ANALYSIS OF WATER-SOLUBLE VITAMINS IN LACTIC ACID BACTERIA

[1]Bekmurodova G.A., [2]Xidirova M.A., [3]Yusupova M.Sh., [4]Rakhimova K.A., [5]Miralimova Sh.M.

[1,2,5]Institute of Microbiology, Academy of Sciences, Uzbekistan
[3]National University of Uzbekistan graduate student of the Faculty of Biology
[4]Tashkent Pharmaceutical Institute

https://doi.org/10.5281/zenodo.12514666

Abstract*. Lactic acid bacteria (LAB) have the ability to synthesize water-soluble vitamins, including B vitamins such as folate, riboflavin, and vitamin B_{12}. The characteristic of effective synthesis of vitamin B_9 from SKB used in the test was L. plantarum KA3 (0.035288 µcg/ml), L. plantarum TK1 (0.028542 µcg/ml), L. lactis TV (0.028500 µcg/ml), A. kunkeei (0.042034 µcg/ml) and E. faecium 1 (0.127141 µcg/ml) strains were determined.*

Keywords*: lactic acid bacteria, water-soluble vitamins, high performance liquid* chromatography.

Lactic acid bacteria (LAB) have the ability to synthesize water-soluble vitamins, including B vitamins such as folate, riboflavin, and vitamin B_{12} [1]. Although most LAB strains are auxotrophic for several vitamins, some strains have the ability to produce these vitamins.

Synthesis of B vitamins by LAB has attracted attention due to its potential applications in food production and in the fortification of cereal products [2,3]. LAB can be used as a starter factor in the fermentation process of various food products, improving their safety, shelf life, nutritional value, taste and overall quality [3]. By incorporating vitamin-producing LAB strains into these products, the B vitamins can be increased, providing additional nutritional benefits [2].

Production of vitamins such as riboflavin by LAB has been studied as an alternative to chemical synthesis. LAB has shown that microbial production of riboflavin has advantages over chemical synthesis. LAB strains have a genetic predisposition for riboflavin biosynthesis, which makes them potential candidates for the production of this vitamin.

In addition to riboflavin, LAB can also synthesize other B vitamins, such as vitamins B_2, B_{11} and folates. The use of genetic strategies to increase vitamin production or create new vitamin-producing LAB strains has also been explored [4].

The ability of LAB to synthesize vitamins has potential applications in alleviating vitamin deficiencies in human populations. Despite the availability of vitamins in various foods, deficiencies still exist due to malnutrition and disordered eating. The use of vitamin-producing LAB may be a cost-effective alternative to current vitamin fortification programs and may contribute to the development of new vitamin-fortified foods [7].

Objective: to analyze the content of water-soluble vitamins in probiotic strains of lactic acid bacteria isolated from local sources.

Research methods: in this research, materials related to *Lactobacillus* family such as *L. delbrueckii* (2 pcs.), *L. plantarum* (2 pcs), *L. reuteri* (2 pcs), and materials related to *Lactococcus* family such as *L. lactis* (2 pcs), *Apilactobacillus kunkeei* and *Enterococcus* family related *E. faecium* strains were used from local sources [5]. All strains are stored frozen at -80°C in the collection of the Laboratory of Microbiology and Biotechnology of Probiotics of the Institute of

Microbiology of the Academy of Sciences of the Republic of Uzbekistan. The same nutrient environment that was used for growing bacteria was selected as the control group.

Preparation of research strains. All strains were activated by inoculation of 1% (v/v) CDM [6] nutrient broth at least three times for 24 h at 37°C.

Used equipments. Vitamin B_{12} was obtained from "Rhydburg Pharmaceuticals" (Germany), vitamins B_1, B_2, B_6, B_9, PP and C from "DSM Nutritional Products GmbH" (Germany). High Performance Liquid Chromatography (HPLC) grade water, acetonitrile, chemically pure grade acetic acid and sodium hydroxide reagents were used.

The amount of water-soluble vitamins in the culture fluid was carried out on a LC-40 Nexera HPLC manufactured by Shimadzu, Japan.

Preparation of standard solutions. Solutions of vitamins PP (CAS 40–60–7) C (CAS 50–81–7), B_1 (CAS 70–16–6), B_6 (CAS 65–23–6) and B_{12} (CAS 68–19–9) (100 mg/l) is prepared by dissolving 5 mg of each vitamin in 50 ml of HPLC grade water. Standard solutions of vitamins B2 (CAS 83-88-5) and B_9 (CAS 59-30-3) were prepared by dissolving 5 mg of these vitamins in 50 ml of 0.025% sodium hydroxide solution. Then, 200 µl of all vitamins were mixed and a stock solution with a concentration of 16.67 mg/l of each vitamin was prepared. By diluting it, solutions with a concentration of 3.333 mg/l, 0.667 mg/l and 0.133 mg/l were prepared, poured into a vial and used for analysis.

