Journal of Animal and Veterinary Advances 8 (12): 2432-2437, 2009
ISSN: 1680-5593

The Effect of Fructose, Prolin, Initial Doses and Different Temperatures on the Growth and Metabolism of *Lactobacillus acidophilus La5*

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Abstract: Probiotics are useful bacteria, which after consumption, inhibit the harmful microorganisms in the intestines and leave useful effects on human health. The biological activity of probiotics is affected by various environmental factors each of which can influence the performance as well as the growth of probiotics for increasing the health of the consumers. This study aims to study the effect of fructose, prolin doses of 0.5, 1, 1.5 and 2% of the primary culture and temperatures of 35, 38, 41 and 44°C on the growth as well as metabolism and metabolism of *Lactobacillus acidophilus La5* in sterilized milk. In order to do this, the milk fermented with *Lactobacillus acidophilus La5* (as the culture) and incubated at 35, 38, 41 and 44°C. The acidity and the pH of the milk samples were measured at 0, 2, 4 and 8 h and the total number of microorganisms were counted at 0, 4 and 8 h after the incubation. In order to measure the effect of fructose and prolin, 0.75% (w v^{-1}) of fructose and 1% (w v^{-1}) prolin was added to the milk samples and samples together with the control sample, was kept in incubator at 41°C and then acidity and pH of was measured and the total number of *lactobacillus acidophilus* was counted at 0, 2, 4 and 8 h after incubation. All the above trials were replicated ten times. The findings of the study using statistical tests indicated that the total number of bacteria and the amount of acidity in incubated milk samples at 41 and 44°C were significantly greater than the other temperatures ($p<0.05$). The multiplication rate of the total hours of bacteria in samples containing 2% of culture during the fourth hour of incubation was significantly greater than the other samples ($p<0.05$). However, this difference in 8 h after the incubation was not significant. Concerning the use of probiotic, fructose and prolin, the total number of bacteria and acidity in the milk samples containing fructose was significantly higher those of prolin and sample containing control sample ($p<0.05$).

Key words: Probiotic, *Lactobacillus acidophilus*, fructose, prolin, growth

INTRODUCTION

It has been documented that probiotics have been used as food supplements to prevent diseases and improve the health of both human beings and animals. Probiotics act differently from antibiotics, probiotics are living micro-organisms that help the useful existed micro-organisms to grow in the gut and thus maintain the of their hosts (Merete and Wicklund, 2004). Nowadays, probiotics are widely used in the production of various kinds of foods not only to boost growth but also to improve the immune system and prevent many diseases. This useful effect can theoretically arise from one of the following mechanisms:

- Weakening reactions, which cause toxic and carcinogenic metabolites
- Enhancing enzymatic reactions involved in detoxifying potentially toxic materials, which either have been swallowed or produced by the body
- Inducing enzymes in primates to digest complicated foods or helping the body provide enzymes through bacteria
- Producing vitamins and other necessary nutrients, which are not adequately found in the food basket (Lourens and Viljeon, 2001; Tamime, 2005; Farkhondeh, 1992; Malekzadeh, 2003)

Lactobacillus acidophilus, which has predominantly been used in the past two decades in producing probiotic foods (Huebner and Wehling, 2007; Saarela *et al*., 2000; Fooler, 1993). The first step in producing fermented products including probiotic ones is identifying technological features and essential needs of the

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micro-rganisms. As the amount of growth and durability of probiotics is low in dairy products, peptide amino acids and indigestible oligosaccharides are also used in order to increase both the time span and the quality of probiotic products. Probiotic products, together with prebiotic ones, play an important role in maintaining micro floral balance in intestines (Mirzai *et al*., 2006). Among the factors, which can greatly affect the growth rate of such bacteria is studying and determining the best dose and also the best temperature at which this microorganism can grow and metabolize (Karim, 2003; Mutlu and Guler, 2007; Gims, 1998). The aim of the present study was to determine the effect of 35, 38, 41 and 44°C temperatures and also to determine the best dose as well as the effect of adding 0.75% (w v^{-1}) fructose and 1% (w v^{-1}) prolin on the growth rate and metabolism of *lactobacillus Acidophilus La5* in sterilized milk.

MATERIALS AND METHODS

UHT sterilized milk containing 1.5% fat, inulin, lactose broth culture medium and MRS agar produced by Merck Co., NAOH produced by ASIA Co. and *Lactobacillus acidophilus La5* strain produced by Cher-hansen Co Danmark.

Preparation of culture starter containing *Lactobacillus acidophilus La5:* It was recommended by the Cher-hansen Co. from which the probiotic species was obtained that 2 g of the strain should added onto an Erlenmeyer flask containing 100 mL of lactose broth and that it should be incubated for 48 h at 37°C. Afterwards, 5 mL was removed from the prepared culture and was added to 1000 mL of sterilized milk containing 1.5% fat heated at 40°C. The resulting sample was then homogenized and incubated at 37°C so that the acidity of the milk reached approximately, 80°C as Dornic's scale. This trial was repeated 3 times in order to meet the necessary conditions for probiotic strain growth. The last fermented milk sample was used as the primary starter culture in various tests.

