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EFFECT OF B VITAMIN ADDITION ON THE BIOSYNTHESIS OF LACTIC ACID BY Lactobacillus delbrueckii

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Aim. The main objective of this work is to investigate the effect of B vitamins on the biosynthesis of lactic acid (LA) by the *Lactobacillus delbrueckii* UY-2/13 strain when using corn-steep liquor as the sole nitrogen source.

Methods. The influence of B vitamins was assessed using a one-factor experimental design. The lactic acid concentration in the culture broth at the end of fermentation was determined using high-performance liquid chromatography, while reducing sugars were analyzed by the modified Bertrand method.

Results. The study revealed that riboflavin (B_2) at a concentration of 1 mg/L increased lactic acid production by approximately 6%, whereas calcium pantothenate (B_5) reduced it by about 4%. Other vitamins (PP, B₉, B₁, B₆, H) had no significant effect on LA biosynthesis.

Conclusions. Vitamins B_2 and B_5 have a specific effect on the biosynthesis of lactic acid by *L. delbrueckii* UY-2/13. Other B vitamins (PP, B₉, B₁, B₆, and H) do not significantly influence the biosynthesis of lactic acid.

Keywords: Lactobacillus delbrueckii, lactic acid biosynthesis, B vitamins, cofactor supplementation, vitamin influence.

Lactic acid, also known as hydroxypropanoic acid, is an industrially significant organic acid primarily produced through biotechnological processes. Lactic acid bacteria (LAB) are the leading microbial producers of lactic acid; however, their cultivation requires complex nutrients that must be incorporated into the fermentation medium [1]. One of the most commonly used components is yeast extract, which can account for up to 38% of the total production costs, making large-scale lactic acid manufacturing economically challenging [2].

To reduce costs, alternative low-cost nitrogen sources can be used instead of yeast extract. However, such substitutions often lead to a significant decrease in process efficiency. The critical role of yeast extract in LA biosynthesis is attributed to its composition, particularly its content of purine and pyrimidine bases, as well as B vitamins [3]. These vitamins are essential cofactors in metabolic pathways responsible for carbohydrate conversion into lactic acid [4].

Thus, the complete or partial replacement of yeast extract with more affordable nitrogen sources, supplemented with B vitamins, may help sustain high lactic acid concentrations while substantially reducing fermentation costs.

Aim. The main objective of this work was to investigate the effect of B vitamin supplementation on LA biosynthesis by Lactobacillus delbrueckii UY-2/13 when using corn-steep liquor as the sole nitrogen source.

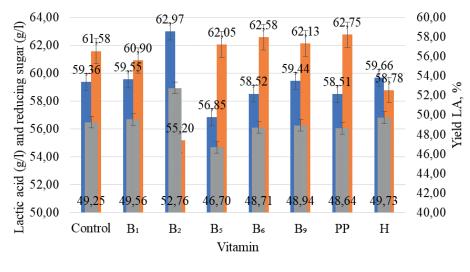
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Methods. Microorganism. L. delbrueckii UY-2/13 was obtained from the industrial collection of production strains (DE "Enzym", Ladyzhyn, Ukraine). Cultivation. The seed culture was grown in MRS medium (De Man, Rogosa, and Sharpe, 1960) in test tubes and flasks at 40 ± 2 °C for 16 hours. Lactic acid biosynthesis was conducted in 300 mL-Erlenmeyer flasks containing 150 mL of medium (glucose — 180 g/L, corn steep liquor — 60 g/L, CaCO₃ — 10 g/L). The media were sterilized at 112 °C for 20 minutes. Fermentation was performed at 40 ± 2 °C without stirring, with an inoculum volume of 10%, and a total process duration of 96 hours. The pH was maintained between 4.5 and 6.5 by adding a 10% Ca(OH)₂. Preparation of vitamin solutions. Sterile vitamin solutions were obtained by filtration through a 0.2 µm cellulose acetate membrane (Sartorius Corporation, New York, USA) and added after medium sterilization. The tested vitamins and their concentrations were as follows: PP (10 mg/L), B₅ (6 mg/L), B₂ (1 mg/L), B₉ (500 µg/L), B₁ (500 µg/L), B₆ (400 µg/L), and H (30 µg/L).

Analysis. The LA concentration was determined using high-performance liquid chromatography (Shimadzu[®] LC-40D; Shimadzu Corporation, Kyoto, Japan). The concentration of reducing sugars was measured using the modified Bertrand method. The lactic acid yield was calculated considering the dilution factor of the culture fluid with a 10% Ca(OH)₂.

Results and Discussion. The selection of vitamin concentrations for the fermentation medium was based on literature data [2, 4] and an approximate calculation of their content in yeast extract at a concentration of 30 g/L. The influence of vitamins was assessed using a one-factor experimental design.

As shown in Figure, riboflavin (B₂) exhibited a positive effect on lactic acid biosynthesis, increasing its concentration by approximately 6%. In contrast, calcium pantothenate (B₅) reduced LA production by approximately 4% (Fig.). Other tested vitamins (PP, B₉, B₁, B₆, H) had no significant impact on lactic acid biosynthesis under the given experimental conditions. According to the results obtained, vitamins B₂ and B₅ in the described concentrations have a specific effect on the biosynthesis of LA.



■ Lactic acid, g/l ■ Reducing sugar, g/l ■ Yield LA, %

Figure. Study of the effect of B vitamins on the biosynthesis of lactic acid by strain L. delbrueckii UY-2/13 Control — without the addition of vitamins

Conclusions. Vitamins B_2 and B_5 have a specific effect on the biosynthesis of lactic acid by *L. delbrueckii* UY-2/13. Other B vitamins (PP, B_9 , B_1 , B_6 , and H) do not significantly influence the biosynthesis of lactic acid.

Authors' contribution

Dmytro Kiiv conducted the literature review, performed the experimental studies and data analysis, and drafted the manuscript. Sofia Vasylyuk provided critical input throughout the research, data analysis, and manuscript preparation. All authors have read and approved the final version of the manuscript.

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