УДК 577.21; 604.4:577.15; 577.15

BIOCHEMICAL PROPERTIES OF RECOMBINANT B-GALACTOSIDASE FROM *STREPTOCOCCUS THERMOPHILUS*

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ABSTRACT

Recombinant β-galactosidase from *Streptococcus thermophilus* was successfully expressed in *Escherichia coli*, purified, and biochemically characterized. The gene encoding β-galactosidase was amplified from the genomic DNA of *St. thermophilus* and cloned into the expression vector pET-28c (+). Using the recombinant vector, a BL21 (DE3)/pLacZST strain-producer was obtained with overexpression of the gene. Optimal culture parameters for producing recombinant β-galactosidase were determined. The recombinant β-galactosidase had an activity of 19 units/mg. Biochemical characterization of recombinant β-galactosidase showed that the enzyme had maximum activity at pH 9.0 and temperature of 60°C. Analysis of the kinetics of lactose hydrolysis gave a Michaelis constant $K_m$of 10.12 ± 2.5 mM and a limiting value of the initial rate of the enzymatic reaction $V_{max}$of 0.47 ± 0.027 mM/min. The β-galactosidase has been used in experiments that simulate commercial production conditions, to producea glucose-galactose syrup.

Keywords: β-galactosidase, *Streptococcus thermophilus,* genomic DNA, lactose.

INTRODUCTION

Lactose (a disaccharide consisting of glucose and galactose connected by a β-(1-4)-O-glycosidic linkage) is hydrolyzed chemically (acid hydrolysis) [1] or using enzymes.Beta-galactosidase belongs to the hydrolase class, which acts on O-glycosyl compounds and cleaves the terminal unreduced β-D-galactose residue, including lactose, to form monosaccharides [2], or transport the β-D-galactose residue on a lactose molecule with the formation of galactooligosaccharides[3].

Traditionally, β-galactosidases are used for production of functional foods and feeds with reduced lactose content, glucose-galactose syrups[4]. Up to 90% of the world's population, including about 70% of adults, do not absorb lactose, which is due to age-related increases of lactose intolerance [5]. Therefore, increasing the production of lactose-free food products and drugs containing β-galactosidase is an urgent task.

In relation to the cell, β-galactosidases are divided into extracellular and intracellular enzymes. Beta-galactosidases of most bacteria and yeast are intracellular enzymes, while in fungi,β-galactosidases are predominantly extracellular enzymes. According to physicochemical parameters, intracellular β-galactosidases have high thermo- and pH-sensitivity, while extracellular glycoprotein β-galactosidases have high acid and temperature lability [6].

Bacterial β-galactosidases are enzymes of intracellular localization that differ in mass, number of subunits, affinity for various substrates, pH optimum and thermal liability. The most fully characterized and studied is β-galactosidase from *E. coli*. Bacterial β-galactosidases show a maximum activity at pH 6.5-7.5. Some bacterial β-galactosidases are characterized by high temperature optimum of activity and thermal stability [7]. For example, the enzyme from *S. solfataricus* has an optimum of activity at +95°C. The maximum activity of β-galactosidase from *Thermusthermophilus* exhibits at +90°C [8, 9]. On the other hand, there is evidence of the catalytic activity of β-galactosidase from *Xanthomas* sp., *B. subtilis* and *Arthrobacterpsychrolactophilus* at +10°C [10, 11]. For β-galactosidase from *Pseudoalteromonashaloplanktis*, which has an optimum of activity at +26°C, 33% of the activity is determined from the maximum at a temperature of +4°C [12].

Yeast β-galactosidases are thermolabile having activity at pH 6.7-7.2 and have a dependence on single and double-charged metal ions. Yeast β-galactosidases do not have a high thermal stability, their temperature optimum usually lies within the physiological temperature, but for yeast β-galactosidase from basidiomycete yeast *Guehomyces pullulans* there is evidence of activity at 0°C, although yeast grow at temperatures not exceeding +5°C [13].

Fungi β-galactosidases are predominantly characterized by increased thermostability associated with the glycolysation of the enzyme. The presence of a large amount of carbohydrate residues causes their acid resistance.

Comparing the activities of β-galactosidases of bacterial, fungal and yeast origin, it can be noted that bacterial β-galactosidases are the most active, and low specific activity is characteristic for β-galactosidases of yeast and fungal origin. For example, for β-galactosidase from *E. coli* activity is 500 U/mg, but for milk yeast *Kluyveromyceslactis* the activity is 4.2 U/mg and for fungi *Aspergillus oryzae*this indicator is 8 U/mg[14].

The analysis confirms the prospects of studying bacterial β-galactosidases for the purpose of their practical application. Previously, we obtained *E. coli* BL21 (DE3)/LacZLP strain, which effectively produced recombinant β-galactosidase from *L. plantarum* (unpublished data). Unfortunately, in this strain, β-galactosidase precipitated into inclusion bodies and passed into the aqueous phase only when 8 molar urea was used. All attempts at refolding were unsuccessful and therefore a decision was made to find another β-galactosidase from lactic acid bacteria.

