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Quality of new *Lactobaillus rhamnosus* like-yoghurt and the effect of using gum Arabic as prebiotic

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ABSTRACT

Lactobacillus rhamnosus is commonly found in several fermented dairy products. The safety of probiotic *L. rhamnosus* OP268117 strain 6481 was evaluated in this study using hemolytic and gelatinase activity tests. Hemolysins or gelatinases were not detected for this probiotic candidate and so it is considered safe for human use. Three different concentrations of gum arabic (GA) were added to the like-yogurt, which was previously inoculated with the probiotic starter stated earlier. The utilization of concentrations of 0.25%, 0.5%, and 1% GA, namely B, C, and D, respectively, resulted in a significant improvement ($p < 0.001$) in the viable count of *L. rhamnosus* compared to the control samples, namely A, with the highest number (18.93×10^9 CFU/ml) observed in treatment D containing 1% GA on the 14th day. Adding GA had a substantial impact on the moisture, protein, and fiber content of the samples, but it did not influence the percentage of fat. The treatment with 1% GA (D) exhibited the highest fiber content. The pH decreased gradually as GA concentration increased during storage. Furthermore, the yogurt with 1% and 0.75% GA (D and C) showed the highest viscosity measurements on the 14th day of storage. The addition of GA had a significant impact on all sensory attributes, except for flavor, which was only minimally affected. The grades for appearance and texture were improved as the GA content increased. Thus, it is recommended to incorporate *L. rhamnosus* in the production of yogurt, together with 1% GA as a prebiotic to enhance the nutritional composition, overall appeal, and survival rate of the probiotics employed in the fermentation process.

***Keywords*:** Gum Arabic, Yogurt, Probiotic, Prebiotic, *L. rhamnosus*

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1. INTRODUCTION

Fermented dairy products are widely consumed globally as healthy and beneficial food products. In recent decades, there has been a substantial soar in the use of fermented dairy products in comparison to liquid milk. Most fermented foods consist of probiotic Lactobacillus, while alternative strains are currently under investigation (Yerlikaya *et al.,* 2020). One of the most popular fermented dairy products worldwide, yogurt is considered a nutritious food (Bouhadi *et al.,* 2021). As yogurt grows more popular, manufacturers and scientists seek value-added ingredients such as probiotics, prebiotics, and plant extracts to make functional yogurts with greater benefits (Fazilah *et al.,* 2018).

Probiotics are live microbial feed supplements that improve the natural balance of intestinal bacteria in the host animal, consequently benefiting it. The technique of adding prebiotics to numerous food products has grown more common in recent years. Bacteria from the genus Lactobacillus are commercially accessible probiotic microorganisms utilized in human nutrition (Muzaffar *et al.,* 2021). The research of novel probiotic strains is important in order to satisfy the increasing request of the market and to obtain functional products in which the probiotic cultures are more active and with better probiotic characteristics than those already present on the market (Bertazzoni *et al.,* 2004).

A prebiotic is a sort of food ingredient that cannot be digested by the body and has a good effect on the host by particularly supporting the growth and/or activity of a small number of bacteria in the colon, thereby increasing the health of the host. Gum Arabic is a form of prebiotic, which is a member of a category of compounds that promote the growth of good bacteria in the gut. Gum Arabic is a complex polymer that has a branching structure. It can be either neutral or slightly acidic. It is frequently seen as a blend of calcium, magnesium, and potassium salts of a polysaccharidic acid called Arabic acid (Ismaiel *et al.,* 2022). Multiple studies have documented the use of gum to improve certain characteristics of yogurt (Daou and Zhang, 2012, Patel and Goyal, 2015). Therefore, this study was carried out to assess the influence of Arabic Gum on the physical properties of yogurt.

When including prebiotics in fermented foods, it is important to carefully assess their stability during the manufacturing stage. Multiple scientific studies have shown that GA possesses prebiotic qualities, making it a conducive environment for the growth of Lactobacilli and Bifidobacteria. This is a result of the presence of fermentable fiber in it as stated by Bisar *et al.* (2014). Adding GA to yoghurt recipe provides two-fold advantages. First and foremost, it functions as a prebiotic compound, stimulating the proliferation of advantageous microorganisms in the gastrointestinal tract. Furthermore, it offers supplementary health benefits to individuals who drink yogurt. The lifespan and efficiency of probiotic bacteria can be considerably influenced by the acidity and storage conditions of the product.

