DETERMINATION OF FREE AMINO ACIDS AND WATER-SOLUBLE VITAMINS IN THE CULTURAL LIQUID OF LACTOBACTERIA

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Abstract. *The presence of free amino acids and water-soluble vitamins in the culture liquid of lactobacilli was determined by HPLC. it was found that during the growth of lactobacilli consume some amino acids and increase the amount of others. It has been established that the amino acid profile of L. plantarum TK1, L. plantarum KA3, L. kunkeeii, Ent. faecalis differs significantly from the medium profile of MPC broth. It was shown that the studied lactic acid bacteria produce vitamin B9, vitamin PP and, in a significant amount, vitamin C.*

Keywords: *essential amino acids, the vitamin-producing gut microbiome, amino acid derivatives, antioxidant activity.*

Microorganisms are a natural source of various metabolites (vitamins, essential amino acids, organic acids, peptides), which may be strain or species specific.

Oxidative resistance as a normal, inevitable and mandatory physiological process plays a positive role in maintaining homeostasis in living organisms. However, when oxidation resistance exceeds the body's immune load, it will inevitably have a harmful effect on the body, even causing various chronic and even life-threatening diseases. To date, we are well aware of various antioxidant systems that exist in living organisms, and these include some enzymes, such as superoxide dismutase,1, 2, 3], albumin, ceruloplasmin, ferritin, and other proteins [4, 5, 6], many compounds of relatively small molecules, for example, ascorbic acid, α-tocopherol, β-carotene, ubiquinol-10, glutathione (GSH), methionine, uric acid, bilirubin [7, 8, 9] and hydroxytyrosol, etc. [10] and some hormones such as estrogen, angiotensin, melatonin, etc. Based on the search for molecules with antioxidant activity, 20 amino acids can be divided into two categories according to their antioxidant capacity. Seven amino acids, including tryptophan, methionine, histidine, lysine, cysteine, arginine, and tyrosine, have the highest total antioxidant capacity compared to 13 other amino acids. [11].

Gut bacteria or groups of bacteria (microbiota) use dietary or body-derived AAs as building blocks for protein synthesis or further metabolize these nutrients through conversion or fermentation to form various metabolites (ammonia, hydrogen sulfide (H 2 s)) [1 2]. In addition, gut bacteria can synthesize some nutritionally essential amino acids (EAAs), which may help regulate AA homeostasis in the body. [13] .

Vitamins are essential micronutrients for every living cell, acting as precursors or participating in many important enzymatic reactions or even in the electron transport chain. Microorganisms can usually biosynthesize B vitamins according to their needs, but humans cannot produce them and therefore must depend on external sources to meet their daily needs [1 4] . The

vitamin-producing gut microbiome can synthesize vitamins and supply them to the human host. These value-added properties have increased the demand for the use of microbes as feed additives due to their ease of production and health benefits. Scientists are trying to find new lactic acid bacteria capable of synthesizing vitamins and amino acids.

Purpose of the study. Determination of the presence of free amino acids and water-soluble vitamins in the culture liquid of lactobacilli by HPLC.

The sedimentation of proteins and peptides from the aqueous extract of the samples was carried out in centrifuge beakers. To do this, 1 ml (exact volume) of 20% TCA was added to 1 ml of the test sample. After 10 minutes the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. After separating 0.1 ml of the supernatant, freeze-dried. The hydrolyzate was evaporated, the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice to neutralize the acid. Reaction with phenylthioisocyanate gave phenylthiocarbamyl derivatives (FTC) of amino acids according to the method of Steven A., Cohen Daviel.

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. Solution A: 0.14 M CH3COONa + 0.05% TEA pH 6.4, B: CH3CN. Flow rate 1.2 ml/min, absorbance 269nm. Gradient %B/min: 1-6%/0-2.5min; 6-30%/2.51-40min; 30-60%/40.1-45min; 60-60%/45.1-50min; 60-0%/50.1-55min. (Table 1).

