Lactobacillus plantarum amino acid and vitamin production properties of bacterial strain СКБ -368

Kamalova H.F., Kutlieva G.J., Turaeva B.I.
Academy of Sciences of the Republic of Uzbekistan
Institute of Microbiology

Abstract: Synthesis of amino acids and vitamins by microorganisms is considered very important and is widely used in various fields. In particular, lactobacilli, which ferment milk, synthesize amino acids and vitamins. In this study, vitamins and amino acids were determined in the culture liquid of *Lactobacillus plantarum* СКБ -368 bacterial strain isolated from mango fruit .

Keywords*:* Lactobacillus plantarum СКБ-368, mango fruit, lactic acid bacteria, primary metabolites, vitamins, amino acid.

Primary metabolites of microorganisms are small molecules that are part of all living cells. They are intermediate products of metabolism, building blocks for macromolecules, some become coenzymes. The most important for industry are amino acids, nucleotides, vitamins, solvents and organic acids. Organisms used for the production of primary metabolites undergo various genetic and physiological mutations to increase their productivity and activity [1]. Amino acids are the building blocks of all plant and animal proteins in nature. Amino acids are essential elements for the development, reproduction and maintenance of all living organisms. Amino acids are amphoteric acids composed of amino and carboxylic acids as functional groups [2]. There is an increasing interest in preparations based on amino acids in the fields of nutrition and animal husbandry. Amino acids have specific physiological properties based on each pathway. For example, alanine and valine increase plant resistance to cold, stimulate chlorophyll synthesis, improve the quality of fruits, regulate the opening of leaf openings, and increase drought resistance. arginine and phenylalanine also increase the cold resistance of plants, stimulate the synthesis of hormones related to flowering and fruit formation, and enhance the development of roots [3]. Amino acids asparagine, glutamine, phenylalanine, glycine and methionine increase the germination capacity of seeds, activate the development of new shoots, participate in the metabolism of amino acids that are a source of organic nitrogen, are growth stimulants, activate pollination, increase the mechanism of resistance to disease-spreading pathogens, ensure the formation of tissues, fruit participates in the growth and improvement of taste. Proline is an antistress effect of plants, increases resistance to osmotic stress, regulates water exchange in plants, increases the amount of chlorophylls and accelerates the process of photosynthesis, activates the generative development of plants [4]. Also, based on amino acids, preparations are developed in the food industry, medicine and pharmaceutical fields [5]. Today, the amino acid market is worth more than 6 billion US dollars in the world and is growing by 5-10% every year. Their production is 3 million tons per year worldwide [6] . Among the substances produced on the basis of biotechnology, amino acids occupy the first place. It ranks second after antibiotics in value. Amino acids are widely used as feed and food additives, pharmaceuticals and even as raw materials of the perfumery industry [7]. The advantage of the microbiological method over the methods of amino acid production is that active state amino acids are obtained due to the fact that the

producing microorganisms only produce active L forms of amino acids. In the chemical synthesis method, a mixture of L- and D-stereoisomeric forms is obtained. D stereoisomeric forms are not used because they are not assimilated by the human and animal body and can be toxic [8]. Different strains of microorganisms are used in obtaining amino acids based on microbiotechnology. Many non-indigenous strains produce only trace amounts of amino acids into the environment [9].

Synthesis of amino acids in microorganisms is a complex biochemical process that requires a lot of energy. They are the building blocks of proteins essential for the growth of living organisms. Currently, creating a safe, effective, environmentally friendly biopreparation for improving ecology and soil fertility is becoming an urgent problem [10]. Amino acid synthesis by lactic acid bacteria hydrolyzes extracellular protein molecules into free amino acids is an important mechanism. The target of the study is *Lactobacillus plantarum* isolated from plants, namely from the local mango fruit analysis of amino acids synthesized by СКБ-368 strain.

