

COMPREHENSIVE EVALUATION OF THE BIOSYNTHETIC POTENTIAL OF *Lactobacillus delbrueckii* STRAINS IN LACTIC ACID SYNTHESIS

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Aim. To perform a comprehensive evaluation of the biosynthetic potential of *Lactobacillus delbrueckii* strains in lactic acid synthesis for potential industrial use.

Methods. The study examined four strains of *Lactobacillus delbrueckii* capable of synthesizing lactic acid (LA). The concentration of lactic acid in the fermentation broth at the end of fermentation was determined using high-performance liquid chromatography. The concentration of free reducing sugars in the nutrient medium was analyzed by a modified Bertrand method at the beginning of the process and by either the Bertrand or Somogyi-Nelson method at the end.

Results. The biosynthesis of lactic acid by four *L. delbrueckii* strains (PB-07, UY-2/13, BMP-92, Q-50) was studied under varying concentrations of carbon sources and nitrogen sources, as well as under optimal (40 °C, without stirring) and suboptimal (30 °C and 50 °C, with stirring) fermentation conditions. One strain, *L. delbrueckii* UY-2/13, demonstrated characteristics suitable for commercial production, achieving a biosynthesis yield of approximately 70 g/dm³ using an inexpensive nitrogen source in batch cultivation with high initial glucose concentrations. The study revealed that physical parameters have a significant influence on the process. A fermentation temperature of 50 °C strongly inhibited lactic acid biosynthesis, while the inhibitory effect at 30 °C was less pronounced. Additionally, pH adjustment and temperature control during fermentation require precise regulation to avoid adverse effects.

Conclusions. The *L. delbrueckii* UY-2/13 strain is a promising candidate for lactic acid production. Its cultivation on cost-effective raw materials, such as low-cost corn extract, could enhance the economic viability of the process.

Key words: *Lactobacillus delbrueckii*, biosynthesis, lactic acid, fermentation, lactic acid bacteria, organic acid production, biotechnology.

Lactic acid or 2-hydroxypropanoic acid (IUPAC) is a naturally occurring organic acid with the molecular formula CH₃CH(OH)COOH, which has two enantiomers (D(–) and L(+)). The Food and Drug Administration (FDA) considers lactic acid (LA) to be a harmless chemical when used under certain conditions [1].

The use of L-lactic acid in industry is due to its antibacterial properties [2] and unique structure, which allows it to be

used in the synthesis of many final and intermediate chemical compounds, namely: ethyl lactate, 1,2-propanediol, acetaldehyde, 2,3-pentanedione, acrylic, pyruvic, and oxalic acids, as well as PLA plastic [3]. Biodegradable polylactide has gained significant popularity due to environmental trends and the shift towards environmentally friendly materials, serving as an alternative to polypropylene and polyethylene. Due to its biosolubility, the use of

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PLA plastic in medicine is also being considered for tissue engineering, drug delivery, and restoration of lost or damaged bones and organs [4]. According to a study by scientists from Ohio State University (Columbus, USA), the rapid growth in the production and use of polylactide has increased the capacity of the LA market by more than 400% between 2016 and 2025 [5]. In addition to the applications described above, lactic acid is also used in the food industry (as a preservative, acidity regulator, and antioxidant) and cosmetics (as part of acne and tartar products, as well as skin lightening and rejuvenation products) [6].

Global trends toward the dynamic expansion the use of lactic acid significantly affect the Ukrainian market. According to research by Ukrainian scientists [7], the domestic lactic acid market is import-dependent, and as of 2023, the volume of LA imports to Ukraine is 685 times higher than exports. Domestic industrial production of lactic acid is at a low level and does not meet domestic needs for lactic acid. Given the large amount of renewable raw materials and agricultural industry waste that can be used as components of the nutrient medium, Ukraine has significant potential for the development of its own large-scale production, which can bring substantial income to the country's economy.

Lactic acid is produced in industry by two methods: chemical and biotechnological synthesis. Today, chemical synthesis is rarely used; however, more than 90% of lactic acid worldwide is produced through biotechnology [8]. Depending on the stereospecificity of lactate dehydrogenases that carry out the reduction of pyruvate, microorganisms can synthesize L(+)-, D(-)-, or a racemic mixture of lactic acid. Industrial value is in this case for producers of L(+)-lactic acid, the natural enantiomer of humans and other higher life forms. Unlike L-lactic acid, D-lactic acid has a neurotoxic effect on the human body, causing encephalopathy, acidosis, and ataxia [9]. Industrial strains producing L-lactic acid include lactic acid bacteria (LAB), genetically modified strains of bacteria *Bacillus spp.*, and microscopic fungi *Rhizopus spp.*, as well as bacteria *Escherichia coli*, *Corynebacterium glutamicum*, and yeast *Saccharomyces cerevisiae* [10–12]. At the same time, the classic industrial producers of lactic acid are homofermentative lactic acid bacteria, which have the following advantages: resistance to acidic pH values, absence of by-products, high yield, optical purity, and LA concentration,

which can reach more than 100 g/l without interfering with the genome of microorganisms [9]. For example, scientists from China isolated and obtained the *Lactobacillus rhamnosus* LA-04-1 strain, which synthesized lactic acid at a concentration of 170 g/dm³ [13]. Despite the facts described above, large-scale production of lactic acid faces specific difficulties related to biochemical, technical, and economic aspects

Biochemical issues

A typical scheme for producing lactic acid is batch fermentation — a simple process from a technological standpoint, which allows for obtaining high concentrations of acid without the risk of contamination by other microorganisms. In addition to this method, various approaches to the fermentation process are also employed, including fed-batch, repeated, and continuous cultivation. However, these methods require specialized equipment and technological support, as well as constant monitoring, qualified personnel, or are characterized by a high level of contamination and low acid yields. The main disadvantages of a typical batch cultivation are the biochemical limitations of microorganisms, namely inhibition of microorganisms by high concentrations of substrates (carbon source) and the final product (lactic acid) [14, 15].

