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Main Microbiological and Biological Properties of Microbial Associations of "*Lactomyces tibeticus*".

M Kukhtyn[1], O Vichko[1]*, O Berhilevych[2], Y Horyuk[3], and V Horyuk[4]

[1]Ternopil Ivan Pul'uj National Technical University, 56, Ruska str. Ternopil, 46001, Ukraine
[2]Sumy State University, Department of Hygiene and Ecology with course of Microbiology, Virology and Immunology, 31, Sanatorna str., 40018, Sumy, Ukraine
[3]Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine, 30, Donetsk str., Kyiv, 03151, Ukraine
[4]Podilskiy State Agrarian-Engineering University, 13, Shevchenko str., Kamyanets-Podilskyi, Khmelnytsky reg., 32300, Ukraine

ABSTRACT

"*Lactomyces tibeticus*" is the associative consortium of different taxonomic groups of microorganisms: *lactomyces*, *Saccharomyces spp*., *Candida kefir*; lactic acid bacteria - *Lactobacillus fermentum*, *Lactobacillus spp.*, *Lactococcus lactis subsp. lactis*, *Leuconostoc lactis*; also acetic acid bacteria - *Gluconobacter oxydans* is found. When fermentation of milk microbiota *Lactomyces tibeticus* at a temperature 28 ± 1 °C for 24 h, the acidity of the product ranged from 80 to 120 ºT, the amount of lactic acid bacteria - (2,9 ± 0,22) x 10^8 CFU/cm^3, *lactomyces* - (3,7 ± 0.27) x 10^4 CFU/cm^3 are defined. During the fermentation of milk *Lactomyces tibeticus* synthesizes 12 amino acids, five of which are irreplaceable - lysine, threonine, methionine, phenylalanine, tryptophan and B vitamins - B_1, B_2, B_3, B_6, which greatly increases the biological value of fermented milks are fixed.

Keywords: *Lactomyces tibeticus,* identification, biotechnological properties, fermented milk.

Corresponding author

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INTRODUCTION

Among the large range of dairy products, products that are based on the use of physiologically active natural symbiotic microflora, deserve special attention.

Production of these products is based on improving processes It includes two points first one is using traditional dairy strains of lactic acid bacteria, the second one is developing new dairy products with new types of microorganisms that show an probiotic properties [1, 2, 3].

In recent years worldwide interest in the study of natural microbial associations such as *Lactomyces tibeticus,* other names are “Indian fungus”, “Indian rice”, “sea rice” is increased [4]. Due to the wide range of biological active substances that are part of beverages, the relative simplicity of cultivation and the possibility of keeping culture for a long time in an active state these natural associations gained widespread in everyday life, and they are interested scientists.

Thus, in the Belarus, polyculture of the microorganisms - *Oryzomys indici* has been deposited. Also biotechnology of non-alcoholic fermented beverages using these microorganisms was proposed [5].

Natural association of microorganisms "Tibetan fungus" is widely used in house conditions to prepare fermented milk beverage that from the perspective of traditional medicine is one of the leading places in activity effects on the human body. However, data about microbial composition of the culture, it’s physiological and biochemical properties, features of metabolic, possibilities of using in an industrial environment are practically absent.

Therefore, comprehensive study of *Lactomyces tibeticus*, as a new symbiotic starters and development of its based biotechnology of production of natural dairy products is important, perspective and has great scientific and practical importance.

The aim of this study was to determine microbiological and some biotechnological *properties* of microbial associations of *Lactomyces tibeticus*.

MATERIALS AND METHODS

To isolate pure cultures of microorganisms that are part of the microbial association of *Lactomyces tibeticus*, we used the following mediums. For isolation of monoculture of lactic acid bacteria MRS medium was used. Saburo medium and beer wort were used for isolation of fungi. Also beer wort with the addition of 3% ethanol and 0.5% $CaCO_3$ was used for the selection of acetic acid bacteria.

For determination of genus of selected strains traditional lab methods were used. They included studying of morphological, cultural and biochemical characteristics. Microscoping of isolated cultures of microorganisms was performed using a light microscope Trinocular MBL2100 ("Krus", Germany) at eyepiece 20 and lens 100.

The final identification of strains of lactic acid bacteria to the type was carried out by range of fermentation of carbohydrates. For this purpose test system for identifying microorganisms API 50SN (Bio Merieux, Inc, France) was performed according to the manufacturer's recommendations.