The aim of this study was to examine the impact of different concentrations of GA on the survival of the primary starter, *L. rhamnosus* OP268117 strain 6481, the physical and chemical characteristics of yogurt-like products, viscosity, and sensory attributes during storage.

2.MATERIALS AND METHODS

2.1. The probiotic *L. rhamnosus* culture

In a previous study conducted by Nasr and Abd-Alhalim (2023), a specific strain of probiotic defined as *L. rhamnosus* OP268117 strain 6481 was obtained in a pure culture. The strain was incubated at 37 °C for 24 hours. Initially, it was activated in MRS broth medium and then transferred to sterilized skim milk with a concentration of 10% (w/v).

2.2. In vitro safety experiments

2.2.1. Hemolytic and gelatinase activity tests

The probiotic strain *L. rhamnosus* was tested for hemolysis as described by Balamurugan *et al*. (2014). Twenty-four old culture was used for streaking plates containing blood agar base supplemented with 5% (v/v) blood. Plates were incubated at 37 °C for 48 hours. The culture's hemolytic activity was characterized by evaluating the various zones of hemolysis. Beta hemolytic activity (β) is defined by the presence of prominent yellow zones surrounding the bacterial colonies. Alpha hemolysis (α) is characterized by a greenish-to-brown zone around the colonies. Non-hemolytic or gamma hemolysis (γ) is found when no apparent zone is present around the colonies.

Gelatinase activity of the probiotic strain *L. rhamnosus* that had been cultured overnight in MRS broth at 37 ◦C. In a Nutrient gelatin medium, Mary *et al*. (2009) 100 µL of strain were added. After incubation 24 hours at 37 ◦C, the culture was cooled for 30 minutes at 4 ◦C. The culture tube was shaken to observe gelatin liquefaction (Fugaban *et al.,* 2021).

2.2.2. Bile salt hydrolase (BSH) activity of the probiotic strain:

The BSH activity of freshly cultivated *L. rhamnosus* was measured, Taurodeoxycholic acid sodium salt (TDCA) supplemented MRS plates were used, while TDCA-free plates were included as negative controls. The plates were incubated at 37 °C for 48 hours. A positive result was identified when precipitated bile acid was found around the spots (Rodríguez *et al.*, 2012).

2.3. Preparation of gum Arabic

The GA was air-dried and manually cleaned to eliminate any extraneous particles and attached barks. Subsequently, the samples were grounded, sieved and stored in glass containers.

2.4. Like-yoghurt processing

Yoghurt was produced by pasteurizing fresh cow milk at 83 °C/15 minutes. The milk was then cooled to 45°C and 3% *L. rhamnosus* starter culture was added. The resulting mixture was divided into four portions, labeled as follows: A = Control (with 0% GA), B = Control + 0.25% GA, C = Control + 0.50% GA and D = Control + 1% GA. Subsequently, all samples were incubated at 37-40°C for 4-5 hours. The yogurt was placed in the refrigerator at 6°C±1. The yoghurt products were assessed for their physicochemical, microbiological, and sensory characteristics at three different time points during storage: fresh, 7, and 14 days.

2.5. The viability of *L. rhamnosus*

To determine the viability of the probiotic *L. rhamnosus* during storage, a selective media (MRS agar) was used. The plates were incubated at 37 °C for 48–72 hours in anaerobic jars.

2.6. Physicochemical analysis

The yoghurt samples obtained were examined for their moisture content using the oven drying method, protein content using the macro Kjeldahal method and fat percentage using the Gerber method, as outlined in the A.O.A.C (2012) guidelines. The pH values were determined using the Thermo Scientific Orion Star pH meter (model A 214).

2.7. Viscosity

The viscosity of the resultant yoghurt was measured according to Çelekli *et al*. (2019) using a Brookfield DV3T™ viscometer. The viscosity was measured using a V-72(72) spindle at 30 rpm at 25 °C. Each trial tested viscosity for 25 seconds using 250 mL of sample. Viscosity was measured in centipoise (cp).