Economic issues

A necessary condition for the growth of lactic acid bacteria, as well as the synthesis of high amounts of lactic acid, is the presence of complex and balanced components of the nutrient medium. One of these components is expensive yeast extract, which fully meets the biochemical needs of the LAB in amino acids, peptides, and B vitamins [16]. Additionally, due to the dependence of glucose concentration on lactic acid concentration at the end of the process, it is necessary to add large amounts of carbon source to the nutrient medium, which also increases the process cost. According to a study by American scientists, the cost of lactic acid accounts for 39 to 45% of the components of the nutrient medium [17]. A promising direction of research in the field of industrial production of LA is the use of alternative and cheap sources of nitrogen. For example, corn extract — an inexpensive by-product of corn processing.

Technical issues

Essential characteristics of the fermentation process are the physical conditions of

the process, namely: partial pressure of oxygen and carbon dioxide, osmotic pressure, pH value, stirring, and temperature [18]. The most significant and unstable parameters among them in large-scale production are temperature and stirring. To the best of our knowledge, lactic acid bacteria are obligate anaerobes. Given this fact, the fermentation process must occur without access to oxygen, i.e., without stirring and aeration. However, in the process of maintaining pH, the fermentation broth must be mixed for uniform distribution of the neutralizing agent.

Additionally, due to the structural features of the fermenters, local overheating of the fermentation broth near the walls is possible, while in the liquid column, a temperature lower than the set temperature is observed. The factors described above negatively affect the biosynthesis process. Therefore, an industrial lactic acid producer must have the flexibility to change the physical conditions of the process.

The purpose of this article is to provide a comprehensive assessment of the biosynthetic potential of *Lactobacillus delbrueckii* strains for lactic acid production, which will contribute to the development of applied research in the field of biotechnological lactic acid synthesis and create the prerequisites for establishing large-scale industrial production in Ukraine.

Materials and Methods

Strains and Inoculation Protocol

Four strains of lactic acid bacteria, *Lactobacillus delbrueckii*, were used in the work: PB-07, UY-2/13, BMP-92, Q-50 (industrial collection, daughter enterprise “Enzim”, Ladyzhyn, Ukraine). The genus and species of microorganisms were confirmed by typical morphology, lactic acid synthesis (confirmed by HPLC), and a negative catalase test [19].

Composition of MRS inoculation medium (g/dm³): glucose, 20; yeast extract, 12; peptone, 7.5; dipotassium phosphate, 2; ammonium citrate, 2; magnesium sulfate heptahydrate, 0.2; manganese sulfate heptahydrate, 0.05; agar-agar, 0.5. Medium was sterilized at 120 °C for 30 min.

The inoculum was grown on MRS medium in test tubes at 40±2 °C for 16–24 hours. The inoculum was prepared by transferring 20 mL of the inoculum into an Erlenmeyer flask containing 200 mL of MRS medium. Four *Lb. delbrueckii* strains were grown at 40±2 °C for 16 hours and then used for research.

Fermentation process protocol

The process of lactic acid biosynthesis was carried out in Erlenmeyer flasks with a capacity of 0.3 dm³, containing 150 ml of fermentation medium (sterilization mode: 112 °C, 20 min). 15 mL of inoculum was added to the medium, cooled after sterilization, and incubated at 40 ± 2 °C without stirring, unless otherwise specified. During the cultivation in the flasks, the pH value was maintained at 4.5–6.5 by adding 10% Ca(OH)₂ solution. Depending on the glucose concentration in the nutrient medium, the duration of the biosynthesis process was from 64 to 96 hours.

Composition of fermentation media:

Stage 1. Study of the biosynthesis of lactic acid depending on the concentration of the carbon source (g/dm³): glucose, 90, 130, 150, 180, and 220; yeast extract, 30; calcium carbonate, 10.

Stage 2. Study of the biosynthesis of lactic acid depending on the nitrogen source (g/dm³): glucose, 150, 180; corn extract, 60; calcium carbonate, 10.

Stage 3. Study of the biosynthesis of lactic acid depending on the change in physical conditions of fermentation (g/dm³): glucose, 100; corn extract, 60; calcium carbonate, 10.

To study the process of LA biosynthesis during stage 3, the fermentation process was carried out at temperatures of 30, 40, and 50 °C without stirring, as well as at 40 °C with constant stirring at 120 rpm.

The numerical results of the study are presented as the arithmetic mean and standard deviation ($M \pm \sigma$). The leading indicators were compared with those of the control group, as indicated in each study in the note, using the Student's t-test; the results were considered reliable at $P < 0.05$. All experiments were performed in duplicate or triplicate. The calculation of all indicators and the construction of graphs and histograms were performed using Microsoft Excel (USA).

Quantitative analysis of lactic acid and monosaccharides

Sample preparation

After completing the lactic acid biosynthesis process in Erlenmeyer flasks, the fermentation broth was diluted three times with distilled water and centrifuged at 4000 g for 10 minutes. The supernatant was used for further studies.

Determination of lactic acid concentration

To determine the concentration of lactic acid in the fermentation broth, a chromatographic separation and analysis method, specifically high-performance liquid chromatography, is employed. The study was performed using a Shimadzu high-performance liquid chromatograph (LC-40D; Shimadzu Corporation, Kyoto, Japan) equipped with a Kromasil chromatographic column (Eka Chemicals AB, Bohus, Sweden) and a SPD-40 spectrophotometric detector.

Determination of free reducing sugar (FRS)

The quantitative determination of free reducing sugars, namely glucose, in the culture broth (CB) before the start of fermentation is carried out using the modified Bertrand method [20]. At the end of the fermentation process, the amount of glucose that has not been converted to lactic acid is measured in the fermentation broth. If the concentration of free reducing sugars exceeds 20 g/dm³, when the modified Bertrand method is used. In cases when the concentration of residual glucose is less than 20 g/dm³, the Somogyi-Nelson method is used [21].

