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Biosynthetic activity study of *Lactobacillus acidophilus* lactic acid bacteria in the lactose fermentation of whey

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Abstract: According to numerous studies, lactic acid bacteria are characterised by the capability of reducing their initial activity given an insufficient quantity of growth factors supplied by a nutrient medium. Conversely, the introduction of additional sources of nutrient into the medium provides favourable conditions for the development of lactic acid-producing microorganisms. The present study was aimed at examining the effect of phosphorus-containing salts on the biosynthetic activity of a specially selected strain of lactic acid bacteria for the biotransformation of acid whey lactose into lactate-containing ingredients. For this purpose, lactic acid bacteria of the *Lactobacillus acidophilus* thermophilic bacilli subgroup applied in cheese and fermented dairy production were used as a producer of lactic acid. In the obtained fermented solutions, the mass fractions of lactose and calcium lactate were determined by the Bertrand method and complexometric techniques, respectively. The variables in the biosynthetic activity study of lactic acid bacteria included the type of phosphorus-containing salt (disodium phosphate dodecahydrate and disubstituted ammonium phosphate) and its mass fraction, which ranged from 1.0 to 3.0 % in increments of 0.5 %. The amount of inoculum introduced for maximum production of lactic acid comprised 2.5 % vol. of the nutrient medium. The titratable acidity of the inoculum ranged from 1.80 to 1.85 g/cm³. In order to produce calcium lactate, the lactic acid accumulating during biosynthesis was neutralised with chalk. The effect caused by the type of phosphorus-containing salt and its mass fraction on the coefficient of whey bioconversion to lactic acid by *L. acidophilus* AT-I lactic acid bacteria was evaluated along with the rate of formation and yield of calcium lactate. The 2.0 % additive of sodium phosphate disubstituted dodecahydrate was established to provide the highest values for the formation rate and yield of the target product, comprising 0.78 g/(dm³·h) and 79.96 %, respectively.

Keywords: lactic acid bacteria, biosynthetic activity, lactose, whey, lactate-containing ingredients

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Исследование биосинтетической активности молочнокислых бактерий *Lactobacillus acidophilus* при сбраживании лактозы молочной сыворотки

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Резюме: Молочнокислые бактерии могут терять первоначальную активность при недостатке факторов роста в питательной среде. Введение в питательную среду дополнительных источников питания обеспечивает благоприятные условия для развития микроорганизмов, продуцирующих

молочную кислоту. Целью данной работы являлось изучение влияния фосфорсодержащих солей на биосинтетическую активность специально подобранного штамма молочнокислых бактерий для биотрансформации лактозы творожной сыворотки в лактатсодержащие ингредиенты. В качестве продуцента молочной кислоты использовали молочнокислые бактерии из подгруппы термофильных палочек *Lactobacillus acidophilus*, которые применяются в производстве кисломолочных продуктов и сыроделии. В полученных сброженных растворах определяли массовую долю лактозы методом Бертрана и лактата кальция комплексонометрическим методом. Исследовали изменение биосинтетической активности молочнокислых бактерий при варьировании вида фосфорсодержащей соли (натрий фосфорнокислый двузамещенный 12-водный, аммоний фосфорнокислый двузамещенный) и массовой доли (от 1,0 до 3,0 % с шагом 0,5 %). Количество вносимого посевного материала для максимального накопления молочной кислоты составило 2,5 % к объему питательной среды. Титруемая кислотность посевного материала составляла от 1,80 до 1,85 г/см³. Накапливающуюся в процессе биосинтеза молочную кислоту нейтрализовали мелом с получением лактата кальция. Установлены зависимости коэффициента биоконверсии сыворотки в молочную кислоту молочнокислыми бактериями *L. acidophilus* AT-I, скорости образования и выхода лактата кальция от вида фосфорсодержащей соли и ее массовой доли. Выявлено, что наибольшие величины скорости образования и выхода целевого продукта (0,78 г/(дм³·ч)) и 79,96 % соответственно) достигаются при внесении 2,0 % двузамещенного 12-водного фосфорнокислого натрия.

Ключевые слова: молочнокислые бактерии, биосинтетическая активность, лактоза, молочная сыворотка, лактатсодержащие ингредиенты

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INTRODUCTION

With an increase in the production of curd products, cheese and casein, the quantity of milk whey, representing a waste product of the dairy industry, significantly increases. A certain part of the whey is used as feed for livestock and for technical and food purposes, while the bulk is drained into the sewer causing environmental pollution [1–6]. For these reasons, whey processing appears to be a significant problem.