The most studied and active β-galactosidase from *E. coli* is not used in the food industry, and β-galactosidases from yeast *K.lactis* and fungi of the genus *Aspergillus* have low activity. Promising are β-galactosidases of lactobacillus bacteria of the genera *Lactobacillus, Lactococcus* and *Streptococcus*[15-20].

Literature data analysis showed that β-galactosidase from *S.thermophilus* is a good candidate. Lactic acid bacteria are an obligate microflora of milk and dairy products, most of them are not pathogenic for humans, a number of them have strong probiotic properties. Obtaining β-galactosidase by cultivation of a bacterial strain seems to be a rather complex and unprofitable process, since problems arise related to the high cost of nutrient media. A promising direction is to obtain β-galactosidases of lactic acid bacteria in recombinant form as industrially important enzymes.

Recombinant DNA technology makes it possible to obtain β-galactosidases in various host cells, in which the level of expression can be much higher than in a natural source. Recombinant β-galactosidase from *A. niger* was obtained in filamentous fungi *Ashbyagossypii*and *Saccharomyces cerevisiae* with a higher level of expression [21, 22]. The resulting recombinant β-galactosidase from *A. niger* in *P. pastoris* is similar in kinetic parameters to the natural one [23].

Thus, bacterial β-galactosidase is a promising target for their use in cheese making and milk processing. In this work, β-galactosidase from *S.thermophilus* was obtained, optimization of biotechnological parameters for maximum production of β-galactosidase, characterization and comparison of biochemical parameters of bacterial β-galactosidase were carried out.

MATERIALS AND METHODS

Strains

The following strains were used: *E.coli* (DH5α, BL21 (DE3), *S.thermophilus*. Chromosomal DNA was isolated from the strain *S. thermophilus* taken from a laboratory collection of bacterial strains.

Media

Luria-Bertani (1% tryptone, 0.5% yeast extract, 0.5% NaCl).For incubation of transformed cells, SOC medium (2% tryptone, 0.5% yeast extract, 0.05% NaCl, 2.5 mMKCl, 20 mM $MgSO_4$, 20 mM glucose, pH 7.5) were used. The concentration of kanamycin antibiotic in the media was 50 μg/ml. The preparation of media was carried out in accordance with Maniatis protocol [24].

Reagents

All reagents used in this work were manufactured by Sigma-Aldrich, AppliChem, Promega, Amresco with the purity category "For molecular biology".

Cloning

The β-galactosidase gene from genomic DNA of *S.thermophilus* was amplified with primers including *BamH*I and *Xho*I restriction sites:

LacZSTBamHI 5′-CGCGGATCCTTATGAACATGACTGAAAAAATTCAAAC-3′ and

LacZSTXhoI 5′-CCGCTCGAGATTTAGTGGTTCAATCATGAAGCTT-3′.

For amplification, Phusion Hot Start II DNA Polymerase (2 U/μL) was used according to the manufacturer's protocol (Thermo Scientific). The length of the gene was 3078 bp. The PCR amplicon was determined by 1% agarose gel in TAE buffer with ethidium bromide.

The resultingamplificate and vector pET-28c(+) were purified by chloroform extraction method for subsequent hydrolysis by specific restriction endonucleases. 30 minutes before the end of the restriction, FastAP phosphatase was added to remove the phosphate groups in the linearized vector. The obtained fragments: the vector and insert were ligated using T4 ligase (Thermo Scientific). The recombinant vector was sequenced using Vector NTI software (Invitrogen, USA).

Expression of β-galactosidase gene from S.thermophilus in E. coli cells

Competent cells of the *Escherichia coli* BL21 (DE3) strain were transformed by the vector containing the β-galactosidase gene. The selected transformants were cultured in Luria-Bertani medium with kanamycin at 200 rpm +37°C. After the cell culture reached OD=0.6, IPTG (Isopropyl β-D-1-thiogalactopyranoside) was added for induction at final concentration of 0.5 mM.

Purification of recombinant protein

Purification of the recombinant β-galactosidase was performed from 400 ml of the induced culture. The cells collected by centrifugation (6000g, +4°C, 7 minutes) were lysed by ultrasonic desintegration (Omni Ruptor 4000 Ultrasonic Homogenizer). Cell lysate was precipitated by centrifugation at 18,200 g, +4°C for 60 minutes. The extract was applied to a HisTrap Chelating HP 1 ml column pre-equilibrated with HisTrap Low Imidasol buffer (500 mMNaCl, 20 mMHepes-NaOH (pH 7.5), 20mM Imidazole (pH 7.5)). After loading, the column was washed with 5 volumes of HisTrap Low Imidasol buffer to remove nonspecifically bound proteins. To search for the eluting imidazole concentration, a linear imidazole gradient was used using the HisTrap Low Imidasol and HisTrap High Imidasol buffer (500 mMNaCl, 20 mMHepes-NaOH (pH 7.5), 500mM Imidazole (pH 7.5). A liquid chromatograph for fast protein purification FPLC (Fast protein liquid chromatography) AKTA model Purifier10 was used in this work. The obtained fractions were analyzed in 12% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Determination of beta-galactosidase activity

The amount of glucose formed was measured by a glucose oxidase-peroxidase method using a commercial kit (Vital). 600 mM lactose (216 g/l) was used as a substrate. To 495 µl of 600 mM lactose in phosphate buffer pH=6.8 solution, 5 µl of the test sample was added. The reaction was carried out at a temperature of +30°C and stopped by heating the sample at 99°C for 5 minutes.

