

Comparing the effect of gums on the growth of lactobacillus species in laboratory medium and fluid milk

Abstract

This study compared the growth of three *Lactobacillus* strains in the presence of gums during a 12-hour incubation period at 37°C in order to determine which gum promoted the most growth. Our results showed that the populations of *Lactobacillus rhamnosus* GGB101 and *Lactobacillus rhamnosus* GGB103 were significantly higher in milk compared to growth in a laboratory medium, whereas *Lactobacillus reuteri* DSM20016 performed better in the medium. The recommended level of log 6 CFU g⁻¹ was exceeded for all tested strains during the incubation period. The addition of xanthan led to the highest growth of *L. rhamnosus* GGB101 (8.81±0.01 log CFU/mL) and *L. rhamnosus* GGB103 (8.32±0.01 log CFU/mL) in milk. Carrageenan-maltodextrin promoted the highest growth (8.30±0.23 log CFU/mL) of *L. reuteri* DSM 20016 in the medium and was found to support significant growth of *Lactobacillus* strains in both milk and medium. Our results showed that carrageenan-maltodextrin, xanthan and carrageenan could thus serve as functional ingredients for the enhanced growth and viability of *Lactobacillus* strains to promote quality probiotic dairy foods and thereby promote human health.

Keywords: gums, *Lactobacillus*, milk, basal medium, probiotics, prebiotics

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Abbreviations: CFU, colony forming unit; MRS, deman rogosa and sharpe; ANOVA, analysis of variance

Introduction

Probiotics are living microorganisms (mainly *Lactobacillus* and *Bifidobacteria*), which confer health benefits to the host when taken in adequate amounts.¹ Most strains of *Lactobacillus* and *Bifidobacteria* are known for their probiotic qualities with associated health benefits.^{2,3} Currently, *Lactobacilli* with probiotics are added to a variety of functional foods, and several studies have demonstrated their beneficial properties in human and animal health. These health benefits include alleviation of lactose intolerance, acceleration of intestinal mobility, reinforcement of gut mucosal immunity, decreased risk associated with mutagenicity and carcinogenicity, hypocholesterolemic effects, reduced duration of diarrhea, prevention of inflammatory bowel disease, prevention of colon cancer, prevention of allergies, and treatment and inhibition of intestinal pathogens.⁴ However, such health benefits can only be attained when the probiotic *Lactobacillus* strains are viable and exceed a population of six million.⁴ These health-promoting microorganisms are vulnerable to various environmental stressors to which they are subjected during fermentation, storage, and digestion. The survival of *Lactobacillus* microorganisms is also affected by food composition and the interaction between different bacterial strains.⁵ To attain the target population level of six million, 100g of food products containing 10⁶–10⁷ live cells must be ingested daily.⁶

Prebiotics are defined as non-digestible food substances that selectively stimulate the growth and activity of limited gut microorganisms and promote health benefits.⁷ Some prebiotic

carbohydrates such as fructooligosaccharides, lactulose, inulin and galactooligosaccharides from lactose (GOS-La) are currently available in the market.^{6,8} However, there is also considerable interest in learning more about new carbohydrates with potential prebiotic qualities. Polysaccharides gums (such as, carrageenan, pectin, xanthan, alginate, gellan, zedo, konjac, starch, cellulose, and chitosan), proteins (casein, whey, gelatin, β-lacto globulin) and wax have been studied as prebiotics that could improve the viability of probiotic microorganisms.^{9–12}

Gums are complex polysaccharides extracted from plant, animal and microbial sources.⁵ Gums impact sensory qualities, contribute fiber to foods¹³ and promote growth in probiotics.¹² Gums are also used as thickeners and binders in cosmetics, medications, inks, paint, paper, and adhesives.¹³ Gums are comprised of food ingredients such as carbohydrates, sugars, salts and minerals that could serve as additional carbon sources to enhance the growth of probiotic organisms in food and in the human gut.

