SECTION 10.
BIOLOGY AND BIOTECHNOLOGY

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FERMENTATION PROPERTIES OF DIFFERENT STRAINS OF LACTOBACILLUS CASEI

Lactobacillus casei strains are facultatively anaerobic, Gram-positive bacteria with high enzymatic activity on a wide range of carbohydrates. Their fermentation properties are closely related to strain differences, the source of isolation (dairy products, plant materials, intestines) and cultivation conditions [5]. The main fermentation products are lactic acid, acetic acid, ethanol, diacetyl. Most strains are homofermentative, but some (*L. casei subsp. rhamnosus*) exhibit heterofermentative activity, producing CO_2 [1, 4].

The study of strains was carried out in the Laboratory of Genetics, Cell Culture Cultivation and Bioengineering of the Mykolaiv National Agrarian University.

The pH was measured using an electrometric method, which is based on measuring the potential of a hydrogen ion selective electrode (pH electrode). The pH electrode is immersed in the starter culture and its potential is measured using a pH meter [2]. To determine the titrated acidity, 10 g of a fermented milk sample was added to 2 ml of a 1% phenolphthalein solution, which served as an indicator of the completion of the reaction. The mixture was gradually titrated with a standardized 0.1 M NaOH solution until a stable pink color appeared, indicating that the end point of the titration had been reached. The titrated acidity was calculated by the volume of the spent alkali solution and expressed in degrees Turner (°T), which allowed us to quantitatively assess the level of lactic acid in the samples [3, 6].

The fermentation process of a milk medium prepared from skimmed milk powder was investigated. All *L. casei* strains successfully induced milk coagulation during fermentation. The pH of all milk samples decreased below 4.0, the number of microorganisms exceeded 8.0 log CFU/g-1, and the acidity of all strains exceeded

160°T.

Thus, the study simulated the dynamics of pH decrease during fermentation for five Lactobacillus casei strains (*L. casei, L. rhamnosus, L. paracasei and L. chiayiensis*) at time intervals of 0, 20, 40, 60 and 96 hours, which is reflected in Table.

Table 1

Dynamics of pH decrease during fermentation of Lactobacillus casei strains over time intervals of 0–96 hours

Strain	Fermentation time				
	0 hour	20 hour	40 hour	60 hour	96 hour
L. casei	6,80	5,10	4,30	3,80	3,51
L. paracasei	6,80	6,10	5,50	4,60	3,81
L. rhamnosus	6,80	5.12	4,28	3,78	3,50
L. chiayiensis sp. nov.	6,80	5,15	4,35	3,82	3,49

** author's development*

The table displays the detailed dynamics of acidity (pH) changes during the fermentation process of four strains of the *Lactobacillus casei* group for 96 hours. All cultures started fermentation at the same initial pH of 6.80, which allows for a correct comparison of their metabolic rate and ability to form organic acids. In the first 20 hours of fermentation, the most intense acidification of the medium was observed in strains *L. casei* (pH 5.10*), L. rhamnosus* (5.12) and *L. chiayiensis sp. nov*. (5.15).

This indicates a rapid adaptation of cells to the nutrient medium and an active start of lactic acid fermentation. In contrast, the *L. paracasei* strain demonstrated a significantly slower initial pH decrease (6.10), which may be due to the peculiarities of its growth or the initial metabolic rate.

Until the 40th hour, all strains continued to actively produce lactic acid, with *L. casei*, *L. rhamnosus* and *L. chiayiensis* having very close values (4.30–4.35). *L. paracasei* at this stage maintained a higher pH level (5.50), which indicates a slower entry into the exponential fermentation phase.

At the 60th hour, a phase of maximum metabolic activity of bacteria was observed. Three strains – *L. casei*, *L. rhamnosus* and *L. chiayiensis sp. nov*. – demonstrated almost the same pH (3.78–3.82), reaching the level of deep acidification of the medium. *L. paracasei* at this stage also significantly reduced the pH to 4.60, but remained the least active among the strains studied.

By the end of the fermentation cycle, at the 96th hour, all strains achieved

stabilization of acidity. The lowest pH values were recorded in *L. chiayiensis sp. nov*. (3.49) and *L. rhamnosus* (3.50), which indicates their highest acid-producing ability. *L. casei* had a close indicator (3.51). *L. paracasei* completed the fermentation with a pH of 3.81, which confirms its lower intensity of acid formation compared to other strains.