Biochemical characteristics of beta-galactosidase

The pH and the temperature optimum were investigated in studying the biochemical characteristics. When studying the dependence of enzyme activity on pH, β-galactosidase was tested under standard conditions at +30°C, only changing the pH from 2.5 to 11.5. During studying the dependence of enzyme activity on temperature, β-galactosidase was tested under standard conditions at the most effective pH value, changing only the temperature from +20°C to +90°C.

To calculate the kinetic parameters, the lactose substrate was titrated from 2.5 to 250 mM (2.5, 5, 10, 20, 40, 80, 160, 250 mM). The enzymatic reaction was carried out under standard conditions at a temperature of +30°C and pH 9.0. The task was to find the relationship between the rate of the enzymatic reaction and the concentration of the substrate. The reaction rate was expressed in mM/min, the concentration of the substrate (lactose) in mM. To calculate the kinetic parameters of β-galactosidase, GraphPad Prism 6 software was used. A unit of enzyme activity was defined as the amount of the enzyme required to liberate one µmole of D-glucose per minute at 30°C.

RESULTS AND DISCUSSION

Cloning

LacZST gene was amplified from the genomic DNA of *S.thermophilus*, then amplicon was successfully integrated into vector pET-28c(+).

Figure 1 shows a plasmid map of vector with a cloned β-galactosidase gene from *S.thermophilus*.

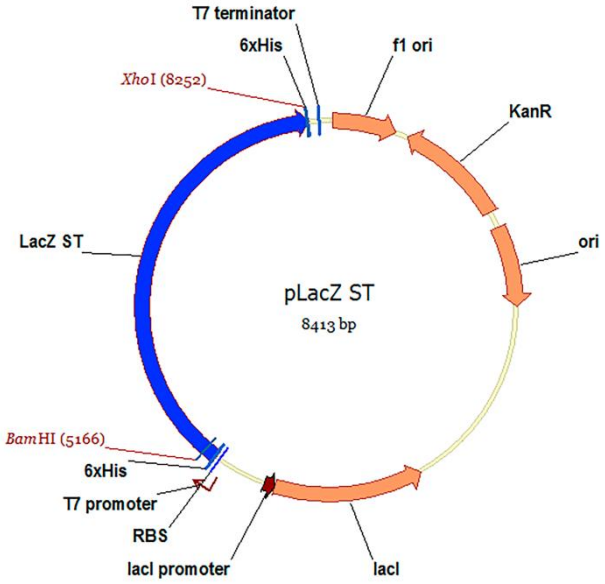
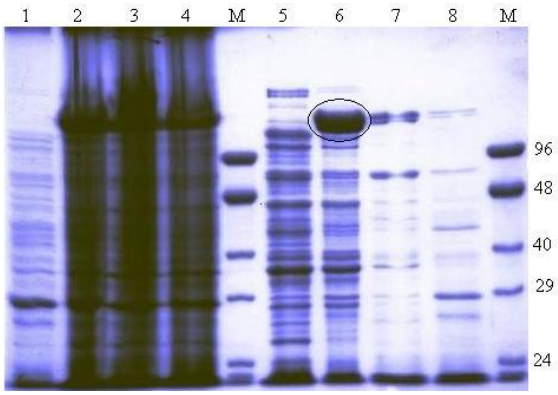


Fig.1. Plasmid map of vector pLacZST

In this vector pLacZST, the β-galactosidase gene is inserted under the control of the T7 promoter. The total length of the protein is 1068 amino acid residues with a calculated mass of 121.7 kDa. In this sequence, 20 a.o. from the N-terminus and 6 a.o. from the C-terminal encode hexahistidine tags. Sequencing of the recombinant vector confirmed the absence of mutations in the gene.

Optimization of β-galactosidase gene expression

The competent cells of *E. coli* BL21 (DE3) strain were transformed by pLacZST. BL21 (DE3)/pLacZST effectively expressed the β-galactosidase gene from *S.thermophilus*(fig.2).

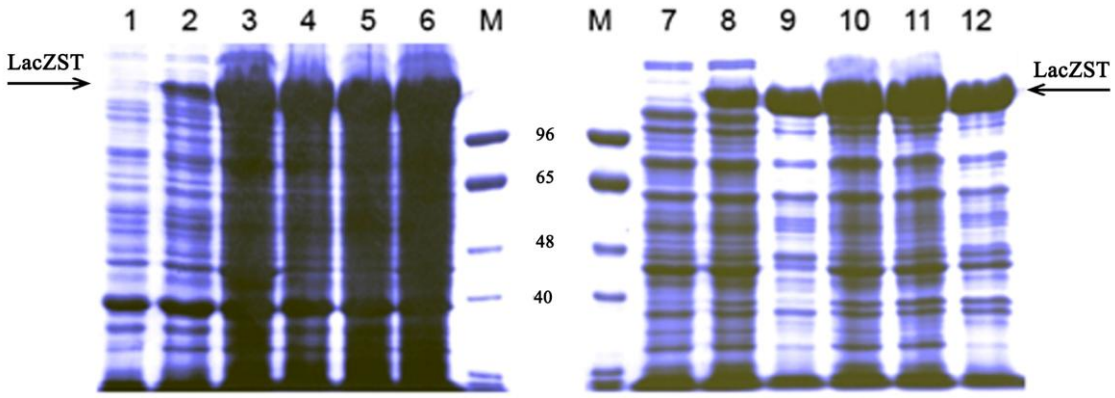


1-4 – pellet; 5-8 – cytosolic fraction;1, 5 – uninduced culture; 2, 6 – 2 hours of induction; 3, 7 – 4 hours of induction; 4, 8 – 16 hours of induction; M – marker

