

UDC 602.16; 577.152.321; 637.14

PRODUCTION OF RECOMBINANT LACTASE FROM *LACTOBACILLUS HELVETICUS* VA FOR APPLICATION IN ADVANCED MILK PROCESSING TECHNOLOGIESShamsiyeva Y. A. ¹, Kostanova A. T. ^{1,2}, Tursunbekova A. E. ^{1,2}, Khassenov B. B. ^{1,3}, Akishev Zh. D. ^{1,3*}¹ "National Center for Biotechnology" Ltd., 13/5 Korgalzhyn road, Astana, 010000, Kazakhstan² S.Seifullin Kazakh Agrotechnical Research University, 62 Zhenis av. Astana, 010011, Kazakhstan³ "GenLab" Ltd, 19/1 M. Gabdullin str., Astana, 010000, Kazakhstan

*Correspondence: Akishev Zhiger akishev@biocenter.kz

ABSTRACT

β -Galactosidases (lactases, EC 3.2.1.23) are widely applied in the production of lactose-free dairy products and whey processing. The aim of the study is to obtain recombinant β -galactosidase and to determine its biochemical and kinetic parameters.

In the present work, eight strains were isolated from goat, sheep, mare, and camel milk, as well as cheeses and fermented dairy products from the Akmola and Almaty regions: *Lactobacillus plantarum* NAR, *Lactobacillus plantarum* VGM1, *Lactobacillus paracasei* VK2, *Lactococcus lactis* ZCM, *Lactococcus lactis* IS2, *Lactobacillus curvatus* ICH3, *Lactobacillus rhamnosus* ICH5, and *Lactobacillus helveticus* VA.

Screening for lactase activity revealed that the cheese-isolated strain *Lactobacillus helveticus* VA exhibited the highest activity compared to the other strains, reaching 212.63 U/mg. The β -galactosidase gene from *Lactobacillus helveticus* was cloned into the pET-28c(+) vector, and the recombinant lactase rLacLM was produced via plasmid expression in the *Escherichia coli* BL21(DE3) strain. Using immobilized metal affinity chromatography (IMAC), rLacLM was purified from the induced recombinant culture. The recombinant β -galactosidase exhibited maximum activity at pH 6.5–7.0 and a temperature of 50°C. The specific activity of the enzyme was 76.1 U/mg, while the K_m , $\max$, k_{cat} , and k_{cat}/K_m values were 21.76 mM, 1015 $\mu\text{M}\cdot\text{min}^{-1}$, 141.48 s^{-1} , and $6.5 \times 10^3 \text{ M}^{-1}\cdot\text{s}^{-1}$, respectively. Thin-layer chromatography confirmed efficient lactose hydrolysis yielding glucose and galactose.

These results indicate that the recombinant β -galactosidase from *Lactobacillus helveticus* VA possesses biochemical and catalytic characteristics compatible with milk processing conditions, rendering it a promising enzyme for the production of lactose-free dairy products and the further development of domestic enzyme preparations.

Keywords: milk, lactose, lactase, β -galactosidase, hydrolysis

INTRODUCTION

Lactose is the primary carbohydrate in milk and dairy products, existing as a disaccharide composed of glucose and galactose residues. Its hydrolysis is mediated by the enzyme β -galactosidase (lactase, EC 3.2.1.23), which catalyzes the cleavage of the β -1,4-glycosidic bond to yield monosaccharides [1]. Due to their high specificity and efficiency, β -galactosidases are widely utilized in the food, pharmaceutical, and biotechnological industries [2]. The most significant application of lactases is in the production of lactose-free dairy products. According to various epidemiological studies, lactase deficiency affects more than half of the global adult population, leading to the development of lactose intolerance [3]. The employment of enzymatic hydrolysis allows for a substantial reduction in the lactose content of milk and dairy products without altering their nutritional value or organoleptic characteristics.

In addition to the production of lactose-free goods, β -galactosidases are applied in the processing of cheese whey—a byproduct of cheese manufacturing that contains up to 5% lactose [4]. Although a portion of whey is utilized in animal feed and the food industry, significant volumes of this raw material still require processing or disposal. Enzymatic hydrolysis of lactose enhances the value of cheese whey, increases carbohydrate digestibility, and expands the potential for its subsequent utilization in the food industry.

The primary industrial sources of β -galactosidases are microorganisms, including yeasts, fungi, and bacteria [1]. Lactic

acid bacteria, particularly representatives of the genus *Lactobacillus*, are of particular interest. They possess a long history of safe use in the food industry, and many strains hold GRAS (Generally Recognized as Safe) or QPS (Qualified Presumption of Safety) status. Furthermore, they are natural inhabitants of the dairy environment and synthesize enzymes adapted to the conditions of processing raw dairy materials [5]. However, the natural productivity of most strains remains insufficient for industrial use, highlighting the relevance of developing recombinant producers that ensure high expression levels of target enzymes [1].

Modern methods of molecular cloning and heterologous expression enable the production of β -galactosidases in high-yield systems, primarily in *Escherichia coli* cells. The use of this expression platform ensures a high level of recombinant protein synthesis, the possibility of obtaining them in preparative quantities, and subsequent purification using metal affinity chromatography [1]. Obtaining recombinant lactases significantly simplifies the scaling of enzyme preparation production and allows for a detailed study of their biochemical properties.

The aim of this work was to obtain recombinant β -galactosidase and determine its biochemical and kinetic parameters. To achieve this goal, the following tasks were set: isolation of lactic acid bacteria from dairy products, screening of strains for lactase activity, cloning of the gene of the most promising β -galactosidase, obtaining the recombinant enzyme in *Escherichia coli* cells, its purification and study of bio-

chemical properties, lactose hydrolysis and determination of hydrolysis products.

