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Growth and effect of garlic (*Allium sativum*) on selected beneficial bacteria

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Abstract

The growing interest of consumers to the new probiotic product has led the industry to focus on it. But viability of probiotic strain has a big challenge during shelf life of food. Therefore, to know the effect of food additives on survival of probiotic is important. The role of probiotics, garlic and yoghurt on the health of humans is widely known. In this study, we focus on finding answers to the question of whether selected beneficial bacteria will survive in the presence of ground garlic solution or fresh garlic juice and obtaining the growth parameters of that strains. Disk diffusion assays revealed that while *Bifidobacterium longum* BB536 is the most susceptible (49.37 ± 1.07 mm), *Lactobacillus acidophilus* 74-2 is the most resistant (no inhibition zone) bacteria to the fresh garlic juice, significantly. However, the strains were not adversely affected in possible usage rates of garlic in some Turkish appetizers. Enumeration assays also confirmed these unexpected findings. The addition of certain quantities of ground garlic or fresh garlic juice that did not show antimicrobial activity for selected probiotic strains are suitable for food matrix, especially yogurt based products.

Keywords: dairy probiotics; effect of garlic; natural antimicrobial compounds; growth study.

Practical Application: The novel dairy foods which contain garlic can be potential for use as a probiotic carrier.

1 Introduction

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts (Food and Agriculture Organization, 2001). By virtue of this definition, foods containing probiotic microorganisms are placed in the functional food classification. Even though probiotic microorganisms have been a part of the daily diet for centuries, human health and the treatment of diseases related to these microorganisms have only been researched in the last two decades (Marco et al., 2017; Haddadin et al., 2012). It has been proven in *in vivo* and *in vitro* experiments that probiotics contribute to lactose digestion (Iqbal et al.,2014), enhance the epithelial barrier (Mikov et al., 2014; Gogineni et al.,2013), prevent diarrhea or decrease diarrhea duration caused by various reasons (Sarowska et al., 2013; Htwe et al., 2008), prevent constipation and allergic reactions (Malaguarnera et al., 2013), and offer alternative medicine to heart and vascular (Thushara et al., 2016), upper respiratory tract (Tapiovaara, 2016), and gingival diseases (Joshi & Pandharbale, 2015).

Bacterial growth is a complex process involving numerous anabolic and catabolic reactions. Lag phase is defined as an adaptation of batch culture, and it is observed that it is the most poorly understood growth phase (Rolfe et al., 2012). The length of the lag phase can vary depending on the bacterial species, environmental conditions, and previous growth conditions. After this phase, cells divide exponentially, and this is known as the exponential phase. The growth rate and the doubling time are obtained during this phase. Then, the growth rate slows down: most of the cells tend to divide, but death starts for others in the deceleration phase. The exhausting of nutrient sources lead to cells entering a stationary phase in which death and growth are equal. As a result of the accumulation of metabolites and the lack of certain nutrition, cells finally die, a state called the death phase (Navarro Llorens et al., 2010).

Despite a great deal of interest in and widespread use of probiotics, the growth curve pattern of certain strains is not generally available. It is thought that the data obtained by the growth curves of the strains used in the production of fermented food are important in determining the quality of the product and in designing proper processing capacity (Maier et al., 2009). Knowledge of the growth curve of a bacteria strain is useful for assessing the behavior of the bacteria in a new food matrix, not only for fermentative or beneficial bacteria but also for pathogenic or spoilage microorganisms. This information can be used to optimize production parameters by determining the conditions that inhibit or support bacterial growth.

The health promoting function of garlic (*Allium sativum*) has been known since its use in ancient Egypt and has been used as a medicinal plant because of its profound effect on some diseases and disorders. When garlic is crushed or mashed, alliin is converted to allicin by the alliinase enzyme. Allicin is known as an antibiotic and antifungal compound, and it gives the garlic specific taste and smell. In addition to the sulfur-containing

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allicin compound, sulfo-free phytoalexin has been shown to be an antioxidant, an antimicrobial, an antitumor, an aflatoxin B2 inhibitor and has a neuropathic effect (Tsai et al., 2012; Valente et al., 2014). For a long time, it has been known that many Gram-negative and Gram-positive bacteria strains are inhibited by garlic (Fani et al., 2007). But the effect of garlic on some probiotic strains has only been discovered recently (Michael et al., 2015; Marhamatizade, 2015; Booyens & Thantsha, 2013).

