

Optimization of β -galactosidase production from lactic acid bacteria

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Abstract

β -galactosidase, commonly known as lactase, represents commercially important enzyme that is prevalently used for lactose hydrolysis in milk and whey. To the date, it has been isolated from various sources. In this study different strains of lactic acid bacteria were assessed for their β -galactosidase productivity, and *Lactobacillus acidophilus* ATCC 4356 resulted with the highest production potential. Thereafter, optimal conditions for accomplishing high yields of β -galactosidase activity were determined. Maximal specific activity (1.01 IU mL⁻¹) was accomplished after 2 days shake flask culture fermentation (150 rpm) at 37 °C, with modified Man Rogosa Sharpe culture broth using lactose (2.5%) as sole carbon source. Finally, in order to intensify release of intracellular β -galactosidase different mechanical and chemical methods were conducted. Nevertheless, vortexing with quartz sand (150 μ m) as abrasive was proven to be the most efficient method of cell disruption. The optimum temperature of obtained β -galactosidase was 45 °C and the optimum range pH 6.5–7.5.

Keywords: *Lactobacillus acidophilus*, β -galactosidase, production, disruption, optimization.

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β -Galactosidase (β -D-galactoside galactohydrolases, EC 3.2.1.23) is an enzyme that catalyzes the cleavage of terminal galactosyl groups from the non-reducing ends of different galactosides. The enzyme is ubiquitous in nature, and can be derived from various sources such as plants, animal organs and microorganisms. In the view of easy production of highly active and stable enzymes, microorganisms are the sources of choice. Nowadays, β -galactosidases are extensively being used in food and pharmaceutical industries due to their capability to hydrolyse lactose, the most abundant sugar in milk and its by-products. This enables alleviation of lactose intolerance problem, prevalent in high share of the human population, and thus broadens consumption of these products [1,2]. It also plays an important role in diminishing some technological difficulties associated with lactose application in food industry such as low solubility, easy crystallization and lack of sweetness [2–4]. In addition, sensorial features of dairy products are improved by disabling lactose strong tendency to absorb flavors and odors [2,5]. Another broad field of β -galactosidase utilization includes the treatments of wastewaters from dairy industries. Namely, disposal of whey and whey

permeates causes huge problems in the environment, owing to the low lactose biodegradability. Hence, hydrolyzed whey lactose provides solution for the problem of pollution [5–7]. On the other hand, under specific conditions such as high lactose concentration, low water activity, and high temperatures, β -galactosidases can catalyze reaction of galactoside synthesis [8,9]. Interest in this aspect of β -galactosidase utilization has been growing rapidly, especially in the field of galacto-oligosaccharides (GOS) production due to their high prebiotic activity and potential positive impacts on human health [8–10].

Additionally, amongst numerous available microbial sources (yeasts, molds and bacteria) lactic acid bacteria strains attract great interest [10–12]. They seem to be the preferable option with respect to their GRAS (generally regarded as safe) status, which provides unhampered usage of their enzymes without any profuse and expensive purification [11,12]. β -Galactosidase derived from lactic acid bacteria (LAB) is found to be a neutral enzyme, suitable for sweet whey and milk lactose hydrolysis as well as GOS production. LAB represents a diverse group of widely applicable industrial microorganisms owing to their GRAS status [11,12]. Although they are already used in large scale lactic acid production, their full potential in the enzyme, predominantly β -galactosidase, commercial production is yet to be achieved. So far, most exploited strains are *Bifidobacterium* species and *Streptococcus thermophiles*, and

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only a few β -galactosidases from *Lactobacillus* sp. have been characterized [1,2,11,12].

The aim of this study was to assure the production of highly active β -galactosidase from LAB. In the first part of experiments potential highest activity producer among available LAB strains was selected. In the next experimental phase focus was on identifying the optimal culture conditions crucial for maximizing the β -galactosidase production such as fermentation time, carbon source and its concentration. In order to achieve satisfactory β -galactosidase exploitation, different cell disruption methods, chemical or mechanical, were investigated. As pointed above, LAB enzymes do not have to be subjected to extensive purification procedures, and can be straightaway used as a crude cell-free extracts in different reactions, thus their characterization (pH and temperature optimum) in reaction with *o*-nitrophenol- β -D-galactopyranoside was performed.