MATERIALS AND METHODS

2.1. Nutrient media, chemicals, strains, and enzymes

The following media were used: MRS (0.5% yeast extract; 1% peptone; 1% meat extract; 2% lactose or glucose; 0.1% Tween-80; 0.5% sodium acetate; 0.2% ammonium citrate; 0.005% manganese sulfate; 0.01% magnesium sulfate; 0.2% KH_2PO_4 ; pH 6.5 ± 0.2), CASO (1.5% casein hydrolysate; 0.5% soy peptone; 0.5% NaCl; 0.1% skim milk; 0.4% yeast extract; 0.5% Tween-80; 0.1% glucose or lactose; pH 7.3 ± 0.2) and their modifications, Luria-Bertani (1% tryptone, 0.5% yeast extract, 0.5% NaCl; pH 7.0 ± 0.2), SOC (2% tryptone, 0.5% yeast extract, 0.05% NaCl, 2.5 mM KCl, 20 mM MgSO_4 , 20 mM glucose, pH 7.5).

The reagents, enzymes, and consumables used in this study were manufactured by Sigma-Aldrich (USA), AppliChem (Germany), Promega (USA), Amresco (USA), Thermo Fisher Scientific (USA), Qiagen (Germany), and New England Biolabs (USA). All reagents were of molecular biology grade.

For genetic engineering work, *Escherichia coli* strains DH5 α (Thermo Fisher Scientific, USA) and BL21(DE3) (Novagen, USA), and the plasmid vector pET-28c(+) (Novagen) were used. The DNA polymerases used were Q5 High-Fidelity DNA Polymerase (NEB, USA) and Taq (GenLab, Kazakhstan); the restriction enzymes used were NdeI and BamHI (Thermo Fisher Scientific, USA); and T4 DNA ligase (Thermo Fisher) was applied.

2.2. Isolation and identification of lactic acid bacteria

Camel, mare, goat, and sheep milk, as well as cheeses and fermented dairy products, were used as sources of lactic acid bacteria. Samples were collected from small farms in the Ak-mola Region (Arshaly settlement, Izhevskoye village, Ilyinka village, Zerenda village) and the Almaty Region. The isolation of lactic acid bacteria was performed by inoculating onto MRS broth and MRS agar. Cultivation was carried out by inoculating 1 μL of the initial sample into 5 mL of MRS broth, followed by incubation at 37 °C for 24 hours. Pure cultures were obtained using consecutive serial dilutions up to 10^{-4} – 10^{-6} , followed by plating onto MRS agar plates and incubation at 37 °C for 48 hours. Morphologically distinct colonies were re-streaked using the streak plate method onto separate MRS agar plates. The colonies were subjected to Gram staining (Sigma-Aldrich, USA).

Strains were identified based on morphological analysis, MALDI-TOF MS (Bruker Biotyper, Germany), and sequencing of the conserved 16S rRNA fragment locus using an ABI 3730XL automated capillary sequencer (Applied Biosystems, USA). The resulting chromatograms were analyzed using SnapGene viewer and Vector NTI software and compared with the GenBank (NCBI) database.

2.3. Screening for β -galactosidase activity

Strains with beta-galactosidase activity were selected on Petri dishes with MRS agar (containing glucose) supplemented with 150 μM X-galactopyranoside. Overnight cultures were applied to the surface of the dish using a bacteriologi-

cal loop. The culture dishes were incubated in an incubator at 37 °C, and color changes were monitored at time points of 12–72 h. Blue colonies were subcultured in MRS broth (containing lactose) and incubated in an incubator at 37 °C for 22–24 h. Overnight cultures were diluted to an optical density $\text{OD}_{600} = 0.1$ and inoculated into flasks with CASO medium containing either glucose or lactose as a carbon source. Cultivation was carried out for 72 h at 37 °C, after which the cells were pelleted by centrifugation ($6,000 \times g$, 10 min, 4 °C) and frozen. The resulting cell pellets were resuspended in phosphate buffer (50 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, pH 6.5; 2 mM β -mercaptoethanol; 10 mM KCl). Lysis was performed by ultrasonic disintegration. Lysates were cleared by centrifugation (15,000 g, 30 min, 4 °C); supernatants were collected for analysis. The protein content in the lysates was determined using the Bradford reagent (Bradford reagent, Bio-Rad, USA) [6].

2.4. Cloning of the β -galactosidase gene

The β -galactosidase gene was amplified from the genomic DNA of *Lactobacillus helveticus* VA by PCR using oligonucleotides HelvLacLMfw (5'-GGAATTCATatgaagcaaatatcaattgg-3') and HelvLacLMrv (5'-CGGGATCCttaattagaataaatgaaattcatgatctaac-3'). The amplified LacLM gene was digested with restriction enzymes NdeI and BamHI in 2 times Tango buffer (Thermo Fisher Scientific). The pET-28c(+) vector was also digested with the corresponding restriction enzymes, and the DNA fragments were ligated using T4 DNA ligase. The T7 region of the resulting construct was sequenced using the Sanger method [7].

2.5. Expression of the lacLM gene and purification of recombinant β -galactosidase

Competent *Escherichia coli* BL21(DE3) cells were transformed with the pET-28/lacLM vector using a MicroPulser electroporator (Bio-Rad, USA). Transformants were selected on LB agar medium containing kanamycin (50 $\mu\text{g}/\text{mL}$). Individual colonies were inoculated into LB with kanamycin and cultured at 37 °C until an optical density (OD_{600}) of 0.6 was reached. Expression was induced by adding isopropyl- β -D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM and incubating for 16 h at 18 °C.

Cells were pelleted by centrifugation at $6,000 \times g$ for 6 min and suspended in lysis buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 2 mM β -mercaptoethanol. Cell disruption was performed using a Ruptor 4000 ultrasonic homogenizer (OMNI, Kennesaw, Georgia, USA). Insoluble cellular components were removed by centrifugation at $40,000 \times g$ for 1 h at 4 °C, after which the water-soluble protein fraction was used for subsequent purification of the recombinant protein. rLacLM was purified by metal affinity chromatography using Ni-NTA (Thermo Scientific) pre-equilibrated with a buffer containing 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 20 mM imidazole. Protein was eluted with a linear gradient of imidazole from 20 to 500 mM in a buffer containing 20 mM Tris-HCl (pH 8.0) and 500 mM NaCl. The resulting fractions were analyzed by SDS-PAGE, the protein concentration was determined, and they were used in subsequent experiments.

