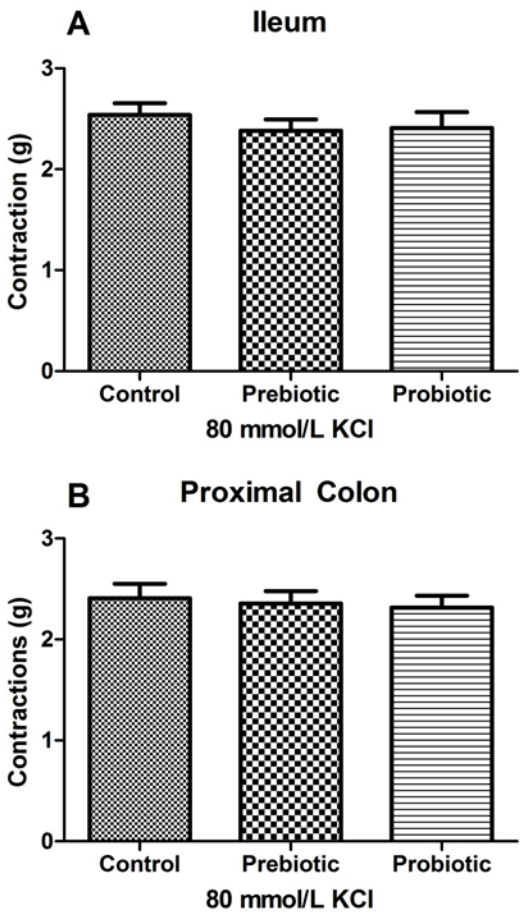

Figure 1. KCl (80 mmol/L) induced contractions of isolated ileum muscle segments in control, prebiotic and probiotic groups. No statistical difference was observed between groups (P > 0.05).

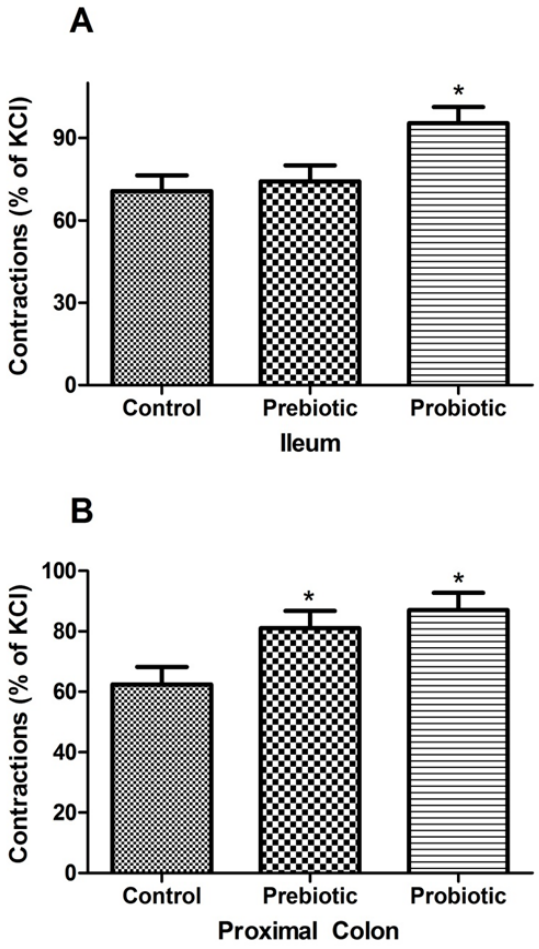

Figure 2. Changes in the spontaneous contraction amplitudes of the isolated smooth muscle segments. A. Ileum B. Proximal Colon.

RESULTS

Contractions induced by 80 mmol/L KCl were not significantly different between control, prebiotic and probiotic groups in isolated ileum smooth muscle segments which indicated that muscle segments from both groups worked properly (Figure 1).