Also species belonging of isolated cultures of lactic acid microorganisms were studied using PCR (polymerase chain reaction). PCR analysis were carried out with genus and specific primers according to research papers [6,7].

RESEARCH FINDINGS AND DISCUSSION

In the process of manufacturing fermented milk product are important qualitative and quantitative composition of microbial association (starters) that determine the conditions of the process and desired product properties. Therefore, at this stage of research we studied microbiological and biotechnological

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properties of microbial associations of *Lactomyces tibeticus*. A photomicrograph of natural association of *Lactomyces tibeticus* is shown in Fig. 1.

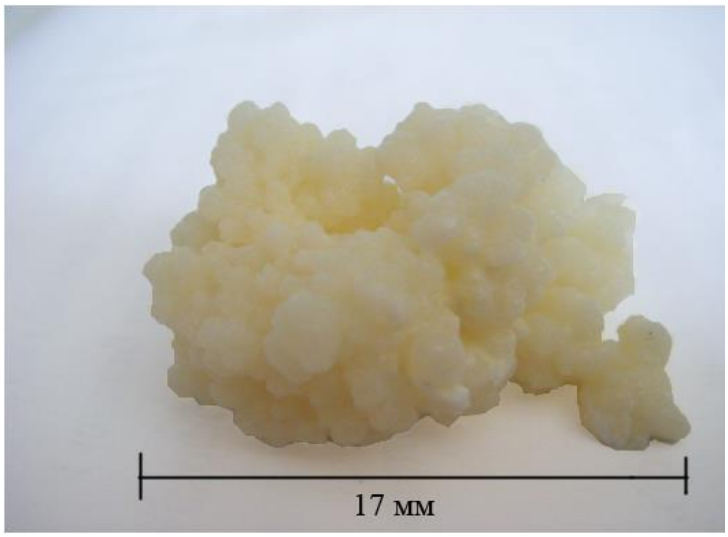

Figure 1: Photomicrography of natural association of *Lactomyces tibeticus* (increase in 5.9 times)

As shown in Fig. 1, lump of *Lactomyces tibeticus* is white and consist of a round or oval chees granule with size of 1 - 3 mm, with a non-homogeneous surface that in water settle to the bottom of the container.

Fig. 2 shows the microscopic picture of *Lactomyces tibeticus* smear that is stained by Gram.

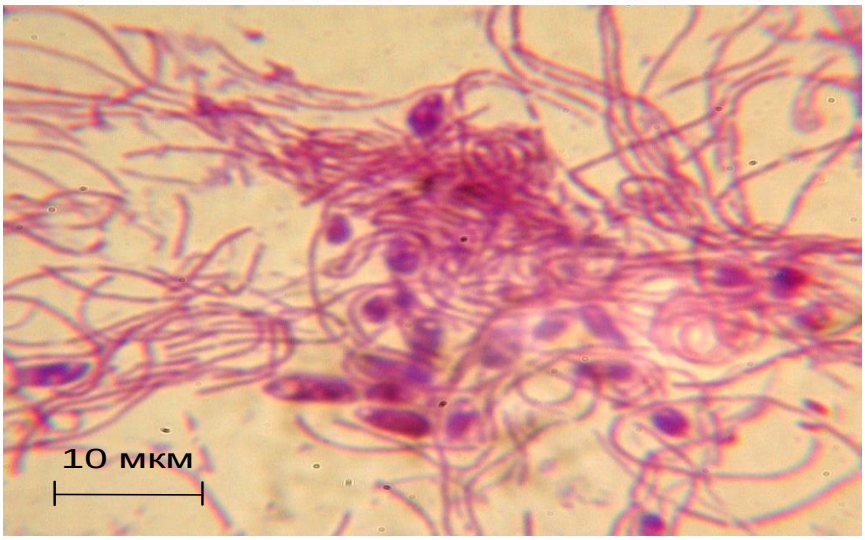

Figure 2: Smears of natural association of *Lactomyces tibeticus*. (Light microscope, increase in 1500 times)

As shown in Fig. 2, microbial association of *Lactomyces tibeticus* , consists of yeast oval and elongated cells which are surrounded by cocci and single and chain rod-shaped bacteria.

