# Impact of some Gums on the Growth and Activity of Bifidobacterium Animalis Subsp. Lactis

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***Abstract*—The present work was conducted to understand the effect of guar gum, gum arabic and tara gum on the growth and acid production of *Bifidobacterium animalis* subsp. *lactis*, the most common used probiotic, by using Tryptone Peptone Yeast Extract broth and reconstituted skim milk. The "growth sustaining ability" of gums was compared with glucose and a commercial inulin powder. Changes in pH and bacteria counts were monitored at the beginning and on 24$^{th}$ hour of incubation. Growth and acidifying activity of the *B. animalis* subsp. *lactis* were observed to be gum type-dependent, and was able to ferment guar gum, gum arabic and tara gum.**

***Index Terms*—gums, growth, *Bifidobacterium lactis***

## I. Introduction

Even though gums are natural polysaccharides, they differ from carbohydrates in chemical composition, carbon chain structure and physical characteristics depending on their origin. They contain cellulose, starches, sugars, calcium, magnesium, potassium and often nitrogen. Gums can be obtained from land/marine plants, microorganisms and animals. Guar gum is obtained from the endosperm of the seeds of the guar plant (*Cyamopsis tetragonolobus*), a member of family of Leguminosae. Tara gum, also known as Peruvian carob bean gum, is obtained by grinding the endosperm of the seeds of the South American tara tree (*Caesalpinia spinose*) which also belongs to the Leguminosae family. Gum Arabic or Acacia gum, is obtained from exudate of *Acacia senegal* and *A. seyal* trees [1], [2].

Since gums have key functional properties including viscosity, stability, suspension, gelation, nutritional and nutraceutical, they have been extensively used in food industry as stabilizers, thickeners and gelling agents as well as to enhance sensory properties. As most of gums are i) non-digestible and non-degradable, ii) capable of imbibing and holding large amounts of water through gel formation, iii) able to be broken down by microorganisms, iv) not digested by the human gut flora due to the lack of appropriate enzymes, v) considered as good source of nutrients for bacterial growth, they are thought to be potential prebiotics that selectively stimulate the growth of bifidogenic and lactic acid bacteria. In addition, the gums have been stated as effective on reduction of blood cholesterol and glucose levels aside with prevention of cardiovascular disease and diabetes [3]-[9].

"Probiotics" have been determined as live microorganisms, which, when administered in adequate amounts, beneficially affect the host by improving the intestinal microbial balance and its properties [10], [11]. Their major health benefits include i) managing lactose intolerance, ii) lowering cholesterol and blood pressure, iii) preventing of colon cancer, iv)improving immune function, v) inhibiting *Helicobacter pylori* and intestinal pathogens, vi) treatment and prevention of allergy. The minimum number of microorganisms to cause the mentioned beneficial health effects is $10^6$-$10^9$ viable cells per gram or milliliter at the moment of consumption. The most common probiotic bacteria associated with food products worldwide are *Lactobacillus* and *Bifidobacterium* species. Bifidobacteria are gram-positive, obligate anaerobe, non spore-forming bacilli and are the third most numerous bacteria of gut microbiota in humans and animals after the *Bacteriodes* and *Eubacterium* species. Bifidobacteria constitute up to 95% of all bacteria in the colons of breast-fed infants and only 10% of children and adults. *Bifidobacterium adolescentis, B. bifidum, B. breve, B. longum* subsp. *infantis* and *B. animalis* subsp. *lactis* are the main species of humans original. Bifidobacteria have gained a lot of attention because number of therapeutic effects including inhibition of pathogenic bacteria and tumor formation, synthesis of B vitamins, lowering of blood ammonia levels and cholesterol absorption [12]-[15].

Nowadays one of the ways of increase the number of beneficiary gut microbiota is the use of prebiotic ingredients. Non-digestible food and feed components that are not digested in the stomach and small intestine

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and selectively stimulate the growth and/or activity of favorable bacteria in the human gut have been described as "prebiotics". By definition, prebiotics must change the overall microbial balance in gut towards healthier microbiota by preventing the growth of gastrointestinal pathogens and stimulating the growth of the limited number of *Lactobacillus* and bifidogenic species present [11], [16]-[18]. Although almost every oligosaccharide and polysaccharide such as dietary fibre may have prebiotic activity, not all dietary carbohydrates are prebiotics. Therefore, to classify as a prebiotic, a component should be resistant to gastric acids, hydrolysis and enzymes of gastro intestinal tract; fermented by intestinal microbiota, selectively stimulate the growth and/or activity of beneficial gastrointestinal microbiota; and display beneficial health effects on the host as result of fermentation.