Determining the optimum temperature for growth: To determine the optimum temperature, one liter of low-fat sterilized milk was incubated in an Erlenmeyer flask for 20 min at 80°C. Then the temperature was reduced to about 40°C by adding cold water. Afterwards 5 mL of the primary starter culture was removed and aseptically, streaked onto the prepared milk and homogenized. The resulting sample was equally distributed into four 250 mL Erlenmeyer flasks to be incubated at 35, 38, 41 and 44°C as exposed to flames. After 0, 2, 4, 6 and 8 h of incubation, the level of pH was measured by means of pH meter and the acidity was measured using titration method total microbial count after 0, 4 and 8 h of incubation was measured using in MRS agar. This process was repeated ten times.

The study of the primary dose effect on the amount of growth and metabolism of *Lactobacillus acidophilus La5*: To reach this goal, a liter of 1.5% fat sterilized milk was equally distributed into four 250 mL Erlenmeyer flasks, where they were exposed to flames. After that 2, 4, 6 and 8 mL of the primary starter culture, which had reached 80°C Dornic were, respectively added to each of the flasks and was incubated at the optimal temperature as measured earlier. The amount of acidity and pH were measured after 0, 2, 4, 6 and 8 h of incubation. The total bacterial count was measured after 0, 4 and 8 h of incubation using surface plate count in MRS agar.

The study of the effect of fructose and prolin on the rate of growth and metabolism of *Lactobacillus acidophilus La5*: To reach this goal, at first 750 mL of 1.5% fat sterilized milk was heated at 80°C for 20 min. Its temperature was later reduced to about 40°C by adding cold water. Then, it was equally distributed into three 250 mL Erlenmeyer flasks to be exposed to flames. Subsequently, an optimal dose of the primary starter culture was added to each of the flasks. After homogenization, 0.75% (w v^{-1}) of fructose was added into one of the flasks, 1% (w v^{-1}) of prolin was added into another flask and nothing was added into the third one, which was to be used as the control sample later on. Then the flasks were incubated at the optimal temperature and the amount of acidity and pH were measured after 0, 2, 4, 6 and 8 h of incubation. The total bacterial count was measured after 0, 4 and 8 h of incubation by means of surface plate count in MRS agar culture environment.

RESULTS AND DISCUSSION

The results obtained from the experiments conducted on determining the optimal temperature for the growth of micro-organism, the effect of initial dose on its growth rate and the effect of adding fructose and prolin are presented in the Fig. 1-3.

As it can be observed from Table 1, in the one-way statistical analysis and the Toki follow-up test in the surface of $\alpha = 0.5$, the average acidity in the incubated samples in 41 and 44°C after 4, 6 and 8 h and that of the 37°C after 4 and 6 h of incubation were significantly higher than other samples ($p<0.05$).

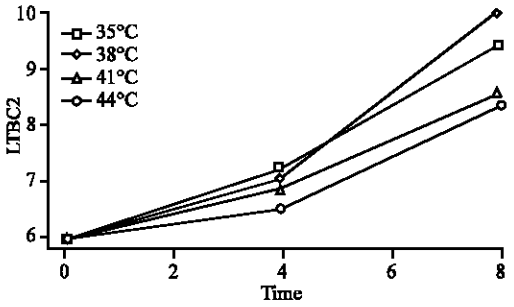

Fig. 1: The total microbial count for milk samples incubated at 35, 38, 41 and 44°C

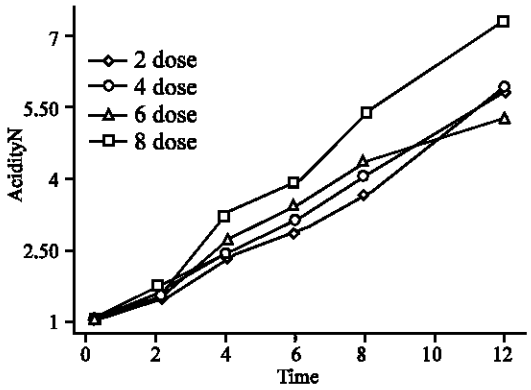

Fig. 2: The sample milk acidity containing different doses of probiotic strain during incubation

