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Research Article

Assessment of the Prebiotic Effect of Gum Arabic on the Growth of Certain Probiotic Bacteria

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ABSTRACT

Probiotics, prebiotics, and symbiotics have been studied extensively in recent years as potential mediators of the maintenance of healthy intestinal flora. The current study investigates the prebiotic effect of gum Arabic on some probiotic bacteria. For this purpose, the growth of three strains (*Lactobacillus rhamnosus*, *Leuconostoc mesenteroides* strain lmn4 (MS11), and *Leuconostoc mesenteroides* strain 1-15 (MR4)) in the basal fermentation medium (MRS broth without glucose) at different concentrations (0.5% and 1%) of gum Arabic and glucose (control) was evaluated by optical density measurement. The findings indicate that *Lactobacillus rhamnosus* ferments gum Arabic better than *Leuconostoc mesenteroides* strains in both 0.5% and 1% concentrations. Moreover, increasing the concentration of gum Arabic had no significant effect on the growth of most of the tested strains. The effect of adding different concentrations of gum Arabic (0.5% and 1%) on the development of probiotic strains in the milk was monitored by pH measurement. The results showed that increasing the concentration of gum Arabic reduces the pH of the prebiotic milk compared to skimmed milk but not significantly. It can be concluded that gum Arabic has been found to promote the growth of *Lactobacillus rhamnosus*, a probiotic used in infants.

INTRODUCTION

Gum Acacia (GA) or Gum Arabic is a type of soluble dietary fibre obtained as exudates from acacia trees. The exudate is a non-viscous liquid rich in soluble fibres that emanates from the stems and branches under stress situations such as drought, poor soil fertility, and injury. GA was widely used in Arabian and African countries and is commonly used as traditional medicine, in which GA has demonstrated significant medical benefits in the digestive system [1, 2]. GA is a complex mixture of macromolecules of varying size and content (mainly carbohydrates and proteins). It is very soluble in water; concentrations of up to 40% are possible without significantly affecting viscosity [1, 3]. Gum exudates are used as an ingredient in many applications, mainly in the food industry. However, there are also considerable non-food applications [4, 5].

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One of the most promising applications linked with gum Arabic concerns its use as a prebiotic, which is 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 and thus improves host health [3, 6]. Several substances have been studied to establish their role as prebiotics. The most prevalent prebiotics are fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and trans-galacto-oligosaccharides (TOS) [6]. It has been reported that GA contains more than 850 g/kg of fermentable dietary fibres, which can increase the amount of probiotic bacteria in the GIT [7]. Probiotics are live bacteria that, when consumed in adequate quantities, can provide health advantages to the host [8]. The target genera for prebiotics that are beneficial to human health include *Lactobacillus* and *Bifidobacteria* [7]. Combinations of probiotics and prebiotics are known as synbiotics, which act synergistically to confer health benefits on the host [9].

The purpose of this study was to evaluate the prebiotic effect of gum Arabic on the growth of certain probiotic strains *in vitro*.

MATERIALS AND METHODES

Preparation of gum Arabic powder

A quantity of Sudanese gum Arabic was purchased from the local market. To obtain raw gum flour, the gum Arabic granules were rinsed with distilled water, dried, ground into fine particles, and sieved (Fig. 1).

a

b

Figure 1: (a) The Granules and (b) powder of gum Arabic

Probiotic Bacteria and Culture Conditions

Two probiotic strains, *Leuconostoc mesenteroides* strain lmn4 (MS11) and *Leuconostoc mesenteroides* strain 1-15 (MR4) were previously isolated from fermented camel milk and identified by sequence analysis of the amplified 16S rRNA gene product [10]. Lactic acid bacteria strains stored in MRS broth with 30% glycerol at -20°C were revivified after transferring a volume of 100 µL of each culture into MRS broth and incubated at 30°C for 24-48h.