Today, traditional foods are very popular, and many Turkish traditional foods involve garlic. Garlic enhances the flavor of foods such as meatballs, lahmacun (Turkish pizza), kebab, yoghurt, cacik, and haydari. As is well known, probiotic foods should contain more than 10^6-10^7 CFU/g at the time of consumption (Liong, 2011). Therefore, it is important to know the effects of garlic, which is used as food additive in especially yoghurt and its derivatives, against probiotics and starters, in order to achieve an effective dose in commercial products.

The objective of this research is to investigate the growth curves of yoghurt starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and three commercial probiotic bacteria (*Lactobacillus acidophilus* 74-2, *Lactobacillus rhamnosus* HN001TM, and *Bifidobacterium longum* BB536). The reason for selecting these strains is that they have the most potential to be used in fermented dairy products containing garlic. Hence, the second aim is to determine the effect of the garlic on selected starter culture and probiotic bacteria strains.

2 Materials and Methods

2.1 Microorganisms

Freeze dried starter co-culture (F-DVS YoFlex), which contains a mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, was provided by Peyma-Chr. Hansen (Hoersholm, Denmark) and probiotic cultures *Bifidobacterium longum* BB536, *Lactobacillus acidophilus* 74-2 and *Lactobacillus rhamnosus* Howaru HN001™ were obtained from Danisco (Dupont Copenhagen, Denmark). Lyophilized bacterial cultures were stored at -40 °C. Cells were activated initially on a Man, Rogosa, and Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 37 °C overnight separately. For only *B. longum*, a MRS broth was sterilized at 118 °C for 15 minutes as instructed by the supplier, and the broth was sterilized at 121 °C for 15 minutes for other bacteria strains.

2.2 Growth conditions and curve

Growth data were obtained by using the plate count method (Oliveira et al., 2011). Each bacteria strain was incubated at 37 °C for 24 h. Samples taken each hour were diluted in Ringer solution (Merck, Darmstadt, Germany). After serial dilutions, *S. thermophilus* in co-culture were counted in M17 agar supplemented sterile 0.5% (v/v) lactose (Oxoid Basingstoke England) solution. To determine the growth curve of *B. longum*, a bacterial culture was added to the MRS broth, and then 24 individual tubes were obtained. This prevented the development of oxidative stress that can occur during sample intake. Strains were enumerated under specific conditions that are given in Table 1.

The colony forming units (CFU) were expressed as Ln CFU/mL and Log CFU/mL. The specific growth rate (μmax) was calculated at an exponential phase according to the method described by Pak et al. (2013). Lag phase duration (λ) was given for each bacterium, and doubling time (td) was calculated according to the equation 1;

$$\mu_{max} = \ln 2 / t_d \quad (1)$$

2.3 pH analysis

The pH values of the media were measured using with a pH meter (Hanna Instruments, HI9025 Ronchi di Villafranca, Padova, Italy) hourly during growth experiment.

2.4 Preparation of Fresh Garlic Juice (FGJ) and 0.02% Ground Garlic (GG) aqueous solution

Peeled garlic cloves were obtained from a local supermarket in Bursa. Cloves (100 g) were ground to fine particles and crushed by a sterile pestle and mortar. Juice was extracted using a sterile cheese cloth (Indu et al., 2006). For our other study, garlic particles were added to sterile deionized water to prepare a 0.02% ground garlic aqueous solution.

2.5 Effect of garlic on test strains

The antimicrobial activity of FGJ and 0.02% GG aqueous solution test microorganisms were specified by disk diffusion assay (García-Díez et al., 2016). The purpose of using FGJ was to determine the resistance of the test microorganisms against undiluted antimicrobial compounds of garlic. The selected ground garlic ratio was determined by reference to the garlic ratio used in commercial products. Yoghurt and probiotic cultures were separately inoculated into the MRS broth. Using the spread plate technique, a 100 μL inoculum (> $2.0x10^7$ CFU/mL) was spread on the MRS agar surface with an L-shaped spreader. After sufficient drying of the medium, sterilized discs (Whatman filter paper No. 1 discs 6 mm in diameter) were placed on the agar surface. Sterile discs were impregnated with 10 μL prepared FGJ and GG aqueous solution separately in different plates. Results

Table 1. Enumeration conditions of strains.