2.8. Sensory analysis

Ten of the academic staff members of the Faculty of Agriculture, Fayoum University performed Hedonic sensory analysis to evaluate the kefir samples based on the following attributes: flavor, appearance, texture and overall acceptability. Each sample was assigned scores based on the standard TS EN ISO 8589:2010: liked-3, normal-2, dislike-1.

2.9. Statistical analysis

The data were subjected to statistical analysis using the General Linear Models procedure of the Statistical Package for Social Sciences (SPSS 2008) version 17.0 software. The means were compared using Duncan's (1955) multiple range tests.

3. RESULTS AND DISCUSSION

3.1. In vitro safety experiments

3.1.1. Hemolytic and gelatinase activity

When choosing a probiotic strain, it is important to ensure that it does not have hemolytic activity. This is because bacteria with hemolytic activity are pathogenic and can potentially cause infectious diseases. Therefore, the absence of hemolysin in a probiotic strain is a safety requirement (FAO/WHO 2002) (Peres *et al.,* 2014). Assessing the hemolytic properties is a crucial factor in determining the suitability of probiotic strains. *Lactobacillus rhamnosus* did not demonstrate β or α-hemolytic activities, and there was no apparent alteration in color. The *L. rhamnosus* strain exhibited no gelatinase activity. The findings of our study were consistent with other prior studies, which indicated that various species of lactic acid bacteria did not exhibit hemolysis (Argyri *et al.,* 2013; Ilavenil *et al.,* 2015). It is important to note that probiotics must meet safety standards. The absence of hemolysins and gelatinases in the *L. rhamnosus* CA15 (DSM 33960) strain indicates that it can be used without any safety concerns. The safety of the *L. rhamnosus* CA15 (DSM 33960) strain was confirmed as it did not exhibit any hemolysis halos when grown on blood agar plates, nor did it display any zones of gelatin hydrolysis on gelatin agar plates (Alessandra *et al*., 2022).

3.1.2. Bile salt hydrolase (BSH) activity

In the process of selecting strains with probiotic potential, it is common to consider the ability to break down bile salts as one of the factors (Rodríguez *et al.,* 2012). However, the occurrence of BSH activity is uncommon in bacteria that have been found in settings lacking bile salts. The *L. rhamnosus* strain exhibited growth on MRS agar plates supplemented with 0.5% (w/v) TDCA sodium salts. However, the *L. rhamnosus* strains did not exhibit BSH activity. This discovery is consistent with the results of earlier research conducted by Bautista-Gallego *et al.* (2013) and Solieri *et al.* (2014).

3.2. Viability of Lactobacillus rhamnosus

The effective use of concentrations of 0.25%, 0.5%, and 1% GA led to a significant increase ($p < 0.001$) in the number of *L. rhamnosus* when compared to the control samples, as depicted in Fig. 1. Beginning on day one and continuing until day 14 of storage, the number of live *L. rhamnosus* bacteria increased in comparison to the control. Following a period of 14 days, the samples that contained 1% GA displayed the highest concentration of *L. rhamnosus*, with a count of 18.93 × 10^9 CFU/ml. Several studies have shown that some gums, which are considered to be prebiotics, have an effect on the survival and development of living bacteria (Ghasempour *et al.*, 2012; Yilmaz-Ersan *et al.*, 2017; Sabooni *et al.*, 2018). These gums have been shown to have this relationship. Gum Arabic is considered to be prebiotic, and studies have shown that it has the ability to improve the symbiotic interaction that exists between probiotics. According to the findings of the research conducted by Niamah *et al.* (2016), who reported that the number of *L. acidophilus* bacteria grew concurrently with the quantities of GA that were present over the 21-day storage period.

Gum Arabic, as a prebiotic, provides promise for several sectors such as food, beverage, medicines, and nutrients. However, there are also challenges to overcome in utilizing GA in these areas (Talib *et al.*, 2018). The growth of beneficial gut bacteria, such as Bifidobacterium and Lactobacillus, is promoted, whereas the growth of harmful bacteria, such as Clostridium, is suppressed (Elnour *et al.*, 2023).