Among the known methods of whey processing, several main areas are distinguished. These include the preparation of concentrates in condensed and dry form, isolation of individual components (protein and lactose hydrolysates, fat, lactulose) and the biological conversion of lactose and whey proteins for production of lactic acid, lactates and probiotic products [5–7].

Whey possess a high biological value due to the presence of soluble proteins, lactose, as well as macro-, micro- and ultramicroelements [1, 8]. On average, 48–52 % of whey accounts for milk solids with the main components presented by milk sugar (70 %), nitrogenous substances (14.5 %), fat (7.5 %) and mineral salts (8 %) [6, 9].

The presence of carbon sources easily digestible by many types of microorganisms, as well as various growth factors, identifies whey to be the among of the most valuable nutrient media for the production of microbial synthesis products. Therefore, the most effective approach to whey pro-

cessing consists in its biotechnological treatment based on the application of microorganisms in a free or immobilised state [10].

For microbiological processing of whey, homofermentative lactic acid streptococci (*Streptococcus lactis*, *Streptococcus cremoris*) and lactobacillus (*Lactobacillus acidophilus*, *Lactobacillus lactis*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*) are of a particular interest. *Streptococcus lactis* are considered to ferment lactose with the formation of L(+) lactic acid in amount of 0.8–1.0 %, and some strains of *Streptococcus cremoris* are believed to form, along with lactic acid, a nisin antibiotic inhibiting the development of other species of lactic acid bacteria, as well as bacilli and butyric acid bacteria. In lactose fermentation, *Lactobacillus bulgaricus* lactobacillus bacteria form up to 3% of lactic acid, mainly of D(-)-form, while *Lactobacillus acidophilus* accumulate the DL form of lactic acid, having the ability to inhibit the development of bacteria of the dysenteric, paratyphoid and *E. coli* group. *L. acidophilus* culture is resistant to adverse environmental influences such as alkaline reaction (pH = 8.0), as well as the presence of phenol, bile (20 %) and sodium chloride (2 %) in the medium.

At the same time, lactic acid bacteria cultures are observed to lose their initial activity in the insufficient amount of growth factors of the nutrient medium. By introducing additional sources of nutrition into the nutrient medium, favourable condi-

tions are provided for the development of starter microorganisms producing lactic acid [11]. According to the developers of a functional supplement with pro- and prebiotic properties based on lactic acid microflora cultivated on whey, the medium must be enriched with germinated wheat grain in an amount of 20 % of the total volume of [4]. In order to provide the normal growth and development of *Lactobacterium jurgurti* bacteria during the fermentation process of deproteinised acid whey, corn extract is applied as an additional food source in an amount ranging from 0.1 to 0.2 % [12]. It is possible to intensify the process of obtaining the combined β -galactosidase enzyme preparation from whey through the mutually stimulating effect of *Kluyveromyces marxianus* fungus, *Candida kefyr* yeast and *Streptococcus thermophilus* thermophilic lactic acid bacteria. In the work [13], *Streptococcus thermophilus* was demonstrated to acidify the medium, stimulating the growth of yeast and releasing, in turn, additional amounts of vitamins and amino acids into the nutrient medium during fermentation to create favourable conditions for the growth of lactic acid bacteria.

The introduction of phosphorus-containing substances into the nutrient medium demonstrates a positive effect on the biosynthetic activity of lactic acid bacteria. The most affordable sources of phosphorus include ammonium phosphate salts providing important nitrogen for the development of bacteria¹ [14]. According to the patent [15], food protein was obtained in the form of a whey concentrate or dry powder containing from 15 to 25 % of solids by *Propionibacterium freudenreichii* VSB-16 и *Lactobacillus casei* GSB-TB1B cultures with an additional introduction of $(\text{NH}_4)_2\text{SO}_4$, KH_2PO_4 , MgSO_2 and ZnSO_2 .