Fig.2. Intracellular accumulation and localization of β-galactosidase from*S.thermophilus* in BL21 (DE3)/pLacZST strain

As can be seen from the figure, the level of protein accumulation strongly depends on the time of incubation of the culture with IPTG. It can be seen that after 4 hours of induction, the level of protein accumulation is greatly reduced.

To determine the incubation time, an experiment was performed with sampling every 30 minutes of induction with IPTG. Figure 3 shows the results of this experiment.



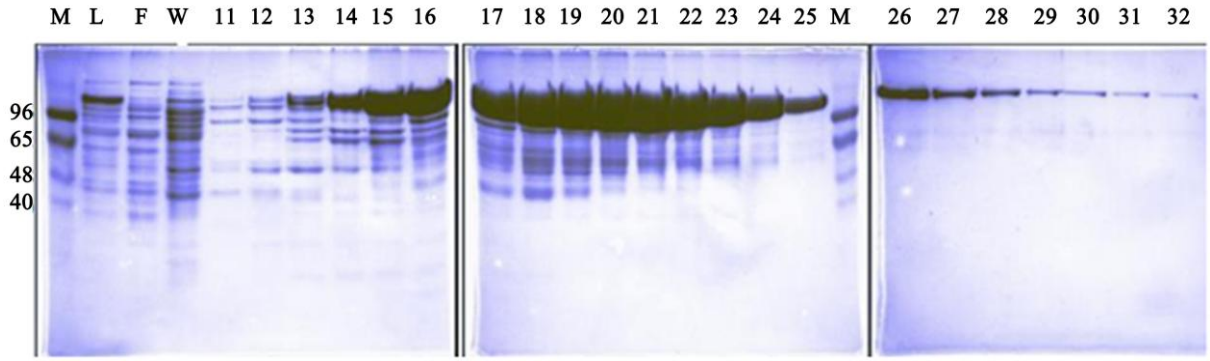
1-6 – pellet; 7-12 – cytosolic (water-soluble) fraction; 1,7 –- uninduced culture; 2,8 – induction of 30 minutes; 3,9 – induction of 60 minutes; 4,10 – induction of 90 minutes; 5,11 – induction of 120 minutes; 6,12 – induction of 180 minutes; M – marker

Fig. 3. Dependence of the accumulation of recombinant β-galactosidase in the cells of BL21 (DE3)/pLacZST on induction time

As follows from the figure 3, the accumulation of recombinant protein in the water-soluble fraction increases in the first two hours, further, the amount of protein decreases due to aggregation and transition into inclusion bodies.

Purification of β-galactosidase

Purification of recombinant β-galactosidase by affinity chromatography showed high efficiency, which is apparently related to the presence of two hexahistidine tags (fig.4).



M –marker; L – loading on the column (load); F – fraction through the column (flowthrough); W – washing off the column; 11-32 – purification fractions

Fig.4. Results of electrophoresis of purified fractions

Biochemical characteristic of the enzyme

The specific activity of purified recombinant β-galactosidase from *S. thermophilus* is 19 units/mg.Results to determine the pH optimum showed that recombinant β-galactosidase is active in the alkaline range with a maximum activity at pH=9.0 (fig.5).

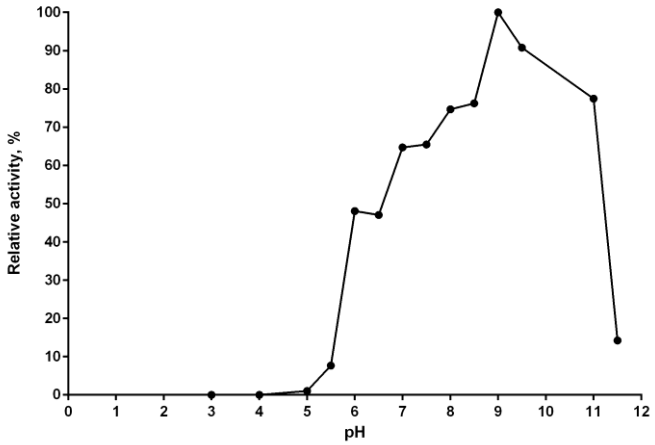


Fig.5.pH dependence of β-galactosidase activity

When studying the dependence of the enzyme activity on temperature, it was found that the activity gradually increased to a maximum at +60°C. After that, a smooth decrease and a sharp decrease to almost 0 at +90°C was observed (fig. 6).

