GROWTH AND ACTIVITY OF BIFIDOBACTERIA IN MIXED CULTURE WITH *LACTOBACILLUS HELVETICUS*

E. Bărăscu* and J. Ciumac**
* Valahia University of Targovite, Faculty of Environment Engineering and Biotechnologies, Department of Food Products Engineering, 18-24 Unirii Street, 130082, Romania
E-mail: elbarascu72@mail.com and elena_barascu@yahoo.com
**Technical University of Moldova, Faculty of Technology and Management in Food Industry, Studentilor Str., 11, TUM, block of study no. 5, Republic of Moldova, MD-2045, Chisinau

Abstract

In this study the growth and activity of bifidobacteria in mixed culture with Lactobacillus helveticus were evaluated in order to use the results in the technology of probiotic dairy products. Bifidobacteria and Lactobacillus helveticus used in this experiment were simultaneously inoculated in milk samples and afterwards at different times. In optimum conditions of incubation the growth rate of bifidobacteria and the changes that occurred in their medium were monitored.
The populations of bifidobacteria in the variants of milk inoculated with Lb. helveticus after 5h from inoculation of bifidobacteria were higher than in variants simultaneously inoculated with the two cultures. The highest growth rate of bifidobacteria was achieved in variant of milk inoculated with bifidobacteria and Lactobacillus helveticus in the proportion of 6:2, where the population of bifidobacteria was 2.29 x 10^9 cfu/ ml.

Key words: probiotic, bifidobacteria, Lactobacillus helveticus, growth rate, metabolic activity

1. INTRODUCTION

Bifidobacteria were first isolated and described in 1899-1900 by Tissier, who described rod-shaped, nongas-producing, anaerobic microorganisms with bifid morphology, present in the faeces of breast-fed infants, witch he termed Bacillus bifidus. Bifidobacteria are generally characterized as gram-positive, non-spore forming, non-motile and catalase-negative anaerobes (Poupard [14], Romond [6], Leveau [4], Gournier-Chateau [3], Gomes [2], Roy [7]). They have various shapes including short curved rods, club-shaped rods and bifurcated Y-shaped rods. The optimum growth temperature for bifidobacteria is 37°C to 41°C (minimum growth temperature: 25-28°C; maximum growth temperature: 43-45°C) and the optimum pH is 6.5 to 7.0 (no growth at 4.5-5.0 at 8.0-8.5). Glucose is degraded exclusively and characteristically by the fructose-6-phosphate shunt (Gournier-Chateau [3], Gomes [2], Roy [7]). Fructose-6-phosphate phosphoketolase is the characteristic key enzyme of the bifid shunt that cleaves fructose-6-phosphate into acetyl phosphate and erythrose-4-phosphate. Acetic and lactic acid are formed primarily in the molar ratio of 3:2 (Gomes [2], Vuyst [13], Roy [7]). Beside glucose, all bifidobacteria from human origin are also able to utilize galactose, lactose and, usually, fructose as carbon sources (Gomes [2]) Many researchers think that bifidobacteria are prophylactic and therapeutic agents that have a potential health benefit, also having good effects on reestablishing and keeping a normal intestinal microbiota balance and reducing the risk of the intestinal cancer and the level of cholesterol and also increasing a certain number of vitamins in our bodies (B_1, B_2, B_6, K) which are vitamins they synthetize (Robinson [5], Sanders [8], Shah [11], Stanton [12]).
Bifidobacteria don't have a proteolytic activity, so they can't get their own assimilable nitrogen sources and necessary growth factors during nutrition. From this point of view cow milk hasn't got appropriate nutritional attributes for bifidobacteria and is considered to be an "artificial medium" for them. There are at least two solutions in order to improve growth of the probiotic bacteria: using some assimilable nitrogen sources or cultivating them in culture mix with proteolytic lactic acid bacteria. At present, the manufacture of probiotic dairy products put bifidobacteria together with

bacteria of yogurt and that could be a benefit for shortening the time needed during thermostation. Yet, growth of yogurt acidity during storage causes an undesired effect on the viability of bifidobacteria because of the post-acidification character of Lb. delbrueckii ssp. Bulgaricus (Dave [1], Shah [10], Stanton [12]).
It is important for them to stay alive during storage of product and to have a concentration at least 10^6 cells/gram when consumed in order to have a contribution on maintaining human body's health (Dave [1], Shah [10]).
In this study the improvement of growth of bifidobacteria in milk was taken, into consideration by cultivating them with Lactobacillus helveticus which is a strain with a moderate proteolitic activity.

2. MATERIALS AND METHODS

Bacterial Cultures

In this study two lyophilized cultures were used a probiotic one Bif species 420 and another one with a moderate proteolitic activity Lactobacillus helveticus 7 (Danisco Cultor, Germany). Prior to inoculation cultures were individually put into base medium (milk reconstituted with 12.4% nonfat dry matter) in order to hydrate cells and get a standardized inoculum.