Table 1

Amount of free amino acids in the culture liquid of lactic acid bacteria

Amino acidstalar nomi	**control**	**L.pl.TK1**	**L.pl.ka3**	**L.kunkeei**	**EF1**
	Concentration mg/ml				
Aspartic acid	2.742692	4.364313	4.276062	3.612796	10.00552
Glutamic acid	1.530007	12.97017	13.53629	7.818388	14.47401
Serine	1.971316	5.116704	5.182087	1.489033	4.172525
Glycine	1.636846	7.466066	4.871668	5.831071	6.028504
Asparagine	1.820143	7.419872	4.027983	5.758136	5.389546
Glutamine	6.949738	2.130777	3.317056	1.861601	7.436875
Cysteine	1.473747	3.206046	3.625696	1.040175	1.549324
Threonine	4.138648	7.042879	1.742539	5.329618	3.698527
Arginine	1.71508	5.92195	4.780879	3.163046	7.120485
Alanine	2.226049	0.652019	0.680127	0.652415	3.637371
Proline	0.782363	1.806661	2.216171	0.588605	4.481567
Tyrosine	16.70839	9.9171	5.894018	7.342328	7.423602
Valine	5.440613	3.44934	5.323542	7.759685	1.408046
Methionine	5.283618	1.002578	2.213407	2.262991	0.574177
Isoleucine	4.907595	10.41519	13.84937	15.61139	16.89367
Leucine	13.22239	11.56866	12.73134	15.26716	15.30149
Histidine	0.536616	3.950758	0.32197	0.434343	2.474747
tryptophan	9.400496	0.855843	9.951764	10.64223	4.386714
Phenylalanine	20.10651	9.794142	1.401065	13.8898	16.92237
Lysine HCl	0.552351	0.211846	0.340506	0.197427	0.461402
Total	**103.1452**	**109.2629**	**100.2835**	**110.5522**	**133.8405**

According to Naijin Xu et al., [15] the amino acids histidine, cysteine, and arginine have a pronounced antioxidant activity. It is possible that the production of these amino acids determines the antioxidant properties of these bacterial strains.

Thus, it was found that during growth, lactobacilli consume some amino acids and increase the amount of others. Thus, *Lactobacillus plantarum TK1* releases aspartic acid, glutamic acid, serine, glycine, asparagine, cysteine, arginine, proline, isoleucine, histidine into the culture fluid in a significant amount. *Lactobacillus plantarum KA3* more than doubles the content of amino acids in the nutrient medium aspartic acid, glutamic acid, serine, glycine, asparagine, cysteine, arginine, proline, isoleucine, *Lactobacillus kunkeeii* produces the amino acids glutamic acid, serine, glycine, asparagine, isoleucine, while *Enterococcus faecalis* - aspartic acid, glutamic acid, serine, glycine, asparagine, arginine, proline, isoleucine, histidine.

HPLC analysis of water-soluble vitamins is carried out on an Agilent Technologies 1200 chromatograph on an Exlipse XDB C18 column (reversed phase), 3.5 μm, 4.6x150 mm. Diode array detector (DAD), 254, 290 nm. Solution A: 0.5% acetic acid, pH 1.7: B:CH3CN (acetonitrile). Flow rate 1 ml/min. Gradient %B/min: 0-5min/96:4%, 6-8min/90:30%, 9-15min/80:20%, 15-17min/96:4%. Thermostat 25 ^{0} C (table 2).

Table 2

Amount of water soluble vitamins in the culture liquid of lactic acid bacteria

vitamins	control	L.plantarum TK1	L. plantarum KA3	L.kunkeei1	E.faecium1
	Concentration, mg/μl				
B-1	0.187865	0.069253	0.070745	0.062912	0.071491
B-2	0.302755	0.061071	0.122141	0.2079	0.06237
B-6	0.026222	0.019519	0.002169	0.003746	0.007886
B-9	0.016243	0.028542	0.035288	0.042034	0.127141
AT 12	0.03135	0	0	0	0
PP	0.029153	0.040143	0.025489	0.036324	0.09385
WITH	0.021757	0.101531	0.129734	0.132957	0.309428

It has been shown that the studied lactic acid bacteria produce vitamin B9, vitamin PP, and vitamin C in a significant amount. It is known that vitamin C is a strong antioxidant [16]; 5-15 times higher than in the control) can play an important role in the formation of the antioxidant activity of each strain and the complex preparation as a whole.

Conclusion. Based on the results obtained, it can be said that lactobacilli in the process of growth consume some amino acids and synthesize others, produce vitamin B9, vitamin PP and vitamin C.

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