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# Selection of Endemic Lactic Acid Bacteria for Lactose-Free Milk Production

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## ABSTRACT

The *β-galactosidase* activity of lactic acid bacteria isolated from traditional lactic acid product matzoon was studied. It has been shown that some endemic lactic acid bacteria isolated from the matzoon samples produced from cow milk have *β-galactosidase* activity. Three Strains have been selected out of 370 studied lactic acid bacteria.

*β-galactosidase* is of great practical importance and is also used for the production of glucose-galactose syrups, sweeteners, as well as for obtaining lactose-free milk and whey processing during waste disposal.

### Introduction

As a traditional and national Caucasian dairy product, matzoon is a symbiotic consortium of lactic acid bacteria and various types of saccharomyces.

Depending on the milk type, its composition and geographical origin, matzoon differs in taste, smell, content of nutrients and density. As a complete chemical food, it contains almost all the nutrients necessary for the human body. Various samples of matzoon differ in milk protein, fat, lactose, vitamins (*A, B, C, D, E*) and other features (Afrikian, 2009). Selected as a "probiotic" during natural evolution, matzoon cleanses the body from toxins, regulates metabolism, reduces the amount of cholesterol in the blood, increases the body's well-being, regulates colitis and intestinal dysfunction neutralizing the effects of drugs, especially antibiotic and hormonal ones (Hakobyan, et al., 2016).

It is well-known that milk contains about 4 % monosaccharide – lactose that some people are unable to digest.

In the absence of lactase which cleaves lactose, people become lactose intolerant, which does not depend on milk, but rather on the specificity of the human body or the lack of β-galactosidase (enzyme) activity (Bellmer, et al. 2005). A real salvation for such people is lactose-free milk, which containing all the useful substances does not have any undesirable effect on the gastrointestinal tract (Bury and Jelen, 2000). Lactase is widespread in nature. The plants

and microorganisms are the natural sources of the enzyme (Cebeci and Gürakan, 2003, Jurado, et al., 2002). Lactases obtained from lactic acid bacteria, which are absolutely safe for humans are of particular interest. Fungal strains are now widely used for the enzyme production, although the focus is on the lactic acid bacteria as they are safe and can be used as starting materials in dairy products (Kim and Rajagopal, 2000, Kreft and Jelen, 2000).

The aim of this research was to conduct a comparative study on the *β-galactosidase* activity of endemic lactic acid bacteria isolated from matzoon samples produced from cow milk.

## Materials and methods

For the research 370 endemic lactic acid bacteria isolated from matzoon samples taken from different regions in Armenia and kept in the Culture Collection of the Microbial Depository Center (MDC) of the SPC "Armbiotechnology" NAS RA were used.

The identification of lactic acid bacteria was performed through API BioMerieux, inc, 50 CHL and 50 CH systems.

Bacteria were grown in MRS nutrient broth at 30-37 $^0$C for 16-24 hours.

*O-nitrophenyl-β-D-galactopyranoside* (ONPG) was used to determine the activity of *β-galactosidase*. One of the final products of ONPG hydrolysis *O-nitrophenol* (ONP) stains the solution yellow, the concentration of which was measured by spectrophotometer (biochemical analyzer-photometer STAT FAX 1904+, USA) at a wavelength of 410 nm. The activity of *beta-galactosidase* was determined according to the well-known method (Le Minor and Ben Hamida, 1962).

The activity of strains was recorded for 20 min, with the intervals of 1, 2 and 24 hours. The amount of enzyme that catalyzed 1 μmol of *O-nitrophenol* per minute under the appropriate conditions has been selected as the unit of *β-galactosidase* activity. The calculation has been conducted according to the following formula (Baran, 1996):

$$U_{/ml} = \frac{\Delta A_{420/\min}}{\varepsilon},$$

where $\Delta A_{420/\min}$ is the rate of absorption change, $\varepsilon$ is the absorption coefficient according to ONP 1.3546 ml $\mu mol^{-1}/cm^{-1}$.

The specific activity was calculated through the following formula:

$$U_{/mg\ protein} = \frac{\Delta A_{420/\min}}{\varepsilon \times mg\ protein / ml\ medium},$$

or

$$U_{/mg\ CDW} = \frac{\Delta A_{420/\min}}{\varepsilon \times mg\ CDW / ml\ medium},$$

where CDW is the dry cell weight.

The amount of lactose in milk and in dairy products was determined through the cyanide method (Bychkova, 2014). The amount of lactose in milk was calculated according to the following formula:

$$\omega = \frac{0.012 \times V_1 \times 100\ \%}{V_2 \times m},$$

where 0.012 is the amount of lactose required to restore 10 ml of 1 % solution of red blood salt $K_3[Fe(CN)_6]$, $V_1$ is the total volume of the test solution, $V_2$ is the volume of the test solution used to titrate 10 ml of $K_3[Fe(CN)_6]$ solution, $m$ is the volume of milk (dairy products).

The statistical analysis of the obtained data was performed by the software of Microsoft Office Word 10 and Microsoft Office Excel 2010. The differences were considered reliable at $p<0.05$.