Parameters	Growth media	Temperature (°C)	Incubation time (h)	Oxygen situation	Reference
L. bulgaricus	MRS	42	72	anaerobic	Dave & Shah (1998)
S. thermophilus	M17 (contains %0.5 lactose)	37	48	aerobic	Ravula & Shah (1998)
L. acidophilus 74-2	MRS	37	72	aerobic	Pyar & Peh (2014)
L. rhamnosus HN001™	MRS	37	48	anaerobic	Bovo et al. (2014)
B. longum BB536	MRS	37	48	anaerobic	Mei et al. (2010)

were given as clear zone diameters. In another experiment, each bacteria strain was inoculated in the 100 mL MRS broths with 0.02% GG and control medium, then were enumerated at the 0 and 24th h. *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus,* and *Bifidobacterium longum* were incubated under anaerobic conditions, while others were incubated under aerobic conditions as indicated in Table 1.

2.6 Statistical analysis

Each assay was run in triplicate. Results were given as the mean ± standard deviation (SD). Statistical analysis was carried out by using the SPSS Software version 22.0. Firstly, data sets were analyzed in terms of conformity to the normal distribution with Kolmogorov Smirnov (if the number of data > 30) or Shapiro Wilk (if the number of data < 30) test. The Kruskal Wallis and Mann-Whitney U tests, which are non-parametric tests, were used for non-normal distribution data. Data sets that provided parametric conditions were analyzed by using One-Way ANOVA and Tukey multiple comparison tests with a level of significance at $p < 0.05$. The relationship of bacterial growth and pH was evaluated using correlation analysis. Kendall's tau b correlation test is used because the data sets did not conform to the normal distribution. Absolute correlation coefficients are classified according to Schober et al. (2018) as very "weak" or "negligible correlation" (0.00-0.10), "weak" (0.10-0.39), "moderate" (0.40-0.69), "strong" (0.70-0.89), and "very strong" (0.90-1.0). Also, the sign of this coefficient indicates whether the correlation is positive or negative (Altuntas et al., 2018).

3 Results and discussion

3.1 Growth characteristics of the test bacteria

Figure 1 illustrates the growth curves of the bacteria and the pH change in the medium during incubation. Since there was no significant difference between triplicates for each bacteria strain, the growth curve data were obtained by taking mean values into account.

