

Effects of Major Ingredients in Cattle Milk on Enzyme Kinetics of Recombinant β -galactosidase (BGalP) Expressed in *Pichia pastoris*

Mohammad Jalili-Nik^{1,3}, Zahra Sharghi Shahri³, Seyed Isaac Hashemy^{2,3}, Baratali Mashkani^{2,3*}

1- Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.

2- Surgical Oncology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

3- Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Background and objective: β -galactosidase enzymes hydrolyze lactose into glucose and galactose for production of lactose free dairy products. However, different ions and fat content in milk may act as the inhibitor or activator for β -galactosidase enzymes. A cold-active β -galactosidase enzyme (BGalP), originally from *Planococcus* sp. *L4*, was previously expressed in *Pichia pastoris* to perform lactose hydrolysis in the refrigerated milk. In this study, the effects of milks major ingredients were evaluated on the enzymatic kinetics to confirm its capacity for hydrolyzing milk lactose.

Material and methods: The activity was determined in different concentrations of NaCl, KCl, MgCl₂, and CaCl₂ as well as in the milk with low, medium or high-fat content. In these experiments ortho-Nitrophenyl β -galactoside was used as the substrate. Additionally, glucose was measured as the product after incubation of milk with BGalP enzyme for 24 h at room temperature.

Results and conclusion: This study demonstrated that ions and fat content did not adversely affect the enzyme activity in the concentration corresponding to the milk contents. Ca (27.5-32.5 mM), Cl (25.3-30.9 mM), Na (15.2-39.1 mM) and Mg (3.75-5.83 mM) had no inhibitory effects, but KCl decreased the enzyme activity. Since Cl existed in MgCl₂, and CaCl₂ exerted no inhibitory effects, it can be concluded that inhibitory effects of KCl resulted from potassium rather than chloride. The results indicate that BGalP enzyme was not inhibited by milks major ingredients and has the potential to be used for the production of lactose-free dairy products.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:

Received 11 July 2018
Revised 14 Aug 2018
Accepted 26 Aug 2018

Keywords:

- BGalP
- Enzyme food biotechnology
- Kinetics
- Milk

*Corresponding author:

Baratali Mashkani,
Department of Medical
Biochemistry,
Mashhad University of Medical
Sciences,
Mashhad, Iran.

Tel: +98-51-3800 2375
Fax: +98-51-3882 8574
Email:
MashkaniBA@mums.ac.ir

1. Introduction

Milk is a rich source of macro and micronutrients; however, lactose intolerance, affecting nearly 70% of the population, discourages many adults from milk consumption [1-5]. The common solution is production of lactose-free milk and other dairy products by using β -galactosidase for hydrolysis of lactose into glucose and galactose [6]. This process increases sweetness of the product and reduces sugar consumption in the dairy products [5]. β -galactosidase enzymes have been also used for synthesis of galacto-oligosaccharides as probiotics [7,8]. These enzymes are naturally expressed in different plants, animal cells and more commonly in microorganisms such as bacteria, yeast, and fungi [5]. The most important industrial sources of β -galactosidase are *Kluyvermyces* (*K.*) *fragilis*, *K. lactis*, *Aspergillus* (*A.*) *niger*

and *Escherichia* (*E.*) *coli* [9,10]. However, due to low levels of expression, its extraction from these natural sources is not economical. Alternatively, production of an enzyme as a recombinant protein can reduce the production costs [11]. Cold-active β -galactosidase (BGalP) enzymes have been considered for lactose hydrolysis in refrigerated milk, which was reported to be highly active for lactose hydrolysis at 5 and 20°C [12,13]. Such psychrophilic enzyme, originally from *Planococcus* sp. *L4*, was previously expressed as a recombinant protein in *Pichia* (*P.*) *pastoris* in our laboratory [14]. However, its kinetic properties were yet to be investigated. The enzymes isolated from various sources have different properties including molecular weight, metal ion requirement, and the pH and temperature for their optimum

activity [15]. Depending on the source of the enzyme, metal ions, glucose and galactose may differently affect enzymatic activity of the β -galactosidase. For example, activity of the enzyme isolated from *K. lactis* was increased in presence of metal ions such as Manganese and was inhibited in the presence of imidazole above 50 mM, as well as galactose and Ca [16,17]. Glucose has been reported as an activator for the β -galactosidase enzyme [18]. While the β -galactosidase extracted from *Bacillus (B.) licheniformis DSM 13* and *E. coli* have been inhibited strongly in the presence of glucose and galactose, their activity was increased with metal ions K and Na (1-100 mM), Mg, Mn and Ca (1 mM) [19].