Vitamins are biologically active organic compounds of low molecular weight, which are necessary in small amounts for the normal functioning and growth of the body, resistance to infections, synthesis of proteins and fats. According to their solubility, they are divided into oil-soluble and water-soluble groups. Plant growth-promoting bacteria can enhance plant growth in a variety of ways that work through multiple physiological, molecular, and biochemical pathways. Vitamins are produced by plants and also by plant growth-promoting bacteria. The main function of vitamins is to act as a cofactor in various metabolic pathways, facilitate the production of important compounds for plants and bacteria, provide resistance to pathogens , directly support plant growth, and participate in energy conversion in the plant from stored compounds. Group B vitamins are important components of cell metabolism, among which riboflavin is one of the most important vitamins necessary for bacteria, plants, animals, and humans [11]. Although vitamins differ in their chemical structure and function, they have many common properties. The reserve of water-soluble vitamins in the body is very small, therefore, it is necessary to replenish it frequently [12]. Because fat-soluble vitamins accumulate in tissues in large quantities, they have the property of having a toxic effect. Vitamins are essential substances for life. Vitamins are organic substances that store a source of carbon [13].

Vitamins directly and indirectly affect the growth of callus, growth of somatic cells, root development. Thiamine, related to cytokinin, plays an important role in stimulating root and callus growth. Biotin, riboflavin, and thiamine facilitate the production of more secondary metabolites such as proteases in pineapple and affect callus development. There are many other vitamins important for the growth of different types of plants [14]. B vitamins and their cofactors are essential for life. B group vitamins are also synthesized in plant, human and animal body. They do not have enough because they produce very little in their body. Therefore, the living organism needs vitamins of group B. Lactobacilli are capable of synthesizing water-soluble vitamins (folates, riboflavin and vitamin B12) belonging to group $_{B.}$ In recent years, a number of biotechnological processes have been developed in order to realize more economical and sustainable vitamin production [15]. One of the main methods

for determining water-soluble vitamins produced by microorganisms is high-performance liquid chromatography (HPLC). This method makes it possible to determine several vitamins of group at the same time .

Materials and methods: The isolated Lactobacillus *plantarum* SKB-368 strain was incubated in liquid GPA medium at 37 ^{o} C for 48 hours. The content of amino acids and vitamins was studied at the Institute of Bioorganic Chemistry of the Academy of Sciences of Uzbekistan using high-performance liquid chromatography analysis. High-performance liquid chromatography (HPLC) analysis of FTK derivatives of amino acids was carried out according to the method of Stephen A., Cohen Daviel. FTK - amino acids were performed on an Agilent Technologies 1200 chromatograph on a 75x4.6 mm Discovery HS C18 column. Solution A: 0.14M CN3SOONa + 0.05% TEA rN 6.4, V:CH3CN . Flow rate 1.2 ml/min, absorbance 269 nm. Gradient %B/min: 1-6%/0-2.5min; 6-30%/2.51-40min; 30-60%/40.1-45 min; 60-60%/45.1-50min; 60-0%/50.1-55min. High-performance liquid chromatography (HPLC) analysis of water-soluble vitamins was performed on an Agilent Technologies 1200 chromatograph on an Exlipse XDB C18 column (reverse phase), 3.5 µm, 4.6x150 mm. Diode array detector (DAD), 230, 265, 254, 285nm. Solution A: 0.5% acetic acid, pH 1.7: V:CH $_{3}$ CN (acetonitrile). The flow rate is 0.6 ml/min. Gradient %B/min: 0-5min/96:4%, 6-8 min/90:30%, 9-15 min/80:20%, 15-17 min/96:4%. Thermostat 25 ^{0} C.

The results obtained. As a result of research *Lactobacillus plantarum* The synthesis of 20 amino acids (alanine, glycine, serine, aspartic acid, glutamic acid, tyrosine, threonine, arginine, cysteine, proline) produced by strain SKB-368 was determined. 6 of them belong to the group of exchangeable, 2 non-exchangeable, and 2 partially exchangeable amino acids (Fig. 1).