The *Lactobacillus rhamnosus* strain was isolated from the product marketed as Ultrabiotic Infantile (Nutrisanté Laboratories, France). A small amount of the probiotic product was aseptically poured into the MRS broth medium that selects the *Lactobacillus rhamnosus* strain and then plated on solid MRS. The purity of the isolates was confirmed after performing three subcultures on MRS agar, followed by microscopic examination after Gram staining.

Probiotic Bacteria Growth in the Basal Fermentation Medium at Different GA Concentrations

A volume of 10 mL of basal fermentation medium (MRS broth without glucose) was supplemented with gum Arabic powder at 0.5% and 1% (w/v) or glucose (0.5% and 1%) used as a control. 100 µL of each fresh bacterial culture is inoculated (10^4 to 10^5 CFU/mL). After incubation for 24 h at 30°C, the growth of the strains was examined by measuring the optical densities (OD) using a spectrophotometer set at 600 nm and equipped with a 1 cm cuvette. The experiment was repeated three times, and the average OD values were calculated [11].

Growth of Probiotic Bacteria in Skimmed Milk and in Prebiotic Milk

Preparation of Prebiotic Milk

Two concentrations of GA 0.5% and 1% (w/v) were added to flasks containing 100 mL of skimmed milk (0% fat) (Candia silhouette). These media were autoclaved for 15 minutes at 115°C [12].

Monitoring of Acidification Kinetics

Bacterial cultures of the exponential phase were inoculated at 2% (v/v) into flasks containing 100 mL of sterile skimmed milk (control) or prebiotic milk. The pH values were measured at 0 h and after 2 h, 4 h, 6 h, 24 h, and 48 h of incubation at 30°C [13].

Statistical Analysis

The results are expressed as means ± SD of three repetitions and processed by analysis of variance (ANOVA) using the R software version 3.5.1 followed by the Tukey test at a significance level of P<0.05.

RESULTS AND DISCUSSION

Probiotic Bacteria Growth in the Basal Fermentation Medium at Different GA Concentrations

The results of OD measurements reflecting the growth of the three probiotic strains in the basal fermentation medium (MRS broth without glucose) at different concentrations of GA (0.5% and 1%) and glucose (0.5% and 1%) are summarized in Fig. 2.

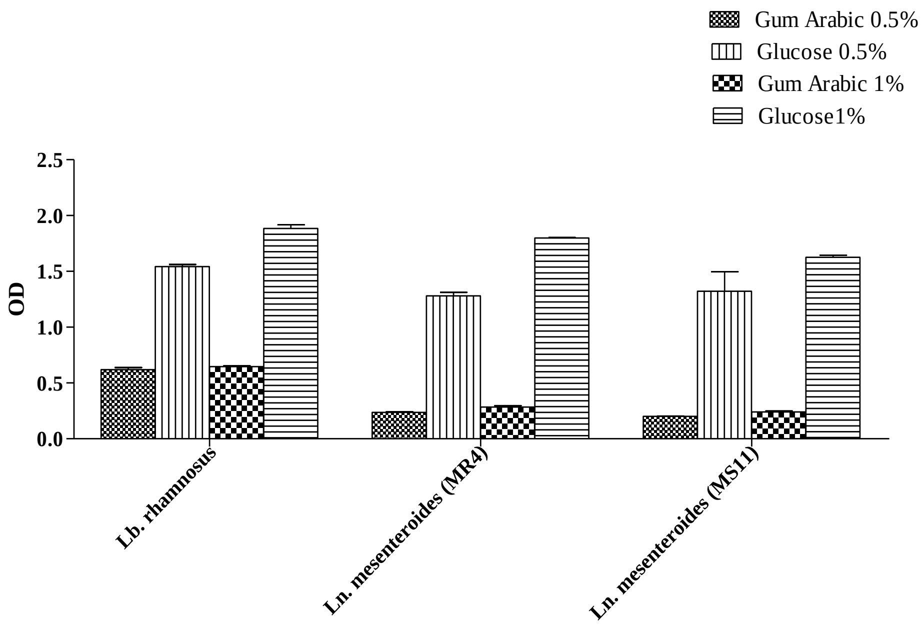

Figure 2: Optical density (OD) measurements (mean±SD) of three probiotic cultures in different concentrations (0.5% and 1%) of prebiotic (GA) and glucose (control).