The activity of the enzyme extracted from *Fusarium oxysporum* is inhibited at the presences of divalent cations as Zn, Mg and Ca [20]. The stability and activity of the β -galactosidase from *Lactobacillus pentosus* was increased at presences of Mg [21]. The enzyme isolated from *Enterobacter cloacae* is activated by Mg, but inhibited by Pb and Zn [22]. Since lactose hydrolysis results in galactose production and Ca is also one of the main naturally occurring ingredients in milk, these enzymes may not be suitable for the production of lactose-free milk [23]. In this study, the effects of different metal ions and fat content were investigated on the kinetics of the BGalP enzyme to evaluate its capacity for production of lactose-free dairy products.

2. Materials and methods

BGalP expression

The *P. pastoris*, previously transformed to express BGalP, was cultured in buffered complex medium with glycerol (BMGY) containing yeast extract (10 g l⁻¹) (Himedia, India), peptone (20 g l⁻¹) (Sigma-Aldrich, USA), yeast nitrogen base (13.4 g l⁻¹) (Sigma-Aldrich, USA), glycerol (10% v v⁻¹) (Merck, Germany), biotin 0.4 mg l⁻¹ (Alborz-daru, Iran) in 100 mM potassium phosphate buffer (pH 6.0) (Merck, Germany) [14]. When the OD₆₀₀ reached 1.5, the medium was replaced by buffered complex medium containing methanol (BMMY). This medium contained the similar ingredients to BMGY except for the glycerol which was replaced by 2% v v⁻¹ methanol (Merck, Germany) to stimulate expression of the BGalP protein. The expression was continued for 5 days and the supernatant concentrated 4-times using a 10 kDa MVCO (Merck, Germany).

Enzyme activity assay using ONPG as the substrate

The enzyme activity in the culture supernatant was measured in Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 50 mM β -mercaptoethanol (Merck, Germany), pH 7.0) and ortho-Nitrophenyl β -galactoside (ONPG) (Sigma-Aldrich, USA) as the substrate as previously described (18). The absorbance was measured using a Victor X5 plate reader

(Perkins Elmer, Waltham, MA). As recommended in the manual for Pichia Expression Kit (Invitrogen, catalog no. K1710-01), β -galactosidase activity (IU ml⁻¹ of the culture supernatant) was calculated by multiplying OD₄₂₀ change in each minute by 380 (a constant value related to the ONPG extinction coefficient). The only modification in the calculation method was to normalize the enzyme activity to the culture supernatant volume (ml⁻¹) rather than its protein content (mg⁻¹).

Evaluating the effect of metal ions on BGalP activity

Stock solutions of KCl (100 mM), NaCl (88 mM), MgCl₂ (21 mM) and CaCl₂ (130 mM) (all from Merck, Germany) were prepared in Z buffer. These stocks contained the concentration of ions nearly 4 times more than milk and were diluted serially in Z buffer for measuring BGalP enzyme activity.

Comparing BGalP activity in Z buffer and deionized water

In a 96-well plate, serial dilutions of lactose (Merck, Germany) were prepared in deionized water and Z buffer. Then 20 μ l of culture supernatant was added to each well. After 24 h incubation at room temperature, the concentration of glucose was evaluated according to the product using Pars Azmun kit (Pars Azmun kit, Iran). The enzyme activity was calculated as micromole(s) of glucose produced per minute.

Investigating the inhibitory effect of lactose on BGalP activity

The BGalP enzyme activity was determined in presence of 5% lactose and different concentrations of ONPG.

Effect of milk fat concentrations on recombinant BGalP activity

The enzyme (20 μ l of the culture supernatant) was added to 100 μ l of commercially packed milk with different concentrations of fat (1.5, 2.5, 3%). After 24 h incubation at room temperature, the concentration of glucose was measured, the enzyme activity (IU min⁻¹) was calculated as micromole(s) of glucose produced per minute.

Statistical analysis

The final results were analyzed by GraphPad Prism software 7 for Windows (GraphPad Software, San Diego, CA). The data was analyzed using nonlinear regression fit and Michaelis-Menten enzyme kinetics model ($V = V_{\max} [S] / (K_m + [S])$) to calculate K_m and V_{\max} values. The mean values calculated from three independent experiments were compared using ANOVA in SPSS 16 software package (SPSS Inc, IL) and considered significantly different if the p-value were ≤ 0.05 . Data presented is expressed as Mean \pm SD.

3. Results and discussion

BGalP was produced in *P. pastoris* in an attempt to preserve nutrients and taste, increasing process flexibility and reducing the cost of manufacturing lactose-free milk through performing lactose hydrolysis at refrigerated temperatures [14]. This is a preliminary investigation aiming to determine whether BGalP is an appropriate enzyme for the production of lactose-free milk.