Most studied prebiotics to be able to generate the bifidogenic effect is necessary that the counting of cultures prebiotics in the colon be higher or equal to $10^6$ $CFUg^{-1}$ of fecal material are fructooligosaccharides (FOS, oligofructose, inulin), galactooligosaccharides (GOS), transgalactooligosaccharides (TOS), lactulose, isomaltooligosaccharides, and xylooligosaccharides, which are dietary carbohydrates. Recent trends about innovative or alternative sources of prebiotics is an area that has dominant featuring in the food industry, and a very promising market, not only to improve access to these ingredients, but also to enhance their usage and consumption by the population in general [19]-[22].

Although there are very few studies conducted on the use of gums to support cell growth and viability of probiotics, extensive studies are necessary to evaluate the use of gum as prebiotics functional ingredients both *in vitro* and *in vivo*. Therefore, the objective of this study was to investigate the effects of the guar gum, gum arabic and tara gum on the growth and pH reducing ability of *B. animalis* subsp. *lactis* by using Tryptone Peptone Yeast Extract (TPY) broth and in reconstituted skim-milk (RSM).

## II. Materials and Methods

### A. Substrates

Guar gum (Maysa Food & Ingredients; Istanbul, Turkey), gum arabic (As Food; İstanbul, Turkey) and tara gum (Tunckaya Chemicals; Istanbul, Turkey) were used at a level of 1% as final concentration. Inulin (Orafti®HSI) was supplied by BENEO-Orafti, Belgium.

### B. Bacteria and Culture Conditions

*Bifidobacterium animalis* subsp. *lactis* was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), was activated according to the method suggested by DSMZ, and incubated at 37℃ for 24 h under anaerobic conditions using an anaerobic atmosphere generation system (Anaerocult A, Merck, Darmstadt, Germany).

Tryptone Peptone Yeast Extract (TPY) was used as the basal medium (Table I). Basal media was prepared by dissolving all of the components in 1 L distilled water. Each batch of basal media with different gums was sterilized at 110℃ for 10 min. Sterilized samples were allowed to cool down to 40℃ and inoculated with *B. animalis* subsp. *lactis.* Skim-milk powder was reconstituted in distilled water at 10.70% (wt/v) to yield reconstituted skim milk (RSM) and was heated to 70℃. Guar gum, gum arabic and tara gum were gradually dissolved in the reconstituted skim milk. Milk samples were then pasteurized in a water bath at 65℃ for 30 min, cooled to 40℃ and inoculated with activated bacterial culture. The inoculated samples were incubated at 37°C for 24 h. Samples with glucose and inulin served as positive controls whereas the negative control was sample containing no carbohydrates.

TABLE I. Composition of TPY Broth

| **TPY** | **g/L** |
|---|---|
| Tryptone | 10.00 |
| Peptone | 5.00 |
| Yeast extract | 2.50 |
| Tween 80 | 1 |
| $K_2HPO_4.3H_2O$ | 2.00 |
| $MgCl_2$ | 0.50 |
| $ZnSO_4.7H_2O$ | 0.2 |
| $CaCl_2$ | 0.15 |
| $FeCl_3.6H_2O$ | 0.003 |
| L-cysteine HCl | 0.5 |

### C. Microbiological Analysis

Viable counts of *B. animalis* subsp. *lactis* in basal media and skim milk were assessed by plate count method using MRS agar (Oxoid Ltd., Basingstoke, Hampshire, UK) at the beginning (0) and 24$^{th}$ h of incubation. The plates were incubated at 37℃ for 72 h in jars under anaerobic conditions. Microbiological colonies from the plates containing 30-300 colony-forming units (cfu) were counted and transformed to $\log_{10}$ cfu/mL.

### D. pH Measurement

The pH of each sample was determined at the beginning and 24$^{th}$ h of incubation using a pH-meter (pH 315i / SET; WTW, Germany) after calibrating with fresh pH 4.0 and 7.0 standard buffers.

### E. Statistical Analysis

Statistical analysis of data was performed using the Minitab statistical package. To significant ($p < 0.01$) differences among substrates were determined by using ANOVA (analysis of variance).