To establish the genus and species of microorganisms in association, after planting of homogenized in sterile saline lump of natural association of *Lactomyces tibeticus* in the series of elective mediums, two types of yeast cells and four kinds of bacteria were identified. However, further studies with using PCR methods for identification of microorganisms allowed to conclude that the microflora of *Lactomyces tibeticus* is more complicated than previously was mentioned.

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Table 1 shows data regarding the qualitative and quantitative composition of microorganisms in *Lactomyces tibeticus* association.

Table 1: Qualitative and quantitative composition of microorganisms in *Lactomyces tibeticus* association, M±m, n=9

№	Microorganisms	The number of cells in 1 g of lumps of *Lactomyces tibeticus* (cfu in gram)	Percentages, %
1	Yeast cells of genus *Saccharomyces* №1	3,56±0,21	0,091
2	Yeast cells of genus *Saccharomyces* №2	2,55±0,17	0,009
3	*Lactobacillus fermentum*	6,55±0,44	88,999
4	*Lactobacillus spp.*	5,56±0,43	9,080
5	*Candida kefir*	2,59±0,17	0,0097
6	*Lactococcus lactis subsp. lactis*	2,56±0,17	0,0091
7	*Leuconostoc lactis*	4,43±0,35	0,680
8	*Gluconobacter oxydans*	4,65±0,37	1,120
Total number of microorganisms		6,60±0,54	100

According to the data given in the Table 1, in lump of *Lactomyces tibeticus* among the eight identified types of microorganisms, microbial cells genus of *Lactobacillus fermentum* are much dominated in 90% of all association. Bacterial cells of genus *Lactobacillus* spp. are found on the second place. Their account was average 9% of all microorganisms of *Lactomyces tibeticus*. Still about 1.12% in lump of *Lactomyces tibeticus* accounts for bacteria genus *Gluconobacter oxydans* and 0.68% for *Leuconostoc lactis*.

Consequently, given the significantly prevailing content of bacteria genus *Lactobacillus* in the structure of microbial associations (about 99%) we can say that they will play a key role in shaping dairy products.

Therefore, further research was aimed to study their metabolityc activity during fermentation of milk in dairy technology product. For probiotic properties of fermented dairy beverage, it is important ratio of yeast and lactic acid bacteria in cultural liquid - dairy products.

Table 2 shows the results of studies determining the number of yeast and bacterial cells in conditions of readiness of the product (acidity), which is cultivated at different temperatures. It is known that functional properties of finished product depends on quantitative correlation of probiotic microorganisms in its. In most cases lactobacilli have a positive impacts on human health because of their increased level may indicate the potential probiotic properties of the beverage.

Table 2: The number of yeast cells and lactobacillus in cultural liquid depends on the temperature of cultivation, M±m, n=15

№	Temperature of cultivation, ºC	time of cultivation, h	Number of yeast cells in 1 ml, cfu / ml	The number of lactobacilli cells in 1 ml, cfu / ml	Acidity, ºT
1	22±1	48	$(5,9±0,42)x10^5$	$(0,5±0,02)x10^2$	96,7±7,2
2	28±1	24	$(3,7±0,27)x10^4$*	$(2,9±0,22)x10^8$**	101,5±8,7
3	35±1	5	$(5,2±0,31)x10^2$	$(5,7±0,30)x10^8$**	85,4±6,5

Note: *–P≤0,05; **–P≤0,001 – compared with cultivation temperature 22 ± 1 ºC

As shown in the Table 2, we observed intensive multiplication of yeast cells, whose number was 5,9 ± 0,42 x 10^5 cfu / ml and complete inhibition of lactic acid bacteria at the fermentation temperature of 22 ° C during 48 hours.

Fermentation of milk at a temperature of 28 ° C caused the multiplication of all microorganisms of *Lactomyces tibeticus.* As a result, the number of yeast cells was 3,7 ± 0,27x10^4 cfu/ml and Lactobacillus cells - 2,9 ± 0,22x10^8 cfu / ml after 24 hours of cultivation.

This proportion of these groups of microorganisms in dairy products - such as kefir complies with the requirements of standard [8]. However, the maximum number of *Lactobacillus* cells (5,7 ± 0,30x10^8 cfu/ml)

was achieved at the fermentation temperature of milk 35 ° C for 5 hours. At the same time the number of yeast cells in such fermented beverage was 5,2 ± 0,31 x10^2 cfu/ml that does not complies with the standard. Consequently, the results indicate that for optimal quantitative accumulation of lactic acid bacteria and yeasts in dairy products, produced with using *Lactomyces tibeticus* favorable temperature for fermentation of milk was 28 ± 1 °C. During the fermentation of milk occurs the qualitative and quantitative changes in its components, for example, proteins are split and amino acids are formed. It is known that amino acids are an essential element of nutrition that determines the direction of development any organism - from microorganisms to humans.