The effects of metal ions on BGalP activity

The enzyme activity at buffer Z [recommended as optimal condition in the manual for Pichia Expression Kit (Invitrogen, catalog no. K1710-01) were 1482 IU ml⁻¹. The BGalP activity was not adversely affected by MgCl₂ at the concentrations up to 21 mM. In contrast, adding KCl led to about 2 fold decrease of enzyme activity (Fig. 1). These results indicate that Mg²⁺ and Cl⁻ ions, unlike K⁺, did not exert any inhibitory effects of BGalP activity at the concentrations up to 4 times higher than those naturally occurring in cow milk.

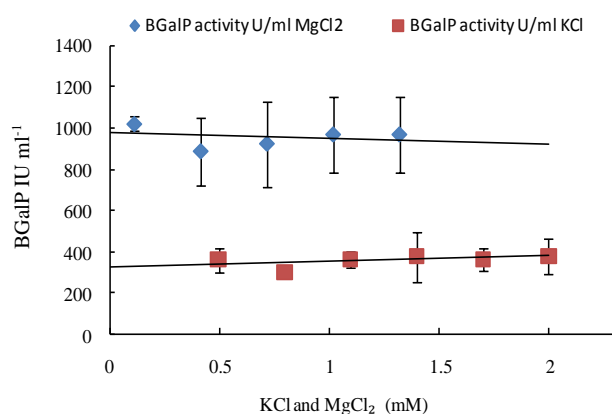


Figure 1. Evaluating the effects of different concentrations of KCl and MgCl₂ on enzyme activity. The enzyme activity was determined in Z buffer containing KCl (3.12-100 mM) and MgCl₂ (0.325-20.8mM). Ortho-Nitrophenyl β-galactoside was used as the substrate. BGalP activity was calculated by multiplying OD₄₂₀ change in each minute by 380 (a constant value related to the ortho-Nitrophenyl β-galactoside extinction coefficient) and finally its normalization to the culture supernatant volume added to the reaction. The experiments were repeated three times and their mean was reported as IU ml⁻¹ of the culture supernatant.

There are some inconsistent reports on effects of magnesium on enzyme activity of the β-galactosidase extracted from *Arthrobacter* sp. Mg²⁺ acted as an inhibitor for the enzyme extracted from *Arthrobacter* sp.32c, but as an activator for the enzyme derived from *Arthrobacter* sp. B7 [12,24].

The β-galactosidase from *Planococcus* sp. L4 was expressed in *E. coli* and its K_m for ONPG was 2.9 mM;

which is consistent with 3.4 observed in this study [17]. When the β-galactosidase from halotolerant *Planococcus* sp. L4 was expressed in *E. coli*, it was resistant to high concentrations of K⁺ and Na⁺ [17]. However, at high concentrations, Na⁺ and Ca²⁺ ions act as inhibitors for BGalP (Fig. 2). The enzyme activity was inversely correlated with NaCl concentrations. CaCl₂ also inhibited the activity of BGalP at the concentrations above 32 mM. The result of this experiment also supported the previous conclusion that the Cl⁻ ion is not a BGalP activity modulator. Previous studies also showed that Ca²⁺ ion inhibited the enzymes derived from *Arthrobacter* sp. B7 and sp. 32c [24,26]. It is consistent with the results of this study, in which high concentrations of Na⁺ and Ca²⁺ ions inhibited the BGalP activity (Fig. 2).

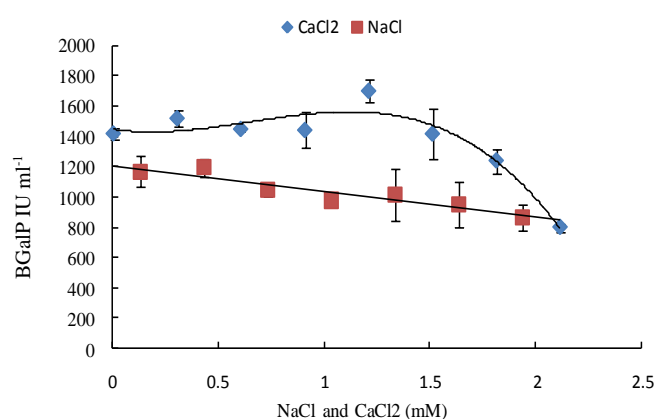


Figure 2. Evaluating the effect of different concentration of NaCl and CaCl₂ on enzyme activity. BGalP enzyme activity was measured in serial dilutions of NaCl (1.3-87 mM) and CaCl₂ (1-130 mM) were prepared in Z buffer. Ortho-Nitrophenyl β-galactoside used as the substrate. BGalP activity was calculated by multiplying OD₄₂₀ change in each minute by 380 (a constant value related to the ortho-Nitrophenyl β-galactoside extinction coefficient) and finally its normalization to the culture supernatant volume added to the reaction. The experiments were repeated three times and their mean was reported as IU ml⁻¹ of the culture supernatant.