Determination of lactic acid yield

The lactic acid yield was calculated using the formula (equation 1):

$$\% \text{LA}_{\text{yield}} = \frac{\text{LA concentration in FB} \times \text{DF}}{\text{FRS initial concentration in CB}} \times 100\%, \quad (1)$$

Equation (1) takes into account the dilution factor (DF) of the fermentation broth (FB) with a 10% calcium hydroxide solution during the pH adjustment process and is calculated by the formula (equation 2):

$$\text{DF} = \frac{m_{\text{beg}}(\text{FB})}{m_{\text{end}}(\text{FB})}, \quad (2)$$

The mass of the fermentation broth (FB) at the beginning and end of the biosynthesis process was determined by the gravimetric method (Technovagy, TVE-2.1-0.01-a, Lviv, Ukraine).

Results and Discussion

Lactic acid bacteria used in the studies belong to the genus *Lactobacillus* and produce lactic acid. Still, for their industrial use, they must have the ability to synthesize lactic acid at a concentration of more than 100 g/dm³. For such an amount of the final metabolite, it is necessary to add more than 100 g/dm³

of glucose to the nutrient medium, which is converted into LA with a theoretical yield of 1 g/g [6].

A solution for neutralizing the acidic pH of the fermentation broth plays a vital role in the fermentation process, which is associated with the synthesis of a significant amount of lactic acid. According to an article by Japanese scientists [22], calcium hydroxide is the most effective agent for adjusting the pH, and the calcium lactate formed during the chemical reaction has the least inhibitory effect. Additionally, calcium hydroxide is a relatively inexpensive reagent.

In order to study the use of *Lb. delbrueckii* strains as industrial producers of lactic acid, the work was divided into three stages. At the first stage, the ability of strains to LA synthesize is determined by the concentration of glucose in the medium, as well as the concentration of glucose at which inhibition of microorganisms by the substrate occurs. The second stage involves studying the biosynthesis of lactic acid using corn extract. As previously indicated, corn extract is a cheap alternative to yeast extract. According to the data provided to us by DE "Enzim", the cost of corn extract can be 40-50 times lower than yeast extract. The purpose of the third stage is to study the biosynthesis process under unstable physical conditions of fermentation.

Stage 1. Study of lactic acid biosynthesis depending on the concentration of the carbon source

Table 1 shows the results of the cultivation of *Lb. delbrueckii* strains on fermentation media with different glucose concentrations. As can be seen, an increase in glucose concentration leads to an increase in the concentration of lactic acid in the fermentation broth, as well as the total duration of the fermentation process, which is entirely consistent with previously published results [23]. In addition all strains are capable of LA biosynthesis on a medium with a glucose concentration of 180 g/dm³. With a further increase in glucose concentration in the fermentation medium, complete inhibition of LAB by the substrate was observed, characterized by lysis of lactic acid bacteria and the absence of lactic acid synthesis.

When strains of *Lb. delbrueckii* PB-07, UY-2/13, and Q-50 were cultivated, and high values of lactic acid yield (> 90%) were obtained regardless of glucose concentration, indicating complete conversion of the carbon

Table 1

Biosynthesis of lactic acid by *Lb. delbrueckii* strains at glucose concentrations of 90, 130, 150, and 180 g/dm³ on medium supplemented with 30 g/dm³ yeast extract

Conc. glucose. g/dm ³	FRS (in.). g/dm ³	Time. (h)	Strain	LA. g/dm ³	FRS (end). g/dm ³	LA yield. %
90	72.38	64	PB-07	67.82±0.01	2.59±0.04	93.69±0.02
			UY-2/13	70.94±1.62	1.97±0.05	95.94±0.17
			BMP-92	65.15±2.25	3.04±1.41	90.01±3.11
			Q-50	68.80±0.62	1.65±0.19	95.05±0.86
130	90.88	72	PB-07	78.18±0.61	3.65±0.11	92.05±0.72
			UY-2/13	78.74±0.48	2.34±0.13	92.70±0.57
			BMP-92	69.83±0.27	10.12±1.27	82.22±0.32
			Q-50	81.09±0.22	2.56±0.09	95.47±0.26
150	118.40	88	PB-07	87.09±2.83	3.10±0.01	94.93±2.93
			UY-2/13	89.21±2.25	3.83±1.49	95.72±1.22
			BMP-92	79.69±1.17	17.61±1.14	82.53±1.30
			Q-50	86.98±2.11	1.60±0.27	91.34±1.09
180	141.70	96	PB-07	98.71±1.95	3.04±0.29	92.77±2.08
			UY-2/13	99.58±1.79	2.34±0.08	90.13±1.29
			BMP-92	80.50±0.73	40.61±1.14	69.66±0.68
			Q-50	99.15±3.99	1.85±0.29	90.50±4.92

Note: Data are presented as mean ± standard error of the mean.

* $P < 0.05$ compared to control (for each strain at a glucose concentration of 90 g/dm³).

source into LA. Strain BMP-92 can synthesize lactic acid at a concentration of not more than 80 g/dm³, probably due to the inhibitory effect of the end product on the producer's metabolic pathway. Compared with other strains, strain *Lb. delbrueckii* BMP-92 turned out to be the least productive; therefore, it was not used in further studies.

The biosynthesis of lactic acid by strains *Lb. delbrueckii* PB-07, UY-2/13, and Q-50 in high concentrations (about 100 g/dm³) can be explained by a sufficient amount of yeast extract in the fermentation medium — 30 g/dm³. The required concentration of the nitrogen source is different for *Lb. delbrueckii* strains. It is known that when using the strain *Lb. delbrueckii* NCIMB 8130 on a fermentation medium with the addition of 5% yeast extract, only about 90 g/dm³ of lactic acid was obtained [24]. The data presented indicate the importance of both the nitrogen source and the carbon source, as well as the differences in the biochemical needs of different strains of *Lb. delbrueckii* for the biosynthesis of high concentrations of LA.