EXPERIMENTAL

Materials

In this study, several lactic acid bacteria (*Lactobacillus acidophilus* ATCC 4356, *Lb. rhamnosus* ATCC 7469, *Lb. reuteri* ATCC 23271, *Lb. helveticus* ATCC 15009, *Lb. delbrueckii* subsp. *bulgaricus* ATCC 11842 and *Streptococcus termophilus* S3) obtained from the American Type Culture Collection (ATCC, Rockville, USA) were screened for β -galactosidase production. Chemicals used for cultivation and fermentation purposes were purchased from Torlak Institute of Immunology and Virology (Belgrade, Serbia). Other chemicals were of analytical grade and obtained from Sigma Aldrich (St. Louis, USA) unless stated otherwise.

Microorganisms and fermentation

Lactic acid bacteria (LAB) strains used in this experiment were maintained at $-20\text{ }^{\circ}\text{C}$ in sterile vials in Man Rogosa Sharpe (MRS) broth medium containing 50% (V/V) glycerol as a cryoprotective agent. The culture activation and propagation was conducted by three successive transfers every 24 h into MRS broth under microaerophilic conditions at $37\text{ }^{\circ}\text{C}$.

Fermentations were performed as batch cultures using modified MRS broth (peptone, 10 g L^{-1} ; meat extract, 10 g L^{-1} ; yeast extract 5 g L^{-1} ; lactose, 20 g L^{-1} ; di-potassium hydrogen phosphate, 2 g L^{-1} ; tri-ammonium citrate, 2 g L^{-1} ; sodium acetate, 5 g L^{-1} ; magnesium sulfate, 0.2 g L^{-1} ; manganese sulfate, 0.04 g L^{-1} ; Tween® 80, 1 mL L^{-1} , distilled water, 1 L) under microaerophilic conditions at $37\text{ }^{\circ}\text{C}$.

First set of experiments was carried out in order to screen for best β -galactosidase producer, and the next ones to optimize fermentation parameters such as incu-

ubation period, carbon source and its preferable concentration, as described later.

Optimization of fermentation

Lactobacillus acidophilus ATCC 4356, a homofermentative L-(+)-lactic acid bacteria strain, chosen as highest activity β -galactosidase producer, was used for further optimization. In order to define optimum fermentation time, fermentation was performed under previously defined conditions for four days. Each day, samples for monitoring bacterial cell concentration and enzyme production, were taken and analyzed. For estimation of bacterial cell concentration plate counting of CFU on MRS agar was used.

Afterwards, modifications of culture medium used for fermentation were introduced. Different carbon sources (overall concentration of 2%) such as glucose, galactose, lactose and their mixtures were applied. Finally, the optimal concentration of the best carbon source was determined. The range of the sugar concentration (1–4%) was chosen on the basis of literature survey.

Extraction of intracellular β -galactosidase

In order to obtain β -galactosidase from *Lb. acidophilus*, bearing in mind its intracellular location, microbial cells were firstly harvested by centrifugation at $12000g$ for 10 min. Then, after being washed twice with 0.1 M sodium phosphate buffer (pH 6.8), they were re-suspended in the same buffer. Finally, the cell suspension was subjected to different chemical and mechanical methods of cell disruption.

Cell disruption with mechanical methods

Effectiveness of several mechanical cell disruption methods was tested. One group of methods included usage of abrasives. In the first experiment, the cell suspension was ground with glass beads for 10 min, using mortar and pestle (GGB). Then, in the second, the mixture of obtained cell suspension and glass beads ($30\text{ }\mu\text{m}$) was stirred vigorously on vortex for 10 min with occasional cooling in the ice bath (VGB). Finally, the same method was used with other kind of abrasive – quartz sand ($150\text{ }\mu\text{m}$), under the same conditions (VS). During the further cell disruption optimization, by vortexing mixture of abrasives and cell suspension, three batches of quartz sand (53 , 150 and $350\text{ }\mu\text{m}$) were used. Activity of released β -galactosidase was measured after removing cell debris by centrifugation ($3000g$ for 10 min).