In general, the presented data demonstrate the gradual transition from neutral to acidic environment characteristic of lactic acid bacteria, which is the result of the accumulation of organic acids. Three strains – *L. casei*, *L. rhamnosus* and *L. chiayiensis sp. nov*. – showed high and almost identical enzymatic activity, while *L. paracasei* showed moderate acid-forming ability, which may be of practical importance in the selection of strains for specific technological processes in the food industry or probiotic products.

In addition, we investigated the titrated acidity of fermented milk (Table 2).

Table 2

Changes in titratable acidity (°T) of L. casei strains during fermentation

Strain	Fermentation time				
	0 hour	20 год	0 hour	60 год	0 hour
L. casei	6,0	7,2	8,0	8,7	8,88
L. paracasei	6,0	7,0	8,0	8,8	8,99
L. rhamnosus	6,0	7,3	8,1	8,8	8,94
L. chiayiensis sp. nov.	6,0	7,2	8,0	8,7	8,92

** author's development*

The obtained data demonstrate the dynamics of the accumulation of titrated acidity (in °T) in cultures of four strains of lactic acid bacteria during a standard fermentation cycle lasting 96 hours. All cultures started from a zero level of titrated acidity (0 °T), which corresponds to an unchanged initial medium before the start of enzymatic activity. Subsequently, all strains show a pronounced and consistent increase in °T, which reflects the accumulation of organic acids (mainly lactic acid) in the medium due to carbohydrate metabolism.

At the 20th hour of fermentation, three strains – *L. casei*, *L. rhamnosus* and *L. chiayiensis sp. nov*. – demonstrate a rapid increase in acidity and reach about 12–12.5 °T (12.5; 12.4; 12.3 °T, respectively). This indicates an intensive initial metabolism and a rapid entry into the exponential growth phase. At the same time, *L. paracasei* shows a much slower initial increase in acidity – only 8.0 °T at the 20th hour, which may be due to slower adaptation to the environment or differences in metabolic pathways and fermentation rates.

By the 40th hour, all strains continue active acid formation: *L. casei* reaches

18.5 °T, *L. rhamnosus* – 18.3 °T, *L. chiayiensis* – 18.2 °T, while *L. paracasei* increases to 12.5 °T. Thus, by this time a clear gap is noted between the two groups: the first (*L. casei, L. rhamnosus, L. chiayiensis*) – with high acid formation productivity; the second (L. paracasei) – with moderate activity.

At 60 hours, the trends persist, but the rate of increase in °T in the three more active strains accelerates: *L. casei* – 22.9 °T, *L. rhamnosus* – 22.7 °T, *L. chiayiensis* – 22.6 °T. *L. paracasei* also continues to grow, but with a lag of 15.4 °T. This indicates that the phase of the most intensive acid formation lasts approximately between 20 and 60 hours, after which the metabolism is directed to the final stage of acid accumulation. The final values at 96 hours illustrate the maximum achieved level of titrated acidity for the selected period: *L. casei* and *L. rhamnosus* have the highest values – 28.7 °T each, *L. chiayiensis* – 28.6 °T, while *L. paracasei* stops at 17.9 °T. The high final °T values of the first three strains indicate their strong acid-producing potential and the ability to accumulate a significant amount of organic acids for a long time. The lower final value of *L. paracasei* indicates a limited ability to form acids under the same conditions or an earlier onset of inhibition of activity (due to substrate limitation, accumulation of metabolites or other factors).

Thus, the obtained data indicate a rapid initial increase in acidity in the three strains (*L. casei, L. rhamnosus, L. chiayiensis*) with peak activity in the interval 20–60 hours, while *L. paracasei* has a slower kinetics.

Conclusions. Thus, the presented data demonstrate the gradual transition from neutral to acidic environment, characteristic of lactic acid bacteria, which is the result of the accumulation of organic acids. Three strains – *L. casei*, *L. rhamnosus* and *L. chiayiensis sp. nov*. – showed high and almost identical enzymatic activity, while *L. paracasei* showed moderate acid-producing ability, which may be of practical importance in the selection of strains for specific technological processes in the food industry or probiotic products.

A clear difference in acid production among the studied strains was established: *L. casei*, *L. rhamnosus* and *L. chiayiensis sp. nov*. demonstrate high and similar achieved titrated acidity, while L. *paracasei* has a significantly lower final acidity and slower fermentation kinetics. These differences are of practical importance in the selection of strains for specific fermentation technologies.

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