## Results and discussions

For the determination of the *β-galactosidase* activity, 370 strains of lactic acid bacteria were studied; 25 Strains with probiotic properties, highly resistant to antibiotics and 4 % NaCl, as well as with thermostability at 45 $^0$C and pH 8.0 were selected. 20 of the strains belong to lactobacilli, 5 to lactococci. As it can be seen from the data presented in Table 1, the selected bacteria belong to different species of the genus Lactobacillus; 8 Strains that become active within 20 minutes belong to *L. fermentum*, *L. delbrueckii* and *L. cremoris*, the remaining strains become active within one hour (Table 1).

In strains with *β-galactosidase* activity, the possibility of lactose cleavage was determined (lactose content in milk was 4.1 %).

Table 2 describes the lactose digestion by the selected LAB in 1 hour.

**Table 1.** *β-galactosidase* activity (IU) of lactic acid bacteria*

| Species of lactic acid bacteria | *β-galactosidase* activity (IU) | |
|---|---|---|
| | 20 min | 1 hour |
| *Lactobacillus fermentum G-4* | 0.09 | 0.09 |
| *L.fermentum G-12-1* | 0.35 | 0.26 |
| *L.fermentum G-12-2* | 0.29 | 0.29 |
| *L.fermentum G-12-3* | 0.13 | 0.27 |
| *L.fermentum 24-7* | 0.46 | 0.47 |
| *L.fermentum 27-1* | 0.57 | 0.57 |
| *L. mesenteroides ssp. cremoris G-6* | 0.07 | 0.05 |
| *L.delbrueckii ssp. lactis 17-13* | 0.31 | 0.48 |
| *Lactobacillus sp. 13-1* | 0 | 0.34 |
| *L. curvatus ssp. curvatus 17-7* | 0 | 0.47 |
| *L.fermentum 27-4* | 0 | 0.66 |
| *L.fermentum 27-6* | 0 | 1.28 |
| *L.salivarvarius 32-4-1* | 0 | 0.58 |
| *Lactobacillus sp. 40-1-4* | 0 | 0.66 |
| *Lactobacillus sp. 35-2-2* | 0 | 0.43 |
| *L.delbrueckii ssp. lactis 40-1-3* | 0 | 0.56 |
| *L.paracasei ssp. paracasei 41-15-1* | 0 | 0.52 |
| *L.delbrueckii ssp. lactis 42-1-1* | 0 | 0.53 |
| *L. plantarum 103-1-3* | 0 | 0.65 |
| *L.delbrueckii ssp. lactis 17-15* | 0 | 0.46 |
| *L.delbrueckii ssp. lactis 17-20* | 0 | 0.59 |
| *L.lindneri 18-2* | 0 | 0.73 |
| *L.delbrueckii ssp. lactis 27-2* | 0 | 0.60 |
| *Leuconostoc lactis 32-4-2* | 0 | 0.59 |
| *Leuconostoc lactis 21-1* | 0 | 0.48 |

**Table 2.** Lactose digestion by lactic acid bacteria*

| Lactic acid bacteria | *β-galactosidase* activity (IU) | Residual amount of lactose in milk ω, % |
|---|---|---|
| *L.fermentum 27-4* | 0.66 | 0.4 |
| *L.fermentum 12-3* | 0.27 | 0.5 |
| *L.fermentum 27-6* | 1.28 | 0.9 |
| *L.delbrueckii ssp. lactis 40-1-3* | 0.56 | 0.6 |
| *L.delbrueckii ssp. lactis 27-2* | 0.60 | 0.8 |
| *L.delbrueckii ssp. lactis 17-13* | 0.48 | 1.1 |
| *L. mesenteroides ssp. cremoris G-6* | 0.05 | 1.5 |

*Composed by the authors.

As it can be seen from the data presented in Table 2, the selected strains differ in *β-galactosidase* activity and the amount of residual lactose in milk. *L. fermentum 27-6* strain has high *β-galactosidase* activity and high residual amount of lactose, whereas *L. mesenteroides ssp. cremoris* G-6 strain with low *β-galactosidase* activity has high residual amount of lactose under the same conditions. It can be assumed that the lack of this symmetry may depend on external factors. As a basis for our future research and for the production of lactose-free milk a consortium of the following strains has been created: *L.fermentum 27-4, L.fermentum 27-6, L.delbrueckii ssp. lactis 27-2*. Preliminary data showed that the average lactose content in the formed consortium was 0.6 % (1 hour after processing milk at 37 $^0$C). Thus, the selected endemic LAB can be the basis for the production of lactose-free milk, the technology of which is currently missing in Armenia.

## Conclusion

The consortium of 3 selected strains (*L. fermentum 27-4, L. fermentum 27-6, L. delbrueckii ssp. lactis 27-2*) can be used for the fermentation of milk and the production of lactose-free milk.

The production of bacterial beta galactosidase is rather widespread. Active in a wide range of temperatures and pHs, lactic acid bacteria are also of particular interest as a potential source of *β-galactosidase*. Apart from using lactic acid bacteria as a probiotic, they are also of vital significance for many people who do not digest lactose, while via non-lactose foods they are able to return to a healthy lifestyle and diet. Therefore, the prospective strains studied by our research group are endowed with high *β-galactosidase* activity and could be possibly used in the production of lactose-free milk, which would be quite beneficial especially for lactose intolerant people using dairy products.

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