Thus, the aim of this study was to investigate the effect of different gums on the growth of three *Lactobacillus* strains in a laboratory medium and fluid milk as a practical means to enhance the viability of probiotics and ultimately improve the quality of dairy products.

Materials and methods

Culture activation

Pure cultures of *L. reuteri* DSM20016, *L. reuteri* SD2112, *L. rhamnosus* GG B101, and *L. rhamnosus* GG B103 strains were obtained from the stock culture collection of the Food Microbiology and Biotechnology Laboratory at North Carolina Agricultural

& Technical State University (Greensboro, NC, USA). Bacterial cultures were activated by transferring 100µl of stock culture to 10mL of deMan Rogosa Sharpe (MRS) broth (Neogen, Lansing, MI) and incubating at 37°C for 24h.

Gum composition, preparation and inoculation

Fluid milk (1% milk fat) and a modified basal medium were prepared by gradually dissolving 0.5% (w/v) of each of the following gums: pectin (PE), carrageenan (CA), carrageenan-maltodextrin (MC), pectin-carrageenan (PC), locust bean (LB), guar (GU), inulin (IN) and guar-locust bean-carrageenan (GL) and xanthan (XA) (Maryland, GA, USA) to a 100mL batch of 1% fat liquid milk at 70°C.

Samples without gums served as a negative control. Milk and media samples were then treated in a water bath at 65°C for 30min and cooled to 42°C before use.

Preparation and composition of basal medium

A basal medium was prepared by dissolving the following: 2g Proteose peptone, 2.0g yeast extract, 1.0ml Tween 80, 1.0 Dextrose, 0.7g L- cysteine, 0.5g L-arginine, 2g Disodium phosphate (Na_2HPO_4), 4.0g sodium acetate (CH_3COONa), 0.1g Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.5g Manganese sulfate ($\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$), in 1ml distilled filtered water (Table 1). Batches of 200ml containing different gums were sterilized at 1100C for 10mins.

Table 1 Composition of modified basal medium for 1L

Component	Supplier	Quantity (g/L)
Proteose peptone #3	Remel	2g
Yeast extract	Neogen	2.0
Tween 80	Fisher Scientific	1.0ml
Dextrose	Fisher Scientific	1.0
L-cysteine	Fisher Scientific	0.5
L-arginine	Fisher Scientific	0.5
Disodium phosphate (Na_2HPO_4)	Fisher Scientific	2g
Sodium acetate (CH_3COONa)	Fisher Scientific	8.0g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	Fisher Scientific	0.1g
Manganese sulphate ($\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$)	Fisher Scientific	0.05g

Legend Basal medium composition was based on the composition of MRS with some modifications

Growth study

Sterilized basal medium samples were inoculated with 1% of inoculum at a final inoculum level of 3 log CFU/ml. Bacterial inoculum was prepared by taking aliquots (1mL) from appropriate serial dilutions from each active culture and aseptically adding the aliquots to each 100mL batch of 1% fat milk and basal medium samples. Samples without gum served as the control. Inoculated samples were serially diluted at a final inoculum level of 3 logs CFU/ml, plated on MRS agar, and incubated at 37°C for 48h to determine initial bacterial counts. Inoculated samples were then incubated at 37°C for 12h. After incubation, samples were serially diluted and aliquots were placed on MRS agar to obtain final bacterial counts.

Data and statistical analysis

Each experiment was conducted three times in a randomized block design. The mean values and standard deviations were calculated from the duplicate tested samples. R-Project for Statistical Computing version R-2.15.2 (<http://www.r-project.org>) was used to determine significant differences between the effect of different gums on the growth of

Lactobacillus strains in milk and medium using one- way ANOVA (analysis of variance) at a significance level of $p < 0.05$.