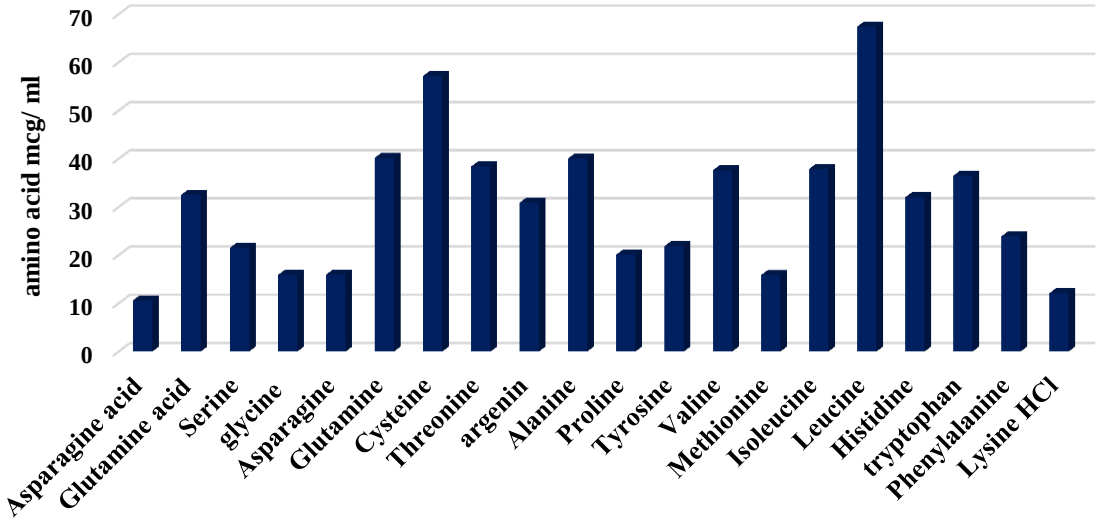

Figure 1. The amount of amino acids in the culture fluid of *Lactobacillus plantarum* СКБ -368 strain (mcg/ml).

Aspartic acid 10.6 mcg/ml, Glutamic acid 32.5, Serine 21.4, Glycine 15.9, Asparagine 16, Glutamine 40, Cysteine 57.1, Threonine 38.4, Arginine 30, 9, Alanine 40.0 Proline 20.1, Tyrosine 21.8, Valine 37.6, Methionine 15.9, Isoleucine 37.9, Leucine 67.4, Histidine 32.0, Tryptophan 36.5, Phenylalanine 23.9 , Lysine 12.1

mcg/ml amino acids were detected. Cysteine was found to produce the highest amounts of 57.1 mcg/ml, Alanine 40.0 mcg/ml, Glutamine 40 mcg/ml, Threonine 38.4 mcg/ml and Isoleucine 37.9 mcg/ml.

During the research, vitamins V1, V6, V12 and RR produced in the culture liquid of *Lactobacillus plantarum* strain were determined.

Table 1 .

***Lactobacillus plantarum* СКБ -368 strain**

Vitamins _	**#1**	**#2**	**Production u-n special nutrient medium**
	Concentration mcg /ml		
B-1	1.51206	1.825062	19.94511
B-2	0.175786	0.200384	0.181186
B-6	2.253155	3.018533	3.321964
B-9	0.741979	1.784759	0
V-12	0.477462	0.055921	0.06382
PP	0.475475	0.196203	0.161392
S	1.85547	0.313153	0.083088

L. plantarum СКБ -368 for the experiment the bacterial strain was created in the laboratory and grown in a patented, production-oriented special nutrient medium, passed through a filter with a capacity of 0.22 mm, and the amount of vitamins in the culture liquid was determined by the YuSSX method. In the experiments, a nutrient medium was obtained in which the selected active bacterial strain was incubated (Production u-n special nutrient medium, PR-S) . B-1. It was found that 1.825062 mg/l, 19.94511 mg/l of PR-S nutrient medium, and 18.120048 mg/l of vitamin V-1 contained in the nutrient medium were absorbed by the bacterial strain. B-2 0.200384 mg/l was determined in the amount of 0.181186 mg/l in the PR-S nutrient medium. The bacterial strain *L. plantarum was found to synthesize* 0.019198 mg/l vitamin B-2 . It was found that B-6 is 3.018533 mg/l, 3.321964 mg/l in PR-S nutrient medium, *L. plantarum* bacterial strain absorbs 0.303431 mg/l vitamin B-6 from nutrient medium . B-9 1.784759 mg/l, vitamin B-9 was not detected in PR-S nutrient medium, The bacterial strain *L. plantarum was found to synthesize* 1.784759 mg/l vitamin B-9. It was found that V-12 0.055921 mg/l, PR-S 0.06382 mg/l, and *L. plantarum* bacterial strain absorb 0.0007899 mg/l vitamin from the nutrient medium . PP 0.196203 mg/l ,

PR-S 0.161392 mg/l, *L. plantarum* bacterial strain 0.034811 mg/l was found to synthesize vitamin RR . It was found that C is 0.313153 mg/l, 0.083088 mg/l of PR-S nutrient medium , *L. plantarum* bacterial strain produces 1.85547 mg/l of vitamin C.