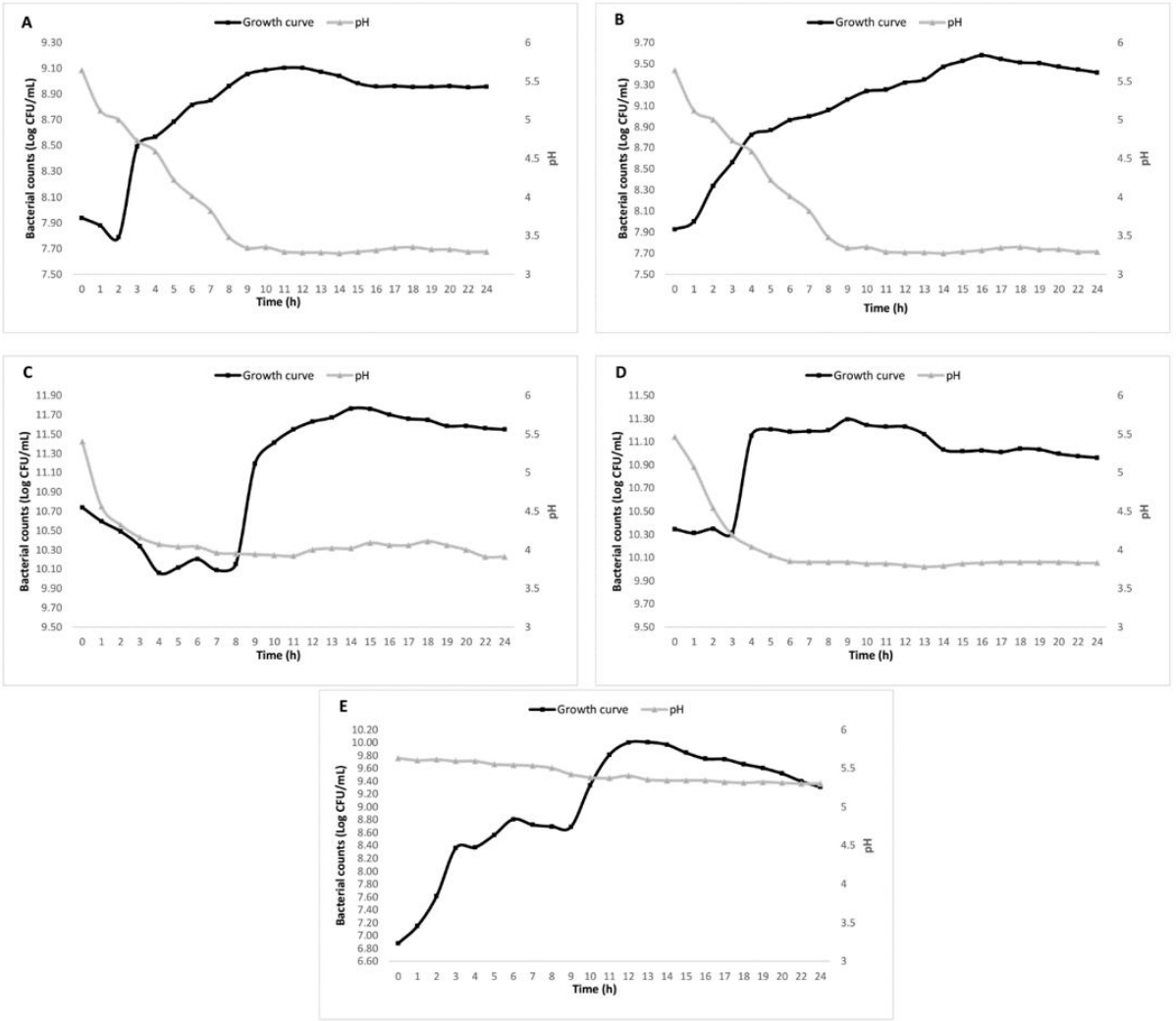

Figure 1. Growth curves of test strains and pH values in MRS broth at 37 °C. **A)** *L. bulgaricus* (Peyma-Chr. Hansen, Hoersholm, Denmark); **B)** *S. thermophilus* (Peyma-Chr. Hansen, Hoersholm, Denmark), yoghurt bacteria were in co-culture; **C)** *L. acidophilus* 74-2 (Dupont Copenhagen, Denmark); **D)** *L. rhamnosus* HN001™ (Dupont Copenhagen, Denmark) and **E)** *B. longum* BB536 (Dupont Copenhagen, Denmark).

Table 2. Growth parameters and decrease of pH from initial to end of incubation.

Parameters	*L. bulgaricus*	*S. thermophilus*	*L. acidophilus*	*L. rhamnosus*	*B. longum*
Growth rate (μmax)(h^{-1})	0.518 ± 0.046*C	0.619 ± 0.026C	1.451 ± 0.129B	1.93 ± 0.098A	0.46 ± 0.024C
Doubling time (t_d)	1.35 ± 0.124A	1.12 ± 0.048A	0.48 ± 0.044B	0.36 ± 0.019C	1.51 ± 0.084A
Lag phase duration (λ) (h)	2	1	8	3	nd **
Initial pH (0 h)	5.64 ± 0.12A	5.64 ± 0.12A	5.40 ± 0.06B	5.46 ± 0.08B	5.63 ± 0.07A
Final pH (24 h)	3.29 ± 0.08C	3.29 ± 0.8C	3.91 ± 0.05B	3.83 ± 0.05B	5.30 ± 0.13A
Unit decrease in pH	2.35	2.35	1.49	1.63	0.33
Correlation coefficient of pH and bacterial growth (r) ***	−0.654(m)	−0.615(m)	−0.007****	−0.275(vw)	−0.478(m)

* Mean values in the same row with different upper-case letters (A-C) are significantly different ($p < 0.05$) (Mean ± S.D.; n=3), ** nd; not detected, *** Correlation coefficient interpretation is indicated as vs: very strong (0.90-1.00) s; strong (0.70-0.89) m; moderate (0.40-0.69) w; weak (0.10-0.39) vw; very weak or negligible correlation (0.00-0.10), **** Correlation is not statistically significant at $p < 0.05$, *L. bulgaricus* (Peyma-Chr. Hansen, Hoersholm, Denmark) and *S. thermophilus* (Peyma-Chr. Hansen, Hoersholm, Denmark) were in co-culture; *L. acidophilus* 74-2, *L. rhamnosus* HN001™ and B. longum BB536 (Dupont Copenhagen, Denmark) were pure culture individually.