Results

Growth of *Lactobacillus* strains

A modified basal medium was prepared using basic components (Table 1) and nine different gums PE, CA, MC, PC, LB, GU, GL, IN and XA (Table 1) to study the effect of selected gums on the growth of *Lactobacillus* strains. The growth of *Lactobacillus* strains in the medium was compared to the growth in one percent fat fluid milk containing selected gums. Figures 1–3 shows the effect of gums on three *Lactobacillus* strains in laboratory media and in milk during 12hours of incubation at 37°C.

The population of the *L.rhamnosus* GGB101 strain was significantly ($p < 0.05$) higher in milk compared to medium in the presence of all tested gums (Figure 1). However, the highest bacterial population was observed in milk containing xanthan at 8.81 ± 0.01 log CFU/mL, which was followed by milk sample containing carrageenan (8.18 ± 0.03 log CFU/mL). The addition of guar-locust bean-carrageenan resulted in

the least growth of *L. rhamnosus* GG B101 ($8.03 \pm 0.01 \log$ CFU/mL) in milk. Similarly, the addition of xanthan led to significantly ($p < 0.05$) higher growth (7.65 ± 0.00 $0.04 \log$ CFU/mL) of *L. rhamnosus* GG B101 in the medium, with locust bean inducing the least growth

($6.89 \pm 0.06 \log$ CFU/mL). However, the population of *L. rhamnosus* GG B101 was 1.16 logs CFU/mL higher in milk than in media in the presence of xanthan (Figure 1).

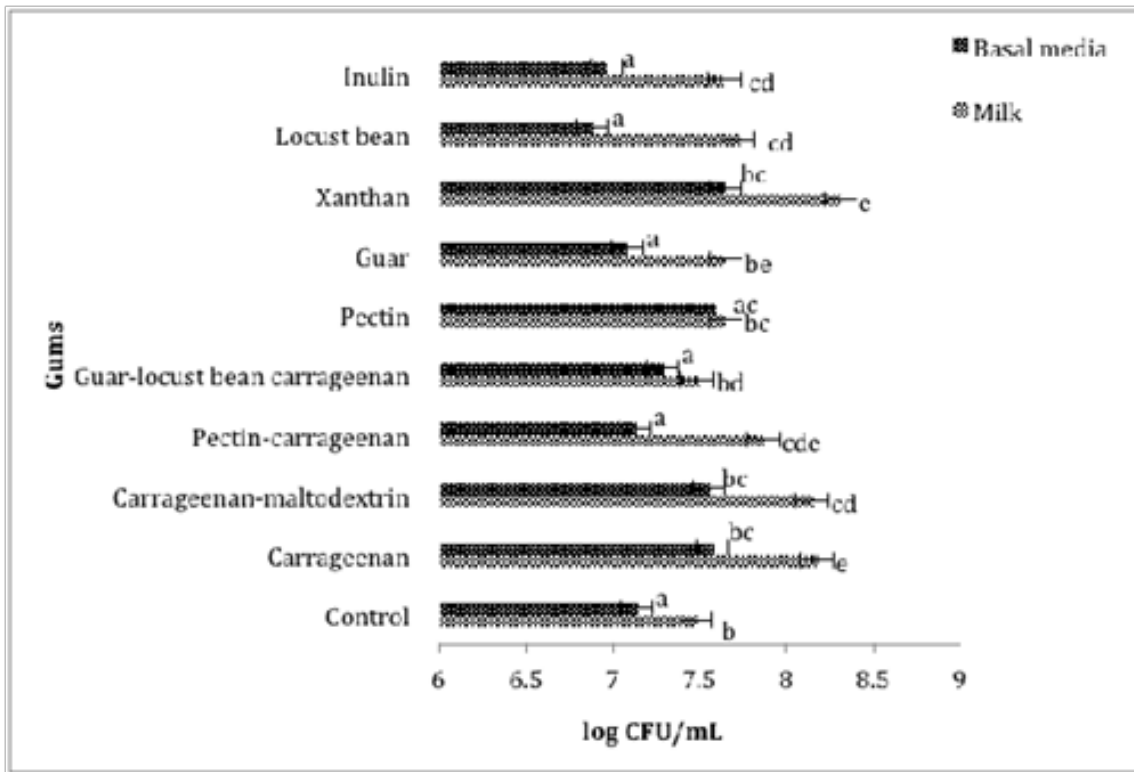


Figure 1 Comparing the effect of gums on the growth of *L. rhamnosus* GG B101 in basal media and milk during 12 hours of incubation at 37°C.