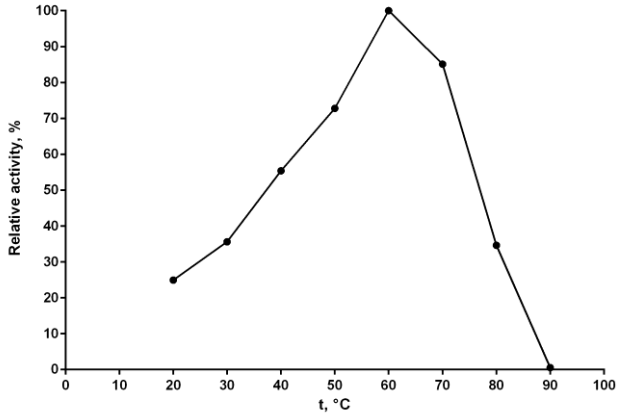


Fig.6. Temperature dependence of β-galactosidase activity

To calculate the kinetic parameters, the lactose substrate was titrated from 2.5 to 250 mM (2.5, 5, 10, 20, 40, 80, 160, 250 mM). Then, the enzymatic reaction was carried out under standard conditions at a temperature of +30°C. A dependence was established between the rate of the enzymatic reaction and the concentration of the substrate. The reaction rate was expressed in relative activity (%), the concentration of the substrate (lactose) in mM (fig.7).

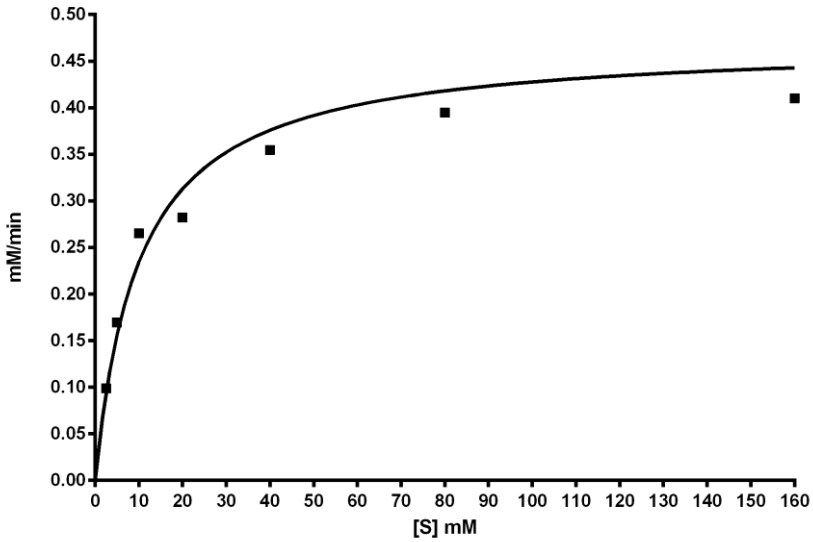


Fig.7. Dependence of β-galactosidase activity on substrate concentration (lactose)

Using the obtained data, the kinetic parameters of β-galactosidase were calculated. According to the obtained data on kinetics, the Michaelis constant is K_m=10.12±2.5 mM, and the limiting value of the rate of the enzymatic reaction is V_{max}=0.47±0.027mM/min. From the obtained data, it follows that recombinant β-galactosidase has a high affinity for the substrate (lactose).

Modeling of production conditions

Experiment 1

An experiment was carried out to evaluate the decomposition of natural raw material – lactose. Whey from milk was used in the hydrolysis of lactose. Analysis of whey on glucose content did not reveal the presence of this monosaccharide. The concentration of lactose as a single reducing sugar was 67 g/l. 200μg of recombinant β-galactosidase was added in 20 ml of whey. For the experiment of decomposition of natural raw materials two levels of temperatures: +37°C and room temperatures of 20-22°C (RT) were chosen. Releasing glucose was monitored every 60 minutes during 6 hours and later during the day. The enzymatic reaction was stopped by 20 μl of concentrated hydrochloric acid on 0.5 ml and then the samples were frozen (fig.8).

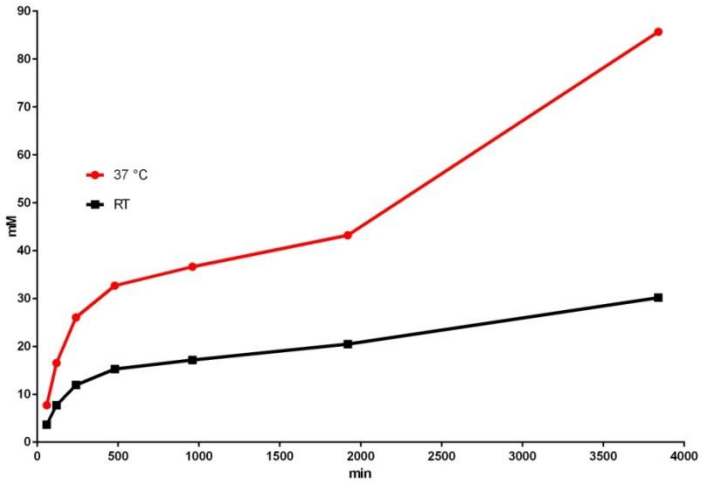


Fig.8. Dynamics of glucose release by hydrolysis of lactose with recombinant β-galactosidase

As can be seen from figure 8, there is a doubling of glucose amount (percentage) during each hour for 4 hours at room temperature and at +37°C. At the temperature of +37°C the reaction rate is two times higher. After 4 hours the reaction rate slows down, which is probably connected with inhibition by the product of the reaction, glucose and/or galactose.