Stage 2. Study of the process of lactic acid biosynthesis depending on the nitrogen source

Fig. 1 and 2 illustrate the results of a study of lactic acid biosynthesis on media supplemented with corn extract as the sole nitrogen source.

As can be seen from the data presented, regardless of the glucose concentration and the duration of the cultivation process, the acid concentration values for the *Lb. delbrueckii* PB-07, UY-2/13 and Q-50 strains are the same.

According to the data provided to us by DE “Enzim”, the content of amine nitrogen in corn extract is half that of yeast. Therefore, corn extract was added to the nutrient medium at a concentration of 60 g/dm³. Despite the fact that the concentration of corn extract in the fermentation medium was twice as high as in yeast, the concentration of LA at the end of the process decreased by approximately 30%. Such data likely indicate that the composition of corn extract contains significantly fewer nutrients (amino acids, B vitamins, mineral compounds) than previously reported in some studies. In particular, the article by J.P. Tan, J.M. Jahim, T.Y. Wu et al. [25] states that

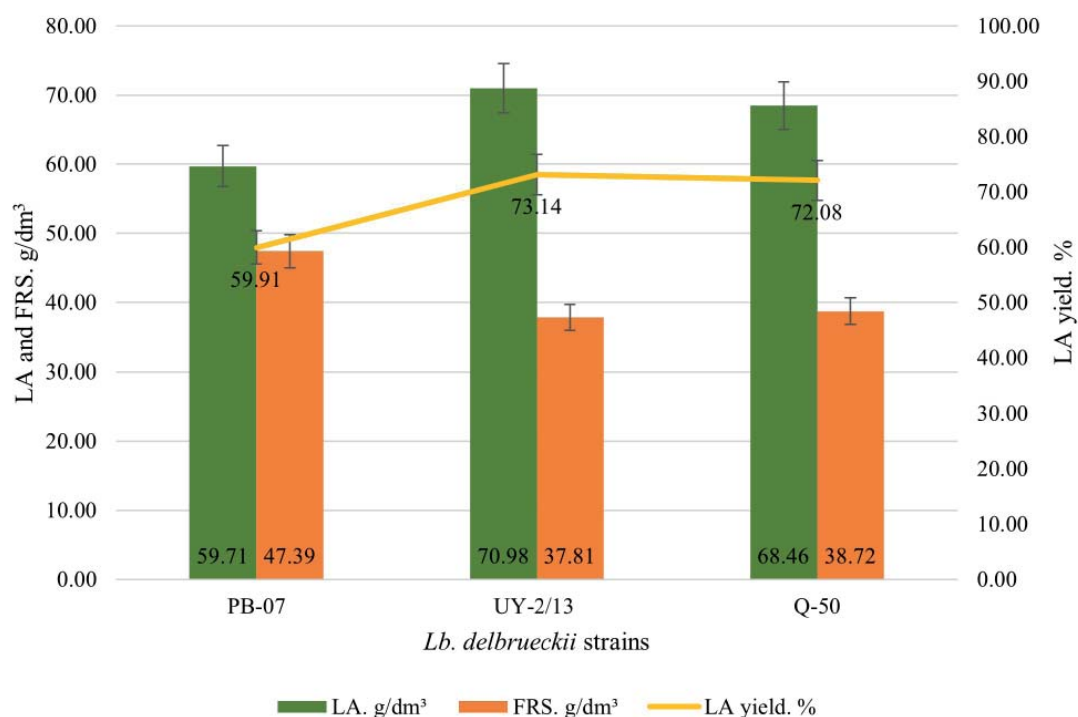


Fig. 1. Values of free reducing sugars, concentration, and yield of lactic acid on the medium: glucose, 180 g/dm³; corn extract, 60 g/dm³

Note: Data are presented as mean $\pm$ standard error. * $P < 0.05$ compared to control (for each strain at a yeast extract concentration of 30 g/dm³).

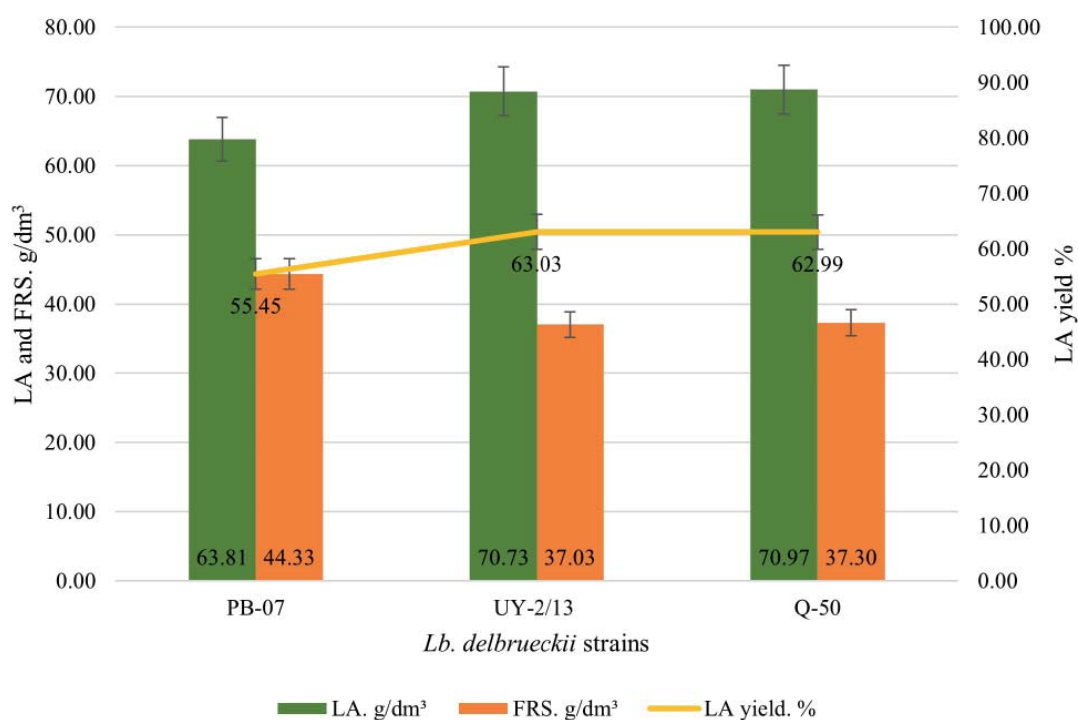


Fig. 2. Values of free reducing sugars, concentration, and yield of lactic acid on the medium: glucose, 150 g/dm³; corn extract, 60 g/dm³