BGalP hydrolyzed lactose with the V_{max} of 0.001 IU ml⁻¹ and its half-saturation coefficient (K_m) was 124.4 mM in Z buffer. Similarly, a higher V_{max} (0.0028 IU ml⁻¹) and lower K_m (94.25 mM) observed in deionized water. This result indicated that Z buffer ingredients could adversely affect the activity of BGalP by decreasing the V_{max} and increasing the K_m (Fig. 3). Additional experiments confirmed that the activity of BGalP enzyme in Z buffer was lower compared to deionized water that may be due to the presence of sodium (as Na₂HPO₄ and NaH₂PO₄) in Z buffer.

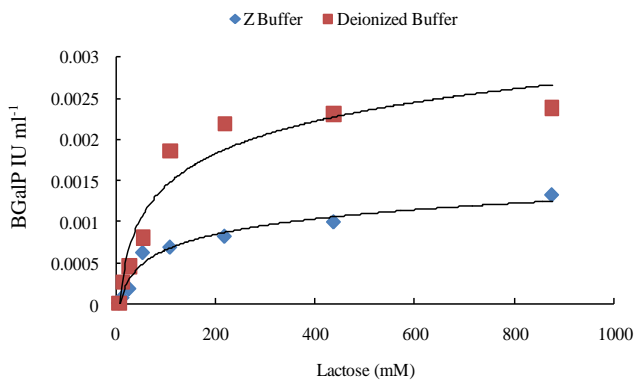


Figure 3. Comparing kinetic parameters of BGalP in Z buffer and deionized water. Glucose concentration was estimated as an indicator of lactose hydrolysis capacity of BGalP in Z buffer and deionized water. Enzyme activity was reported as micromole glucose produced per minute of reaction.

It was previously reported that MgCl₂ (10 mM), CaCl₂ (10 mM) and MnCl₂ (10 mM) did not have any adverse effects on the activity of the enzyme isolated from *E. coli*. KCl and NaCl activated the enzyme at low concentrations (250 mM) but acted as inhibitors at higher concentrations. The enzyme activity decreased in the presence of 1 mM concentrations of NiCl₂, CuSO₄, ZnSO₄ and CaCl₂ [27]. Some of these ions do not occur in significant concentrations in the cattle milk; therefore they were exempted from the current study. BGalP, however, was highly sensitive to the inhibitory effects of the Na and K ions which are different from the enzyme extracted from *E. coli*. Sequence alignment revealed only 10% identity between these two enzymes, which may be the cause of these variations in kinetics properties (Fig. 4).

Substrate specificity of BGalP

BGalP demonstrated relatively high affinity toward ONPG (indicated by its low K_m) when compared to the other β-galactosidase enzyme extracted from different sources (Table 1).

Table 1. K_m valuse reported for β-galactosidase enzymes originated from different organisms. The assay was performed using ONPG as the substrate.

Microorganisms	K _m (mM)	Reference
BGalP expressed in <i>Pichia pastoris</i>	3.97	Current work
<i>Lactococcus lactis</i>	65.36	[28]
<i>Planococcus</i> sp. <i>L4</i>	2.9	[17]
<i>Arthrobacter</i> sp. <i>B7 gene 15</i>	0.4	[24]
<i>Arthrobacter psychrolactophilus F2</i>	2.8	[29]
<i>Paracoccus</i> sp. <i>32d</i>	1.17	[30]
<i>Pseudoalteromonas</i> sp. <i>TAE 79b</i>	0.16	[31]
<i>Flavobacterium</i> sp. <i>4214</i>	0.65	[32]
<i>Guehomyces pullulans 17-1</i>	3.3	[33]
<i>Halomonas</i> sp. <i>S62</i>	2.9	[34]

To reveal the substrate specificity of the enzyme, K_m and V_{max} of BGalP were determined using ONPG as the substrate in presence of 5% lactose. Lactose increased the K_m for ONPG from 3.97 to 14.56 mM. In lactose-free assay, at ONPG concentration of 0.8 mM, the enzyme activity was 321 IU ml⁻¹; which declined to 66 IU ml⁻¹ in presence of 5% lactose which could only be restored by 6.25 mM ONPG. This suggests that lactose may compete with ONPG for binding to the BGalP catalytic site (Fig. 5).

These results attested that BGalP enzyme may have higher affinity for binding to lactose than ONPG.

