The ability of enterococci extracted from traditional Carpathian cheese bryndza to produce biologically active substances

I. I. Kushnir¹, O. Y. Tsisaryk¹, S. H. Shalovylo¹, B. V. Gutyj¹, G. V. Kushnir², I. M. Slyvka¹, L. Y. Musiy¹

¹Stepan Gzhitsky National University of Veterinary Medicine and Biotechnologies, Pekarska Str., 50, Lviv, 79010, Ukraine
²State Scientific-Research Control Institute of Veterinary Medicinal Products and Feed Additives, Donetska Str., 11, Lviv, 79019, Ukraine

Abstract

The article presents the results of determining the ability of enterococci extracted from traditional Carpathian cheese bryndza to produce biologically active substances, in particular, amino acids, B vitamins and cations (ammonium, potassium, sodium, magnesium, calcium). It was found that the studied strains of enterococci in different quantities synthesized both essential and essential amino acids. Thus, the essential amino acid lysine was found in the cultivation of strains of E. durans SB18, E. durans SB20, in particular, its concentration was significantly increased by 15.6 and 10.4 %, respectively (P < 0.05) compared to the control. A probable increase in the essential amino acid histidine by 20 and 53.3 % (P < 0.05) was detected in the cultivation of only E. faecium SB12 and E. durans SB18. In addition, it was found a probable increase in threonine synthesis by enterococci E. durans SB6 and E. durans SB18, respectively – 33.3 and 39.6 % (P < 0.05). The replacement amino acid serine was able to synthesize strains of E. faecium SB12, E. durans SB18 and E. durans SB20, while its concentration increased by 40.0 (P < 0.001), 30.0 and 35.0 %, respectively < 0.01), and strains of E. durans, SB6, and E. durans SB18 synthesized glycine, the concentration of which increased by – 10.2 and 16.2 %, respectively (P < 0.01). In addition, it was found that the studied strains in small quantities synthesized B vitamins, or not synthesized at all. In all experimental samples the most vitamin B1 was detected, its concentration increased from 8.5 to 10.0 times (P < 0.001). Riboflavin was synthesized by three strains of enterococci – E. durans SB6, E. durans SB18, E. durans SB20, so the concentration of vitamin B2 probably increased, respectively, 4.1, 2.0 and 2.0 times (P < 0.05). Enterococci E. durans SB6, E. faecium SB12, E. durans SB18 and E. durans SB20 synthesized in significant quantities only vitamin B3, in particular, its concentration probably increased by 1.8, 1.6, 1.5 and 1.4 times (P < 0.05). Analysis of the macroelement composition of the supernatant of enterococci showed that strains of E. durans, SB6, E. faecium SB12, E. durans SB18 and E. durans SB20 are able to produce only Calcium, in particular, found a probable increase, respectively, in 1.8, 2.4, 1.6 and 1.4 times (P < 0.05).

Key words: Enterococcus durans, E. faecium, vitamins, amino acids, macronutrients.

1. Introduction

Human health largely depends on nutrition, as a lack or deficiency of nutrients causes a violation of the general condition and a decrease in the body's immune system (Omelchuk et al., 2010; Musiy et al., 2017; Tsisaryk et al., 2018; Gachak et al., 2019). When developing innovative and improving existing technologies for the production of dairy products, it is important to introduce functional products into human nutrition in order to prevent disease and premature aging (Didukh, 2008). Functional products derived from natural ingredients contain a large number of biologically active substances that can stimulate immune responses in the body and prevent disease (Richardson, 2002; Kapreliants & Iorhachova, 2003; Hachak et al., 2018).

Taking into account abovementioned, an important task of food biotechnology is to obtain functional products that contain and promote the development of beneficial bacteria. The normal intestinal microflora of the macroorganism plays an extremely important role in the prevention of beriberi and enzymatic disorders. In addition, the normoflora promotes endogenous synthesis of nucleotides, essential amino acids and peptides, regulates adaptation processes and the formation of a protective barrier of the intestinal mucosa (Heyman et al., 2005). However, under adverse conditions, there are changes in the ratio of normal microflora of the digestive tract, which leads to dysbacteriosis (Tkach et al., 2014). In such cases, it is advisable to use probiotics, and the strains of bacteria that are part of them, are selected according to the ability to produce various bio-
logically active substances and they must show synergism (Tarasenko & Filippova, 2014). The advantages of probiotics are the prevention of infectious diseases and food allergies, lowering serum cholesterol, anticancer activity, immunoadjuvant properties and improving the absorption of lactose (Soccol et al., 2010). That is why the processes of microbial fermentation in the large intestine affect not only the normal functioning of the digestive system, but also the state of the body as a whole (Lahti et al., 2008). Normal microflora, due to fermentation activity, is able to synthesize biologically active substances, in particular, organic acids, alcohols, lipids, B vitamins, etc (Gottshal, 1982).