For *L. bulgaricus*, it was found that the first 2 h were the lag phase, and in the next 3 h microorganisms exhibited rapid growth, which was called the exponential phase (Figure 1A). The specific growth rate (h^{-1}) and the doubling time (h) (Table 2) were calculated as 0.518 h^{-1} and 1.35 h, respectively. After 24 h of incubation, the decrease in pH was determined as 2.35 units, which reached a more acidic pH than others, and the correlation between growth and pH was determined as moderately negative. In another study, the specific growth rate of *L. bulgaricus* was found to be 0.014 h^{-1} by using optical density at 37 °C in the MRS broth medium (Meleigy & Hendawy, 2009). It was observed that this value is considerably lower than those obtained in this study. Despite the use of the same temperature and medium, different results can be explained by the use of different strains of *L. bulgaricus* and the co-culture situation. Elsayed et al. (2014) determined that, after the first 2 h, *L. bulgaricus* entered the exponential phase as in our study, and the specific growth rate was found to be 0.47 h^{-1} in SPY-10 (Soy Peptone Yeast) medium at 37 °C.

It was observed that, after the first h, *S. thermophilus* grew rapidly until the 4th h in the exponential phase, and the number of bacteria continued to increase until the 16th h by the deceleration of growth (Figure 1B). *S. thermophilus* and *L. bulgaricus* cultures were inoculated with mixed cultures as mentioned previously, and the pH value of 5.64 was reduced to 3.29 after 24 h (Table 2). A study by Soydemir (2008) reported that the specific growth rate of four different strains of *S. thermophilus*, which were activated in the M17 broth at 43 °C, ranged between 0.610-0.674 h^{-1}. Similarly, the specific growth rate obtained in this study was 0, 619 h^{-1}. In contrast, according to Adamberg et al. (2003), the specific growth rate of *S. thermophilus* St20 was defined 2.2 h^{-1} at 45 °C in modified medium. In another experiment, it was reported that *S. thermophilus* Sfi39 and its three mutants had no lag phase, and the specific growth rate changed between 1.25 – 2.31 h^{-1} in M17 broth supplemented with 1% (w/v) lactose (LM17) at 42 °C (Zotta et al., 2009). These findings suggest that medium, temperature, and strain can influence the growth parameters (Czárán et. al., 2018). A symbiotic relationship between *L. bulgaricus* and *S. thermophilus* has been well documented (Wang et al., 2016). It is known that at the beginning of the incubation, *S. thermophilus* grow faster than *L. bulgaricus*. A similar tendency was observed in the current study.

In a study by Pyar & Peh (2014), *L. acidophilus* 74-2 was enumerated aerobically because there was no statistically significant difference in media under both aerobic and anaerobic conditions. In the growth curve of *L. acidophilus* 74-2, which had the longest adaptation period among the bacterial cultures, the lag phase lasted for the first 8 h (Figure 1C). The MRS broth medium decreased from 5.40 to 3.91 pH at the end of 24 h, and the correlation between pH and growth between pH and growth was not found statistically significant. A previous study demonstrated that *L. acidophilus* reached a stationary phase at the same time as in the present study (Nurhajati et al., 2010). Brizuela et al. (2001) stated that *L. acidophilus*' (B / 103-1-5) growth curve was obtained by optical density measurement at 2 h intervals at 37 °C for 24 hours in a MRS liquid medium, the specific growth rate was 0.493 h^{-1}, and the doubling time was 1.4 h. The shorter doubling time of *L. acidophilus* 74-2 used in the study shows that the values are different in a strain-by-strain comparison despite having the same temperature and medium. But by comparing the study, which was published by Ahn et al. (2002), *L. acidophilus* strains were found to be similar in terms of growth parameters under the same growth conditions.

As shown in Figure 1D, with *L. rhamnosus* HN001™, the lag phase continued for the first 3 h after the exponential phase was observed, between 3 and 4 h. The specific growth rate was 1.93 h^{-1}, and the doubling time was 0.36 h. MedveDova´ et al. (2008) investigated the growth of *L. rhamnosus* GG (ATCC 53103) strain in milk and MRS broth at different temperatures (6-41 °C). The results revealed that the influence of different temperature and media on growth was significant, and the doubling time decreased when the temperature increased. In another study by Makras et al. (2006), the specific growth rate in the MRS broth medium at 37 °C was 0.82 h^{-1}. The fact that the results obtained in the study were different from the literature indicated that the values were variable among the strains strains (Pellegrino et al., 2018; Tarrah et al., 2018). In the current study, the growth patterns of *L. acidophilus* and *L. rhamnosus* are similar in terms of statistics under the same growth conditions.