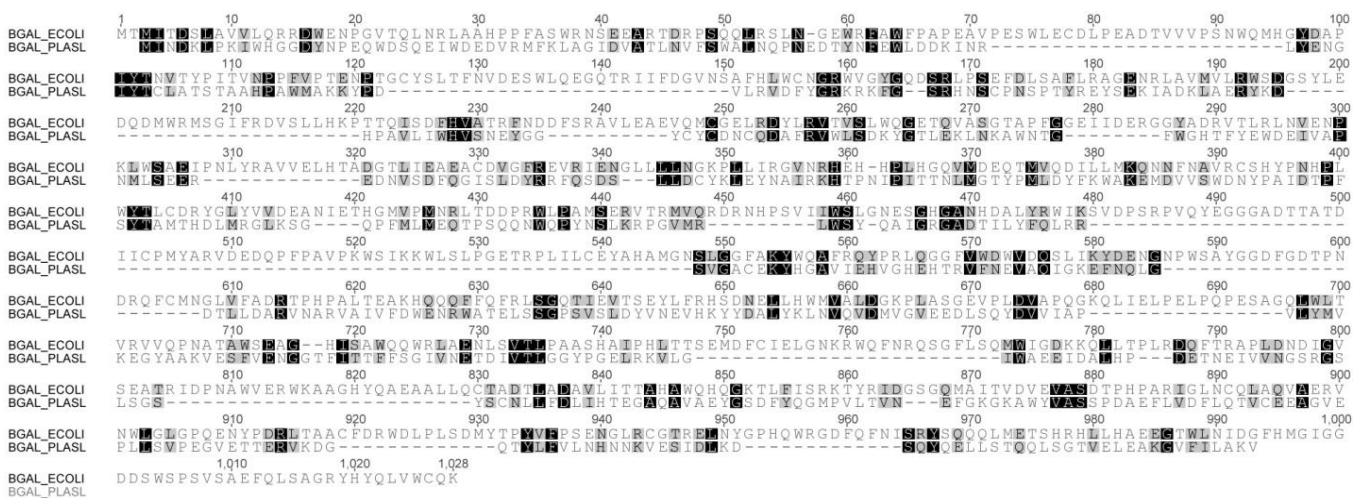


Figure 4. Amino acid sequences alignment of the enzyme from *Escherichia coli* (BGAL-ECOLI) with BGalP (BGAL-PLASL); It was performed using Geneious software suite. Identical and similar amino acids are indicated by black and gray backgrounds, respectively.

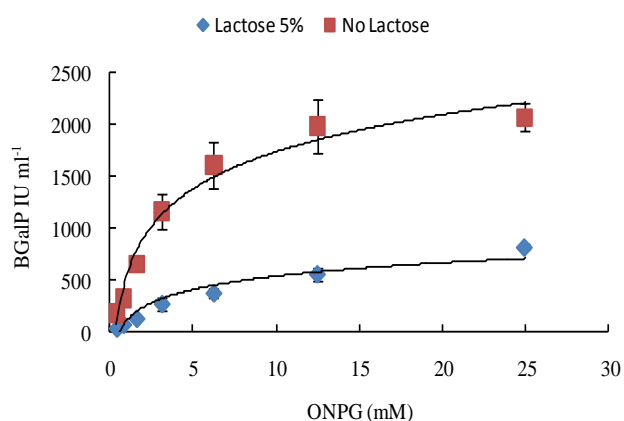


Figure 5. Substrate specificity of BGalP. BGalP activity was measured in serially dilution concentration of ONPG (0.4-25 MM) as the substrate in the reaction medium with or without 5% lactose.

Effect of milk fat content on BGalP activity

As shown in Fig. 6, there was no significant difference in enzyme activity values in the milk containing 0, 1.5, 2.5, or 3% fat ($p > 0.05$). This can be considered as an important advantage for BGalP which is going to be used for lactose hydrolysis in milks containing different levels of fat.

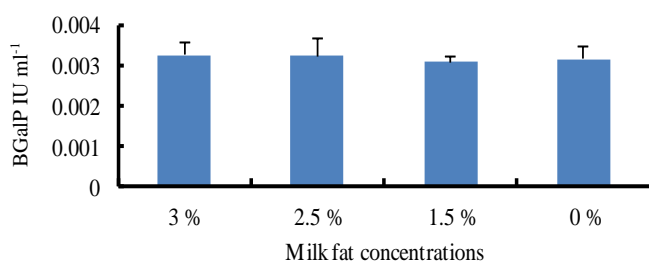


Figure 6. Effect of milk fat on enzyme activity. The enzyme activity (IU min^{-1}) was calculated as micromole(s) of glucose produced per minute.

4. Conclusion

It seems that none of the naturally occurring β -galactosidase enzymes have all the ideal properties to be used for production of lactose-free dairy products. The main drawbacks include inappropriate optimum pH and being adversely affected by the milk ingredients. Interestingly, in this study, it was confirmed that at naturally occurring concentrations of milk ingredients, the fat content and metal ions, excluding potassium, did not have any significant inhibitory effects on BGalP.

Therefore, BGalP can potentially be used for production of lactose-free dairy products. Its kinetic properties can also be improved by removing the glycosylation or even protein engineering techniques, if required.

5. Acknowledgements

Authors would like to thank Mashhad University of Medical Sciences for the financial support (grant No. 931431).