Preparation of Fermented Milk

Reconstituted skim milk with 12.4% nonfat dry matter was heated at 85^0C for 15 minutes and cooled to 40^0C afterwards Bifidobacteria and Lactobacillus helveticus were simultaneously or at different times inoculated after 5 and 13 hours.
In the culture mix bifidobacteria and Lactobacillus helveticus existed in a ratio of 4: 2 and 6: 2 and a sample containing only bifidobacteria was used as a control (6%). The samples were thermostated at 37^0C until pH decreased to 4.5 in the mix. Then they were kept at 4^0C.

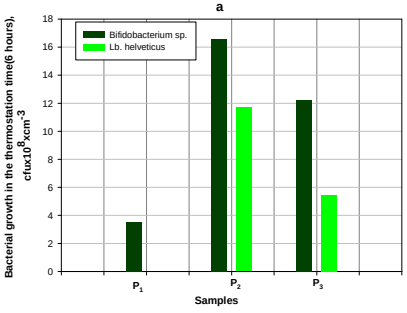
Microbiological and Physicochemical Determination

Milk samples aseptic harvested were immediately tested after inoculation, after 4 hours (in simultaneous inoculation) or after adding culture of Lactobacillus helveticus (in altered time inoculation) and the end of the fermentation process. Bacteria were counted after having made decimal dilutions through the agency of Breed Method. Titratable acidity expressed in grams of lactic acid/dm^3, was evaluated by making a titration with NaOH, 0.1 N, using phenolphthalein, as indicator until it got a shade of pink. A Denver pH-meter was used for determining the pH after a calibration made with protecting standard solutions recently prepared having a pH of 4 and 7. The score method was used for a sensory analysis of fermented milk samples (Segal [9]). A sensory evaluation of fermented milk sample was made by 10 tasters after 24 hours of storage in cold places.

3. RESULTS AND DISCUSSION

3.1. Simultaneous Inoculation of Bifidobacteria and Lactobacillus helveticus Cultures

After cultivating simultaneously such cultures in a ratio of 4: 2 (bifidobacteria Lactobacillus helveticus) a stimulation of bifidobacterial growth was noticed and their number was 4.7 higher than was in the control (figure 1a). During the same experiment acidity of the mix, increased from 1.46 to 6.26 grams of lactic acid/dm^3, after six hours and this increase was 2.17 bigger than was in the control (figure 1b). Proportionally to the growth of acidity the level of pH decreased and after a thermostation lasting 6 hours reached a smaller score (one unit less) than it did in the control as showed in figure 1c.



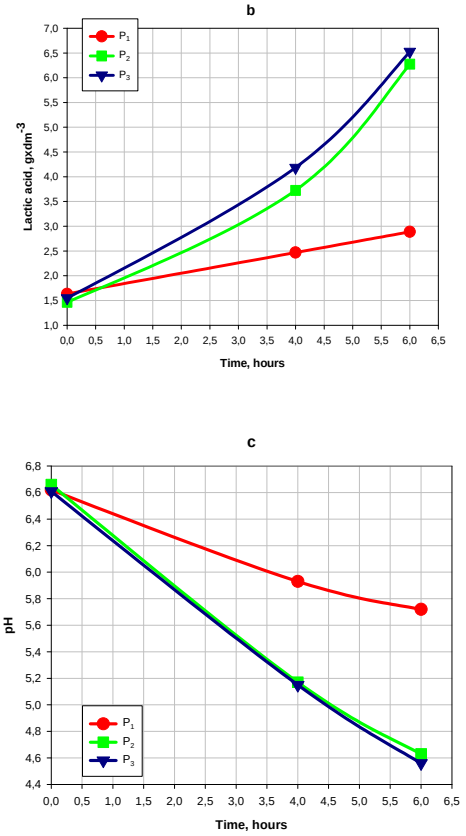
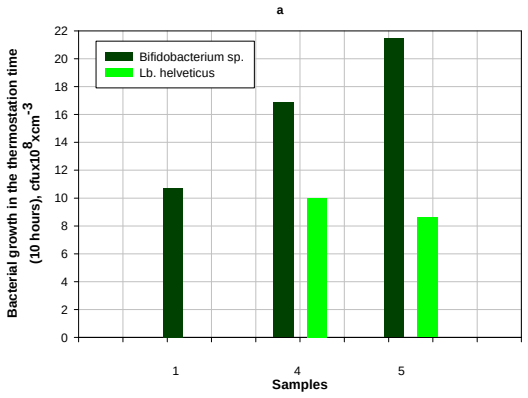


Figure 1. Growth and activity of bifidobacteria in case of simultaneous inoculation
a) growth dynamics of bifidobacteria in singular cultures or in culture mix
b) evolution of acidity in samples testated during thermostation
c) evolution of pH during this process
P1-control (Bif 6%);
P2- Bif 4% and Lactobacillus helveticus2%;
P3-Bif 6% si Lactobacillus helveticus 2%.