2.6. Determination of β -galactosidase activity

β -galactosidase activity was determined using the natural substrate, lactose. The reaction mixture (100 μL) contained 1 mM MgCl_2 , 50 mM Britton-Robinson buffer (20 mM ace-

tic acid, 20 mM phosphoric acid, and 20 mM boric acid, pH 6.5), and 220 mM lactose. The reaction was carried out for 30 min at 37 °C. The reaction was stopped by heating the mixture to 99 °C for 5 min. The amount of glucose formed was determined using the D-Glucose Assay Kit (Megazyme, Ireland) according to the manufacturer's instructions. One unit of β -galactosidase activity was defined as the amount of enzyme required to produce 1 μ mol of glucose in 1 min under the specified conditions.

2.7. Determining Optimal Conditions for Activity and Kinetic Parameters

The effect of temperature was studied in the range of 20–80 °C. The temperature optimum was determined based on the residual enzyme activity after incubation at a given temperature. The pH optimum was determined in the pH range of 3.0–10.0 using 50 mM Britton-Robinson buffer. Relative activity was calculated relative to the maximum value, which was taken as 100%.

Kinetic measurements were performed at the optimal temperature and pH of maximum activity using lactose as a substrate in 50 mM Britton-Robinson buffer. Lactose concentrations ranged from 1 mM to 585 mM. Kinetic parameters and constants were calculated by nonlinear regression, and experimental data were approximated by the Henry-Michaelis-Menten equation using SigmaPlot software (SPSS Inc., USA).

2.8. Lactose hydrolysis

Hydrolysis efficiency was studied using lactose or pasteurized milk solutions. The reaction was conducted under optimal enzyme activity conditions. Samples were collected at specified time intervals. The degree of hydrolysis was assessed by the reduction in lactose concentration and the accumulation of glucose and galactose using enzymatic or thin-layer chromatography using Silica gel 60 F254 plates (Supelco, Merck, Darmstadt, Germany).

2.9. Statistical data analysis

All experiments and measurements were performed in independent triplicates. Mean values were calculated using GraphPad Prism software, version 8.0.1. The calculation of

protein molecular weights and their isoelectric points, the analysis of sequencing chromatograms, and primer design were performed using Vector NTI Advance 11 and SnapGene Viewer software.

RESULTS AND DISCUSSION

Screening and Identification of Isolates with Beta-Galactosidase Activity

Lactic acid strains were isolated from milk, cheese, and dairy product samples collected on small farms in the Akmola and Almaty regions. Eight isolates were obtained from the dairy and fermented milk product samples. Based on morphological, proteomic, and genomic analysis, the isolated strains were identified as *Lactobacillus plantarum* NAR, *Lactobacillus plantarum* VGM1, *Lactobacillus paracasei* VK2, *Lactococcus lactis* ZCM, *Lactococcus lactis* IS2, *Lactobacillus curvatus* ICH3, *Lactobacillus rhamnosus* ICH5, and *Lactobacillus helveticus* VA (Table 1).

By plating the strains on MRS agar plates with X-gal, it

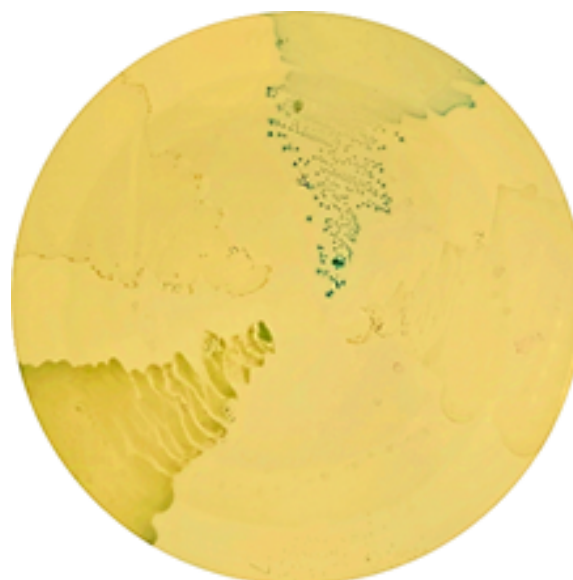


Figure 1. Results of the X-gal beta-galactosidase activity test

Table 1- Information on isolated isolates

Name of isolates	Place of collection	Source	Bacteria	X-gal test	Activity, U/mg
NAR	Astana	Fermented milk drink	<i>Lactobacillus plantarum</i>	++++	0.68
VGM1	Arshaly village	Goat milk	<i>Lactobacillus plantarum</i>	+	5.09
VK2	Arshaly village	Kumiss	<i>Lactobacillus paracasei</i> ,	-	0
ZCM	Zerenda village	Camel milk	<i>Lactococcus lactis</i>	-	13.25
IS2	Ilyinka village	Mare's milk	<i>Lactococcus lactis</i>	-	0
ICH3	Izhevskoye village	Homemade cheese	<i>Lactobacillus curvatus</i>	-	0
ICH5	Izhevskoye village	Homemade cheese	<i>Lactobacillus rhamnosus</i>	++	0
VA	Almaty	Homemade cheese	<i>Lactobacillus helveticus</i>	+++	212.63