According to the results, all probiotic bacteria prefer glucose as a source of carbon and energy for their growth, as it is easily assimilated.

An increase in the concentration of gum arabic significantly affected the growth of the MR4 strain. However, it had no effect on the growth of MS11 or *Lactobacillus rhamnosus* strains.

Comparing the fermentation of GA by the three probiotic strains, it appears that *Lb. rhamnosus* ferments it more efficiently than *Leuconostoc mesenteroides* strains at both concentrations of GA (0.5% and 1%). This could be due to the presence of the *Lactobacillus* enzymes galactosidase and arabino-furanosidase, which degrade the arabinogalactan contained in GA, giving it its prebiotic potential [2].

A Mohamed et al. (2014) investigated the use of gum arabic as a prebiotic when added at two levels of 0.5% and 1% to some fermented milk products, using two types of probiotic bacteria: *Lactobacillus plantarum* (LBP) and *Lactobacillus acidophilus* (LBA). They found that gum Arabic is a good prebiotic for *Lactobacilli* by increasing their number, which is consistent with the findings for the *Lactobacillus rhamnosus* strain used in this study. Ahallil et al. (2019) also demonstrated that *in vitro* fermentation of gum Arabic in a colon model promoted the growth of probiotic bacteria such as those in the genera *Bifidobacterium*, *Lactobacillus* and the family *Bacteroidaceae* in humans [14].

Growth of Probiotic Bacteria in Skimmed Milk and in Prebiotic Milk

Gum Arabic contains various carbohydrates that probiotic bacteria can ferment [15]. Fig. 3, 4, and 5 shows the acidification kinetics of skimmed and prebiotic milk (0.5% and 1% of GA) inoculated with *Lactobacillus rhamnosus*, MR4 and MS11 strains respectively.

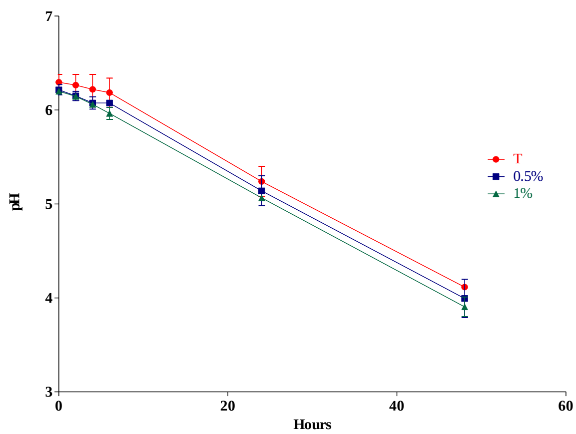

Figure 3: Evolution of acidification kinetics of *Lactobacillus rhamnosus* on the skimmed milk (0% fat) in the absence and presence of gum Arabic (0.5% and 1%).

Upon inoculation of the milks with 2% of each probiotic culture (at 0 h), the pH of all samples (including the control) is close to 6.30. Demirci and Hemme (1995) reported that the pH after 6 h of incubation is important because it allows the distinction between different strains and provides some indication of acidification kinetics. In all the strains tested, the decrease in pH after 6 h of incubation at 30°C was not considerable (pH close to 6). After 24 h of incubation, the reduction in pH was significant in *Lb. rhamnosus* (Fig. 3), and the acidification point (pH 4.6) [16] was reached before 48 h of incubation. All milk samples were coagulated with *Lb. rhamnosus*, which is considered to be an acidifying bacterium and can be used as a starter in milk lactic fermentations. This strain is a homofermentative bacterium that essentially produces L (+) lactic acid from different carbon sources. It is thermophilic and resists high concentrations of lactic acid, hence its great interest in the industry [17].