Experiment 2

Using the obtained data for temperature and pH optimum a concentration of lactose was hydrolyzed by 1.25 mg of recombinant β-galactosidase in 7 ml of concentrate at pH=9.0 and a temperature of +65°C. A hydrolysis was carried out in a water bath, the pH of the lacticconcentrate was increased by adding sodium bicarbonate. After a 3-hour hydrolysis, the liquid (with crystallized pellet from the supersaturated solution of lactose) became transparent, the precipitate disappeared, and it was found that the concentration of glucose in the sample was 145 mM.

Experiment 3

An experiment was conducted simulating production conditions. As a production raw material, defatted protein-free whey was chosen as a solution of lactose in buffer with pH=9,0 at a concentration 180 mM. The selected concentration corresponds to milk with a high lactose content.

It was formed 3 groups of samples:

1) The reaction mixture contained 10 ml of 180 mM lactose and 50 µl of the enzyme fraction (2.5 mg/ml) or a dilution of 1/200.

2) The reaction mixture contained 10 ml of 180 mM lactose and 250 µl of the enzyme fraction (2.5 mg/ml) or a dilution of 1/40.

3) The reaction mixture contained 10 ml of 180 mM lactose and 250 µl the enzyme fraction (2.5 mg/ml) or a dilution of 1/40. The only difference that the enzyme was added fractionally – 50 µl for 8 hours (5 times).

The samples were incubated at pH=9.0 and a temperature of +30°C for two days. Aliquots were taken from all three groups every 2 hours for analysis of enzymatic degradation of lactose at the level of one of the growing products - glucose. Monitoring was carried out for 8 hours every 2 hours and, then, after the first day and second day. The results are presented in figure 9.

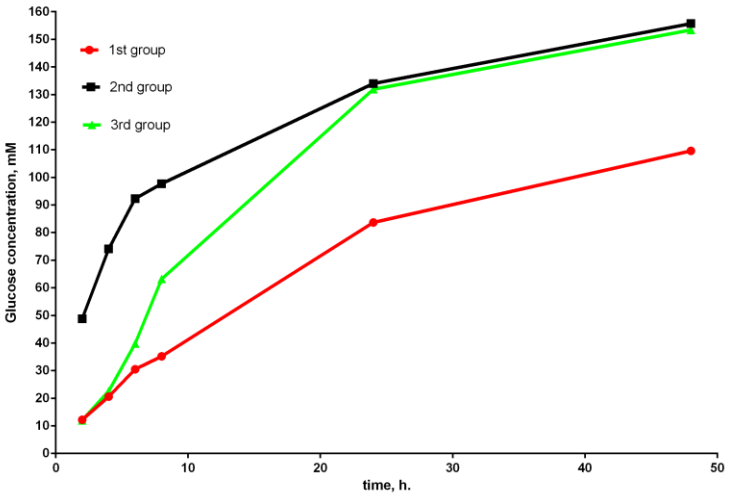


Fig.9. Incubation of recombinant β-galactosidase at various dilutions

The results of the experiment shows that only matters the amount of added enzyme, and not the manner of the addition, indicating that there is no decrease in the activity of recombinant β-galactosidase over time. The graph shows that after fractional adding, when the concentration of the enzyme reaches the level of sample no. 2 after 8 hours the conversion of lactose is the same.

The results of the experiment a smooth increase of the final product-glucose in all groups was observed. The fastest growth was observed during the first 8 hours in the second group with a one-dose injection of 250 µl enzyme. After the first day (24 hours) quantity of the final product – glucose is approximately the same in the second and third group and is 145 mM which is approximately 75% conversion of lactose. In the first group, the conversion is about 50%.

After 2 days the conversion of the lactose in the first group is about 63%, whereas in the second and third group of 85-87%.

As a result after adding 250 µl/10 ml substrate (1/40 dilution) about 75% conversion of lactose occurs after 24 hours at a temperature of +30°C and pH=9,0 and after 48 hours is nearly 90% conversion of lactose (fig.10).

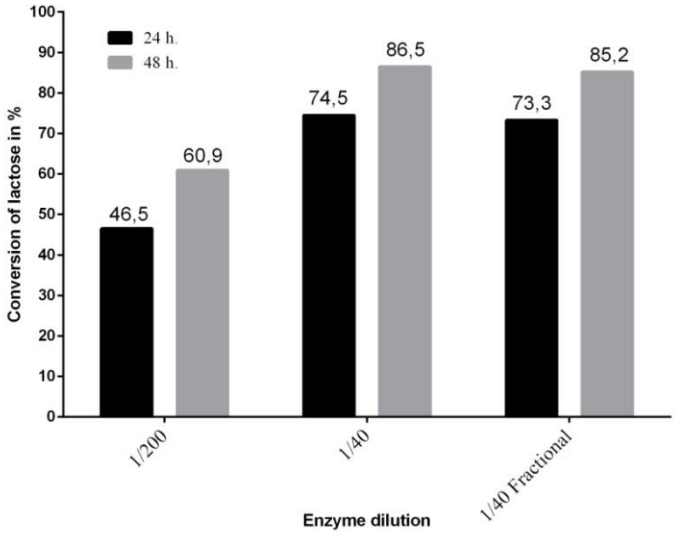


Fig.10. Lactose conversion

CONCLUSION

The β-galactosidase gene from *S.thermophilus* was isolated and cloned in pET-28c(+) vector. Using the recombinant vector, the expression strain BL21 (DE3)/pLacZST was obtained with overexpression of the β-galactosidase gene in water-soluble fraction.