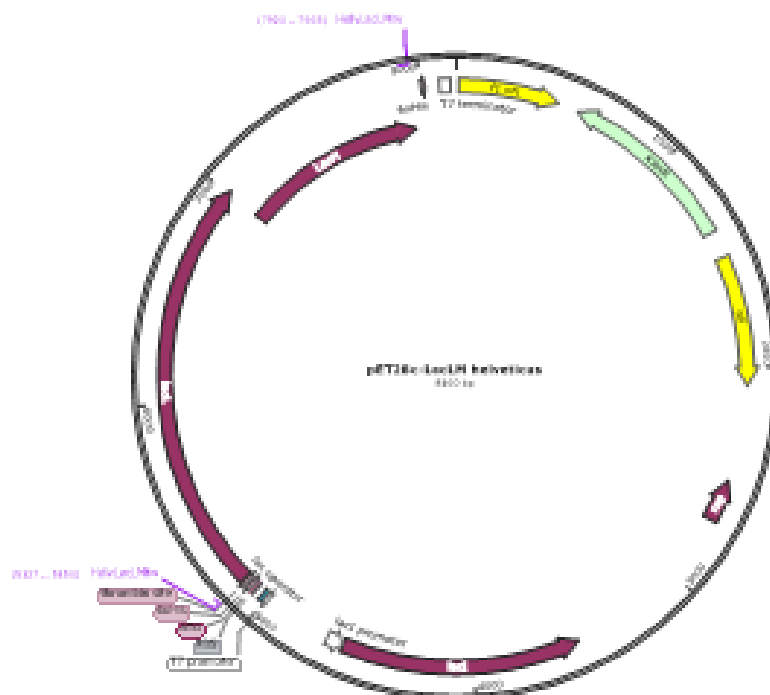
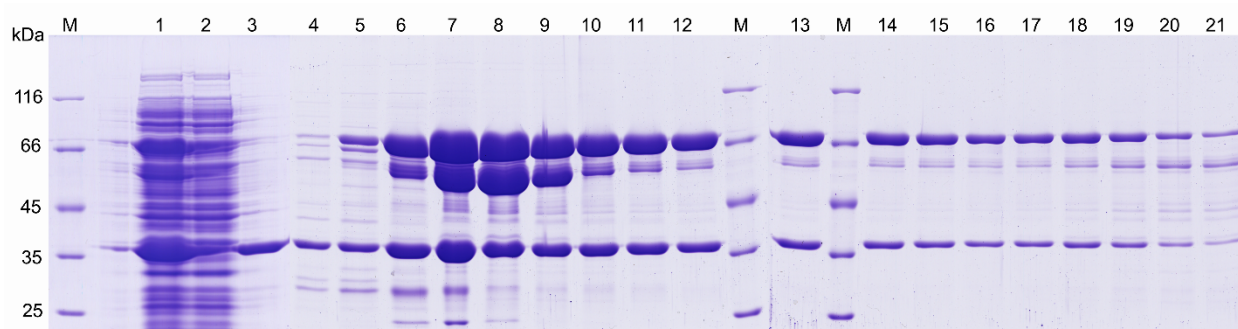


Figure 2. Plasmid map of pET-28/lacLM



M – protein marker (26610, Thermo Scientific), 1,2-lysates, 3-21-eluates

Figure 3. Results of chromatographic purification of rLacLM on Ni-NTA.

was found that four of the eight strains possessed β -galactosidase activity. Measurement of the β -galactosidase activity of the lysates revealed that the *Lactobacillus helveticus* VA strain, isolated from homemade cheese, possessed

the highest activity (212.63 U/mg).

Production of recombinant β -galactosidase from *Lactobacillus helveticus* VA

Based on an analysis of *Lactobacillus helveticus* genomes from the NCBI GenBank database, oligonucleotides were selected to amplified the β -galactosidase gene and then cloned into the pET-28c(+) vector. Sequencing of the lacLM gene revealed that it contains 2827 base pairs. Figure 2 shows the plasmid map of the pET-28/lacLM vector.

The open reading frame of the vector encodes two proteins, 648 and 318 amino acid residues long, with estimated molecular weights of 75.6 and 35.9 kDa. At the N-terminus, the recombinant rLacLM protein contains a hexahistidine tag linked to β -galactosidase via a glycine-serine hinge. Transformation of competent *E. coli* BL21(DE3) cells with this vector yielded a recombinant strain exhibiting intracellular accumulation of the rLacLM

protein. Using metal affinity chromatography on Ni-NTA with an imidazole gradient, the rLacLM protein was eluted (Figure 3) at a concentration of 150-200 mM.

Determination of protein concentration by the Bradford method showed that the *E. coli* strain BL21(DE3)/pET-28/lacLM produces recombinant β -galactosidase with a yield of 45 mg/l.

Determination of biochemical characteristics of rLacLM

Biochemical analysis showed that the activity of the recombinant β -galactosidase rLacLM depends on the pH of the reaction medium (Figure 4). In the acidic region (pH 3.0–5.5), the enzyme exhibited only insignificant activity, not exceeding 10% of the maximum, but with an increase in pH to 6.0, a sharp increase in enzymatic activity was observed, which reached a maximum value at pH 6.5–7.0. In this range, the relative activity was 98–100%, indicating an optimum in the neutral region. A further increase in pH led to a decrease in rLacLM activity. At pH 7.5, approximately 75% of the maximum activity was retained, at pH 8.0 – 70%, and at pH 8.5 – no more than 65%. A more pronounced decrease was ob-

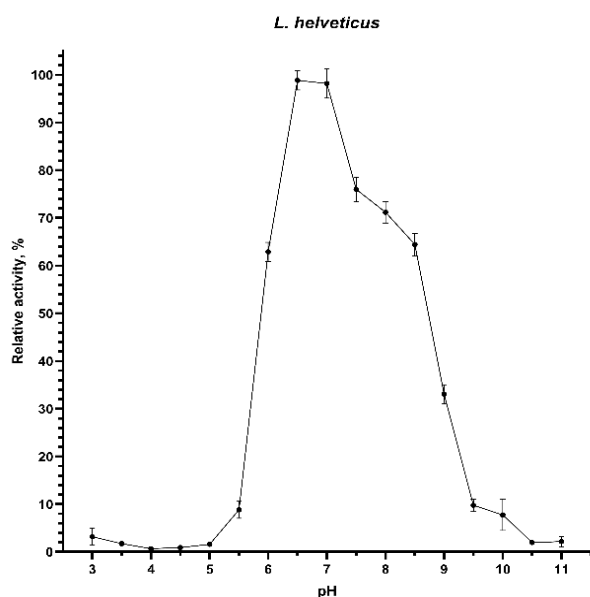


Figure 4. Effect of pH on rLacLM activity in the range of 3.0–11.0

served at a pH above 9.0: at pH 9.5, the activity was about 35%, while at pH 10.0–10.5 it did not exceed 10%. In a highly alkaline environment (pH 11.0), residual activity was practically undetectable.