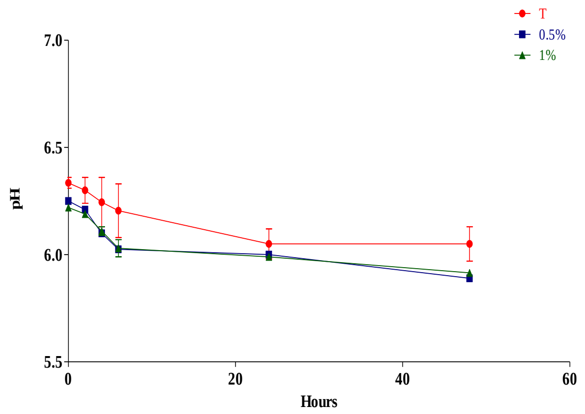

Figure 4: Evolution of acidification kinetics *Leuconostoc mesenteroides* strain 1-15 (MR4) on the skimmed milk (0% fat) in the absence and presence of gum Arabic (0.5% and 1%).

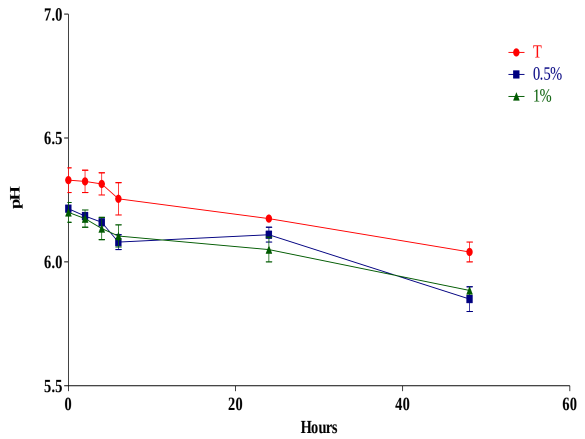

Figure 5: Evolution of acidification kinetics of *Leuconostoc mesenteroides* strain lmn4 (MS11) on the skimmed milk (0% MG) in the absence and presence of gum Arabic (0.5% and 1%).

The addition of gum Arabic to milk fermented with *Lb. rhamnosus* lowered the pH proportionally with increasing concentration but not significantly. This result is in line with that of A Mohamed et al. (2014), who reported that the pH values of the treatments are decreased due to the increase in the concentration of gum arabic for both types of *Lactobacillus* bacteria (*Lactobacillus plantarum* (LBP) and *Lactobacillus acidophilus* (LBA)). Thus, the pH values of the LBA bacteria treatments of 0.5 and 0.1 % GA are (4.1 and 4.0), respectively, compared to the control, which is (4.3), while the pH values of the LBP bacteria are (4.0 and 3.8), respectively, for the 0.5 and 1.0 % GA treatments compared to the control (4.2). After 48 h of incubation at 30°C, the pH of all milk samples inoculated with *Leuconstoc mesenteroides* (MS11) and (MR4) strains was above 5.5 in the absence or presence of gum arabic (0.5 and 1%) (Figs. 4 & 5). It can be seen that these strains are not acidifying. The slow development of *Ln. mesenteroides* strains in milk can explain this result. Furthermore, strains of the genus *Leuconostoc* have a hetero fermentative metabolism that produces lactic acid, CO_2, as well as ethanol or acetate. For this, *Leuconostoc* isolates should be combined with acid-producing *Lactococcus* spp. in mixed cultures for cheese production, given the flavours and textures they develop [18, 19].

CONCLUSION

GA is the most important gum, with applications in both food and medicine. It contains soluble fiber, or prebiotics, that stimulate the growth of healthy bacteria in the gut. This study found that GA has a greater *in vitro* prebiotic impact in *Lactobacillus rhamnosus* strains than in *Ln. mesenteroides* strains. Furthermore, raising the amounts of GA had no significant effect on the growth of the majority of the probiotic strains tested.

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