6. Conflict of interest

The authors declare that they have no conflict of interest with this publication.

References

- Haug A, Hostmark AT, Harstad OM. Bovine milk in human nutrition-a review. *Lipids Health Dis.* 2007; 6: 25. doi: /10.1186/1476-511X-6-25
- Ugidos-Rodriguez S, Matallana-Gonzalez MC, Sanchez-Mata MC. Lactose malabsorption and intolerance: a review. *Food Funct.* 2018; 9(8): 4056-4068. doi: /10.1039/c8fo00555a
- Baadkar SV, Mukherjee MS, Lele SS. Study on influence of age, gender and genetic variants on lactose intolerance and its impact on milk intake in adult Asian Indians. *Ann Hum Biol.* 2014; 41(6): 548-553. doi: /10.3109/03014460.2014.902992
- Qiao R, Huang C, Du H, Zeng G, Li L, Ye S. Milk consumption and lactose intolerance in adults. *Biomed Environ Sci.* 2011; 24(5):512-517. doi: /10.3967/0895-3988.2011.05.009
- Abbasalazadeh S, Hejazi MA, Hajiabbas MP. Kinetics of β -galactosidase production by *Lactobacillus bulgaricus* during pH controlled batch fermentation in three commercial bulk starter media. *Appl Food Biotechnol.* 2015; 2(4): 39-47. doi: /10.22037/afb.v2i4.9512
- Rosado JL. Lactose intolerance. *Gac Med Mex.* 2016; 1: 67-73.
- Juers DH, Matthews BW, Huber RE. LacZ β -galactosidase: Structure and function of an enzyme of historical and molecular biological importance. *Protein Sci.* 2012;21(12):1792-1807. doi: /10.1002/pro.2165.
- Van Laere KM, Abee T, Schols HA, Beldman G, Voragen AG. Characterization of a novel beta-galactosidase from *Bifidobacterium adolescentis* DSM 20083 active towards transgalactooligosaccharides. *Appl Environ Microbiol.* 2000; 66(4):1379-1384.
- Husain Q. Beta galactosidases and their potential applications: a review. *Crit Rev Biotechnol.* 2010; 30 (1):41-62. doi: /10.3109/07388550903330497