The obtained results show that the studied β -galactosidase is a neutral enzyme with an optimum activity at pH 6.5–7.0. A similar profile is characteristic of most β -galactosidases from lactic acid bacteria, including representatives of the genus *Lactobacillus*, whose enzymes are evolutionarily adapted to function in the cell cytoplasm where a near-neutral pH is maintained. A sharp decrease in activity in the acidic region is probably due to a change in the degree of protonation of amino acid residues in the catalytic center, which disrupts enzyme-substrate complex formation and catalysis efficiency [9]. Similarly, in an alkaline medium, deprotonation of catalytically significant residues and disruption of intramolecular interactions lead to gradual inactivation of the enzyme. The obtained pH optimum corresponds well to the conditions of lactose hydrolysis in milk, the natural pH of which is about 6.6–6.8 [10]. This demonstrates the potential of using recombinant β -galactosidase for the production of lactose-free dairy products without the need for additional adjustments to the acidity of the raw materials. This feature will reduce process costs and minimize changes in the organoleptic properties of the finished product. Similar pH optimum values have previously been described for β -galactosidases from various strains of *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactocaseibacillus casei*, confirming that the enzyme under study belongs to the group of neutral bacterial β -galactosidases [8, 11]. Furthermore, maximum activity at pH 6.5–7.0 makes the lactase under study particularly attractive for direct use in milk processing.

The effect of temperature on the activity of recombinant β -galactosidase was studied in the range from 0 to 70 °C (Figure 5). The enzyme retained low activity at low temperatures, amounting to approximately 10–20% of the maximum in the range of 0–15 °C. With a further increase in temperature, a gradual increase in catalytic activity was observed. At 25–35

°C, the relative activity reached 30–55%, and at 40 °C and 45 °C it increased to 62% and 74%, respectively. The maximum activity of recombinant β -galactosidase was recorded at 50 °C. With a further increase in temperature, the activity rapidly decreased: at 55 °C, approximately 63% of the maximum activity was retained, at 60 °C – 50%, at 65 °C – 20%, whereas at 70 °C the enzyme almost completely lost its catalytic activity.

The studied β -galactosidase is characterized by a temperature optimum of 50 °C, indicating its moderate thermal stability. This temperature profile is typical of β -galactosidases from lactic acid bacteria, which maintain high catalytic efficiency at temperatures used in milk processing. The gradual increase in activity with increasing temperature is due to an increase in the kinetic energy of the molecules and an in-

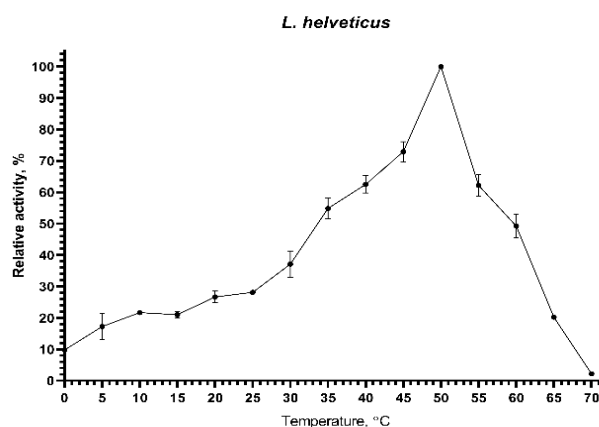


Figure 5. Effect of temperature on rLacLM activity in the range of 0–75 °C

crease in the frequency of effective collisions of the enzyme with the substrate. However, at temperatures above the optimum, the spatial structure of the protein is disrupted, accompanied by a decrease in catalytic activity due to partial thermal denaturation and a change in the conformation of the active center [9]. The optimum temperature of 50 °C is of practical interest for the industrial use of the enzyme. Carrying out lactose hydrolysis at elevated temperatures helps to increase the rate of the enzymatic reaction, reduce the viscosity of the milk raw material, and reduce the likelihood of the development of foreign microflora. Furthermore, the use of temperatures close to 50 °C is well suited to individual stages of the dairy production process and allows for a reduction in the duration of enzymatic treatment. The obtained results are consistent with literature data, according to which β -galactosidases from various representatives of the genus *Lactobacillus* typically exhibit maximum activity at temperatures of 40–55 °C [8, 11, 12]. Furthermore, the identified temperature optimum is somewhat higher than those of several previously described lactases from lactic acid bacteria, making the studied recombinant enzyme a promising candidate for industrial lactose hydrolysis at moderately elevated temperatures.

The kinetic parameters of the recombinant β -galactosidase were determined using lactose as a substrate (Table 2). The enzyme exhibited a specific activity of 76.1 U/mg⁻¹. Kinetic analysis revealed K_m value of 21.76 mM, indicating a moderate affinity of the enzyme for lactose. The maximum reaction velocity V_{max} reached 1015 $\mu\text{mol}\cdot\text{min}^{-1}$, while the turn-

Table 2. Kinetic parameters of the recombinant β -galactosidase rLacLM»

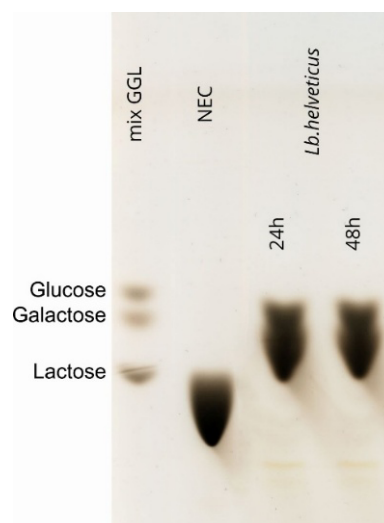
Kinetic parameter	Value
Specific activity, U/mg	76.1
K_m , mM/L	21.76
V_{max} , $\mu\text{mol}\cdot\text{min}^{-1}$	1015
k_{cat} , s^{-1}	141.479
k_{cat}/K_m ($\text{M}^{-1}\text{s}^{-1}$)	6.5×10^3

over number (kcat) was 141.48 s^{-1} . The catalytic efficiency of the enzyme (kcat/ K_m) was equal to $6.5 \times 10^3 \text{ M}^{-1} \cdot \text{s}^{-1}$, which reflects efficient lactose hydrolysis under substrate-saturating conditions.

