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

I. I. Kushnir[1], O. Y. Tsisaryk[1], S. H. Shalovylo[1], B. V. Gutyj[1], G. V. Kushnir[2], I. M. Slyvka[1], L. Y. Musiy[1]

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

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Correspondence author
Iryna Kushnir
Tel.: +38-098-290-16-94
E-mail: irynakushn@gmail.com

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 1.5, 1.5 ($P < 0.05$), respectively, 1.5 ($P < 0.01$) and 1.6 ($P < 0.001$) times, and vitamin B5 was produced by *E. faecium* SB12, *E. durans* SB18 and *E. durans* SB20, the concentration of nicotinic acid increased, respectively, 2.9 ($P < 0.05$), 8.4 and 9.5 ($P < 0.001$) times. 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.

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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 (Lahtin 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 (Gottshalk, 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, J, 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 (Korzun 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 cm^3 of supernatant of the corresponding enterococci grown on liquid MPC medium for 48 h at a temperature of 37 °C was added to the hydrolysis vials and 9 cm^3 were added. hydrochloric acid. They were sealed, stirred and hydrolyzed at 110 °C for 14–16 hours. After hydrolysis, the contents of the vial 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 cm^3 of hydrolyzate and evaporated in a stream of warm air. 0.15 cm^3 of sodium carbonate solution and 0.3 cm^3 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 cm^3 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 cm^3 of hydrolyzate was taken and evaporated in a stream of warm air. The dry residue was dissolved in 0.50 cm^3 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 cm^3 of the supernatant of the corresponding enterococci grown on liquid MPC medium for 48 h was added to the dark glass vials and 4 cm^3 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 electrophoregrams.

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 trethionine synthesis by enterococci *E. durans* SB6 and *E. durans* SB18, respectively, by 33.3 and 39.6 % ($P < 0.05$). The replacement amino acid serine was able to be synthesized by strains of *E. faecium* SB12, *E. durans* SB18 and *E. durans* SB20, and its concentration increased by 40.0 ($P < 0.001$), 30.0 and 35.0 %, respectively ($P < 0.01$), and strains of *E. durans* SB6 and *E. durans* SB18 synthesized glycine, the concentration of which increased by 10.2 and 16.2 %, respectively ($P < 0.01$).

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 B_6 and Sun. Vitamin B_6 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 B_1 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 B_2 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 B_3, 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 B_5, 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 B_1, %	0.066 ± 0.003	0.633 ± 0.032***	0.566 ± 0.027***	0.586 ± 0.042***	0.586 ± 0.023***
Vitamin B_2, %	0.013 ± 0.003	0.053 ± 0.009*	nd	0.026 ± 0.001*	0.026 ± 0.003*
Vitamin B_3, %	0.443 ± 0.023	0.643 ± 0.055*	0.643 ± 0.027**	0.663 ± 0.041**	0.710 ± 0.01***
Vitamin B_5, %	0.046 ± 0.003	nd	0.133 ± 0.018*	0.386 ± 0.031***	0.436 ± 0.042**
Vitamin B_6, %	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

Table 3
Macronutrients of experimental samples (M ± m, n = 3)

Macroelements	Control	Strains of enterococci			
		SB 6	*SB 12*	*SB 18*	*SB 20*
Am, %	0.226 ± 0.002	0.032 ± 0.001	0.032 ± 0.002	0.031 ± 0.0006	0.030 ± 0.0003
K, %	0.112 ± 0.005	0.108 ± 0.004	0.115 ± 0.003	0.112 ± 0.003	0.111 ± 0.004
Na, %	0.249 ± 0.004	0.243 ± 0.009	0.247 ± 0.008	0.259 ± 0.007	0.248 ± 0.009
Mg, %	0.005 ± 0.0003	0.005 ± 0.0003	0.005 ± 0.0003	0.005 ± 0.0003	0.004 ± 0.0003
Ca, %	0.005 ± 0.0003	0.009 ± 0.002*	0.012 ± 0.004*	0.008 ± 0.002*	0.007 ± 0.001*

Note: * – P < 0.05

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 higher, respectively, by 10.3, 39.5 (P < 0.05) and 5.8 % than in the control, as well as the amino acids serine and glycine, the concentration of which was, respectively, 30.0 and 16.2 % (P < 0.01) higher compared to the control.

2. In all experimental samples the most vitamin B_1 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 B_3, in particular, its concentration is likely to increase, respectively, in 1.5 (P < 0.05), 1.5 (P < 0.05), 1.5 (P < 0.05) 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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