A second group of mechanical methods were ultrasonication methods. In the first case, the cell suspension was treated for 5 min in ultrasonication bath (Ei Niš-Ro-VEP, Niš, Serbia) with intermittent cooling in 5 cycles (US), while in second, the cell suspension was frozen, and then thawed in ultrasonication bath

(FT/US), under the same conditions. The disrupted cell suspension was centrifuged (3000g for 10 min) and enzyme activity was assayed in obtained cell extract.

Extraction with solvents

The cell suspension was treated with 2 % chloroform (CH) and 80% ethanol (ET). After being incubated at 37 °C for 9 and 20 h, respectively, mixtures were centrifuged (3000g for 10 min), and the enzymatic activity of released β -galactosidase in supernatants was determined.

β -Galactosidase assays

β -Galactosidase activity was determined using 10 mM *o*-nitrophenol- β -D-galactopyranoside (*o*-NPG) in 0.1 M sodium phosphate buffer (pH 6.8) as a substrate. The reaction course was monitored for 2 min, by measuring the concentration of released *o*-nitrophenol (*o*-NP) spectrophotometrically at 410 nm. One unit (IU) is defined as the amount of the enzyme that catalyzes the liberation of 1 μ mol *o*-NP per min under the specified assay conditions. The molar extinction coefficient for *o*-NP was found to be 1357 dm³ mol⁻¹ cm⁻¹.

The amount of proteins in the crude cell-free extracts was determined using Bradford assay with bovine serum albumin as the standard [13].

Effects of temperature and pH on enzymatic activity

The effect of temperature on β -galactosidase activity was examined by incubation of the enzyme and the substrate (10 mM *o*-NPG in 0.1 M sodium phosphate buffer pH 6.8) at temperatures ranging from 30 to 60 °C. Further, the effect of pH was determined on 45 °C by varying buffers used for substrate preparation. Sodium acetate buffer (pH 4.0–5.5) and sodium phosphate buffer (pH 6–8) were used for this purpose. The enzyme activity measurement was conducted in the previously described manner.

RESULTS AND DISCUSSION

Screening for β -galactosidase producing microorganisms

In this paper, several lactic acid bacteria strains (listed in Table 1) were tested for capability of high yield β -galactosidase production. Fermentation was carried out in shake flask on a rotary shaker (150 rpm), using modified MRS broth as a medium for two days. Other fermentation parameters such as temperature (37 °C), initial pH (6.5) and inoculum size (5%) were fixed and chosen on the basis of literature survey.

The results of these experiments have shown that *Lb. acidophilus* (0.671 IU mL⁻¹) is the most efficient β -galactosidase activity producer. In addition, significantly active β -galactosidases were extracted from *Lb. reuteri* (0.420 IU mL⁻¹) and *Lb. helveticus* (0.344 IU mL⁻¹),

while *Lb. rhamnosus* and *Lb. bulgaricus* showed insignificant β -galactosidase productivity (Table 1). Consequently, in the later stages of this study, *Lb. acidophilus* was used for further optimization of fermentation conditions with the aim of accomplishing greater yields of β -galactosidase.

Table 1. Production of β -galactosidase using LAB

Lactic acid bacteria strain	Activity, IU mL ⁻¹
<i>Lb. acidophilus</i> ATCC 4356	0.671
<i>Lb. rhamnosus</i> ATCC 7469	0
<i>Lb. reuteri</i> ATCC 23271	0.420
<i>Lb. helveticus</i> ATCC 15009	0.344
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> ATCC 11842	0.011