The obtained kinetic parameters indicate the high catalytic activity of the recombinant β -galactosidase for lactose. The K_m value (21.76 mM) reflects a moderate affinity of the enzyme for the substrate and is within the range characteristic of most bacterial β -galactosidases produced by members of the genus *Lactobacillus*. It should be noted that the lactose concentration in milk is typically 130-150 mM, which significantly exceeds the obtained K_m value. Therefore, during lactose hydrolysis in natural milk, the enzyme will function under conditions close to substrate saturation, ensuring a high reaction rate. The kcat value of 141.48 s^{-1} indicates a high rate of catalytic conversion. Combined with the catalytic efficiency, this indicates the ability of the recombinant β -galactosidase to effectively cleave lactose even with moderate affinity for the substrate. The obtained results are in good agreement with the previously established activity optima of the studied enzyme (pH 6.5-7.0 and temperature 50°C), which makes it promising for use in the technology of producing lactose-free dairy products.

The ability of recombinant β -galactosidase to hydrolyze lactose was confirmed by thin-layer chromatography (Figure 6). A mixture of glucose, galactose, and lactose was used as standards, allowing the enzymatic reaction products to be identified by Rf values. The negative control (NEC), which did not contain the enzyme, showed only one intense spot corresponding to lactose, indicating the absence of spontaneous hydrolysis under the experimental conditions. After incubating lactose with recombinant β -galactosidase for 24 h, the intensity of the lactose spot significantly decreased, while spots corresponding to glucose and galactose appeared, indicating enzymatic cleavage of the disaccharide. After 48 h of incubation, the chromatogram profile remained similar, but the monosaccharide spots became more pronounced, while the amount of residual lactose further decreased, indicating further progress of the hydrolysis reaction.

The obtained results confirm that the recombinant β -galactosidase efficiently hydrolyzes lactose into glucose and galactose, consistent with the classical mechanism of enzyme action. Catalysis proceeds via the cleavage of the β -1,4-glycosidic bond between the galactose and glucose residues, yielding the corresponding monosaccharides. Notably, a residual amount of lactose was detected on the chromatogram even after 48 h of incubation. This finding may be attributed to the reaction reaching equilibrium, incomplete hydrolysis driven by

**Figure 6.** Dynamics of the process of enzymatic hydrolysis of lactose using recombinant β -galactosidase rLacLM after 24 and 48 h of incubation

a decrease in substrate concentration, or partial enzyme inactivation during prolonged incubation. These findings agree with the literature, which indicates that β -galactosidases from lactic acid bacteria are highly effective in lactose hydrolysis and hold strong potential for the production of lactose-free dairy products and whey processing.

CONCLUSION

Lactobacillus helveticus strain isolated from fermented dairy products demonstrated the highest β -galactosidase activity, and its gene was successfully cloned and expressed in *Escherichia coli* BL21(DE3) for purification via metal affinity chromatography. Characterization showed that the recombinant enzyme is highly active at pH 6.5-7.0 and 50°C , making it promising for efficient lactose hydrolysis in dairy processing, as validated by thin-layer chromatography.

The obtained kinetic parameters indicate efficient lactose hydrolysis, and the high catalytic activity confirms the enzyme's suitability for practical use. The efficiency of lactose hydrolysis was confirmed by thin-layer chromatography, demonstrating the formation of glucose and galactose as a result of the action of recombinant β -galactosidase. Taken together, the obtained results demonstrate the potential of this enzyme for developing technologies to produce lactose-free dairy products and the integrated processing of whey.

AUTHOR CONTRIBUTIONS

Conceptualization and analysis - K.B., T.A.; writing and editing – T.A.; experimental work - A.Z., S.Y., K.A. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP23489692).

LITERATURE

1. Zhou Y., Liu Y., Gao F., Xia Z., Zhang Z., Addai F. P.,

Zhu Y., Chen J., Lin F., Chen D. β -Galactosidase: Insights into source variability, genetic engineering, immobilisation and diverse applications in food, industry and medicine// *International Journal of Dairy Technology*. - 2024. - Vol.77. - No 3. - P. 651-679. <http://doi.org/10.1111/1471-0307.13098>

2. Ruiz-Ramírez S., Jiménez-Flores R. Invited review: Properties of β -galactosidases derived from *Lactobacillaceae* species and their capacity for galacto-oligosaccharide production // *Journal of Dairy Science*. - 2023. - Vol.106. - No.12. - P. 8193-8206. <http://doi.org/10.3168/jds.2023-23392>

3. Misselwitz B., Butter M., Verbeke K., Fox M. R. Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management// *Gut*. - 2019. - Vol.68. - No.11. - P.2080-2091. <http://doi.org/10.1136/gutjnl-2019-318404>

4. Prazeres A. R., Carvalho F., Rivas J. Cheese whey management: a review // *J Environ Manage*. - 2012. - Vol. 110. P. 48-68. <http://doi.org/10.1016/j.jenvman.2012.05.018>

5. Bourdichon F., Casaregola S., Farrokh C., Frisvad J. C., Gerds M. L., Hammes W. P., Harnett J., Huys G., Laulund S., Ouwehand A., Powell I. B., Prajapati J. B., Seto Y., Ter Schure E., Van Boven A., Vankerckhoven V., Zgoda A., Tuijtelars S., Hansen E. B. Food fermentations: microorganisms with technological beneficial use // *Int J Food Microbiol*. - 2012. - Vol.154. - No.3. - P. 87-97. <http://doi.org/10.1016/j.ijfoodmicro.2011.12.030>

6. Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // *Anal Biochem*. - 1976. - Vol. 72. P.248-54. [http://doi.org/10.1016/0003-2697\(76\)90527-3](http://doi.org/10.1016/0003-2697(76)90527-3)

7. Sanger F., Nicklen S., Coulson A. R. DNA sequencing with chain-terminating inhibitors// *Proc Natl Acad Sci USA*. - 1977. - Vol.74. - 12. - P. 5463-7. <http://doi.org/10.1073/pnas.74.12.5463>

8. Kittibunchakul S., Pham M. L., Tran A. M., Nguyen T. H. β -Galactosidase from *Lactobacillus helveticus* DSM 20075: Biochemical Characterization and Recombinant Expression for Applications in Dairy Industry // *Int J Mol Sci*. - 2019. - Vol. 20. - No.4. - 947 <http://doi.org/10.3390/ijms20040947>

9. Juers D. H., Matthews B. W., Huber R. E. LacZ β -galactosidase: structure and function of an enzyme of historical and molecular biological importance // *Protein Sci*. - 2012. - Vol.21. - No.12. - P. 1792-807. <http://doi.org/10.1002/pro.2165>