10. Zhou QZK, Chen XD. Effects of temperature and pH on the catalytic activity of the immobilized β -galactosidase from *Kluyveromyces lactis*. *Biochem Eng J*. 2001; 9(1):33-40. doi: /10.1016/S1369-703X(01)00118-8
11. Katrolia P, Yan Q, Jia H, Li Y, Jiang Z, Song C. Molecular cloning and high-level expression of a β -galactosidase gene from *Paecilomyces aeruginus* in *Pichia pastoris*. *J Mol Catal B Enzym*. 2011; 69(3):112-119. doi: /10.1016/j.molcatb.2011.01.004
12. Hildebrandt P, Wanarska M, Kur J. A new cold-adapted β -D-galactosidase from the Antarctic *Arthrobacter* sp. 32c-gene cloning, overexpression, purification and properties. *BMC Microbiol*. 2009;9:151. doi: /10.1186/1471-2180-9-151
13. Xia Y, He L, Mao J, Fang P, Ma X, Wang Z. Purification, characterization, and gene cloning of a new cold-adapted beta-galactosidase from *Erwinia* sp. E602 isolated in northeast China. *J Dairy Sci*. 2018; 101(8):6946-6954. doi: /10.3168/jds.2018-14605
14. Mahdian SM, Karimi E, Tanipour MH, Parizadeh SM, Ghayour-Mobarhan M, Bazaz MM, Mashkani B Expression of a functional cold active beta-galactosidase from *Planococcus* sp-L4 in *Pichia pastoris*. *Protein Expr Purif*. 2016; 125: 19-25. doi: /10.1016/j.pep.2015.09.008
15. Van Laere KM, Abee T, Schols HA, Beldman G, Voragen AG. Characterization of a novel β -Galactosidase from *Bifidobacterium adolescentis* DSM 20083 active towards transgalactooligosaccharides. *Appl Environ Microbiol*. 2000; 66 (4):1379-1384. doi: 10.1128/AEM.66.4.1379-1384.2000
16. Kim CS, Ji ES, Oh DK. Expression and characterization of *Kluyveromyces lactis* beta-galactosidase in *Escherichia coli*. *Biotechnol Lett*. 2003; 25(20):1769-1774. doi: /10.1023/A:1026092029785
17. Hu JM, Li H, Cao LX, Wu PC, Zhang CT, Sang SL, Xiao Y, Zhang†, Min J, Chen†, Jia Q, Lu†, Yu H, Liu. Molecular cloning and characterization of the gene encoding cold-active beta-galactosidase from a psychrotrophic and halotolerant *Planococcus* sp. L4. *J Agric Food Chem*. 2007; 55(6):2217-2224. doi: /10.1021/jf062910r.
18. Kim CS, Ji ES, Oh DK. A new kinetic model of recombinant beta-galactosidase from *Kluyveromyces lactis* for both hydrolysis and transgalactosylation reactions. *Biochem Biophys Res Commun*. 2004; 316(3):738-743. doi: /10.1016/j.bbrc.2004.02.118
19. Juajun O, Nguyen TH, Maischberger T, Iqbal S, Haltrich D, Yamabhai M. Cloning, purification, and characterization of beta-galactosidase from *Bacillus licheniformis* DSM 13. *Appl Microbiol Biotechnol*. 2011; 89(3):645-654. doi: /10.1007/s00253-010-2862-2
20. Brandao RL, Nicoli JR, Figueiredo AF. Purification and characterization of a beta-galactosidase from *Fusarium oxysporum* var. lini. *J Dairy Sci*. 1987; 70(7): 1331-1337.
21. Maischberger T, Leitner E, Nitisinprasert S, Juajun O, Yamabhai M, Nguyen TH, Haltrich D. Beta-galactosidase from *Lactobacillus pentosus*: Purification, characterization and formation of galacto-oligosaccharides. *Biotechnol J*. 2010; 5(8):838-847. doi: /10.1002/biot.201000126
22. Vella M, Greenwell P. Purification and partial characterization of beta-galactosidase from *Tritrichomonas foetus*. *Glycoconj J*. 1997; 14(7):883-887.
23. Lomer MC, Parkes GC, Sanderson JD. Review article: Lactose intolerance in clinical practice-myths and realities. *Aliment Pharmacol Ther*. 2008; 27(2): 93-103. doi: /10.1111/j.1365-2036.2007.03557.x
24. Trimbur DE, Gutshall KR, Prema P, Brenchley JE. Characterization of a psychrotrophic *Arthrobacter* gene and its cold-active beta-galactosidase. *Appl Environ Microbiol*. 1994; 60(12): 4544-4552.
25. Faraji H, Ramezani M, Sadeghnia HR, Abnous K, Soltani F, Mashkani B. High-level expression of a biologically active staphylokinase in *Pichia pastoris*. *Prep Biochem Biotechnol*. 2017; 47(4):379-387. doi: /10.1080/10826068.2016.1252924
26. Pawlak-Szukalska A, Wanarska M, Popinigis AT, Kur J. A novel cold-active β -d-galactosidase with transglycosylation activity from the Antarctic *Arthrobacter* sp. 32cB-Gene cloning, purification and characterization. *Process Biochem*. 2014; 49 (12):2122-2133. doi:10.1016/j.procbio.2014.09.018
27. Reithel FJ, Kim JC. Studies on the β -galactosidase isolated from *Escherichia coli* ML 308. I. The effect of some ions on enzymic activity. *Arch Biochem Biophys*. 1960; 90 (2): 271-277.
28. Yin S, Zhu H, Shen M, Li G, Lu S, Zhao Y, Le S1, Tan Y1, Peng Y2, Hu F1, Wang J. Surface display of heterologous beta-Galactosidase in Food-Grade Recombinant *Lactococcus lactis*. *Curr Microbiol*. 2018. doi: /10.1007/s00284-018-1531-z
29. Nakagawa T, Fujimoto Y, Ikehata R, Miyaji T, Tomizuka N. Purification and molecular characterization of cold-active beta-galactosidase from *Arthrobacter psychrolactophilus* strain F2. *Appl Microbiol Biotechnol*. 2006; 72(4):720-725. doi: /10.1007/s00253-006-0339-0
30. Wierzbicka-Wos A, Cieslinski H, Wanarska M, Kozłowska-Tylingo K, Hildebrandt P, Kur J. A novel cold-active beta-D-galactosidase from the *Paracoccus* sp. 32d-gene cloning, purification and characterization. *Microb Cell Fact*. 2011; 10: 108. doi: /10.1186/1475-2859-10-108
31. Fernandes S, Gueke B, Delgado O, Coleman J, Hatti-Kaul R. Beta-galactosidase from a cold-adapted bacterium: Purification, characterization and application for lactose hydrolysis. *Appl Microbiol Biotechnol*. 2002; 58(3): 313-321. doi: /10.1007/s00253-001-0905-4.

32. Sorensen HP, Porsgaard TK, Kahn RA, Stougaard P, Mortensen KK, Johnsen MG. Secreted beta-galactosidase from a *Flavobacterium* sp. isolated from a low-temperature environment. *Appl Microbiol Biotechnol.* 2006; 70(5): 548-557.
doi: /10.1007/s00253-005-0153-0
33. Song C, Liu GL, Xu JL, Chi ZM. Purification and characterization of extracellular β -galactosidase from the psychrotolerant yeast *Guehomyces pullulans* 17-1 isolated from sea sediment in Antarctica. *Process Biochem.* 2010; 45(6):954-960.
doi: /10.1016/j.procbio.2010.02.025
34. Wang GX, Gao Y, Hu B, Lu XL, Liu XY, Jiao BH. A novel cold-adapted beta-galactosidase isolated from *Halomonas* sp. S62: gene cloning, purification and enzymatic characterization. *World J Microbiol Biotechnol.* 2013; 29(8):1473-1480.
doi: /10.1007/s11274-013-1311-7.