Table 3 shows the data of amino acid composition of milk and fermented product, which was produced with *Lactomyces tibeticus*.

Table 3: Amino acid composition of milk and fermented dairy producti , that was produced with *Lactomyces tibeticus* , mg/100 g protein, M±m, n=5

Amino acids	The content of amino acids in proteins of milk	The content of amino acids in proteins fermented milk product after 48 hours of fermentation
Nonessential amino acids	*16,37*	*25,89**
Alanine	1,05±0,010	0,71±0,03
Arginine	1,2±0,04	2,28±0,012*
Aspartic acid	2,45±0,014	3,67±0,04*
Histidine	1,05±0,02	0,86±0,07
Glycine	0,54±0,03	0,91±0,05*
Glutamic acid	4,13±0,05	7,84±0,23*
Proline	2,73±0,04	1,82±0,07
Serine	1,67±0,05	3,34±0,16*
Tyrosine	1,40±0,03	4,35±0,21**
Cystine	0,15±0,009	0,18±0,01
Essential amino acids	*11,99*	*17,47**
Valine	0,99±0,011	0,95±0,001
Isoleucine	1,14±0,07	1,22±0,08
Leucine	2,85±0,14	2,97±0,15
Lysine	2,44±0,09	5,85±0,14**
Methionine	0,92±0,03	1,28±0,08
Threonine	1,33±0,05	2,52±0,09*
Phenylalanine	1,73±0,007	2,07±0,09
Tryptophan	0,52±0,02	0,63±0,05
Total amount of all amino acids	28,29	43,36*

Note: *−P≤0,05; **−P≤0,001 − compared to the amino acid composition of milk.

According to the presented data in Table 3, fermented dairy beverage that was produced with *Lactomyces tibeticus* had all spectrum of amino acids. Moreover, the total content of amino acids in the milk and fermented dairy product were somewhat different. Thus, it was found an increase in 1.6 times (R≤0,05) essential aminoacids in dairy products in compare with milk. Number of essential amino acids in the fermented dairy beverage was in 1.5 times higher (R≤0,05) than in milk.

It was particularly marked increase in the number such essential amino acid, as lysine in 2.4 times (P ≤ 0,001), the lack of which can cause anemia, miodistrofiey, osteoporosis and other. Also, it was noted that threonine are increased in 1.9 times (R≤0,05), methionine - 1.4 times, and phenylalanine, tryptophan - in 1,2 times. The lack of these amino acids in the body can cause abnormal functioning of various organs and systems.

Thus, conducting research of the amino acid composition of milk and fermented dairy products indicate that microorganisms of *Lactomyces tibeticus* during their life was produced 12 amino acids, five of which are essential. This increases the biological value of the product.

Table 4 shows the results of research concerning the content of some vitamins in milk and fermented dairy products from it using *Lactomyces tibeticus* .

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Table 4: Vitamin composition of milk and fermented dairy products that was produced with *Lactomyces tibeticus* , mg/%, M±m, n=5

Vitamins	The content of vitamins in milk	The content of vitamins in fermented dairy products, after 48 hours of fermentation
Vitamin A	0,03±0,001	0,027±0,002
Vitamin C	1,52±0,06	1,49±0,007
Vitamin B_1	0,04±0,002	0,128±0,007**
Vitamin B_2	0,15±0,02	0,315±0,008*
Vitamin B_6	0,05±0,001	0,09±0,01*
Vitamin B_3 (PP)	0,31±0,02	0,46±0,02*

Note: *–P≤0,05; **–P≤0,001 – compared to the vitamin content of milk.

According to data that are presented in Table 4, amount of vitamin A and vitamin C has not practically changed the in fermented milk products in compare to milk. However, we noticed an increase number of vitamins of group B in dairy products. Thus, content of vitamin B_1 has increased in 3,2 times ($P \leq 0.001$), vitamin B_2 – in 2,1 times (R≤0,05), B_6 – in 1,8 times (R≤0,05) and B_3 – in 1.5 times (R≤0,05). This indicates that the microorganisms of *Lactomyces tibeticus* during its multiplying ,synthesized vitamins of group B, which is an important indicator of the biological value of any dairy products.