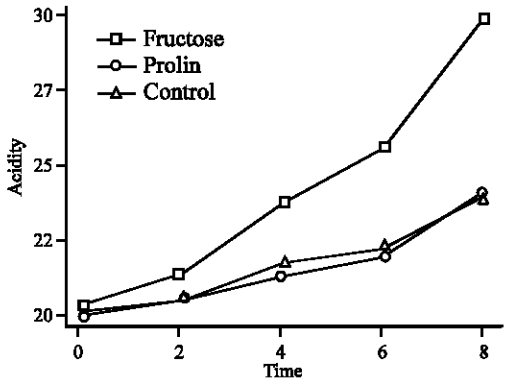

Fig. 3: The acidity of probiotic milk samples containing fructose and prolin compared with the control milk sample during incubation

As it can be seen from Table 2, in the one-way statistical analysis and Toki follow-up test in the surface of $\alpha = 0.5$, the average log of the number of *L. acidophilus* in the milk samples incubated at 41 and 44°C for 8 h was significantly higher than that of other samples ($p<0.05$).

As shown in Table 3, in the one-way statistical analysis and Toki follow-up test in the surface of $\alpha = 0.5$, the average increase in the acidity of the samples containing 2% of the culture of *L. acidophilus* after 6, 8 and 12 h of incubation was significantly higher than that of samples containing 0.5 of *Lactobacillus acidophilus* starter culture ($p<0.05$).

As shown in Table 4, in the one-way statistical analysis and the Toki follow-up test in the surface of $\alpha = 0.5$, the average log of the number of *L. acidophilus* in samples containing 1.5 and 2% *L. acidophilus* starter culture after 4 h of incubation was significantly higher than that of samples containing 0.5 and 1% starter ($p<0.05$).

As shown in Table 5, in the one-way statistical analysis and Toki follow-up test in the surface of $\alpha = 0.5$, the average acidity of the milk samples containing fructose after 2, 4, 6 and 8 h of incubation was significantly higher than that of the control samples and prolin-containing samples ($p<0.05$).

As shown in Table 6, in the one-way statistical analysis and Toki follow-up test in the surface of $\alpha = 0.5$, the average log of the number of *L. acidophilus* in the samples containing fructose after 4 and 8 h of incubation was significantly higher than that of the control and prolin-containing samples ($p<0.05$).

One of the external factors affecting the rate of growth, metabolism and multiplication of bacteria is determining the optimal growth temperature. This is of course of utmost importance in supplying probiotic products and simultaneous growth of bacteria. By definition, in probiotic products there should at least 6 log CFU mL^{-1} of microorganism, so that such products will have useful effects on consumers (Razavilar, 2003; Mirzai, 2005; Vinderola *et al*., 2000).

The results from the present study showed that metabolism and growth rate of *L. acidophilus La5* at 41 and 44°C were significantly higher than that of 35 and 38°C ($p<0.05$) (Table 1, 2 and Fig. 1).

Of the various tested temperatures, 37°C was the best temperature for growth and metabolism of a number probiotic *L. acidophilus* strains for producing probiotic yoghurt.

In another research study, it was reported that 37-40°C incubation temperature are the best temperatures for increasing the growth rate of probiotic strains. In another study run on a number of probiotic strains including *L. acidophilus* 1748 and *L. rhamnosus GG*, it was observed that of the 20, 30, 37 and 45°C temperatures, which were experimented for growth of species, the best was 37°C.

The results obtained from the test on the best microbial dose for the growth and metabolism of

Table 1: The average acidity of milk samples incubated al 35, 38, 41 and 44°C for different periods

	Incubation time (h)				
Temp. (°C)	0	2	4	6	8
35	15.9±0.31a	17.6±0.52a	18.00±0.00a	19.35±0.47a	21.40±0.99a
37	16.00±0.23a	17.75±0.54a	18.70±0.42a	20.5±0.47a	22.35±0.81a
41	1 5.95±0.15a	18.10±0.61a	19.95±1.15b	23.40±1.17b	30.90±4.3b
44	16.00±0.00a	18.5±0.64a	20.30±1.03b	21.0±2.01b	27.50±3.9b

Table 2: The average log of the number of *L. acidophilus* in the samples incubated at 35, 38, 41 and 44°C for different lengths of incubation

	Incubation time (h)		
Temp. (°C)	0	4	8
35	5.78±0.72a	6.53±0.38a	8.36±0.57a
37	5.52±0.66a	6.90±1.0a	8.6±0.76a
41	6.1±0.50a	7.04±0.94a	10.0±49.00b
44	6.2±0.70a	7.2±0.95a	9.5±0.26b

Table 3: The average increase in the acidity of the samples containing 0.5, 1, 1.5 and 2% doses of *Lactobacillus acidophilus* culture for different periods of incubation time