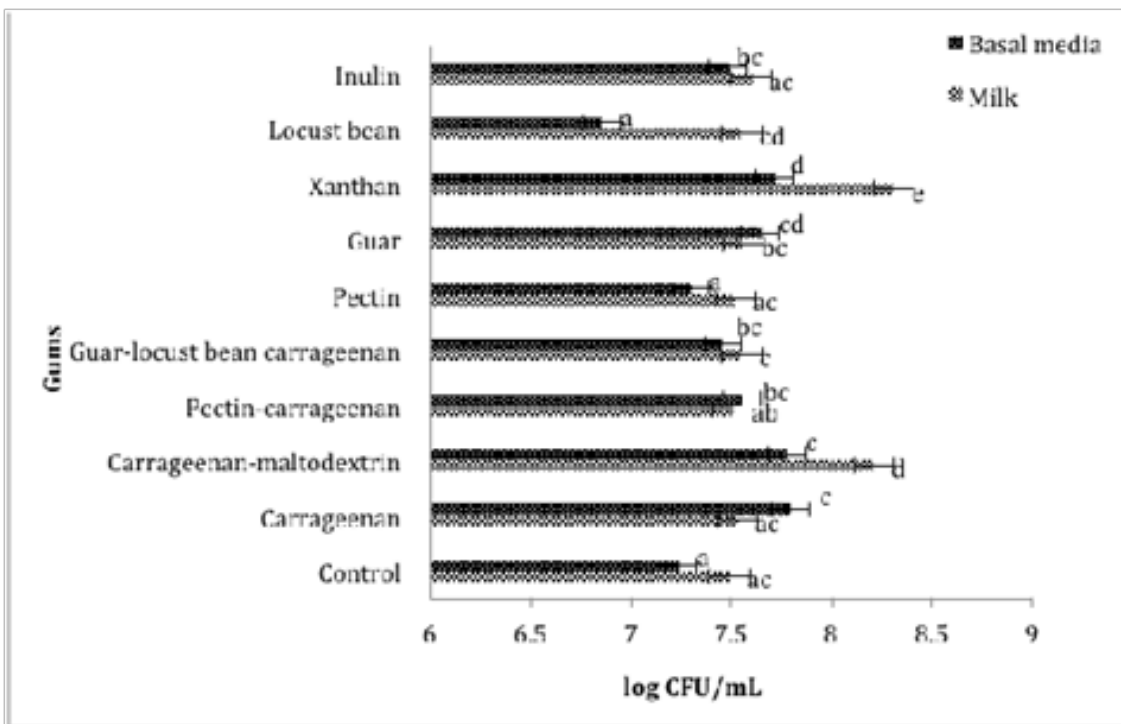


Figure 2 Comparing the effect of gums on the growth of *L. rhamnosus* GG B103 in basal media and milk during 12 hours of incubation at 37°C.