In the smooth muscle segments from ileum, the mean amplitude of the spontaneous contractions was 70.6 ± 4.6 in the control group, 74.2 ± 5.2 in prebiotic group and 95.5 ± 7.1 in probiotic group, respectively. There was no significant difference between the amplitude responses of control and prebiotic groups ($p > 0.05$). But the amplitude of probiotic group was significantly higher than both control and prebiotic groups ($p < 0.05$) (Figure 2A).

In the smooth muscle segments from proximal colon, the mean amplitude of the spontaneous contractions was 62.4 ± 3.5 in the control group, 81.4 ± 4.4 in prebiotic group and 87.5 ± 5.2 in probiotic group, respectively. Both amplitude responses of spontaneous contractions of prebiotic and probiotic groups were significantly high when compared to the control group ($p < 0.05$). There was significant difference between prebiotic and probiotic groups ($p > 0.05$) (Figure 2B).

In the smooth muscle segments from ileum, the mean frequency of the spontaneous contractions was 27.2 ± 1.6 in the control group, 28.8 ± 2.2 in prebiotic group and 35.7 ± 4.1 in probiotic group, respectively. There was no significant difference between the frequency responses of control and prebiotic groups ($p > 0.05$). But the frequency of probiotic group was significantly higher than both control and prebiotic groups ($p < 0.05$) (Figure 3A).

In the smooth muscle segments from proximal colon, the mean frequency of the spontaneous contractions was 12.3 ± 1.5 in the control group, 11.1 ± 1.4 in prebiotic group and 17.5 ± 2.2 in probiotic group, respectively. Although there was no significant difference between the frequency responses of control and prebiotic groups ($p > 0.05$), spontaneous contraction amplitude responses of probiotic group was significantly high when compared to the control and prebiotic groups ($p < 0.05$) (Figure 3B).

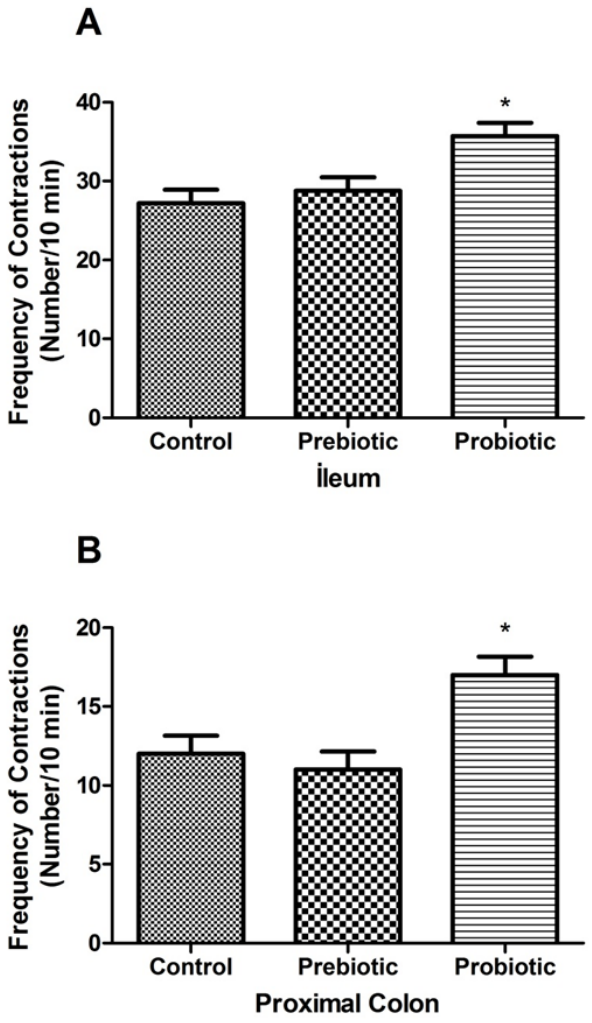

Figure 3. Changes in the spontaneous contraction frequency of the isolated smooth muscle segments. A. Ileum B. Proximal Colon.