Vitamins are precursors of intracellular coenzymes needed to regulate biochemical reactions in the cell. Humans and animals are not able to synthesize most vitamins, so they must come with food. Although most vitamins are part of a variety of foods, however, vitamin deficiencies still exist. For this reason, many countries have adopted laws on the forced fortification of certain products with appropriate vitamins and minerals, however, some countries have not adopted this program due to possible side effects (Asrar & O’Connor, 2005; Blencowe et al., 2010).

That is why the alternative to chemically synthesized products are vitamins synthesized by microorganisms, because they do not cause side effects. Along with the positive effect of vitamins on the body of humans and animals, an important role is played by macro- and microelements, so of the 118 known chemical elements, 81 were found in the human body (Levitin et al., 2017). Among them are indispensable (calcium, potassium, sodium, manganese, magnesium, sulfur, iron) imbalance which causes clinical symptoms. Trace elements in the body act as cofactors of enzymes (Zn, Mg, Mn, Mo, Cu, Fe) and can be structural components of molecules (Ca, Cr, Co, etc.). Aging, various diseases, intense physical activity and bad habits cause a decrease in the content of macro- and microelements in the human body, in particular, deficiency of J, Fe, Ca, F and Se is found in 90 % of the population of Ukraine (Korzn et al., 2007). Macro- and microelements in the human body are not synthesized, and their balance is maintained by the receipt of food (Orlov, 1998).

Taking into account abovementioned, while the creation of new bacterial fermenting drugs an important ability of probiotic microorganisms is the production of biologically active substances (Tarasenko & Filippova, 2014).

Therefore, the aim of the research was to establish the ability of enterococci E. durans SB6, E. faecium SB12, E. durans SB18, E. durans SB20, isolated from traditional Carpathian cheese bryndza (Slyvka et al., 2018), to produce biologically active substances and some macronutrients.

2. Materials and methods

Determination of qualitative and quantitative content of biologically active substances and some macronutrients in the experimental samples was performed by capillary electrophoresis using the device “Kapel-105/105M”, which is equipped with special software. The method is based on electrokinetic phenomena, electromigration of ions and other charged particles and electroosmosis, which occur in solutions when they are placed in an electric field, mainly high voltage. When the solution is in a thin capillary, the electric field along the capillary causes it to move charged particles and passive fluid flow, causing the sample to split into individual components, as the electromigration parameters are specific to each type of charged particle.

When determining the qualitative and quantitative composition of amino acids and cations (ammonium, potassium, sodium, magnesium, calcium), 1 cm³ of supernatant of the corresponding enterococci grown on liquid MPC medium for 48 h at a temperature of 37 °C was added to the hydrolysates vials and 9 cm³ were added. Hydrochloric acid. They were sealed, stirred and hydrolyzed at 110 °C for 16 hours. After hydrolysis, the contents of the vials were cooled to room temperature and filtered through a blue tape filter, discarding the first portions. To determine amino acids in glass vials were taken 0.05 cm³ of hydrolyzate and evaporated in a stream of warm air. The dry residue was dissolved in 0.15 cm³ of sodium carbonate solution and 0.3 cm³ of FITC solution were added to each dry residue vial, mixed thoroughly to dissolve the precipitate and left for 35 minutes at room temperature. Then the solutions were dried dry in a stream of warm air. The dry residue was dissolved in 0.5 cm³ of double-distilled water and used for the study. Detection of amino acids was performed at a wavelength of 254 nm.

To determine the mass fraction of cations in glass boxes, 0.50 cm³ of hydrolyzate was taken and evaporated in a stream of warm air. The dry residue was dissolved in 0.50 cm³ of distilled water and cations were determined in the test samples. Detection was performed at a wavelength of 267 nm.

To determine the qualitative and quantitative composition of B vitamins, 1 cm³ of the supernatant of the corresponding enterococci grown on liquid MPC medium for 48 h was added to the dark glass vials and 4 cm³ of working solution for vitamin extraction was added. The test vials were placed on a boiling water bath for 5 minutes, then cooled, centrifuged in Eppendorf tubes, and vitamins detected at 200 and 267 nm wavelengths.

When determining the amino acid composition, the content of B vitamins and the content of cations (ammonium, potassium, sodium, magnesium, calcium) in the studied samples at the initial stage of work, calibration graphs were constructed and the stability of control solutions was checked. In addition, the width of the identification window was set and the automatic identification of the studied indicators was checked on the received electrophorograms.