اثرات اجزای مهم شیر گاو بر کینتیک آنزیم نو ترکیب بتا-گالاکتوزیداز (BGalP) تولید شده در مخمر پیشیا پاستوریس

محمد جلیلی نیک^۱، زهرا شرقی شهری^۲، سید اسحاق هاشمی^۳، براتعلی مشکانی^{۳*}

۱- کمیته تحقیقات دانشجویی، دانشگاه علوم پزشکی مشهد، مشهد، ایران.

۲- مرکز تحقیقات جراحی سرطان، دانشگاه علوم پزشکی مشهد، مشهد، ایران.

۳- گروه بیوشیمی بالینی، دانشکده پزشکی، دانشگاه علوم پزشکی مشهد، مشهد، ایران.

چکیده

سابقه و هدف: آنزیم بتا-گالاکتوزیداز موجب آبکافت لاکتوز به گلوکز و گالاکتوز و تولید فرآورده‌های شیری بدون لاکتوز می‌شود. میزان چربی و یون‌های گوناگون در شیر می‌توانند به عنوان مهارکننده یا فعال‌کننده آنزیم‌های بتا-گالاکتوزیداز فعالیت کنند. در مطالعه قبلی ژن آنزیم بتا-گالاکتوزیداز فعال در سرما (BGalP) حاصل از پلانوکوکوس sp. *L4* در پیشیا پاستوریس بیان شد تا لاکتوز شیر ضمن نگهداری یخچالی آبکافت شود. در این مطالعه، برای تایید ظرفیت این آنزیم در آبکافت لاکتوز شیر، اثر ترکیبات عمده موجود در شیر بر کینتیک آنزیمی بررسی شده است.

مواد و روش‌ها: فعالیت آنزیم BGalP در غلظت‌های گوناگون از یون‌های سدیم کلرید (NaCl)، پتاسیم کلرید (KCl)، منیزیم کلرید (MgCl₂) و کلسیم کلرید (CaCl₂) و در شیر حاوی مقادیر کم، متوسط و زیاد چربی تعیین شد. در این آزمون‌ها اورتو-نیتروفنیل بتا-گالاکتوزید به عنوان رشد مایه (substrate) مورد استفاده قرار گرفت. همچنین، بعد از ۲۴ ساعت گرمخانه‌گذاری در دمای اتاق میزان گلوکز تولید شده به عنوان فرآورده به دست آمده اندازه‌گیری شد.

یافته‌ها و نتیجه‌گیری: این مطالعه نشان داد که میزان چربی و یون‌ها اثر نامطلوبی بر فعالیت آنزیمی در غلظت‌های گوناگون شیر ندارند. کلسیم (۳۲/۵-۲۷/۵ mM)، کلر (۳۰/۹-۲۵/۳ mM)، سدیم (۳۹/۱-۱۵/۲ mM) و منیزیم (۵/۸۳-۳/۷۵ mM) اثر مهارکنندگی نداشتند اما پتاسیم کلرید فعالیت آنزیمی را کاهش داد. از آنجا که کلر موجود در منیزیم کلرید و کلسیم کلرید اثرات مهارکنندگی ندارد می‌توان نتیجه‌گیری کرد که اثرات مهارکنندگی پتاسیم کلرید بیشتر حاصل از پتاسیم است تا کلر. نتایج دلالت بر این دارد که آنزیم BGalP توسط ترکیبات عمده شیر مهار نمی‌شود و امکان استفاده از آن برای تولید فرآورده‌های شیری بدون لاکتوز وجود دارد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

دریافت ۱۱ جولای ۲۰۱۸

داوری ۱۴ آگوست ۲۰۱۸

پذیرش ۲۶ آگوست ۲۰۱۸

واژگان کلیدی

• آنزیم بتاگالاکتوزیداز (BGalP)

• زیست‌فناوری غذایی آنزیمی

• کینتیک

• شیر

*نویسنده مسئول

براتعلی مشکانی، گروه بیوشیمی بالینی، دانشکده پزشکی، دانشگاه علوم پزشکی مشهد، مشهد، ایران.

تلفن: +۹۸-۵۱-۳۸۰۰۲۳۷۵

دورنگار: +۹۸-۵۱-۳۸۸۲۸۵۷۴

پست الکترونیک:

MashkaniBA@mums.ac.ir