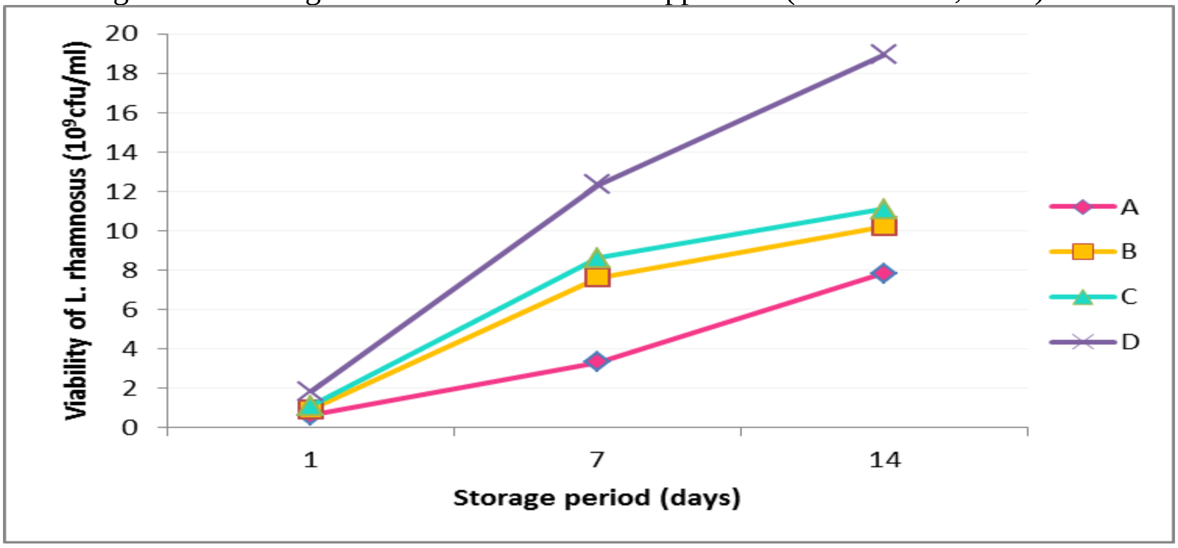

Fig. 1. Changes in *L. rhamnosus* counts (cfu/ml) as affected by adding different concentrations of GA and storage periods at 5±1°C. A: Control (with 0% GA), B: Control + 0.25% GA, C: Control + 0.50% GA, and D: Control + 1% GA.

3.3. Physicochemical analysis

The results in Table 1 demonstrate how the addition of various amounts of GA influenced the chemical composition of *L. rhamnosus*-like yoghurt in terms of moisture, fat, and protein. Compared to the yoghurt that had been treated with 1% GA (D), which had the lowest moisture level (85.05%), the control (A) yoghurt, which contained 0% GA, had a greater moisture content of 86.75%. Furthermore, it is evident that the addition of GA has a significant effect ($p < 0.01$) on the ratio of protein to total protein. The control (A) had the lowest protein content, which was recorded at 3.52%. On the other hand, the highest protein content was found in probiotic yoghurt that contained 1% GA (D; 3.78), followed by samples that were supplemented with 0.75% GA (3.72%). On the other hand, the addition of GA had insignificant effect on the percentage of fat that was present in the yogurt. There was a range of 3.10 to 3.12% fat content in all the treatments.

The addition of GA has a significant effect ($p < 0.001$) on the fiber content, which increases as the concentration of gum increases. The treatment containing 1% GA (D) had the highest fiber content, reaching 0.78%. In contrast, the control (A) had no fibers present. This might be due to the fact that GA is a profitable natural wellspring of dissolvable dietary fiber that reaches 85% of its weight (Food and Drug Administration, Jarrar *et al.*, 2021, Rawi *et al*., 2021).

3.4. pH values

The results presented in Figure 2 illustrate the changes in pH levels seen in both fresh samples and throughout a 14-day storage period. Upon examining fresh samples, it is evident that the addition of GA resulted in a reduction in pH when compared to the control. Simultaneously, the results obtained demonstrated an adverse relationship between the increase in GA and a decrease in pH values. The findings accord to Shenana's (2021) study, which indicated that the inclusion of GA can enhance lactic acid production, resulting in a decrease in pH level. The increase in buffering capacity slowly dropped the pH and improved the level of acidification by the starting bacteria (El-Alfy, 2021). During storage there was a progressive reduction in pH values reaching 3.90, 3.77, 3.45 and 3.20 in A, B, C and D after 14 days of storage, correspondingly. The pH values in B, C and D were lower than in A, which prove the influence of GA in accelerating the pace of drop in pH values. These findings are similar to the ones reported by Najafi *et al.* (2019). Furthermore, the incorporation of GA into milk before inoculation with starting bacteria can enhance the activity of the starter bacteria in producing lactic acid. The drop in the pH value can be attributed to the ongoing fermentation of lactose and its conversion into lactic acid, which occurs as a consequence of the sluggish fermentation activity of the starting cultures during cold storage (Shenana, 2021 and Fadawy *et al*., 2022).