## III. Results and Discussion

Changes in the pH of TPY broth and RSM containing guar gum, gum arabic and tara gum incubated at 37°C for 24 h are displayed in Fig. 1. The drop in pH was similar for all samples, showing significant differences in the pH of TPY broth and RSM containing different substrates ($p<0.01$). The rate of pH fall was observed as substrate-dependent.

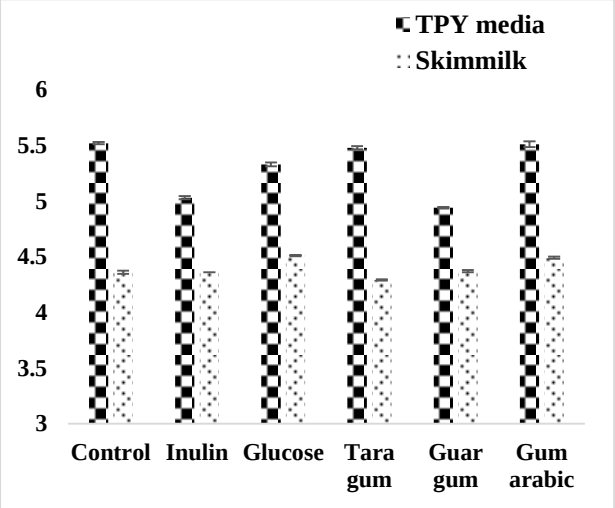


Figure 1. The pH values in TPY media and RSM containing gums after 24 h of fermentation

At the beginning of fermentation the average pH of samples in TPY media was detected as varying between 5.63 and 5.37 for all substrates. The pH reduction was slower in gum containing samples and decreased gradually from 5.50 to 4.94 after 24 h of incubation at 37 ℃ in TPY broth. In general, due to the alkaline nature of most gums, the pH reduction was slightly lower than the negative control.

The initial pH values for RSM samples with gums ranged from an average of 6.51 to 6.43. The pH of all samples decreased during 24 h-incubation. The pH of samples with gums after 24 h incubation ranged from pH 4.51 ± 0.008 (glucose) to pH 4.29 ± 0.005 (tara gum) These results are consistent with the findings of Dave and Shah [23] who reported that, if optimum growth conditions are provided, most strains of Bifidobacteria would decrease the pH of sterile RSM to 4.5 in 24 h. Although the *Bifidobacterium* spp. are acid-tolerant microorganisms, the optimal growth pH is between pH 6.5 to 7.0. The acid tolerance responses of *Bifidobacterium* spp. to either simulated pH of human stomach (1.0-3.0) or low pH of fermented dairy products around 4.0 have been reported by several researchers [23], [24]. The weakest acidifying activity was noted for control and inulin on TPY media and inulin for RSM, whilst the highest acidifying activity was obtained with guar gum for TPY broth and with tara gum and guar gum for RSM. In general, it could be seen that the acidifying activity of *B. animalis subsp. lactis* increases with gum supplementation.

In case of a prebiotic to perform its health effects it has to reach the colon undigested and selectively utilized by beneficial gut microbiota. Some preliminary work on gum fermentation by Lactobacilli and Bifidobacteria have suggested that they could be designated as potential/innovative prebiotics [25]-[29]. Taking into account these considerations, it appears that production of a synbiotic product with *Bifidobacterium* spp. and gums would be a feasible approach in administering beneficial bacteria. In order to comply a fermented synbiotic dairy product, the growth of *B. animalis* subsp. *lactis* in the presence of gums in both TPY media and RSM was depicted in Fig. 2.

The effect of gums (guar gum, gum arabic and tara gum) supplementation to basal media/RSM on the growth of *B. animalis* subsp. *lactis* was found to be significant ($p<0.01$) (Fig. 2). The numbers of *B. animalis* subsp. *lactis* in the gum-supplemented samples were higher than both the negative control and positive controls with inulin and glucose ($p<0.01$). Gum arabic and tara gum were found to support good growth of *B. animalis* subsp. *lactis* during incubation at 37 ℃ in TPY broth. On the other hand, inulin and glucose supplementation, when compared to the gums used in the present work, was found less stimulating for *B. animalis* subsp. *lactis* growth. It was observed that *B. animalis* subsp. *lactis* was able to metabolize these gums as much as or even higher than glucose, a readily available carbon source for bacterial metabolism. The findings were in agreement with a research conducted by Karlton-Senaye and Ibrahim [7] who mentioned that pectin and carrageenan-maltodextrin have enhanced the growth of *L. reuteri.*