Time course of β -galactosidase production

In order to define an optimum fermentation time for maximum β -galactosidase production, the kinetics of β -galactosidase production, as well as cell concentration of *Lb. acidophilus* through time, were studied (Fig. 1). For the purpose of this experiment, fermentation was carried out for 4 days under the previously defined conditions. As shown in Fig. 1, the enzyme activity production exhibited a similar trend as the cell concentration curve, and increased gradually as the fermentation started, reaching the maximum activity (0.718 IU mL⁻¹) after two days. Thereafter, a continuous decrease in the enzyme activity was observed. This is presumably closely related to the fact that the culture has reached the stationary phase after two days. Similar trends, with a maximum production of the enzyme at the beginning of the stationary phase, have previously been reported on production of β -galactosidase from various microorganisms [14,15]. Most probably, such a behavior of *Lb. acidophilus* could be explained by previous findings that during stationary phase, changes in cell wall structure and composition occur, which leads to hampered intracellular enzyme extraction [16]. Besides, reduced lactose concentration and larger amounts of liberated glucose, as a product of lactose hydrolysis, present in the medium during this phase were believed to cause repression of β -galactosidase production. And finally, intracellular enzyme instability is increased by greater amount of produced proteases [15,17].

Simultaneously with intracellular enzyme activity measurements, samples of fermentation medium were screened for implied extracellular activity. It has been previously reported that some LAB, namely *Lb. brevis* and *Lb. plantarum*, excrete β -galactosidase into the fermentation medium by alleged cell autolysis [18]. However, the extracellular activity was not observed in our experiments (results not shown).

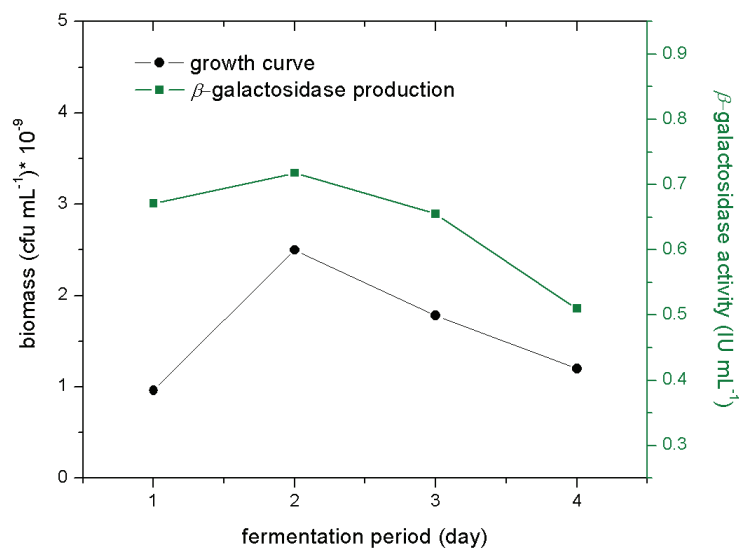


Figure 1. Time course of β -galactosidase production.

Optimization of carbon sources

Several studies reported the major influence of the carbon sources on the β -galactosidase production by various microorganisms. It was concluded that the role of carbon source in this biosynthesis may vary greatly depending upon the used microorganisms [19,20]. Literature data concerning carbon source regulation within *Lb. acidophilus* are rather scarce [20]. Therefore, in this study, different carbohydrates (glucose, galactose, lactose and their mixtures) were tested in order to select the best carbon source. Initial concentrations of above mentioned carbohydrates (2%) corresponded to the one used in previous experiments.

Results presented in Fig. 2 undoubtedly indicate that lactose tends to promote high β -galactosidase activities, since three mediums containing lactose showed considerable enzymatic activity production. Lactose, as sole source, proved to be the inducer of the