Using another variant of simultaneous culture of bifidobacteria with Lactobacillus helveticus associated in a ratio of 6: 2 a better growth of bifidobacteria was noticed (3.8 times better than in the control) but this was not as good as using a ratio of 4: 2 . As for the results got by evaluating the level of acidity and pH, no significant differences from previous evaluation were recorded. Consequently, speaking a boat growth the best results in the study of simultaneous cultures of bifidobacteria with Lactobacillus helveticus was got using an association of 4 to 2.

3.2. Inoculation of Lactobacillus helveticus after 5 hours from inoculation of bifidobacteria

Inoculating Lb. helveticus five hours after the inoculation of bifidobacteria, associated in a ratio of 4: 2 determined almost the same number of bifidobacteria ($1.82x10^9$cfu/ml) as the one got in simultaneous cultures, in the same range but is had the advantage of getting a lower acidity (with 1 gram of lactic acid /dm^3 less) and having a good effect on its sensory attributes. At the end of the thermostation process the growth rate of bifidobacteria was 43.6% greater then the one got by evaluating the control sample.
Altering the time needed for the operation but using a range of 6 to 2 a larger number of bifidobacteria was got in comparison with the variant previously discussed (figure 2a) and variants discussing about simultaneous cultures in the inoculation process. In this case bifidobacteria reached a maximum number of $2.29x10^9$ cfu/ml, which was 1.8 higher than in the control and 1.25 times higher than in the variant previously discussed. Speaking about acidity and pH, no significant differences from the results got in this variant to the inoculated in a range of 4 to 2 were noticed in conditions of altering the time necessary for this process. (figures 2b and 2c).



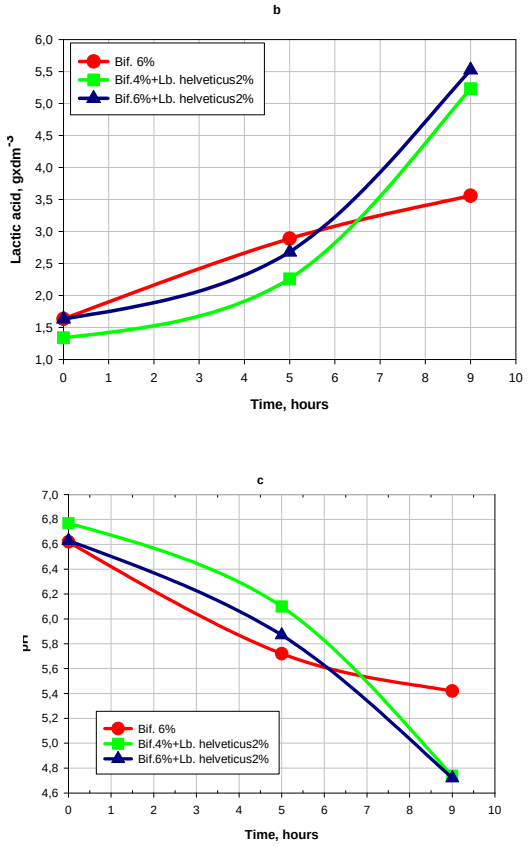


Figure 2. Growth and activity of bifidobacteria in case of 5 hours altered time inoculation
a) evolution of bifidobacterial number in singular and mixed cultures with Lactobacillus helveticus inoculated 5 hours after the first culture
b) dynamics of lactic acid accumulation during the fermentation process
c) variation of pH during thermostation

The acidity of samples inoculated at different times increased considerably after addition of Lactobacillus helveticus culture and reached a maximum score (5.52g lactic acid/dm^3) in the inoculated in a range of 6 to 2 which was 1.55 higher than in the control. Inoculating Lactobacillus helveticus culture to 13 hours after inoculating bifidobacteria, no satisfying results were obtained, so we won't talk about them in this study.

4. CONCLUSIONS

Making this study, we draw the conclusion that cultivating bifidobacteria with Lactobacillus helveticus would be a benefit for stimulating growth of bifidobacteria during the proteolytic activity and improving the connection existing between them.
Taking into account the sensory attributes and the number of bifidobacteria, the best result, was got by inoculating Lactobacillus helveticus 5 hours after inoculating bifidobacteria in a ratio of 6: 2.
The mix between bifidobacteria and Lactobacillus helveticus is more profitable than is the singular culture as it determines a maximum number of cells in a shorter time.

5. REFERENCES

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