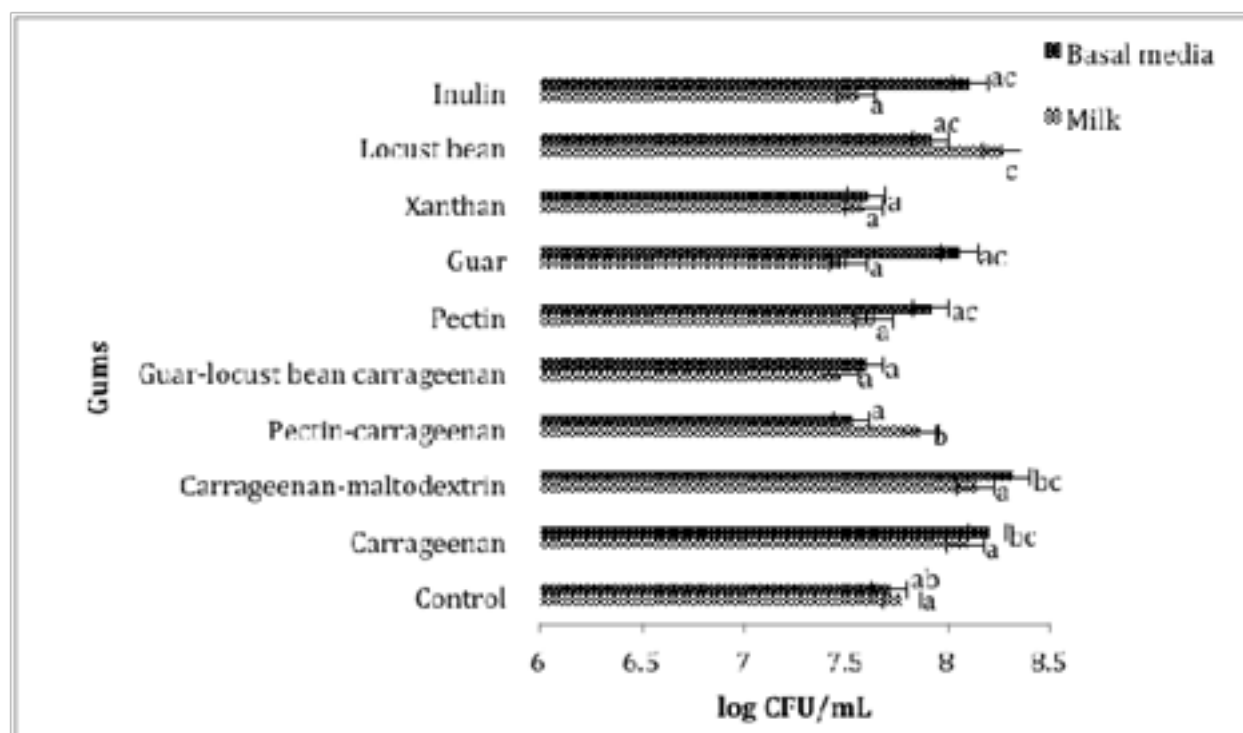


Figure 3 Comparing the effect of gums on the growth of *L. reuteri* DSM20016 in basal media and milk during 12 hours of incubation at 37°C.

Significantly ($p < 0.05$) higher population of *L. rhamnosus* GG B103 was observed in milk compared to medium samples in the presence of all tested gums, except in the milk sample containing guar, pectin-carrageenan, and carrageenan where a slightly higher population of *L. rhamnosus* GG103 was found (Figure 2). The addition of xanthan led to the highest population of *L. rhamnosus* GG B103 ($8.32 \pm 0.01 \log \text{CFU/mL}$) in milk during the incubation period. Compared to the control, the population of *L. rhamnosus* GG B103 was significantly ($p < 0.05$) lower in the presence of pectin and locust beans in the medium, indicating an inhibiting effect of these gums on the growth of *L. rhamnosus* GG B103. The addition of tested gums led to a positive effect on the population of *L. rhamnosus* GG B103 in all milk samples compared to medium (Figure 2).