10. Foroutan A., Guo A. C., Vazquez-Fresno R., Lipfert M., Zhang L., Zheng J., Badran H., Budinski Z., Mandal R., Ametaj B. N., Wishart D. S. Chemical Composition of Commercial Cow's Milk // *J Agric Food Chem*. - 2019. - Vol.67. - No.17. - P. 4897-4914. <http://doi.org/10.1021/acs.jafc.9b00204>

11. Nguyen T. T., Nguyen H. A., Arreola S. L., Mlynek G., Djinić-Carugo K., Mathiesen G., Nguyen T. H., Haltrich D. Homodimeric β -galactosidase from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081: expression in *Lactobacillus plantarum* and biochemical characterization // *J Agric Food Chem*. - 2012. - Vol.60. - No.7. - P. 1713-21. <http://doi.org/10.1021/jf203909e>

12. Ruiz-Ramírez S., Jiménez-Flores R. Isolation and characterization of a β -galactosidase from *Lactobacillus helveticus* for industrial processing // *JDS Communications*. - 2025. - Vol.6. - No.1. - P.19-23. <http://doi.org/10.3168/jdsc.2024-0563>

REFERENCES

1. Zhou Y., Liu Y., Gao F., Xia Z., Zhang Z., Addai F. P., Zhu Y., Chen J., Lin F., Chen D. β -Galactosidase: Insights into source variability, genetic engineering, immobilization and diverse applications in food, industry and medicine// *International Journal of Dairy Technology*. - 2024. - Vol.77. - No 3. - P. 651-679. <http://doi.org/10.1111/1471-0307.13098>

2. Ruiz-Ramírez S., Jiménez-Flores R. Invited review: Properties of β -galactosidases derived from *Lactobacillaceae* species and their capacity for galacto-oligosaccharide production // *Journal of Dairy Science*. - 2023. - Vol.106. - No.12. - P. 8193-8206. <http://doi.org/10.3168/jds.2023-23392>

3. Misselwitz B., Butter M., Verbeke K., Fox M. R. Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management// *Gut*. - 2019. - Vol.68. - No.11. - P.2080-2091. <http://doi.org/10.1136/gutjnl-2019-318404>

4. Prazeres A. R., Carvalho F., Rivas J. Cheese whey management: a review // *J Environ Manage*. - 2012. - Vol. 110. P. 48-68. <http://doi.org/10.1016/j.jenvman.2012.05.018>

5. Bourdichon F., Casaregola S., Farrokh C., Frisvad J. C., Gerds M. L., Hammes W. P., Harnett J., Huys G., Laulund S., Ouwehand A., Powell I. B., Prajapati J. B., Seto Y., Ter Schure E., Van Boven A., Vankerckhoven V., Zgoda A., Tuijtelars S., Hansen E. B. Food fermentations: microorganisms with technological beneficial use // *Int J Food Microbiol*. - 2012. - Vol.154. - No.3. - P. 87-97. <http://doi.org/10.1016/j.ijfoodmicro.2011.12.030>

6. Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // *Anal Biochem*. - 1976. - Vol. 72. P.248-54. [http://doi.org/10.1016/0003-2697\(76\)90527-3](http://doi.org/10.1016/0003-2697(76)90527-3)

7. Sanger F., Nicklen S., Coulson A. R. DNA sequencing with chain-terminating inhibitors// *Proc Natl Acad Sci USA*. - 1977. - Vol.74. - 12. - P. 5463-7. <http://doi.org/10.1073/pnas.74.12.5463>

8. Kittibunchakul S., Pham M. L., Tran A. M., Nguyen T. H. β -Galactosidase from *Lactobacillus helveticus* DSM 20075: Biochemical Characterization and Recombinant Expression for Applications in Dairy Industry // *Int J Mol Sci*. - 2019. - Vol. 20. - No.4. - 947 <http://doi.org/10.3390/ijms20040947>

9. Juers D. H., Matthews B. W., Huber R. E. LacZ β -galactosidase: structure and function of an enzyme of historical and molecular biological importance // *Protein Sci*. - 2012. - Vol.21. - No.12. - P. 1792-807. <http://doi.org/10.1002/pro.2165>

10. Foroutan A., Guo A. C., Vazquez-Fresno R., Lipfert M., Zhang L., Zheng J., Badran H., Budinski Z., Mandal R., Ametaj B. N., Wishart D. S. Chemical Composition of Commercial Cow's Milk // *J Agric Food Chem*. - 2019. -

Vol.67. – No.17. – P. 4897-4914. <http://doi.org/10.1021/acs.jafc.9b00204>

11. Nguyen T. T., Nguyen H. A., Arreola S. L., Mlynek G., Djinočić-Carugo K., Mathiesen G., Nguyen T. H., Haltrich D. Homodimeric β -galactosidase from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081: expression in *Lactobacillus plantarum* and biochemical characterization // *J Agric*

Food Chem. - 2012. – Vol.60. – No.7. – P. 1713-21. <http://doi.org/10.1021/jf203909e>

12. Ruiz-Ramírez S., Jiménez-Flores R. Isolation and characterization of a β -galactosidase from *Lactobacillus helveticus* for industrial processing // *JDS Communications*. - 2025. – Vol.6. – No.1. -P.19-23. <http://doi.org/10.3168/jdsc.2024-0563>

ӘОЖ: 602.16; 577.152.321; 637.14

СҮТТІ ТЕРЕҢ ӨНДЕУ ТЕХНОЛОГИЯЛАРЫНДА ҚОЛДАНУҒА АРНАЛҒАН *LACTOBACILLUS HELVETICUS* VA-ДАН РЕКОМБИНАНТТЫ ЛАКТАЗА АЛУШамсиева Ю.А.¹, Костанова А.Т.^{1,2}, Турсунбекова А.Е.², Хасенов Б.Б.^{1,3}, Акишев Ж.Д.^{1,3}

«Ұлттық биотехнология орталығы» ЖШС, Қорғалжын тасжолы, 13/5, Астана, 010000, Қазақстан

«С. Сейфуллин атындағы Қазақ агротехникалық зерттеу университеті» КЕАҚ, Жеңіс даңғылы, 62, Астана, 010011, Қазақстан

«GenLab» ЖШС, М. Ғабдуллин көшесі, 19/1, Астана, 010000, Қазақстан

ТҮЙІН

β-Галактозидазалар (лактазалар, ЕС 3.2.1.23) лактозасыз сүт өнімдерін өндіруде және сүт сарысуын өндеуде кеңінен қолданылады. Зерттеудің мақсаты – рекомбинантты β-галактозидаза алу және оның биохимиялық әрі кинетикалық сипаттамаларын анықтау.