Summary.

According to the results of the study 20 non-exchangeable amino acid groups were identified in the culture fluid of *Lactobacillus plantarum* . Also *Lactobacillus plantarum In the* culture liquid of strain СКБ-368, 5 vitamins of group V, vitamins RR and C, were detected. The activity of synthesis of amino acids and vitamins of *Lactobacillus plantarum* СКБ-368 strain, whose primary metabolites were studied , was determined. Such microorganism strains are important for the field of biotechnology and production. Among the biologically active substances, the microorganism strains active in the synthesis of amino acids and vitamins have the potential to be used in the fields of pharmaceuticals, agriculture, and animal husbandry. Microorganism strains active in amino acid and vitamin synthesis can be used in the form of biologically active additives to improve human health, as a feed additive for animal feed in all fields of animal husbandry, and as part of organic fertilizers in agriculture. In the course of research, it is planned to carry out experiments on increasing the productivity of amino acid and vitamin synthesis of strains by optimizing fermentation conditions and choosing the optimal composition of nutrient media used in the incubation of bacteria.

Used literature list

1. Enuo Liu. Amino Acid Biosynthesis and Proteolysis in Lactobacillus Bulgaricus // Computational Molecular Bioscience.- Vol.2 - No. 3.- 2012 .
2. Glagoleva L.E., Ryaskina L.O., Rodionov A.A., Pastukhova N.A. Prospects for innovative healthy food products based on dietary supplement "Vitazar" // Bulletin of the Voronezh State University of Engineering Technology 2016. No. 1 (67). S. 122–127.
3. JD Tammam Amino acid fermentation in non-starter Lactobacillus spp. isolated from Cheddar cheese 25 December 2001 https://doi.org/10.1046/j.1472-765x.2000.00727 .
4. Genchi G. An overview on D-amino acids. Amino Acids. 2017;49:1521–1533. doi: 10.1007/s00726-017-2459-5.
5. Shpak V. S. Ways to obtain and use synthetic amino acids // Bulletin of the Academy of Sciences of the USSR. 1983. No. 2. S. 107
6. Burdaeva K. Amino acid market in the Russian Federation: news and trends // Agricultural Review of Tsenovykh. 2015. No. 12. S. 16–25
7. Woodworth Dj. Immobilized cells and enzymes. Method. M.: Mir. 1988. 215 p.
8. Grinstein Dj., Vinits M. Chemical amino acids and peptides. M.: Mir. 1965. 824 c .
9. Kovaleva T. A., Slivkin A. I., Belenova A. S. Suslina S. N. Biotechnology. Voronezh: Publishing House of Voronezh State University. 2011. 89 p
10. S. Kralj, 1 Glucan synthesis in the genus Lactobacillus: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains Microbiology (2004)

11. Kiran Thakur, Sudhir Kumar Tomar, Sachinandan De Lactic acid bacteria as a cell factory for riboflavin production First published: 21. 2015. https://doi.org/10.1111/1751-7915.12335
12. Oscar A. Palacios, Yoav Bashan & Luz E. de-Bashan Proven and potential involvement of vitamins in interactions of plants with plant growth-promoting bacteria—an overview Published: 2014 .
13. Vitamins & Plant GrowthBy: Charles Pearson 21. 2017.
14. R. Tomar. Role of Vitamins in Plant Growth and their Impact on Regeneration of Plants under In vitro Condition Published 31 March 2018 Biology International Journal for Research in Applied Science and Engineering Technology DOI:10.22214/IJRASET.2018.3068 Corpus ID: 90168930
15. Svetlana Gerdes, Claudia Lerma-Ortiz, Océane Frelin, Samuel MD Seaver, Christopher S. Henry, Valérie de Crécy-Lagard, Andrew D. Hanson Plant B Vitamin Pathways and their Compartmentation: a Guide for the Perplexed Journal of Experimental Botany, V. 63, Issue 15. 2012 , P. https://doi.org/10.1093/jxb/ers208