Table 1. Chemical composition of *L. rhamnosus* yoghurt-like products as affected by adding different concentrations of gum Arabic (GA)

Component (%)	Treatment					
	A	B	C	D	Sig.	SE±
Moisture	86.75^{a}	86.11^{b}	85.48^{c}	85.05^{d}	**	0.03
Fat	3.10	3.12	3.12	3.13	NS	0.05
Protein	3.52^{b}	3.65^{ab}	3.72^{a}	3.78^{a}	*	0.11
Fibers	0.00^{d}	0.22^{c}	0.61^{b}	0.78^{a}	***	0.01

Means having different superscripted letters within each column are significantly different (p <0.001). A: Control (with 0% GA), B: Control + 0.25% GA, C: Control + 0.50% GA, and D: Control + 1% GA. SE: Standard error Sig.: Significance. NS: Not significant.

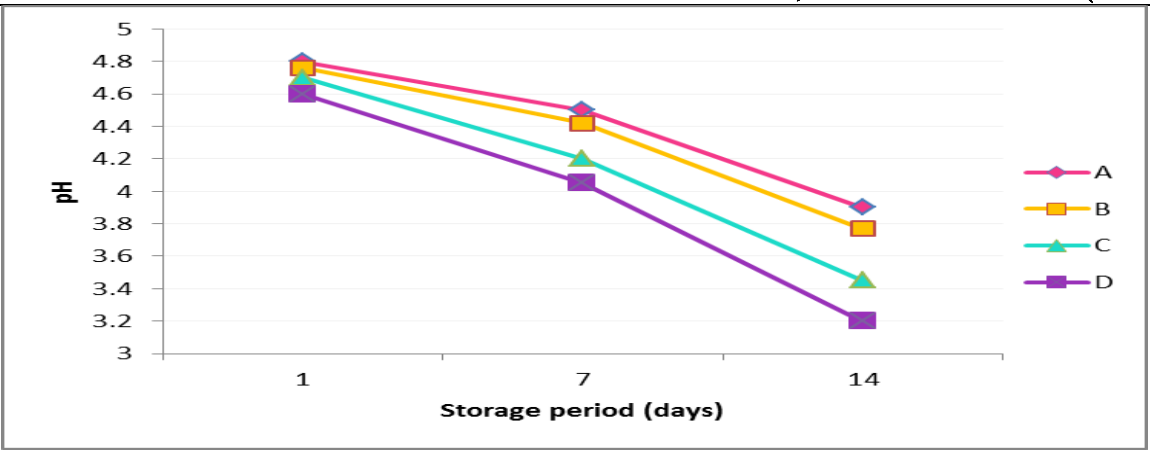

Fig. 2. Changes in pH values of *L. rhamnosus* yoghurt-like products as affected by adding different concentrations of Gum Arabic (GA) and storage periods at 5±1°C. A: Control (with 0% GA), B: Control + 0.25% GA, C: Control + 0.50% GA, and D: Control + 1% GA.

3.5. Viscosity

Viscosity is an important attribute necessary for achieving the desired texture of yogurt. The statistical analysis revealed that both the concentration of GA and the storage period had a significant effect ($p < 0.001$) on the viscosity value presented in Table 2. The samples with a concentration of 1% GA exhibited the highest viscosity value during storage. The yogurt containing 1% and 0.75% GA (D and C) had the highest viscosity values, measuring 2917 and 2894 cp, respectively, on the 14th day of storage. The lowest values were for control samples during storage period. Similar results were reported by Ismaiel *et al.* (2022). Also, Abdelgadir (2018) found that there was significant ($p \leq 0.05$) increment in indices of viscosity as gum level increased. Gum Arabic could raise the viscosity value, which could be a result of its functional characteristics of its soluble and insoluble fractions (Azarikia and Abbasi, 2010). It is obvious from studies that there is a positive correlation between viscosity and time. The observed variation in viscosity may be attributed to alterations in the dimensions of casein micelles during storage, which can be influenced by factors such as the presence and quantity of lipids and proteins, or micelle-binding (Alhssan *et al.*, 2023).