Note: Data are presented as mean $\pm$ standard error. * $P < 0.05$ compared to control (for each strain at a yeast extract concentration of 30 g/dm³).

corn extract has a composition similar to yeast, but contains more mineral salts and growth factors. The differences between corn and yeast extract, the effect of corn extract concentration on LA biosynthesis, and the study of the levels of consumption of corn extract components require detailed research. They will be the goal of future work.

When *Lb. delbrueckii* UY-2/13 and Q-50 were cultivated on media with glucose concentrations of 150 and 180 g/dm³, almost the same amount of lactic acid was obtained (~70 g/dm³). However, when using strain PB-07, the amount of LA was approximately 12% lower, amounting to approximately 60 g/dm³. The results obtained indicate that strain *Lb. delbrueckii* PB-07 has significantly higher needs for amino acids, mineral salts, vitamins, and other growth factors for the synthesis of lactic acid. Therefore, strain *Lb. delbrueckii* PB-07 was not used in further studies.

Stage 3. Study of the process of lactic acid biosynthesis depending on changes in physical fermentation conditions.

Fig. 3 and 4 present the results of a study of the ability of lactic acid bacteria strains to biosynthesize LA at different temperatures.

As shown in the graphs above, the optimal fermentation temperature for both strains

of lactic acid bacteria is 40 °C. Increasing the process temperature to 50 °C results in a significant decrease in lactic acid biosynthesis, which may be attributed to the lysis of microorganisms and a reduction in the enzymatic activity of metabolic pathways. At the same time, reducing the fermentation temperature to 30 °C was characterized by a significantly smaller adverse effect on lactic acid biosynthesis compared to increasing it to 50 °C. The results obtained are entirely consistent with previously published data by Sarkar D. and Qin H. [26, 27]. In addition, according to the data presented in Figs. 3 and 4, at temperatures of 30 °C and 40 °C, the *Lb. delbrueckii* UY-2/13 strain was more productive than the Q-50 strain. At the same time, at a temperature of 50 °C, no differences were observed between the studied strains.

According to the studies of Diptendu Sarkar [26] and Susan Michelz Beitel [28], the optimal conditions for the biosynthesis of lactic acid by *Lactobacillus* spp. Strains are stirring at a level of 100 to 150 rpm. In addition, it is known that the presence or absence of mixing does not affect the acid concentration at the end of the process [29]. However, according to the study's results (Fig. 5), stable stirring at 120 rpm leads to a decrease in LA biosynthesis. The acid concentration in this case decreases

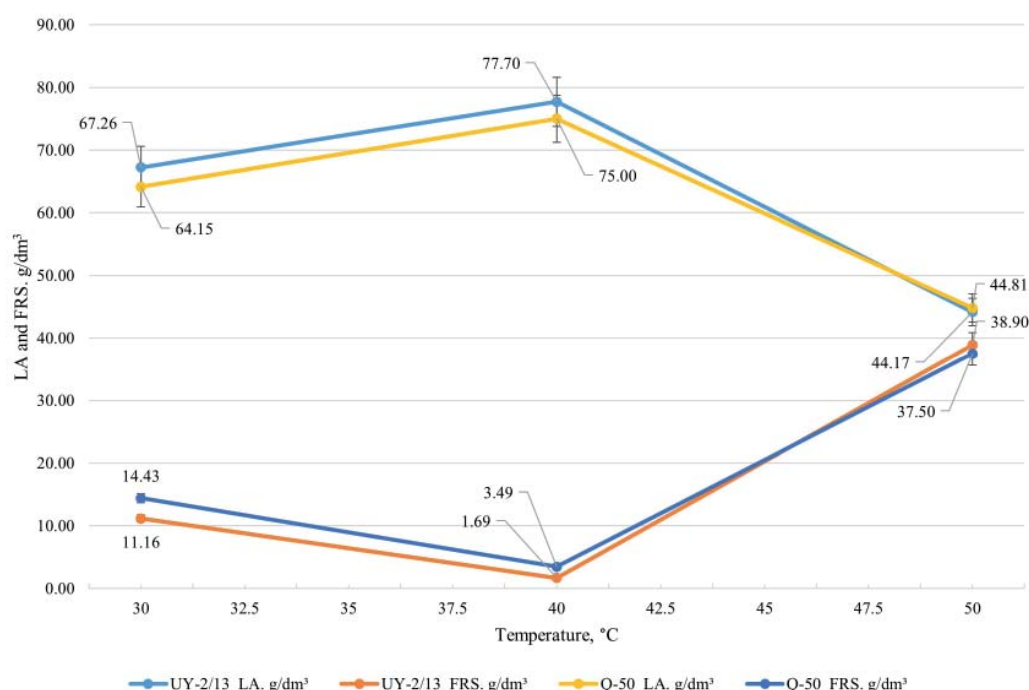


Fig. 3. Values of free reducing sugars and lactic acid concentration at different fermentation temperatures

Note: Data are presented as mean ± standard error.

* $P < 0.05$ compared to control (for each strain at 40 °C).