Optimum conditions for the maximum expression of recombinant β-galactosidase were determined: Luria-Bertani medium, cultivation/induction temperature +37°C, IPTG concentration 0.5 mM; 2 hours induction time. Under these conditions, the yield of recombinant β-galactosidase is 40 mg from 1 liter of culture.

Optimal conditions were selected and recombinant β-galactosidase was purified by affinity chromatography. It has been established that β-galactosidase has maximum activity at pH=9.0 and temperature +60°C. Additional hexahistidine tags from both ends of the recombinant protein led to a shift in the maximum activity to the alkaline side, in contrast to the native protein [25]. The activity of the recombinant β-galactosidase is 19 units/mg. The kinetics of the enzymatic reaction of lactose hydrolysis using recombinant β-galactosidase was studied, the Michaelis constant K_m=10.12±2.5mM was determined and the limiting value of the initial rate of the enzymatic reaction V_{max}=0.47±0.027mM/min.

Vacuum concentration and hydrolysis of whey with recombinant β-galactosidase was performed and a glucose-galactose syrup was obtained.

Acknowledgements

This work was performed within the framework of grant financing "Obtaining genetically engineered enzyme preparations of chymosin and β-galactosidase for deep milk processing" financed by the Ministry of Education and Science of the Republic of Kazakhstan (Grant no. 5134/GF4).

REFERENCES

1. Arribas J.C., Herrero A.G., Martin-Lomas M., Canada F.J., He S., Withers S.G. Differential mechanism-based labeling and unequivocal activity assignment of the two active sites of intestinal lactase/phlorizin hydrolase.*Eur J Biochem*., 2000,vol. 267, no. 24, pp. 6996-7005.

2. Braunstein A. Recommendations of the International Biochemical Union on the nomenclature and classification of enzymes, as well as on the units of enzymes and symbols of the kinetics of enzymatic reactions. *Nomenclature of enzymes.*, 1979, pp. 320.

3. Panesar P.S., Kumari S., Panesar R. Potential Applications of Immobilized beta-Galactosidase in Food Processing Industries.*Enzyme Res.*, 2010, vol. 2010, pp. 473-137.

4. Husain Q. Beta galactosidases and their potential applications: a review.*Crit Rev Biotechnol.*, 2010,vol. 30, no. 1, pp. 41-62.

5. Heyman M.B. Lactose intolerance in infants, children, and adolescents.*Pediatrics.*, 2006,vol. 118, no. 3, pp. 1279-1286.

6. V. Gekas M.L.-L. Hydrolysis of lactose: a literature review.*Process Biochemistry*, 1985,vol. 20, pp. 2-12.

7. Pisani F.M., Rella R., Raia C.A., Rozzo C., et al. Thermostable beta-galactosidase from the archaebacteriumSulfolobussolfataricus. Purification and properties.*Eur J Biochem*., 1990,vol. 187, no. 2, pp. 321-328.

8. Ohtsu N., Motoshima H., Goto K., Tsukasaki F., Matsuzawa H. Thermostable beta-galactosidase from an extreme thermophile, Thermus sp. A4: enzyme purification and characterization, and gene cloning and sequencing.*BiosciBiotechnolBiochem*., 1998,vol. 62, no. 8, pp. 1539-1545.

9. Hidaka M., Fushinobu S., Ohtsu N., et al. Trimeric crystal structure of the glycoside hydrolase family 42 beta-galactosidase from Thermusthermophilus A4 and the structure of its complex with galactose.*J Mol Biol.*, 2002,vol. 322, no. 1, pp. 79-91.

10. Wong-Madden S.T., Landry D. Purification and characterization of novel glycosidases from the bacterial genus Xanthomonas.*Glycobiology*, 1995, vol. 5, no. 1, pp. 19-28.

11. Nakagawa T., Fujimoto Y., Ikehata R., Miyaji T., Tomizuka N. Purification and molecular characterization of cold-active beta-galactosidase from Arthrobacterpsychrolactophilus strain F2.*ApplMicrobiolBiotechnol.*, 2006,vol. 72, no. 4, pp. 720-725.

12. Fernandes S., Geueke B., Delgado O., Coleman J., Hatti-Kaul R. Beta-galactosidase from a cold-adapted bacterium: purification, characterization and application for lactose hydrolysis.*ApplMicrobiolBiotechnol*., 2002,vol. 58, no. 3, pp. 313-321.

13. Song C., Chi Z., Li J., Wang X. beta-Galactosidase production by the psychrotolerant yeast Guehomyces pullulans 17-1 isolated from sea sediment in Antarctica and lactose hydrolysis.*Bioprocess Biosyst Eng*., 2010,vol. 33, no. 9, pp. 1025-1031.