The growth curve of the *B. longum* BB536 strain displayed an increase in viable count from the first h of incubation, and a lag phase was not detected (Figure 1E). The specific growth rate and doubling time were 0.46 h^{-1} and 1.51 h respectively, and the obtained data were in agreement with Mahmoudi et al. (2013). The growth curve fluctuated, and these results were confirmed by the replicates. The pH decreased from 5.63 to 5.30. Compared to yoghurt bacteria and other probiotics, it was determined that *B. longum* was the bacteria that minimally changes the pH value in the MRS liquid medium which was statistically significant (Table 2). Previous experimental data have shown that *Bifidobacterium* spp. could not grow at pH values of 4.50-5.00, and the optimum growth pH is 5.50-6.50 with a temperature of 37 °C (Meena et al., 2011; Garro et al., 2006). In this case, the growth conditions are suitable for *B. longum*. Amaretti et al. (2007), studying the growth kinetics of *Bifidobacterium adolescentis* MB239 strain with different carbohydrate sources, found that the maximum biomass yield and specific growth rate were at pH 5.50, but the acid production did not have linear correlation with these parameters; the lowest level was found to be pH 5.50. This research may explain specifically why in this study the development of acidity was slow despite the high bacterial growth.

3.2 Effect of garlic on probiotics and yoghurt bacteria

The enumeration of each bacteria in the control and the broth containing 0.02% *GG* revealed that the probiotic and yoghurt bacteria have not been adversely affected in a significant way by the given ratio of garlic. On the contrary, it is possible to say that at the end of 24 h the counts of some strains increased even though they were not statistically significant (Table 3). Also, Zhang et al. (2013) stated that garlic fructan can be used as a carbon source for bifidobacteria due to its being classified as non-digestible carbohydrates. Similarly, Shalini et al. (2017) reported that garlic fructan is an effective prebiotic for *L. casei*. Therefore; certain quantities of garlic, especially those below the antimicrobial activity concentration, may help to selectively promote the growth of beneficial bacteria in the gut. Michael et al. (2010) investigated the effect of a plant extract (olive, garlic, onion, and citrus with sodium acetate) on a yoghurt starter culture and showed that it improved the viability of *L. bulgaricus* in particular over 29 days (maintained 6 log CFU/mL).

In a study in which extracts of 37 fruits, vegetables, and spices obtained by four different extraction methods were used to monitor the growth curve of probiotics along with the control group, it was discovered that as aqueous extract of garlic has a growth promoting effect on *Lactobacillus reuteri* and *L. rhamnosus* (Sutherland et al., 2009). However, Booyens et al. (2014), using scanning electron microscopy micrographs, showed that the amount of allicin in minimal bactericidal concentrations had a killer effect on bifidobacteria similar to that exhibited in pathogens.

All strains except *L. acidophilus* 74-2 were inhibited at different levels by 10 µL FGJ. A similar finding was obtained by Booyens & Thantsha (2013) where *L. acidophilus* La14 150B did not show an inhibition zone against a 30 µL garlic extract. This result is important because at least 10^6 CFU /g viable cells are expected from probiotic intake throughout the shelf life of the product (Settachaimongkon et al., 2014). However, Tas (2008) reported that the diameter of the inhibition zone for *L. acidophilus* LAB108 was 27 mm when 100 µL garlic extract is applied. One of the possible explanation for these differences could be that the amount of garlic extract used in the experiment was 10 times more than our usage.