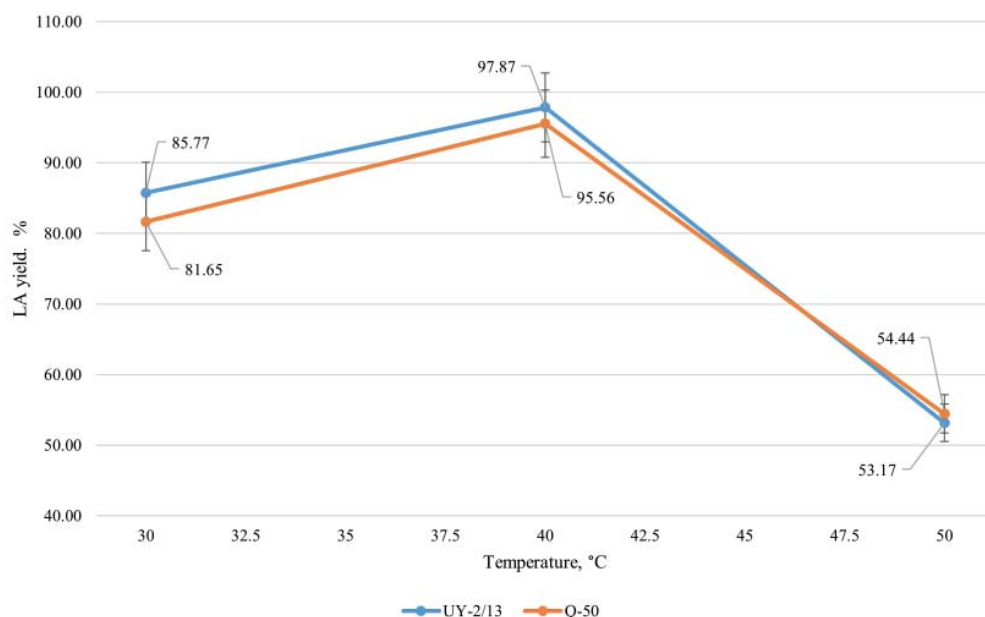


Fig. 4. Lactic acid yield values at different fermentation temperatures

Note: Data are presented as mean $\pm$ standard error.

* $P < 0.05$ compared to control (for each strain at 40 °C).

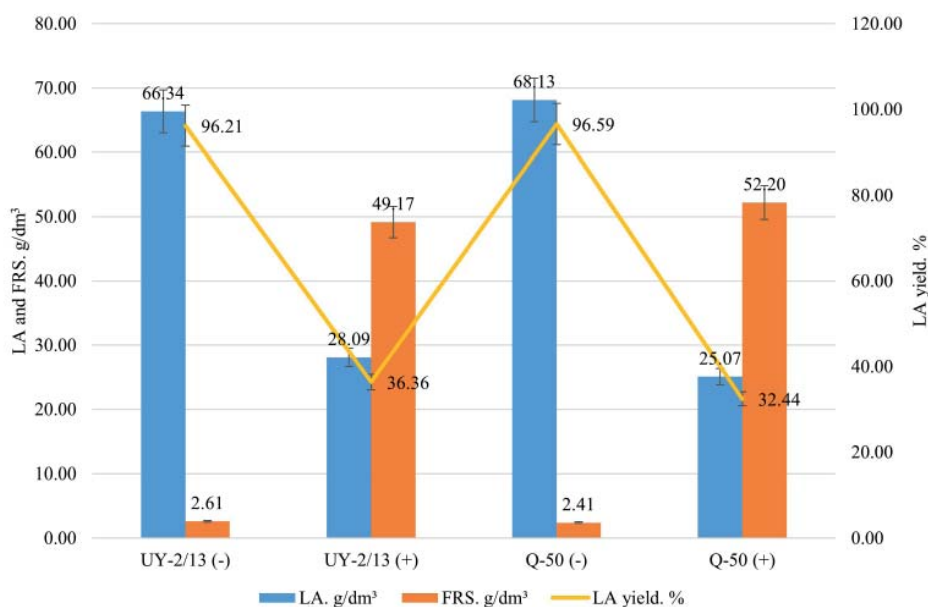


Fig. 5. Values of free reducing sugars, concentration, and yield of lactic acid in the absence of stirring (-) and with constant stirring (+)

Note: Data are presented as mean $\pm$ standard error.

* $P < 0.05$ compared to control (for each strain in the absence of stirring).

to 28–25 g/dm³, and the yield to 36–32%, which indicates a significant adverse effect of stirring and contradicts previously published results.

It is also worth noting that in studies on the influence of stirring on the lactic acid

biosynthesis process, the *Lb. delbrueckii* UY-2/13 strain, as well as in the study of the impact of temperature, turned out to be more productive compared to the Q-50 strain. The concentration of lactic acid was higher by 3 g/dm³, and the yield was higher by 4%.

Therefore, the *Lb. delbrueckii* UY-2/13 strain is more resistant to changes in the temperature regime of the fermentation process and the presence of stirring.

Thus, according to the research results, a potential industrial producer of lactic acid is the strain *Lb. delbrueckii* UY-2/13, which, compared to other studied strains, is characterized by a significantly higher degree of bioconversion, the ability to synthesize high amounts of lactic acid on a cheap nitrogen source (corn extract), and is also more resistant to adverse conditions of the fermentation process.

Conclusions

Thus, a comprehensive assessment of the biosynthetic potential of *Lactobacillus delbrueckii* strains for lactic acid production was conducted, encompassing the economic, biochemical, and technological aspects of the process.

During the study, one strain was selected, namely *Lb. delbrueckii* UY-2/13, which has the necessary qualities for a production producer. The chosen strain is capable of biosynthesizing approximately 100 g/dm³ of lactic acid in batch fermentation at high initial glucose concentrations in the nutrient medium (approximately 180 g/dm³). Despite the decrease in lactic acid concentration when using corn extract, the strain is capable of synthesizing about 70 g/dm³ of acid. It is characterized by lower metabolic requirements for the nitrogen source compared to others.