DISCUSSION

The gut represents a complex and dynamic microbial ecosystem in which intestinal micro flora has an important and specific metabolic, trophic, and protective function. Normal gut structure and function are the end-point of a complex set of interactions between the host and microorganisms colonizing the gut (Guarner and Malagelada, 2003). Bacteria can be used to improve human health. A bacterium that provides specific health benefits when consumed as a food component or supplement would be called a probiotic. A consensus definition of the term was issued a few years ago and states that oral probiotics are living microorganisms that upon ingestion in specific numbers exert health benefits beyond those of inherent basic nutrition (Guarner and Schaafsma, 1998; Guarner et al., 2005). While probiotics are the live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989); prebiotics are defined as food ingredients that promote the growth or activity of a limited number of bacterial species for the benefit of host health (Gibson and Roberfroid, 1995). Organisms used as probiotics are most frequently of the *Lactobacillus* or *Bifidobacterium* species, and clinically beneficial effects of probiotics have been described in travellers' diarrhea, irritable bowel syndrome and inflammatory bowel disease (Walker and Buckley, 2006; Shanahan, 2007).

There are many conflicting studies about the effects of pro and prebiotics on gastrointestinal motility. While some of these studies suggest that pro and prebiotics increase intestinal motility, others suggest opposite. It has been shown that *Lactobacillus reuteri* ingestion consistently alters the motility of colon segments in an *ex vivo* organ bath recording setup. The effect is a decrease in the amplitudes of contractions at constant luminal filling preasure, and an increase in the threshold luminal pressure required to evoke rhythmic contractions (Wang et al., 2010). On the other hand, an *in vivo* study showed that administration of probiotics induces increased colonic propulsive contractions and defecation rate in pigs (Ohashi et al., 2001). It has been shown in a human clinical study that probiotic supplements may have a positive effect on bowel movements among orthopedic rehabilitation elderly patients (Zaharoni et al., 2011). In addition, Tabbers et al. (2009) suggested that *B. lactis* strain DN-173 010 is effective in increasing stool frequency after 3 weeks of product consumption in children with functional constipation and a defecation frequency less than 3 weeks.

There are little data available related to the influence of prebiotics on gastrointestinal motility in preterm infants. In a study in healthy preterm infants, Boehm et al. (2002) demonstrated that preterm infants fed with mother's milk had lower stool consistency and higher stool frequency than infants fed a preterm bovine milk formula. Supplementation of the same formula with a mixture of scGOS and lcFOS resulted in a reduction in stool consistency and an increase in stool frequency. More recently, Mihatsch et al. (2006) demonstrated a clinically relevant reduction in the gastrointestinal transit time in preterm infants fed a formula supplemented with these prebiotics.

In this study, consistent with these positive studies, we found that probiotics increased spontaneous contraction amplitude and frequency of both ileum and proximal colon. On the other side, while prebiotics increasing spontaneous contraction amplitudes of proximal colon, did not changed spontaneous contraction amplitude of ileum. Also prebiotics did change neither spontaneous contraction frequency of ileum nor spontaneous contraction frequency of proximal colon. The difference between the effect of pro- and prebiotics on ileum spontaneous contraction amplitude may be related to the difference in physiology and bacterial colonization between ileum and proximal colon. It is clear that a complex, resident gut microflora is present in human

subjects. While the transit of residual foodstuffs through the stomach and small intestine is probably too rapid for the microbiota to exert a significant impact, this slows markedly in the colon. Colonic micro-organisms have ample opportunity to degrade available substrates (Cherbut, 2003; Gibson et al., 2004; Flint et al., 2008). Due to the high residence time of colonic contents, as well as a diverse and profuse flora, the colonic microbiota plays a more important role in host health and well-being than is the case in the small intestine. As a result, it has been defined that both prebiotics and probiotics increased intestinal motility. While probiotics have effects on both ileum and proximal colon, prebiotics seem to be effective in colon. The difference possibly related to the microbial flora. It is well known that changes in gastrointestinal micro flora exhibit an intestinal motility response and that such change can be initiated by addition of synbiotics to the diet. According to these findings it seems that food supplemented with probiotic and prebiotics would prevent impared motility seen in lots of gastrointestinal diseases. Further work is necessary in order to identify the underlying mechanisms responsible for diet/bacterial induced changes in gastrointestinal motility.

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