Investigation of ethanol content in fermented dairy products on the basis of *Lactomyces tibeticus* during its produce and the period of storage at a temperature of 6 ° C ± 2 are shown in Figure 3.

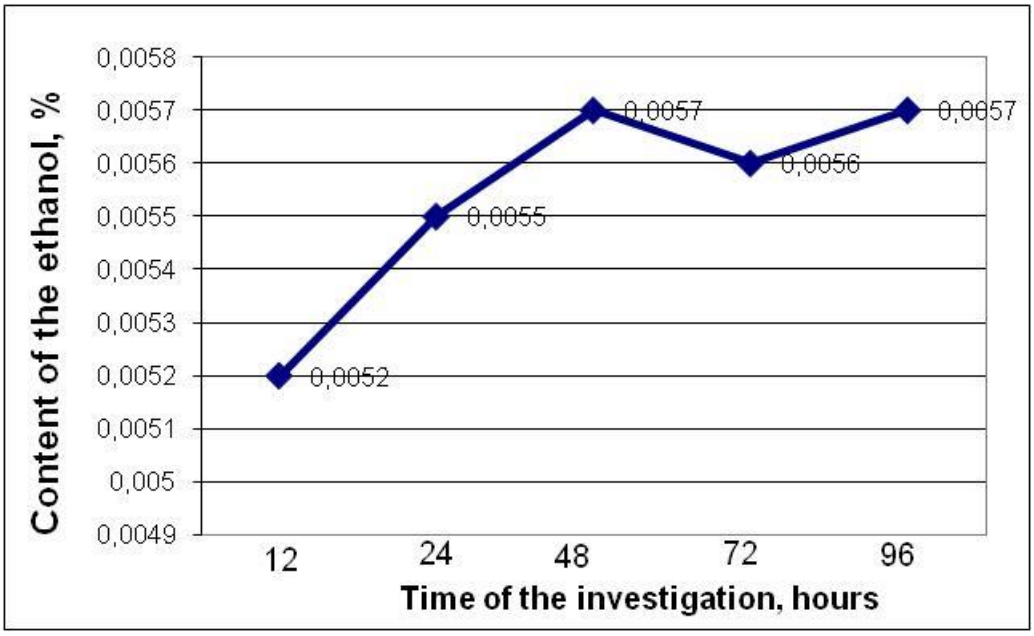

Figure 3: The content of ethanol in fermented dairy products

From the data of Fig. 3 are shown that the content of ethanol in dairy products with *Lactomyces tibeticus* has not exceed 0,0057 ± 0,0001% within a 96 hour shelf life.

This indicates that ethanol, which was produced by yeast almost fully was utilized by acetic acid bacteria of *Lactomyces tibeticus* . It allows using of fermented dairy products for dietary nutrition and both for people of different ages and for animals.

CONCLUSIONS

- Found that biocultura of *Lactomyces tibeticus* is an associative consortium of different taxonomic groups of organisms: fungi *Saccharomyces* spp., *Candida kefir*; lactic acid bacteria - *Lactobacillus*

fermentum, Lactobacillus spp., Lactococcus lactis subsp. lactis, Leuconostoc lactis; acetic acid bacteria - *Gluconobacter oxydans*.

- Determined the optimal technological parameters of fermentation of milk by microbiota of *Lactomyces tibeticus,* which ensures compliance with desirable passage metabolityc processes and obtaining fermented dairy products with good organoleptic properties: fermentation temperature 28 ± 1 ° C for 24 h, the acidity of the product from 80 to 120 ºT, the amount of lactic acid bacteria - (2,9 ± 0,22) $x10^8$ cfu/cm^3, fungi - (3,7 ± 0,27) $x10^4$ cfu/cm^3.
- At fermentation of milk by *Lactomyces tibeticus* 12 amino acids were synthesized, five of which are essential - lysine, threonine, methionine, phenylalanine, tryptophan and vitamins of group B - B_1, B_2, B_3, B_6, which significantly increases the biological value of dairy products. Obtained product contains a small amount of ethanol (0,0057 ± 0,0001%), which does not affect the basic metabolic functions of the macroorganism.

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