EXPLORING THE BIOGENIC SYNTHESIS OF BRANCH-CHAIN AMINO ACIDS BY DIVERSE LACTIC ACID BACTERIA STRAINS

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https://doi.org/10.5281/zenodo.10515193

Abstract. *The study focused on the capacity of indigenous strains of lactic acid bacteria to synthesize branched-chain amino acids, a pivotal factor in determining the formulation of a biopreparation designed for the prevention and treatment of obesity. Notably, within the array of strains examined, L. casei K7/3 demonstrated a remarkable twofold increase in branched-chain amino acid production when cultivated in MRS-bouillon medium. Specifically, there was a significant elevation in leucine content, a deficiency or absence of which in the human body can precipitate a decline in body weight, impede development and growth, and lead to metabolic disturbances.*

Keywords: *lacto- and bifidobacteria in homeostasis, "ProbiokeirAWL", "LatopropolisAWL", produced by "AllWellLab" LLC, and "Probiokolit".*

The proven importance of lacto- and bifidobacteria in homeostasis and health maintenance of the human body determines the relevance of the development of new technical and technological solutions in the design of food products and biopreparations [1]. Currently, the pharmaceutical industry of Uzbekistan presents a range of domestic biologic supplements - "ProbiokeirAWL", "LatopropolisAWL", produced by "AllWellLab" LLC, and "Probiokolit" (Institute of Microbiology, Academy of Sciences of Uzbekistan) [2, 3].

Achievement of the specified concentrations of bacteria in the active form leads to an increase in the mass fraction of protein in the product and changes in its amino acid composition. The accumulation of amino acids of bacterial origin is an important criterion for estimating the amount of microbial biosynthesis products - biologically active substances produced by bifido- and lactoflora [4].The study of changes in amino acid composition and assessment of biological value of dairy fermented products with high content of probiotic microflora biomass is an actual scientific task.

The three branched-chain amino acids (BCAAs) are valine, leucine and isoleucine. They are part of the 20 amino acids the body needs to make proteins and essential amino acids. These amino acids are considered branched because they have a "branched" side chain consisting of one carbon atom and three hydrogen atoms. Supplements containing leucine can inhibit the activation of AMP-activated protein kinase (AMPK), which is a signal transducer for maintaining energy homeostasis [5].

BCAAs even in small amounts inhibit fatty acid synthesis and improve β-oxidation of fatty acids by modulating hepatic lipogenic gene expression in female broiler chickens, and this modulation is probably through the AMPK-mTOR-FoxO1 pathway [6].

Addition of glutamine (a metabolite of BCAA) to liraglutide regimen in diabetic rats enhances insulin production and hence glycemic control, which is associated with increased

expression of sodium-dependent neutral amino acid transporter-2 (which transports glutamine for insulin regulation and glucagon secretion) in the pancreas [7].

Further results show that different ratios of BCAAs can regulate synthesis, transport, oxidation, lipolysis and secretion of fatty acid adipokines, which is associated with the expression of adipose tissue function genes such as AMPKα, mTOR, silent information regulator transcript 1 (SIRT1) and peroxisome proliferator-activated receptor-G receptor coactivator-1α (PGC-1α) [8].

The aim of this work is to investigate the ability of local strains of lactobacilli to form branched-chain amino acids and to design bioproducts based on them for the prevention and treatment of obesity.

Quantitative analysis of branched chain amino acid (BCAA) in the culture fluid of lactobacilli by HPLC. We quantitatively analyzed 3 branched-chain amino acids (BCAAs) in the culture fluid of Lactobacillus strains. Uncultured MRS-bouillon medium was used as control. 50 μl of reconstituted *Lactobacillus* culture was inoculated in 5 ml of MRS-bouillon and incubated at 37º C for 18 hours. The proteins and peptides of the test samples were precipitated by adding 1 ml of 10% trichloroacetic acid (TCA) solution to 1 ml of the test sample and further centrifugation at 8000 rpm for 15 minutes. 0.1 ml of the supernatant was lyophilically dried and free amino acid derivatives of FTC (phenylthiocarbamyl)-derivatives were synthesized according to the method described in Steven A. et al. (1988).

Identification of amino acid derivatives was carried out by HPLC. HPLC conditions: Agilent Technologies 1200 chromatograph with DAD-detector, 75x4.6 mm Discovery HS C18 column, 3 μm. Solution A: 0.14 M CH3COO-Na + 0.05 TEA, pH 6.4; B:CH3CN. Flow rate 1.2 mL/min, detection at 269 nm. Qualitative analysis and quantitative calculation of the concentration of the free amino acids under study were performed by comparing the retention times and peak areas of standard and FTC derivatives of amino acids under study [9].

Quantitative analysis of the content of branched-chain amino acids (BCAAs) in the culture fluid of lactobacilli. The change in the amino acid composition of essential branched-chain amino acids for the strains under study is presented in Table 1.

The experimental results presented in Table 1 show that the change in the amino acid profile is specific for the studied consortium and for the studied Lactobacillus strain, which indicates the unique metabolic activity of the studied microorganisms in the fermentation process. Changes in the amino acid profile of the studied fermented systems entail changes not only in their functional properties, the study of which is difficult due to the variety of corrected functions, but also changes in the biological value of fermented products (Table 1).

The obtained results indicate that as a result of peptone fermentation in MRS-bouillon medium there is an uneven accumulation of amino acids, which are part of protein substances synthesized by microorganisms and possessing different biological value and functional effect on the organism. During fermentation, preferential synthesis of all 3 amino acids was established in the culture liquid of *L.casei* K7/3 strain.

Thus, in the culture liquid of strain *L.casei* K7/3 the content of free amino acids amounted to 2.78mg/mL, which is 2 times more than the amount of amino acids in the control medium MRS-bouillon (1.28mg/mL). In the culture fluid of strain *L.plantarum* AB-1, the content of free branched-chain amino acids was 2.01 mg/ml; the culture of *L.plantarum* ET-2 synthesized amino acids in the amount of 2.02 mg/ml. The presented data show that the amino acid composition of the cultivation medium is highly dependent on the metabolic activity of the studied strains.

Table 1

Quantitative content of free amino acids in Lactobacillus culture fluid

№	Aminoacids	Quantitative analysis (mg/mL)					
		MRS-bouillon	*L.casei* K7/3	*L.plantarum* AB-1	*L.plantarum* ET-2	*L.casei* CO1	*Product B*
1	Valine	0,26	0,44	0,42	0,40	0,31	0,25
2	Isoleucine	0,33	0,78	0,49	0,74	0,61	0,67
3	Leucine	0,69	1,56	1,10	0,88	1,01	1,22
	Total	1,28	2,78	2,01	2,02	1,93	2,14

L.casei CO1 culture synthesized BCAAs in the amount of 1.93 mg/mL, which is 1.5 times more than in the control medium.

The change of amino acid composition of the culture fluid of the strains under study due to proteolytic properties of lactobacilli is a very valuable property. The final dairy product is enriched with essential amino acids, which are very necessary for the human body.

Thus, the obtained data indicate that as a result of fermentation of MRS-bouillon by local strains of lactobacilli it is possible to obtain different amino acid composition of products with different biological value and functional properties. The difference in amino acid activity of the studied strains of lactobacilli can be expressed in the formation of functional properties of biopreparations proposed for use in the diet of athletes and obese patients.The difference in amino acid activity of the studied strains and products based on them can be expressed in the formation of different functional biocorrective properties of bioproducts produced with the use of these microorganisms, which necessitates the development and implementation of complex schemes for the use of fermented products for therapeutic purposes, taking into account the metabolic amino acid activity of different consortia.

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