The effect of temperature and the presence of agitation on the biosynthetic capacity of the *Lb. delbrueckii* UY-2/13 strain was established. It was confirmed that the fermentation temperature of 50 °C has a significant inhibitory effect on the

biosynthesis of lactic acid. At the same time, at 30 °C, the adverse effect is less pronounced, and the need for exceptional control over agitation when adjusting the pH and maintaining the fermentation temperature was also revealed.

Thus, the use of the *Lb. delbrueckii* UY-2/13 strain under the established technological parameters of its cultivation using economically advantageous raw materials (cheaper corn extract) can increase the profitability of the lactic acid production process, and is promising for introduction into production.

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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Competing interests

The authors declare that they have no competing interests.

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REFERENCES

- Castillo Martinez, F. A., Balciunas, E. M., Salgado, J. M., Domínguez González, J. M., Converti, A., Oliveira, R. P. d. S. (2013). Lactic acid properties, applications and production: A review. *Trends in Food Science & Technology*, 30(1), 70–83. <https://doi.org/10.1016/j.tifs.2012.11.007>
- Wang, C., Chang, T., Yang, H., Cui, M. (2015). Antibacterial mechanism of lactic acid on physiological and morphological properties of *Salmonella Enteritidis*, *Escherichia coli* and *Listeria monocytogenes*. *Food Control*, 47, 231–236. <https://doi.org/10.1016/j.foodcont.2014.06.034>
- Gezae Daful, A., Loidon, M., R. Chandraratne, M. (2024). Lactic Acid Production from Lignocellulosic Biomass. *IntechOpen*. <https://doi.org/10.5772/intechopen.112739>
- Sentharamaikkannan, C., Akash, K., Amanullah, S., Barath, M., Manojkumar, R., Jagadeeshwaran, J. (2020). Overview of Polylactic acid and its derivatives in medicinal applications. *IOP Conference Series: Materials Science and Engineering*, 47, 231–236. <https://doi.org/10.1016/j.foodcont.2014.06.034>

- 988, 012003. <https://doi.org/10.1088/1757-899x/988/1/012003>
5. Manandhar, A., Shah, A. (2023). Techno-Economic analysis of the production of lactic acid from lignocellulosic biomass. *Fermentation*, 9(7), 641. <https://doi.org/10.3390/fermentation9070641>
 6. Abedi, E., Hashemi, S. M. B. (2020). Lactic acid production — producing microorganisms and substrates sources-state of art. *Heliyon*, 6(10). <https://doi.org/10.1016/j.heliyon.2020.e04974>
 7. Okhmat, O., Zhaldak, M., Mokrousov, M. (2024). The global lactic acid market. Herald of Khmelnytskyi National University. *Technical Sciences*, 1(6), 215–222. <https://doi.org/10.31891/2307-5732-2024-343-6-33>
 8. Saavedra, S., Alejandro-Paredes, L., Flores-Santos, J. C., Flores-Fernández, C. N., Arellano-García, H., Zavaleta, A. I. (2021). Optimization of lactic acid production by *Lactobacillus plantarum* strain Huil in a medium containing sugar cane molasses. *Agronomía Colombiana*, 39(1), 98–107. <https://doi.org/10.15446/agron.colomb.v39n1.89674>
 9. Kiiv, D., Vasylyuk, S., Lubenets, V. (2024). Lactic acid: Industrial synthesis, microorganisms-producers and substrates: A review. *Chemistry & Chemical Technology*, 18(2), 157–169. <https://doi.org/10.23939/chcht18.02.157>
 10. Okano, K., Tanaka, T., Ogino, C., Fukuda, H., Kondo, A. (2009). Biotechnological production of enantiomeric pure lactic acid from renewable resources: Recent achievements, perspectives, and limits. *Applied Microbiology and Biotechnology*, 85(3), 413–423. <https://doi.org/10.1007/s00253-009-2280-5>
 11. Taskila, S., Ojamo, H. (2013). The Current Status and Future Expectations in Industrial Production of Lactic Acid by Lactic Acid Bacteria. *InTech*. <https://doi.org/10.5772/51282>
 12. Battula, S.K., Narayana, S.K.V., Sarva, S.N.G., Besetty, T., Gopinadh, R. (2019). Industrial production of lactic acid and its applications. *International Journal of Biotech Research*. 1(1), 42–54.
 13. Li, Z., Lu, J., Zhao, L., Xiao, K., Tan, T. (2010). Improvement of l-lactic acid production under glucose feedback controlled culture by *Lactobacillus rhamnosus*. *Applied Biochemistry and Biotechnology*, 162(6), 1762–1767. <https://doi.org/10.1007/s12010-010-8957-5>
 14. Abdel-Rahman, M. A., Tashiro, Y., Sonomoto, K. (2013). Recent advances in lactic acid production by microbial fermentation processes. *Biotechnology Advances*, 31(6), 877–902. <https://doi.org/10.1016/j.biotechadv.2013.04.002>
 15. Ojo, A. O., de Smidt, O. (2023). Lactic acid: A comprehensive review of production to purification. *Processes*, 11(3), 688. <https://doi.org/10.3390/pr11030688>
 16. Coelho, L. F., Lima, C. J. B. d., Rodovalho, C. M., Bernardo, M. P., Contiero, J. (2011). Lactic acid production by new *Lactobacillus plantarum* LMISM6 grown in molasses: Optimization of medium composition. *Brazilian Journal of Chemical Engineering*, 28(1), 27–36. <https://doi.org/10.1590/s0104-66322011000100004>
 17. Manandhar, A., Shah, A. (2020). Techno-Economic analysis of bio-based lactic acid production utilizing corn grain as feedstock. *Processes*, 8(2), 199. <https://doi.org/10.3390/pr8020199>
 18. de Oliveira, P. M., Santos, L. P., Coelho, L. F., Avila Neto, P. M., Sass, D. C., Contiero, J. (2021). Production of L(+) lactic acid by *Lactobacillus casei* ke11: Fed batch fermentation strategies. *Fermentation*, 7(3), 151. <https://doi.org/10.3390/fermentation7030151>
 19. Garrity, G. M., De Vos, P., Jones, D., Krieg, N., Ludwig, W., Rainey, F., Schleifer, K., Whitman, W. (2010). Bergey's manual of systematic bacteriology (Vol. 3, The Firmicutes). *Springer*. <https://doi.org/10.1007/978-0-387-68489-5>
 20. Bertrand M. (1906). The dosage of reducing sugars. In *Memoirs presented to the Chemical Society*. Masson. 1285–1299
 21. Somogyi M. (1952). Notes on sugar determination. *Journal of Biological Chemistry*. 195(1), 19–23.
 22. Nakano, S., Ugwu, C. U., Tokiwa, Y. (2012). Efficient production of d-(–)-lactic acid from broken rice by *Lactobacillus delbrueckii* using $\text{Ca}(\text{OH})_2$ as a neutralizing agent. *Bioresource Technology*, 104, 791–794. <https://doi.org/10.1016/j.biortech.2011.10.017>
 23. Farooq, U., Anjum, F., Zahoor, T., Rahman, S., Randhawa, M., Ahmed, Dr A., Akram, K. (2012). Optimization of lactic acid production from cheap raw material: Sugarcane molasses. *Pakistan Journal of Botany*. 44(1), 333–338
 24. Kotzamanidis, C., Roukas, T., Skaracis, G. (2002). Optimization of lactic acid production from beet molasses by *Lactobacillus delbrueckii* NCIMB 8130. *World Journal of Microbiology and Biotechnology*, 18(5), 441–448. <https://doi.org/10.1023/a:1015523126741>
 25. Thakur, A., Panesar, P. S., Saini, M. S. (2017). L(+)-Lactic acid production by immobilized *Lactobacillus casei* using low cost agro-industrial waste as carbon and nitrogen sources. *Waste and Biomass Valorization*,