14. Hirata H., Negoro S., Okada H. High Production of Thermostable beta-Galactosidase of Bacillus stearothermophilus in Bacillus subtilis.*Appl Environ Microbiol*., 1985,vol. 49, no. 6, pp. 1547-1549.

15. Sriphannam W., Lumyong S., Niumsap P., et al. A selected probiotic strain of Lactobacillus fermentum CM33 isolated from breast-fed infants as a potential source of beta-galactosidase for prebiotic oligosaccharide synthesis.*J Microbiol.*, 2012,vol. 50, no. 1, pp. 119-126.

16. Liu G.X., Kong J., Lu W.W., Kong W.T., et al. beta-Galactosidase with transgalactosylation activity from Lactobacillus fermentum K4.*J Dairy Sci.*, 2011,vol. 94, no. 12, pp. 5811-5820.

17. Benavente R., Pessela B.C., Curiel J.A., et al. Improving Properties of a Novel beta-Galactosidase from Lactobacillus plantarum by Covalent Immobilization.*Molecules.*, 2015,vol. 20, no. 5, pp. 7874-7889.

18. Acebron I., Curiel J.A., de Las Rivas B., Munoz R., Mancheno J.M. Cloning, production, purification and preliminary crystallographic analysis of a glycosidase from the food lactic acid bacterium Lactobacillus plantarum CECT 748(T).*Protein Expr Purif.*, 2009,vol. 68,no., pp. 177-182.

19. Schroeder C.J., Robert C., Lenzen G., McKay L.L., Mercenier A. Analysis of the lacZ sequences from two Streptococcus thermophilus strains: comparison with the Escherichia coli and Lactobacillus bulgaricus beta-galactosidase sequences.*J Gen Microbiol.*, 1991,vol. 137, no. 2, pp. 369-380.

20. Vincent V., Aghajari N., Pollet N., et al. The acid tolerant and cold-active beta-galactosidase from Lactococcuslactis strain is an attractive biocatalyst for lactose hydrolysis. *Antonie Van Leeuwenhoek. Journal of Microbiology***,** 2013,vol. 103, no. 4, pp. 701-712.

21. Magalhes F., Aguiar T.Q., Oliveira C., Domingues L. High-level expression of Aspergillus niger b-galactosidase in Ashbyagossypii.*BiotechnolProg.*, 2014,vol. 30, no. 2, pp. 261-268.

22. Oliveira C., Teixeira J.A., Lima N., Da Silva N.A., Domingues L. Development of stable flocculent Saccharomyces cerevisiae strain for continuous Aspergillus nigerbeta-galactosidase production.*J BiosciBioeng*., 2007,vol. 103, no. 4, pp. 318-324.

23. Hu X., Robin S., O'Connell S., Walsh G., Wall J.G. Engineering of a fungal beta-galactosidase to remove product inhibition by galactose.*ApplMicrobiolBiotechnol*., 2010,vol. 87, no. 5, pp. 1773-1782.

24. Molecular cloning. A laboratory manual / T. Maniatis E., J. Sambrook.New York,Cold Spring Harbor Laboratory, 1982, 545 p.

25. Greenberg N.A., R. M. R. Production and Characterization of β-Galactosidase from Streptococcus thermophiles.*Food Science,* 1982,vol. 47, no. 6, pp. 1824-1835.

STREPTOCOCCUS THERMOPHILUS-ТАН БӨЛІНГЕН РЕКОМБИНАНТТЫ В-ГАЛАКТОЗИДАЗАНЫҢ БИОХИМИЯЛЫҚ ҚАСИЕТТЕТРІ

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ТҮЙІН

β-галактозидаза лактозаны глюкоза-галактозалы шәрбатқа айналдыруда қолданатын маңызды фермент болып табылады. Жүргізілген жұмыс нәтижесінде қол жеткізілді: *Streptococcus thermophilus* – тан pET-28c(+) векторы құрамында β-галактозидаза гені бөлініп алынып, клондалды. Алынған гендік-инженерлік құрылымды қолданып, суда ерігіш фракцияға гені асқын экспрессияланатын BL21(DE3)/pLacZST штамм-продуценті алынды. Рекомбинантты β-галактозидаза алу үшін оңтайлы параметрлер анықталды. Рекомбинантты β-галактозидазаның биохимиялық сипаттамалары зерттелінді. β-галактозидаза pH=9,0 және температура +60°C болғанда, ең жоғары белсенді болатыны анықталды. Рекомбинантты β-галактозидаза көмегімен лактоза гидролизі ферменттік реакциясының кинетикасы зерттелініп, Михаэлис константасы K_m= 10,13±2,5мМ және ферменттік реакцияның алғашқы жылдамдығының шектік мәні V_{max}=23,5±1,372 μМ/мин анықталды. Рекомбинантты β-галактозидаза көмегімен ірімшіктен алынған сарысуды вакуумдық қойылту мен гидролизі бойынша жұмыс жүргізіліп, глюкоза-галактозалы шәрбат алынды.

Негізгі сөздер: β-галактозидаза, *Streptococcus thermophilus,* геномдық ДНҚ, лактоза.