Осы зерттеу барысында Ақмола және Алматы облыстарынан жиналған ешкі, қой, бие және түйе сүтінен, сондай-ақ ірімшік пен ашытылған сүт өнімдерінен сегіз штамм бөлініп алынды: *Lactobacillus plantarum* NAR, *Lactobacillus plantarum* VGM1, *Lactobacillus paracasei* VK2, *Lactococcus lactis* ZCM, *Lactococcus lactis* IS2, *Lactobacillus curvatus* ICH3, *Lactobacillus rhamnosus* ICH5, *Lactobacillus helveticus* VA.

Лактазалық белсенділікке жүргізілген скрининг нәтижесінде ірімшіктен бөлініп алынған *Lactobacillus helveticus* VA штаммы басқа штаммдармен салыстырғанда ең жоғары белсенділік көрсетті – 212,63 бірлік/мг. *Lactobacillus helveticus*-тен бөлінген β-галактозидаза гені pET-28c(+) векторына клондалып, *Escherichia coli* BL21(DE3) штаммында плазмидалық экспрессия арқылы rLacLM рекомбинантты лактазасы алынды. Металл-аффинді хроматография көмегімен rLacLM индукцияланған рекомбинантты культураны тазартылды. Рекомбинантты β-галактозидаза ең жоғары белсенділікті рН 6,5–7,0 аралығында және 50°C температурада көрсетті. Ферменттің меншікті белсенділігі 76,1 бірлік/мг болды, ал Km, Vmax, kcat және kcat/Km көрсеткіштері сәйкесінше 21,76 mM, 1015 мкМ·мин⁻¹, 141,48 с⁻¹ және 6,5 × 10³ М⁻¹·с⁻¹ құрады. Жұқа қабатты хроматография лактозаның глюкоза мен галактоза түзе отырып тиімді гидролизденетінін растады.

Алынған нәтижелер *Lactobacillus helveticus* VA-дан алынған рекомбинантты β-галактозидазаның сүтті өндеу жағдайларына сәйкес келетін биохимиялық және каталитикалық қасиеттерге ие екенін көрсетті. Бұл фермент лактозасыз сүт өнімдерін өндіруде және отандық ферменттік препараттарды әзірлеуде перспективалы биокатализатор ретінде қарастырылуы мүмкін.

Кілт сөздер: сүт, лактоза, лактаза, β-галактозидаза, гидролиз.

УДК: 602.16; 577.152.321; 637.14

ПОЛУЧЕНИЕ РЕКОМБИНАНТНОЙ ЛАКТАЗЫ ИЗ *LACTOBACILLUS HELVETICUS* VA ДЛЯ ПРИМЕНЕНИЯ В ТЕХНОЛОГИЯХ ГЛУБОКОЙ ПЕРЕРАБОТКИ МОЛОКАШамсиева Ю.А.¹, Костанова А.Т.^{1,2}, Турсунбекова А.Е.², Хасенов Б.Б.^{1,3}, Акишев Ж.Д.^{1,3}¹ ТОО «Национальный центр биотехнологии», Кургальжинское шоссе, 13/5, Астана, 010000, Казахстан² НАО «Казахский агротехнический исследовательский университет им. С.Сейфуллина», пр. Женис, 62, Астана, 010011, Казахстан³ ТОО «GenLab», ул. М. Ғабдуллина, 19/1, Астана, 010000, Казахстан

АННОТАЦИЯ

β-Галактозидазы (лактазы, ЕС 3.2.1.23) широко применяются для производства безлактозных молочных продуктов и переработки молочной сыворотки. Целью исследования является получение рекомбинантной β-галактозидазы и определение её биохимических и кинетических параметров.

В настоящей работе из козьего, овечьего, кобыльего, верблюжьего молока, сыров и кисломолочных продуктов Акмолинской и Алматинской областей было выделено 8 штаммов: *Lactobacillus plantarum* NAR, *Lactobacillus plantarum* VGM1, *Lactobacillus paracasei* VK2, *Lactococcus lactis* ZCM, *Lactococcus lactis* IS2, *Lactobacillus curvatus* ICH3, *Lactobacillus rhamnosus* ICH5, *Lactobacillus helveticus* VA.

Проведенный скрининг на лактазную активность показал, что штамм *Lactobacillus helveticus* VA, выделенный из сыра обладает наибольшей активностью в сравнении с другими штаммами – 212,63 Ед/мг. Ген β-галактозидазы из *Lactobacillus helveticus* был клонирован в векторе pET-28c(+) и методом плазмидной экспрессии в штамме *Escherichia coli* BL21(DE3) была получена рекомбинантная лактаза rLacLM. С помощью металл-аффинной хроматографии rLacLM была очищена из индуцированной рекомбинантной культуры. Рекомбинантная β-галактозидаза проявляла максимальную активность при рН 6,5–7,0 и температуре 50°C. Удельная активность фермента составила 76,1

Ед/мг, значения K_m , V_{max} , k_{cat} и k_{cat}/K_m были равны 21,76 мМ, 1015 мкМ·мин⁻¹, 141,48 с⁻¹ и $6,5 \times 10^3$ М⁻¹·с⁻¹, соответственно. Тонкослойная хроматография подтвердила эффективный гидролиз лактозы с образованием глюкозы и галактозы.

Полученные результаты свидетельствуют, что рекомбинантная β -галактозидаза из *Lactobacillus helveticus* VA обладает биохимическими и каталитическими характеристиками, соответствующими условиям переработки молока, и может рассматриваться как перспективный фермент для производства безлактозных молочных продуктов и дальнейшей разработки отечественных ферментных препаратов.

Ключевые слова: молоко, лактоза, лактаза, β -галактозидаза, гидролиз.