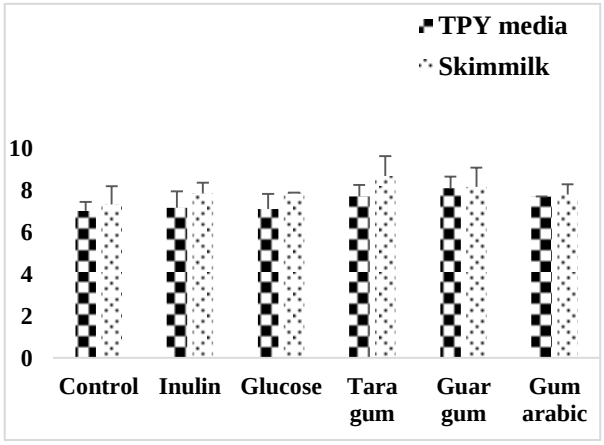


Figure 2. The counts of *B. animalis* subsp. *lactis* in basal TPY medium and RSM after 24 h fermentation (log cfu/mL)

The initial numbers of *B. animalis* subsp. *lactis* strains varied from an average of 6.65 to 7.65 log cfu/mL and 5.50 to 7.52 for TPY broth and RSM, respectively. After the 24 h-incubation with gums, viable cell numbers were found between 7.00-8.08 log cfu/mL for TPY broth and 7.30 to 8.67 log cfu/mL for RSM. The increase in the viable cell numbers on TPY broth with gums varied within the range of 1.08-1.74 log cycles. The addition of tara gum and gum arabic led to the highest (1.74 log cycles increase) growth in *B. animalis* subsp. *lactis* for TPY broth, whereas guar gum had relatively lower supporting growth effect. On the other hand, on RSM

guar gum displayed the highest growth supporting effect (2.95 log cycles).

Mumcu and Temiz [30] reported that the increase of *B. animalis* subsp. *lactis* BB-12 counts with prebiotics (FOS, XOS, GOS, SOS, LAC) varied within the range of 1.7-2.3 log cycles after incubation at 37 ℃ for 24 h. In general, bacterial counts were slightly higher in RSM compared to TPY media, in the presence of gums, with tara gum detected as the highest (8.67 log cfu/mL) in RSM. This was an indication that the metabolic activity of the *B. animalis* subsp. *lactis* was gum type-dependent, used as both carbon and energy source for growth. Similar results have been reported by Kartlon-Senaye et al. [31]. These researchers indicated that the numbers of *Lactobacillus rhamnosus* GGB101 and *L. rhamnosus* GGB103 were significantly higher in milk than the growth on deMan Rogosa Sharpe (MRS) broth, supplemented with pectin, carrageenan, carrageenan-maltodextrin, pectin-carrageenan, locust bean, guar, inulin, guar-locust bean-carrageenan and xanthan. Ramnani et al. [32] stated that *Gelidium* seaweed CC2253 and alginate powder CC2238 induced the counts of Bifidobacteria (0.49 log) and total bacterial numbers (0.57 log) after 24 h fermentation, respectively. Cherbut et al. [5] reported that acacia gum was able to selectively increase the proportions of lactic acid bacteria and bifidobacteria in healthy subjects. Wang et al. [33] reported that alginate-oligosaccharides stimulated the growths of *B. bifidum* ATCC 29521 and *B. animalis* subsp. *lactis* SMU 27001 more significantly in comparison to fructo-oligosaccharides (FOS).

In these experiments, the numbers of *B. animalis* subsp. *lactis* were significantly higher in the presence of all tested gums than the control samples. Gum arabic and tara gum were found to be the best gums to enhance growth for both on TPY broth and RSM. Fermentation of the gums used by *B. animalis* subsp. *lactis* indicated that these substrates may act as a novel source of prebiotics. However, more-detailed studies should be conducted on gum supplementation whether i) they support the growth and activity of other probiotic microorganisms with *in vitro* and *in vivo* tests, and ii) has any adverse influence on physico-chemical, textural and sensory characteristics of the synbiotic food.

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