The obtained values were satisfactory, because according to the certified research methodology, the deviation at each point of the graduated characteristic did not exceed 5–8 %, which further allowed to carry out the planned research.

3. Results and discussion

The obtained data on the determination of amino acids in the experimental samples (Table 1) indicate that the studied strains of enterococci in different quantities synthesized both essential and non-essential amino acids. It was found that all four strains of enterococci used for their diet such essential amino acids as tyrosine and proline, as well as essential methionine. The essential amino acid lysine was synthesized in various amounts by all strains of enterococci. However, a probable increase in the level of this amino acid was observed in the cultivation of strains of E. durans SB18, E. durans SB20, in particular, its concentration was significantly increased by 15.6 and 10.4 % (P < 0.05), respectively, compared to the control. Phenylalanine and alanine were not
synthesized by all studied strains of enterococci, but were not used in the diet. A probable increase in the essential amino acid histidine by 20 and 53.3% (P < 0.05) was detected in the cultivation of strains of E. faecium SB12 and E. durans SB18.

A slight increase in the essential amino acids leucine + isoleucine occurred in the cultivation of E. durans SB6 and E. faecium SB12 and E. durans SB18. There was also a probable increase in treithion ine synthesis by enterococci SB12 and E. faecium E. durans from 8.5 to 10.0 times (P < 0.001). Riboflavin was aminized.

### Table 1
Amino acid composition of test samples (M ± m, n = 3)

| Amino acids       | Control          | Strains of enterococci |
|-------------------|------------------|-------------------------|
|                   |                  | SB 6 | SB 12 | SB 18 | SB 20 |
| Arginine, %       | 0.051 ± 0.002    | 0.032 ± 0.001           | 0.055 ± 0.002 | 0.029 ± 0.001 | 0.029 ± 0.003 |
| Lysine, %         | 0.077 ± 0.003    | 0.081 ± 0.001           | 0.079 ± 0.004 | 0.089 ± 0.003* | 0.085 ± 0.001* |
| Tyrosine, %       | 0.030 ± 0.012    | 0.019 ± 0.002**         | 0.019 ± 0.001** | 0.016 ± 0.001** | 0.016 ± 0.001** |
| Phenylalanine, %  | 0.048 ± 0.001    | 0.049 ± 0.002           | 0.050 ± 0.004 | 0.049 ± 0.004 | 0.050 ± 0.002 |
| Histidine, %      | 0.015 ± 0.001    | 0.014 ± 0.002           | 0.018 ± 0.001* | 0.023 ± 0.004* | 0.016 ± 0.001 |
| Leucine + isoleucine, % | 0.120 ± 0.001 | 0.123 ± 0.001           | 0.112 ± 0.001 | 0.127 ± 0.002* | 0.103 ± 0.004* |
| Methionine, %     | 0.033 ± 0.008    | 0.019 ± 0.002           | 0.016 ± 0.003 | 0.028 ± 0.004 | 0.016 ± 0.002 |
| Valine, %         | 0.049 ± 0.002    | 0.043 ± 0.002           | 0.031 ± 0.002** | 0.056 ± 0.006 | 0.025 ± 0.001*** |
| Proline, %        | 0.103 ± 0.002    | 0.101 ± 0.002           | 0.092 ± 0.004 | 0.095 ± 0.0024 | 0.080 ± 0.003** |
| Threonine, %      | 0.048 ± 0.002    | 0.064 ± 0.005*          | 0.047 ± 0.005 | 0.067 ± 0.004* | 0.053 ± 0.006 |
| Serine, %         | 0.040 ± 0.0007   | 0.039 ± 0.002           | 0.056 ± 0.001*** | 0.052 ± 0.001** | 0.054 ± 0.001** |
| Alanine, %        | 0.095 ± 0.001    | 0.094 ± 0.001           | 0.0899 ± 0.001* | 0.113 ± 0.006 | 0.099 ± 0.003 |
| Glycine, %        | 0.167 ± 0.002    | 0.184 ± 0.002**         | 0.167 ± 0.007 | 0.194 ± 0.011** | 0.171 ± 0.004 |