Primary starter dosage	Incubation time (h)				
starter (%)	2	4	6	8	12
0.5	1.05±0.49a	2.00±0.66a	2.75±0.48a	3.90±0.80a	7.00±0.50a
1	1.00±0.52a	2.10±0.73a	3.50±0.64ab	4.40±0.99a	6.80±0.75ab
1.5	0.70±0.58a	2.45±0.54a	3.50±1.26ab	4.80±1.1a	6.10±0.89ab
2	0.80±0.67a	3.25±1.33a	4.25±1.56b	6.37±1.37b	7.22±1.75b

a and b: The difference between the averages, which do not have common letters in significant

Table 4: The average log of the number of *Lb. acidophilus* (CFU mL^{-1}) in the samples containing 0.5, 1.5 and 2% of *Lactobacillus acidophilus* starter culture for different periods of incubation time

Primary dosage	Incubation time (h)		
starter (%)	0	4	8
0.5	6.99±0.61a	7/43±0.77a	9.41±1.21a
1	6.92±0.95a	8.28±1.07ab	9.77±0.82a
1.5	7.20±0.52a	8.74±0.62b	10.09±0.58a
2	7.49±0.94	9.12±1.11b	10.30±0.11a

a and b: The difference between the averages, which do not have common letters in significant

L. acidophilus La5 showed that if the probiotic dose is higher at the first stage, a shorter incubation period will be needed and as a result the product will attain the target pH for producing probiotic products.

However, it has to be considered that a higher dose should not only be justified economically, but also it should be clarified, whether this higher dose and shorter incubation period have any effects on the orgnoloptic properties of the products or not.

Based on the results obtained from this study after 6 h of incubation, the number of bacteria in the samples containing 1.5 and 2% were significantly higher than those in the samples containing 0.5 of primary culture starter. However, after 8 h of incubation, this difference was insignificant (Table 3, 4 and Fig. 2).

This study also showed that the amount of the primary starter dose at early stages of incubation can affect the number of bacteria and the acidity of the cultured samples. Nevertheless, this effect will in the long run fade away gradually, which can be due to the production of limiting metabolites including all kinds of organic acids and a considerable decrease of the pH.

This process will in turn cause a drop in the amount of the rising growth of the probiotic strain. Hild and Heland (2003) showed that if the pH in the probiotic products reaches levels 4.1-4.4, the amount of strain growth in lactobacillus rhamnosus GG, *L. acidophilus* 1748 and bifidobacterium bifidium will fall drastically due to the production of acidic metabolites and a dramatic fall in pH.

The results obtained from assessing the influence of fructose and prolin on the growth rate and metabolism of *Lactobacillus acidophilus La5* showed that by adding 0.75% (w v^{-1}) fructose to the sterilized samples of milk containing probiotic strains will bring about a significant change in the amount of acidity rise, total microbial load and a decreased pH relative to the control sample and the sample containing 1% (w v^{-1}) prolin (Table 5, 6 and Fig. 3).

In a research study, Hild and Treimo (2005) observed that *L. acidophilus La5* and rhamnosus GG will not grow well in milk enriched by tryptone and other prebiotics. Lourens and Viljoen (2001) reported that fructose and prolin compounds cause lactobacilli to grow.

Table 5: The average log of the number of *L. acidophilus* (CFU mL^{-1}) in control samples and samples containing fructose and prolin for different lengths of incubation time

	Incubation time (h)				
Prebiotic	0	2	4	6	8
Fructose	20.20±0.94a	21.30±0.71b	23.75±0.97b	25.70±0.91b	30.10±1.99b
Prolin	19.95±0.90a	20.35±0.57a	21.20±0.42a	21.90±0.80a	24/05±0.51a
Control	19.80±0.78a	20.40±0.5a	21.75±0.59a	22.25±0.42a	23.95±0.59a

a and b: The difference between the averages, which do not have common letters in significant

Table 6: The average log of the number of *Lactobacillus acidophilus* (CFU mL^{-1}) in control samples and samples containing fructose and prolin for different lengths of incubation time

	Incubation time (h)		
Prebiotic	0	4	8
Fructose	6.99±1.23a	8.41±0.57b	9.67±0.70b
Prolin	6.67±0.77a	7.21±0.55a	7.96±0.84a
Control	6.60±0.93a	7.70±0.60a	7.91±0.80a

a and b: the difference between the averages, which do not have common letters in significant

Hild and Heland (2003) used fructose to enhance growth and increase metabolism in different probiotic strains.

Fructoglicosachrids caused an increase in the growth of bifid bacterium strains and prevent the growth of pathogen microbes such as cholestridium and icholi in intestines due to fermentation and production of organic acids with a short chain.

CONCLUSION

It is concluded from this study that when fructose is added to milk, the growth of *Lactobacillus acidophilus La5* is significantly increased, whereas this is not true when prolin is added.

The results from the present study show that metabolism and growth rate of *L. acidophilus La5* at 41 and 44°C were significantly higher than other temperatures under study. The results obtained from the test on the best microbial dose showed that if the probiotic dose is higher at the first stage, a shorter incubation period will be needed.

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