In the present work, acid whey was fermented by a specially selected probiotic strain of *Lactobacillus acidophilus* lactic acid bacteria characterised by a fairly high productivity and the ability to release exopolysaccharides (EPS) into the environment. Among the advantages of using strains of lactic acid bacteria capable of producing EPS are included increased time of refrigerated storage, reduced syneresis, as well as the improved texture and consistency of products. The fermentation of whey by EPS-producing strains of *Lactobacillus acidophilus* increases the nutritional value of the target product, improving its organoleptic and rheological characteristics [16–19].

Thus, the current study was aimed at the effect of phosphorus-containing salts on the biosynthetic activity of a specially selected strain of lactic acid bacteria for biotransformation of acid whey lactose into lactate-containing ingredients.

¹ Kuznetsov A.E., Gradova N.B., Lushnikov S.V., Engelhart M., Weisser T. Applied Ecobiotechnology: Textbook; in 2 V. Moscow: BINOM. Laboratoriya Znaniya, 2010. Vol. 1 – 629 p.; Vol. 2 – 485 p.

EXPERIMENTAL PART

The objects of study were:

- “Letnyaya” acid whey produced by Losevo Dairy Plant LLC;
- *Lactobacillus acidophilus* AT-I lactic acid bacteria (*L. acidophilus* AT-I – producer of lactate-containing ingredients);
- sodium phosphate disubstituted dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) according to GOST 4172-76;
- disubstituted ammonium phosphate $(\text{NH}_4)_2\text{HPO}_4$ according to GOST 3772-74.

Experimental studies were performed by the laboratory equipment of the All-Russian Scientific Research Institute of Food Additives (VNIIPD), a branch of the Gorbатов Research Centre for Food Systems RAS, using the methods adopted in research practice and according to the current technical documentation.

In the experimental samples, the mass fractions of both lactose and calcium lactate were determined along with titratable acidity.

Three methods were applied to the evaluation of the studied parameters including the Bertrand method [20], complexometric technique and acid-base titration for determination of mass fraction of lactose and calcium lactate, as well as titratable acidity, respectively.

RESULTS AND DISCUSSION

A change in the biosynthetic activity of lactic acid bacteria was examined under varying type of phosphorus-containing salt ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$) and its mass fraction (from 1.0 to 3.0 % in increments of 0.5 %).

The fermentation process of acid whey containing from 3.9 to 4.3 % of lactose was performed by *L. acidophilus* AT-I lactic acid bacteria in a thermostat at a temperature of $37 \pm 1^\circ\text{C}$.

A pure culture of lactic acid bacteria was stored in freeze-dried form. The producer was activated at a temperature of $37 \pm 1^\circ\text{C}$ for 10–12 hours. Primary starter culture and inoculum was prepared with sterilised skimmed milk and acid whey, respectively.

The initial caseous whey was pasteurised in a water bath at a temperature range of $70\text{--}75^\circ\text{C}$ for 1 h. The amount of inoculum introduced for maximum accumulation of lactic acid comprised 2.5 % of the volume of the nutrient medium; further increases in the amount were observed to be technologically ineffective. The titratable acidity of the inoculum ranged from 1.80 to 1.85 g/cm^3 .

The lactic acid accumulating during biosynthesis was regularly neutralised with chalk to produce calcium lactate.

Figures 1 and 2 demonstrate the effect of the phosphorus-containing salt type and its mass fraction on the coefficient of whey bioconversion into lactic acid by *L. acidophilus* AT-I lactic acid bacteria after 24 hours. The maximum efficiency in

the biotransformation of lactose into lactic acid is observed when 1.5 to 2.0 % sodium phosphate and 2.0 % ammonium phosphate are added. An increase in the mass fraction of phosphorus-containing salts leads to a decrease in the biosynthetic activity of lactic acid bacteria.

Table 1 presents data on the change in the rate of formation and yield of calcium lactate depending on the type and mass fraction of phosphorus-containing salt, subsequently characterising the change in the biosynthetic activity of lactic acid

bacteria. With the addition of 1.0 and 3.0 % of sodium phosphate, the biosynthetic activity of bacteria was established to be equal to 0.64 g/(dm³·h), while, at introduced amount of 1.5–2.5 %, the value of about 0.78 g/(dm³·h) was obtained. Depending on the mass fraction of sodium phosphate equal to 1.0, 1.5 and 2.0 %, the fermentation rate of acid whey lactose was 0.43, 0.47 and 0.53 g/(dm³·h), respectively. With 2.0 % of phosphorus-containing salts added, the yield of calcium lactate reached the value of about 80 %.