It was observed that *B. longum* BB536 was the most sensitive bacteria, and the susceptibility pattern was *S. thermophilus*, *L. rhamnosus* HN001™, and *L. bulgaricus* with 28.16 ± 1.17, 18.81 ± 0.82, and 15.11 ± 1.49 mm zone diameter, respectively (Table 3). Results showed that there were statistically significant differences between the groups. Booyens & Thantsha (2013) determined that *Bifidobacterium lactis* Bi-07 300B was the most resistant, and *B. longum* BB536 was the most susceptible bacteria in the study on the effect of garlic among *Bifidobacterium* spp. by disk diffusion assay. In that study, it is stated that the zone diameter of *B. longum* BB536 was 31.3 ± 2.3 mm against 30 µL garlic extract which is higher zone diameter than our result. This result proves that different garlic origin or extraction method may have different antimicrobial effects on the same strain. Therefore, the comparison of the amount of antimicrobial compound may be a more accurate approach. The our disk diffusion assay results of 0.02% GG aqueous solution showed that a possible usage rate of garlic in (an appetizer, is made of strained yoghurt, cucumber, salt, grounded garlic, mint and olive oil) or haydari (an appetizer, is made of very thick yoghurt, feta cheese, chopped dill, mint, grounded garlic and olive oil)

Table 3. The effect of garlic on yoghurt and probiotic strains.

Strain	Enumeration assay (log CFU/mL)*				Disk diffusion assay(mm)**	
	0.h		24.h		FGJ****	GG
	Control	0.02% GG	Control	0.02% GG		
L. bulgaricus	7.94 ± 0.04***B	7.94 ± 0.03B	9.55 ± 0.40A	10.22 ± 0.16A	15.11 ± 1.49d	nd
S. thermophilus	7.93 ± 0.02B	7.91 ± 0.09B	9.26 ± 0.19A	9.46 ± 0.28A	28.16 ± 1.17b	nd
L. acidophilus 74-2	10.74 ± 0.02B	10.72 ± 0.09B	11.61 ± 0.13A	11.47 ± 0.12A	nd*****	nd
L. rhamnosus HN001™	10.35 ± 0.01B	10.36 ± 0.21B	10.86 ± 0.12A	10.57 ± 0.02A	18.81 ± 0.82c	nd
B. longum BB536	6.88 ± 0.07B	6.86 ± 0.10B	9.51 ± 0.27A	9.86 ± 0.12A	49.37 ± 1.07a	nd

* Significant difference ($p < 0.05$) did not found between control and 0.02% GG (ground garlic) for 0 h and 24 h separately by Mann Whitney U test; ** Significant difference ($p < 0.05$) found between groups by One-way ANOVA; *** Mean values in the same row with different upper-case letters (A-B) are significantly different ($p < 0.05$) (Mean ± S.D.; n=3); **** Mean values in the same column with different lower-case letters (a-b) for FGJ (fresh garlic juice) are significantly different ($p < 0.05$) *****nd; not detected. *L. bulgaricus* (Peyma-Chr. Hansen, Hoersholm, Denmark) and *S. thermophilus* (Peyma-Chr. Hansen, Hoersholm, Denmark) were in co-culture; *L. acidophilus* 74-2, *L. rhamnosus* HN001™ and *B. longum* BB536 (Dupont Copenhagen, Denmark) were pure culture individually.

did not have an adverse effect on test strains. This finding is remarkable regarding the production of new probiotic foods by improving palatability.

As far as we know, this is the first study researching the effect of garlic on yoghurt bacteria by disk diffusion assay although there are numerous studies examining the inhibitory effect of garlic on some pathogenic microorganisms. In this study, it was determined that both bacteria were adversely affected, and *S. thermophilus* was more sensitive than *L. bulgaricus* against FGJ, but a 0.02% GG aqueous solution was not effective enough to inhibit growth.

4 Conclusion

In this paper, we have demonstrated that, as expected, yoghurt and some probiotic strains have different growth parameters and abilities to decrease pH in same medium and temperature. While the lag phase duration of *L. acidophilus* 74-2 took the longest time of all the test strains, *S. thermophilus* and *B. longum* adapted for a short time. It is remarkable that the pH of medium containing *B. longum* maintained at 5.30 at the end of incubation despite the fact that growth had occurred. Further study is required to evaluate the growth mechanism of *B. longum* and its relationship to pH.

We also concluded that garlic has an antimicrobial effect on probiotic and yoghurt bacteria, but it may play a role in improving the growth of certain strains or be ineffective depending on the usage rate of garlic. Yoghurt is still the most commonly used vehicle for incorporation of probiotic microorganisms into the diet. Therefore, the growth characteristics of certain strains and the effect of food additives on the strains before usage in a new food matrix is important knowledge. This study contributes to our understanding of the probable behavior of probiotic strains against garlic. The promising results obtained in this study encourage further research on the food matrix in order to evaluate the survival of probiotic strains during storage.

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