Note: * – P < 0.05; ** – P < 0.01***; – P < 0.001

As can be seen from the results of studies of B vitamins (Table 2), the studied strains synthesized vitamins in small quantities, or not synthesized at all, in particular, this applies to vitamin B6 and Sun. Vitamin B6 in small quantities was synthesized only by the strain E. durans SB6, and folic acid – E. durans SB18, and all other strains, apparently, used them for their nutrition. In all experimental samples the most vitamin B1 was detected, its concentration increased from 8.5 to 10.0 times (P < 0.001). Riboflavin was synthesized by three strains of enterococci – E. durans SB6, E. durans SB18, E. durans SB20, so the concentration of vitamin B2 probably increased, respectively, 4.1, 2.0 and 2.0 times (P < 0.05), and the E. faecium SB12 strain apparently used this vitamin, so it was not detected. It should be noted that all four strains of enterococci E. durans, SB6, E. faecium SB12, E. durans SB18 and E. durans SB20 synthesized a significant amount of vitamin B3, in particular, its concentration probably increased by 1.5, 1.5, respectively (P < 0.05), 1.5 (P < 0.01) and 1.6 (P < 0.001) times. The three strains of enterococci E. faecium SB12, E. durans SB18 and E. durans SB20 were able to synthesize large amounts of vitamin B1, so the concentration of nicotinic acid increased, respectively, in 2.9 (P < 0.05), 8.4 and 9.5 (P < 0.001) times.

### Table 2
Vitamin composition of experimental samples, (M±m, n=3)

| Vitamin | Control          | Strains of enterococci |
|---------|------------------|-------------------------|
|         |                  | SB 6 | SB 12 | SB 18 | SB 20 |
| Vitamin B1, % | 0.066 ± 0.003    | 0.633 ± 0.032***      | 0.566 ± 0.027*** | 0.586 ± 0.042*** | 0.586 ± 0.023*** |
| Vitamin B2, % | 0.013 ± 0.003    | 0.053 ± 0.009*        | nd               | 0.026 ± 0.001*   | 0.026 ± 0.003*   |
| Vitamin B3, % | 0.443 ± 0.023    | 0.643 ± 0.055*        | 0.643 ± 0.027**  | 0.663 ± 0.041**  | 0.710 ± 0.011**  |
| Vitamin B4, % | 0.046 ± 0.003    | nd               | 0.133 ± 0.018*   | 0.386 ± 0.031*** | 0.436 ± 0.042**  |
| Vitamin B6, % | 0.036 ± 0.003    | 0.023 ± 0.0088       | nd               | nd               | nd               |
| Vitamin Bc, % | 0.27 ± 0.007     | nd               | nd               | 0.31 ± 0.020     | nd               |

Note: * – P < 0.05; ** – P < 0.01***; – P < 0.001

nd – not detected
Analysis of the results of studies to determine the content of cations (ammonium, potassium, sodium, magnesium, calcium) in the supernatant of enterococci, which are shown in table 3, showed that only calcium was present in significant quantities. In particular, a probable increase of 1.8, 2.4, 1.6 and 1.4 times (P < 0.05) in the content of Calcium in the supernatant, where cultured, respectively, strains of E. _durans_ SB6, _E. faecium_ SB12, _E. durans_ SB18 and _E. durans_ SB20. For other macronutrients, no significant differences compared to the control were found.

### 4. Conclusions

1. The strains of enterococci _E. durans_, SB6, _E. faecium_ SB12, _E. durans_ SB18 and _E. durans_ SB20 extracted from traditional Carpathian cheese bryndza are capable of synthesis of biologically active substances. The studied strains in different quantities synthesize both essential and essential amino acids. It was found that the strain _E. durans_ SB18, synthesizes the largest number of amino acids, in particular essential amino acids – lysine, trethionine and leucine + isoleucine, the concentration of which was, respectively, 3.0, 0.0 and 16.2% (P < 0.01) higher compared to the control. Additionally, it was found a probable increase of 1.8, 2.4, 1.6 and 1.4 times (P < 0.05).

2. In all experimental samples the most vitamin B1 was detected, its concentration increased from 8.5 to 10.0 times (P < 0.001). Enterococci _E. durans_, SB6, _E. faecium_ SB12, _E. durans_ SB18 and _E. durans_ SB20 are able to synthesize vitamin B2, in particular, its concentration is likely to increase, respectively, in 1.5 (P < 0.05), 1.5 (P < 0.05), 1.5 (P < 0.001) and 1.6 (P < 0.001) times. Enterococci _E. faecium_ SB12, _E. durans_ SB18 and _E. durans_ SB20 synthesize vitamin B5, the concentration of which increased, respectively, in 2.9 (P < 0.05), 8.4 (P < 0.05) and 9.5 (P < 0.001) times compared to control.

3. When determining the macronutrients in the supernatant, where cultured strains of _E. durans_, SB6, _E. faecium_ SB12, _E. durans_ SB18 and _E. durans_ SB20, found a probable increase in calcium levels, respectively, 1.8, 2.4, 1.6 and 1.4 times (P < 0.05).

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