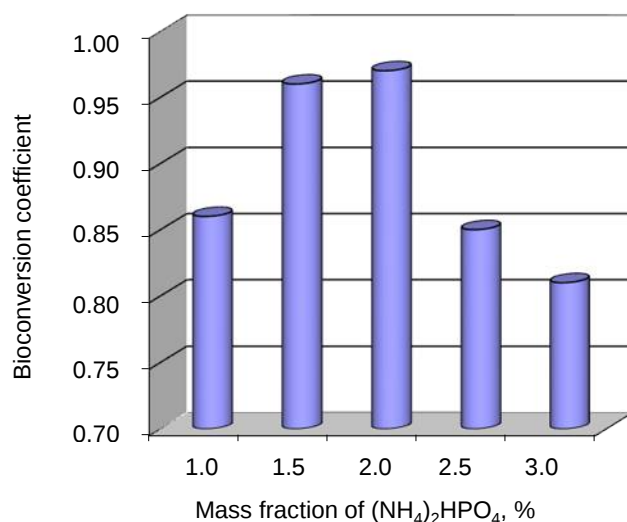


Fig. 1. Relationship between the bioconversion coefficient of whey to lactic acid by *L. acidophilus* AT-I and the mass concentration of Na₂HPO₄·12H₂O after 24 hours

Рис. 1. Зависимость коэффициента биоконверсии сыворотки в молочную кислоту молочнокислыми бактериями *L. acidophilus* AT-I от массовой доли соли Na₂HPO₄·12H₂O через 24 ч

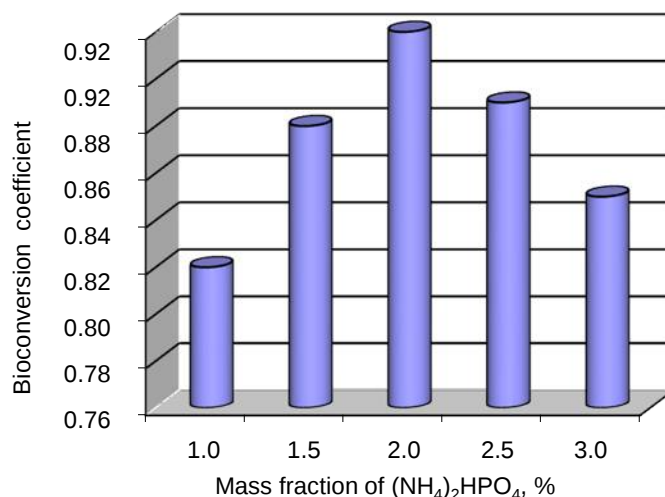


Fig. 2. Relationship between the bioconversion coefficient of whey to lactic acid by *L. acidophilus* AT-I and the mass concentration of (NH₄)₂HPO₄ after 24 hours

Рис. 2. Зависимость коэффициента биоконверсии сыворотки в молочную кислоту молочнокислыми бактериями *L. acidophilus* AT-I от массовой доли соли (NH₄)₂HPO₄ через 24 ч

Table 1

Indicators of biosynthetic activity of lactic acid bacteria *L. acidophilus* depending on the type of nutrient salt and its mass concentration

Таблица 1

Показатели биосинтетической активности молочнокислых бактерий *L. acidophilus* в зависимости от вида питательной соли и ее массовой доли

Mass fraction of phosphorus-containing salt, %	Formation rate of calcium lactate, g/(dm ³ ·h)				Calcium lactate yield, %			
	Na ₂ HPO ₄ ·12H ₂ O		(NH ₄) ₂ HPO ₄		Na ₂ HPO ₄ ·12H ₂ O		(NH ₄) ₂ HPO ₄	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1.0	0.64±0.09	0.39±0.04	0.68±0.06	0.40±0.01	40.32±1.81	76.80±1.00	42.00±0.50	79.21±3.79
1.5	0.77±0.05	0.40±0.02	0.80±0.06	0.41±0.02	40.65±1.03	76.33±0.30	40.81±0.90	76.56±1.40
2.0	0.78±0.03	0.40±0.15	0.84±0.07	0.40±0.01	41.20±1.50	79.96±6.85	42.48±1.48	79.18±2.83
2.5	0.79±0.06	0.39±0.03	0.93±0.05	0.39±0.03	36.74±1.30	71.58±2.65	41.93±1.06	76.56±1.68
3.0	0.64±0.02	0.27±0.01	0.73±0.06	0.29±0.01	34.50±1.45	61.8±1.79	38.80±0.04	67.80±1.37

From the above, an increase in the mass fraction of the phosphorus-containing salt introduced up to 2.5 % contributes to an increase in the biosynthetic activity of lactic acid bacteria. A further increase in the dosage of salt acts on the growth of bacteria as an inhibitory factor.