Preparation of sample solution. For the extraction of water-soluble vitamins, 1 ml of the tested sample was measured, placed in a 50 ml conical flask, and 25 ml of 0.1 N HCl solution was added. The mixture was extracted in an ultrasonic bath of GT SONIC-D3 (China) at a temperature of 60°C for 20 minutes. Then the mixture was cooled, filtered and made up to 25 ml with water in a volumetric flask. 1.5 ml of the extract was filtered through a 0.45 µm syringe filter and placed in a vial and used for analysis.

Identification of vitamins. Standard solutions and sample extracts were analyzed using the following items such as LC-40 Nexera Lite high performance liquid chromatograph consisting of LC-40D pump, SIL-40 autosampler, SPD-M40 photo-diode array detector (PDA) and LabSolutions ver. 6.92 software. Also, the following were used in the process as well such as, Shim pack GIST C18 (150 × 4.6 mm; 5 µm, Shimadzu, Japan) reversed-phase column and a gradient mobile phase consisting of acetonitrile (A) and a 0.25% solution of acetic acid in water (B). The injection volume was set at 10 µl, the flow rate at 0.9 ml/min, and the column thermostat temperature at 35°C. Analytical signal (peak area) of each vitamin was recorded at three wavelengths 265, 291, 550 nm.

The results obtained. Analysis of water-soluble vitamins in their culture fluids was performed strains isolated from local sources in the study were used such as *Lactobacillus delbrueckii D, Lactobacillus delbrueckii R2, Lactiplantibacillus plantarum KA3, L. plantarum TK1, Limosilactobacillus reuteri 1, L. reuteri 2, Lactococcus lactis R2, L. lactis TV, Apilactobacillus kunkeei, Enterococcus faecium 1* (Table 1).

When quantitative indicators are compared with CDM control indicators, vitamin B9 is present in all bacterial strains used in the test (6), but the most effective indicator compared to the control is *L. plantarum KA3* (0.035288 µcg/ml), *L. plantarum* TK1 (0 ,028542 µcg/ml), *L. lactis* TV (0.028500 µcg/ml), *A. kunkeei* (0.042034 µcg/ml) and *E. faecium* 1 (0.127141 µcg/ml) were recovered in LAB strains. PP vitamin *A. kunkeei* (0.132957 µg/ml) and E. faecium1 (0.309428 µcg/ml) LAB strains, *L. plantarum KA3* (0.129734 µcg/ml), *L. plantarum TK1* (0.101531 µcg/ml)

and *A. kunkeei* (0.132957 µcg/ml) and *E. faecium1* (0.309428 µg/ml) LAB strains, vitamin B_{12} was not recovered in almost all strains tested. Recovered *L. reuteri* 1 (0.00385 µcg/ml), *L. reuteri* 2 (0.000451 µcg/ml) was evaluated as low in effectiveness. Vitamins B1, B2 and B6 showed lower results compared to the control.

Table 1

The number of water-soluble vitamins in the culture fluid of Lactic acid bacteria

	B_1	B_2	B_6	B_9	B_{12}	PP	C
	Concentration µcg/µl						
Nazorat (TTOM)	0,187865	0,302755	0,026222	0,016243	0,031350	0,029153	0,021757
L.delbrueckii D	0.022501	0.012511	0.003523	0.018210	0	0,006324	0
L.delbrueckii R2	0.005575	0.0.4752	0	0.002725	0	0,000851	0
L. plantarum KA3	0,070745	0,122141	0,002169	0,035288	0	0	0,129734
L. plantarumTK1	0,069253	0,061071	0,019519	0,028542	0	0	0.101531
L.reuteri 1	0.042500	0.057510	0	0,016851	0,003854	0	0
L.reuteri 2	0.041240	0.042520	0	0,013850	0,000451	0	0
L.lactisR2	0.087530	0.002521	0	0,012401	0	0	0
L.lactis TV	0.018625	0.002512	0	0,028500	0	0	0
A.kunkeei	0,062912	0,207912	0,003746	0,042034	0	0,036324	0,132957
E. faecium1	0,071491	0,062370	0,007886	0,127141	0	0,093850	0,309428

Summary. Lactic acid bacteria have the ability to synthesize a number of vitamins during metabolism. Among the lactic acid bacteria used in the test, *L. plantarum* KA3 (0.035288 µcg/ml), *L. plantarum* TK1 (0.028542 µcg/ml), *L. lactis* TV (0.028500 µcg/ml), *A. kunkeei* (0.042034 µcg/ml) and *E. faecium 1* (0.127141 µcg/ml) were found to be able to efficiently synthesize vitamin B9.

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