Table 2. Effect of addition of gum Arabic (GA) at different concentration on viscosity value (cp) to *L. rhamnosus* yoghurt-like products samples during storage time

Treatments*	Storage period (days)		
	1	7	14
A	1205^{h}	1489^{g}	1585^{f}
B	1569^{f}	2029^{d}	2310^{c}
C	1896^{e}	2318^{c}	2894^{a}
D	1910^{e}	2403^{b}	2917^{a}
Sig.		***	
SE±		35.16	

Means having different superscripted letters within each column are significantly different ($p < 0.001$). A: Control (with 0% GA), B: Control + 0.25% GA, C: Control + 0.50% GA and D: Control + 1% GA. SE: Standard error. Sig.: Significance.

3.6. Sensory evaluation

The addition of GA to yoghurt samples that resembled *L. rhamnosus* had a significant effect on the sensory scores, including flavor, appearance, and texture, and therefore, the overall acceptability (Fig. 3). The inclusion of GA had a substantial influence on all sensory attributes, except for flavor, which was only minimally impacted by the addition of GA. The scores for appearance and texture (consistency) were enhanced with the increase of GA concentration. However, even after increasing the concentration, the control samples (A) still had the lowest scores. The samples containing GA and the control showed a comparatively similar range of flavor scores, ranging from 2.8 to 3.

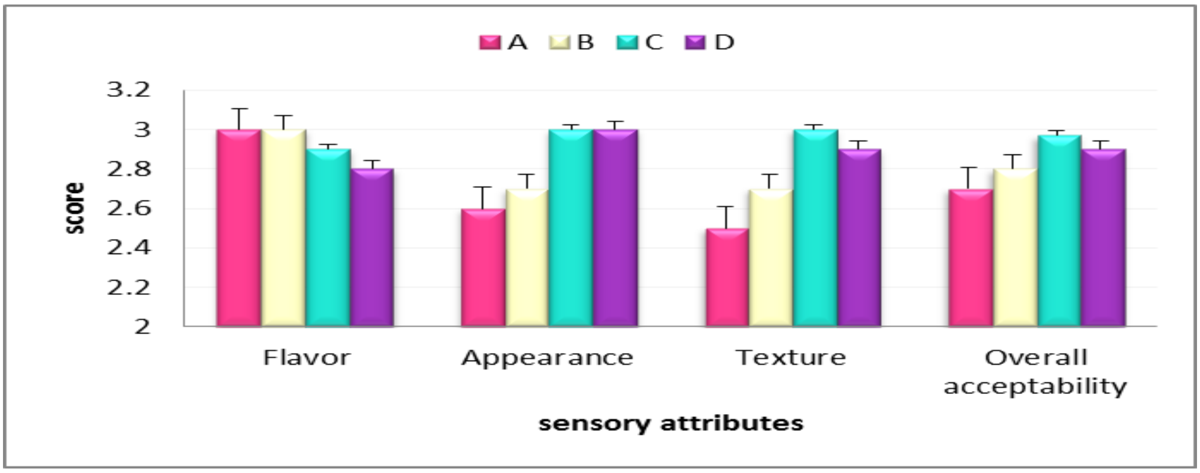

Fig. 3. Changes in sensory attributes of *L. rhamnosus* like-yoghurt as affected by adding different concentrations of gum Arabic and storage periods at 5±1°C. A: Control (with 0% GA), B: Control + 0.25% GA, C: Control + 0.50% GA and D: Control + 1% GA.

4. CONCLUSION

The addition of GA to *L. rhamnosus*-like yogurt had a substantial positive impact on the viability of *L. rhamnosus* OP268117 strain 6481, which has been confirmed to be safe. Additionally, it influenced many chemical, rheological, and sensory characteristics. Thus, it can be concluded that GA has significant potential to enhance the quality of fermented milk in terms of its health-promoting properties. The symbiotic blend of specific probiotic bacteria and prebiotic GA can enhance health by promoting the growth of probiotics in the gastrointestinal tract through synergistic effects.

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