Figure 3 presents the dynamics of the lac-

tose fermentation and the accumulation of calcium lactate with varying mass fraction of sodium phosphate and fermentation time. As can be seen from Fig. 3, the application of 2.0 % of Na₂HPO₄·12H₂O allows the highest values for the fermentation rate of whey lactose and the accumulation of calcium lactate to be achieved.

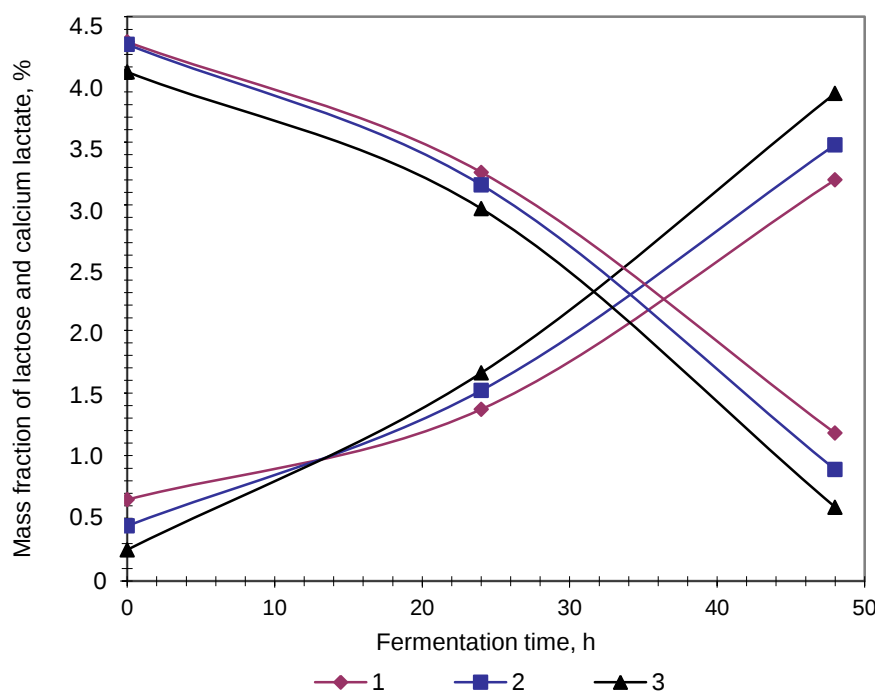


Fig. 3. Mass concentration of calcium lactate and lactose during whey fermentation by *L. acidophilus* AT-I when varying the mass concentration of sodium phosphate, %:
1 – 1.0; 2 – 1.5; 3 – 2.0

Рис. 3. Изменение массовой доли лактата кальция и лактозы в процессе сбраживания творожной сыворотки молочнокислыми бактериями *L. acidophilus* AT-I при варьировании массовой доли фосфорнокислого натрия, %:
1 – 1,0; 2 – 1,5; 3 – 2,0

CONCLUSION

According to the set of indicators characterising the biosynthetic activity of *L. acidophilus* AT-I lactic acid bacteria, the application of sodium phosphate disubstituted dodecahydrate in an amount of 2.0 % increases the efficiency of

obtaining calcium lactate from acid whey. The *Lactobacillus acidophilus* AT-I producer is specified by rather high formation rate for calcium lactate and fermentation rate for lactose whey equal to 0.78 g/(dm³·h) and 0.53 g/(dm³·h), respectively.

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Elena A. Shipovskaya, Vera V. Eveleva, Tatyana M. Cherpalova carried out the experimental work, analyzed the experimental results and prepared the text of the manuscript. Elena A. Shipovskaya, Vera V. Eveleva, Tatyana M. Cherpalova have equal author's rights and bear equal responsibility for plagiarism.

Conflict of interests

The authors declare no conflict of interests regarding the publication of this article.

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