The presence of carrageenan-maltodextrin resulted in the highest population of *L. reuteri* DSM20016 ($8.3 \pm 0.23 \log \text{CFU/mL}$) in modified basal media during the incubation period (Figure 3). It was notable that carrageenan, carrageenan-maltodextrin, guar, and inulin promoted higher growth in the media compared to milk and also promoted higher growth in treatments than in the control. In contrast, gums (pectin-carrageenan, pectin, xanthan, guar-locust bean-carrageenan and carrageenan) that led to a higher population of *L. reuteri* DSM20016 in treated samples than in control samples also enhanced bacterial growth in milk. This would indicate that the metabolic activity of *Lactobacillus* strains was affected directly by gums and indirectly by the medium of growth. This effect could be due to the interaction between the gums and the strains. *L. rhamnosus* GG that grew best in milk compared to the *L. reuteri* strain.

Carrageenan, carrageenan-maltodextrin, guar, inulin and locust bean enhanced the growth *L. reuteri* DSM20016 in media. However, the addition of pectin-carrageenan, pectin, guar-locust bean-carrageenan and xanthan resulted in a slightly higher population

of *L. reuteri* DSM20016 in milk (Figure 3). The presence of carrageenan-maltodextrin led to the highest population of *L. reuteri* DSM20016 ($8.3 \pm 0.23 \log \text{CFU/mL}$) in media. It was also observed that carrageenan, carrageenan-maltodextrin, guar, inulin and locust bean which induced higher growth in the treatment group than in the control, also promoted higher growth in media compared to milk. Similarly, gums (pectin-carrageenan, pectin, xanthan, guar-locust bean-carrageenan and carrageenan) that led to higher populations of strains in treated samples than in control samples also improved the growth of *L. reuteri* DSM20016 in media compared to milk. Xanthan significantly ($p < 0.05$) stimulated the highest growth of *L. rhamnosus* GG strains in both milk and media whereas the addition of xanthan resulted in a slight inhibition in the *L. reuteri* strain in both milk and media. The presence of carrageenan-maltodextrin and carrageenan significantly ($p < 0.05$) promoted the growth of all tested *Lactobacillus* strains in both media and milk.

Discussion

The results from this study demonstrated that gums could promote the growth of *Lactobacillus* strains. These results support previous findings^{12,14} that showed carrageenan-maltodextrin could promote growth and viability of *Lactobacillus* strains.

According to Su et al.,⁴ the addition of certain prebiotics such as fructose oligosaccharides (FOS) and soybean oligosaccharides (SOS) at 1.5% enhanced the growth of *Lactobacillus* in a basal medium at about 1 log CFU/mL. Buttermilk and whey supplemented with yeast extract also improved the growth of *Lactobacillus* strains, although the growth was lower compared to MRS.¹⁵ The ability of gums to impact the growth of *Lactobacillus* could be due to the interaction between gums and the tested *Lactobacillus* strains as the metabolic activity of *Lactobacillus* strains tend to respond to the composition of the medium

of growth.¹⁶ Additionally, the structure and composition of gums could be a key factor in the enhancement of bacterial growth. Gums vary in chemical composition and carbon chains which impacts on their digestibility and availability to bacteria. According to Hernandez-Hernandez et al.,¹⁰ the length of carbon chains in carbohydrates such as galactooligosaccharides affects digestibility. The longer the carbon chains as in xanthan, the slower the digestibility, which subsequently impacts the growth of lactobacilli. We would therefore expect to find the same trend in the digestibility of selected gums due to their composition and structure as well as carbon chain length with their consequent effect on growth and viability.¹⁰ Additionally, gums added carbon and energy sources for the hetero fermentative metabolic activity by *L. reuteri* and *L. rhamnosus* strains leading to enhance growth in the milk and media containing gums.¹⁶

Conclusion

Our results indicated that the tested gums could enhance the growth of *Lactobacillus* strains in milk compared to media as the addition of gums significantly improved the population of *Lactobacillus* strains in milk. In addition, carrageenan-maltodextrin and carrageenan showed enhanced growth of *Lactobacillus* strains in both milk and media. Xanthan stimulated the highest growth of *L. rhamnosus* GG strains but led to a slight inhibition in the growth *L. reuteri* strain in both milk and media.

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Conflict of interest

Author declares that there is no conflict of interest.

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