- 10(5), 1119–1129. <https://doi.org/10.1007/s12649-017-0129-1>
26. Sarkar, D., Paul, G. (2019). A study on optimization of lactic acid production from whey by lactobacillus sp isolated from curd sample. *Research Journal of Life Science, Bioinformatics, Pharmaceutical and Chemical Sciences*, 5, 816–824. <https://doi.org/10.26479/2019.0502.6>.
27. Qin, H., Gong, S.-S., Ge, X.-Y., Zhang, W.-G. (2012). The effect of temperature on l-lactic acid production and metabolite distribution of *Lactobacillus casei*. *Preparative Biochemistry and Biotechnology*, 42(6), 564–573. <https://doi.org/10.1080/10826068.2012.665114>
28. Beitel, S. M., Coelho, L. F., Contiero, J. (2020). Efficient conversion of agroindustrial waste into D(–) lactic acid by *Lactobacillus delbrueckii* using fed-batch fermentation. *BioMed Research International*, 2020, 1–13. <https://doi.org/10.1155/2020/4194052>
29. Panesar, P. S., Kennedy, J. F., Knill, C. J., Kosseva, M. (2010). Production of L(+) lactic acid using *Lactobacillus casei* from whey. *Brazilian Archives of Biology and Technology*, 53(1), 219–226. <https://doi.org/10.1590/s1516-89132010000100027>

БІОСИНТЕТИЧНИЙ ПОТЕНЦІАЛ ШТАМІВ *Lactobacillus delbrueckii* У ВИРОБНИЦТВІ МОЛОЧНОЇ КИСЛОТИ: КОМПЛЕКСНИЙ АНАЛІЗ

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Мета. Провести комплексне оцінювання біосинтетичного потенціалу штамів *Lactobacillus delbrueckii* щодо синтезу молочної кислоти для використання в промисловості.

Методи. Об'єктом дослідження були чотири штами *Lactobacillus delbrueckii*, що синтезують молочну кислоту (МК). Концентрацію молочної кислоти у культуральній рідині наприкінці процесу ферментації визначали за допомогою високоефективної рідинної хроматографії. Концентрацію вільних редуковувальних речовин у живильному середовищі визначали модифікованим методом Бертрана, а наприкінці процесу — методом Бертрана чи Сомоджи-Нельсона.

Результати. Досліджено біосинтез молочної кислоти чотирма штамми *Lb. delbrueckii* PB-07, UY-2/13, BMR-92, Q-50 за різних концентрацій джерела вуглецю, типів джерела азоту, а також за оптимальних (температура 40 °C, без перемішування) та несприятливих (температура 30 та 50 °C, наявність перемішування) умовах ферментації. Відібрано один штам (*Lb. delbrueckii* UY-2/13) з необхідними для виробничого продуцента якостями (біосинтез близько 70 г/дм³ на дешевому джерелі азоту в режимі періодичного культивування за високих початкових концентрацій глюкози в живильному середовищі). Виявлено, що вагому роль відіграють фізичні параметри. Температура ферментації 50 °C має значний гальмівний ефект на біосинтез молочної кислоти, тоді як при 30 °C негативний вплив є менш вираженим. Крім того, перемішування при коригуванні pH та підтриманні температури ферментації потребує особливого контролю.

Висновки. Перспективним штамом для впровадження у виробництво є *Lb. delbrueckii* UY-2/13, культивування якого із застосуванням економічно вигідної сировини (дешевшого кукурудзяного екстракту) може підвищити рентабельність процесу одержання молочної кислоти.

Ключові слова: *Lactobacillus delbrueckii*, біосинтез, молочна кислота, ферментація, лактобактерії, виробництво органічних кислот, біотехнології.