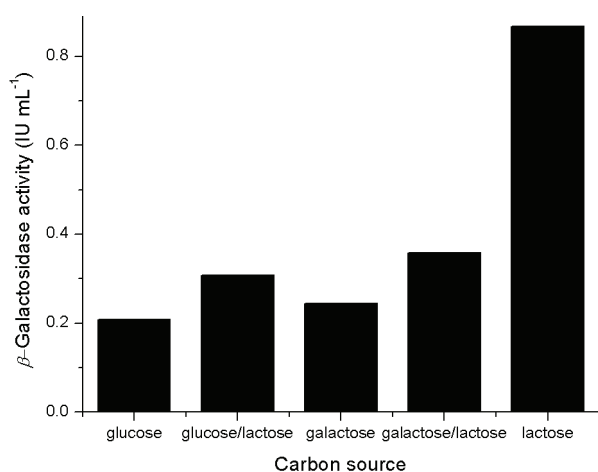


Figure 2. Effect of different carbon sources on β -galactosidase production.

maximum β -galactosidase production, while lactose mixtures with glucose and galactose displayed moderate levels of β -galactosidase activity induction. Our results were compatible with those reported by Hsu *et al.* claiming that highest β -galactosidase activity was detected in cultures containing lactose as the sole carbon source, followed by galactose and the lowest activity with glucose as the carbon source [19]. These results could be explained on the basis of catabolic repression with intracellular accumulation of glucose by-products, and the fact that bacteria primarily consume simpler sugars, thus does not induce significant β -galactosidase production [17].

Besides the nature of carbon sources in the culture media, the amount of it proved to be equally important from the aspect of the β -galactosidase production yield [20,17]. Different lactose concentrations (1.0–3.5% w/V) were used as substrates for β -galactosidase production. The β -galactosidase activity increased progressively with increasing initial lactose concentrations up to 2.5% (Fig. 3). Further growth of the lactose content resulted in the reduction of β -galactosidase activity, which implies that together with the rise of lactose content, there is an increase in degradation products concentrations leading to repression of inducible β -galactosidase production. Similar phenomenon was observed by Murad *et al.* who investigated *Lb. reuteri* and *St. thermophilus* [17]. Their results showed that enzyme activity increased with the increase of lactose concentration (up to 6%), reaching 2.54 and 2.59 IU mL⁻¹, respectively. Further increase of lactose concentration up to 10% decreased the *Lb. reuteri* enzyme production dramatically, while it increased the *St. thermophilus* enzyme production (3.01 IU mL⁻¹). These results suggest that there is clear pattern, regardless of microorganism used, in β -galactosidase production.

β -Galactosidase is lactose inducible enzyme, and its activity yield represents a compromise between lactose induction and intracellular glucose repression.

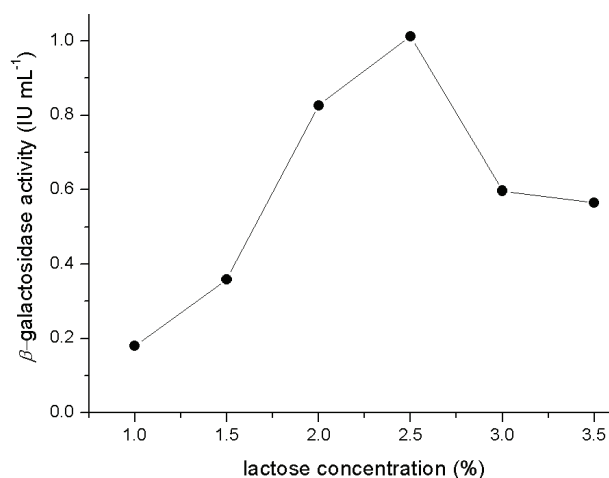


Figure 3. Effect of lactose concentration on β -galactosidase production.

Extraction of intracellular β -galactosidase activity

Since the results of preliminary experiments had shown that significant β -galactosidase activity could be detected only inside the cell, following experimental series was focused on optimization of the cell disruption process. Various methods can be used to release intracellular products depending on their location, stability and purpose [16]. In previous studies, a wide range of mechanical, physical, chemical, enzymatic and combined methods have been applied for β -galactosidase extraction from various microorganisms [21–24]. Nevertheless, systematic study comprising a wide selection of extraction methods and comparing their

effects on released activity has not yet been undertaken. Therefore, throughout this study, the effects of different mechanical (vortexing with abrasives, grinding with abrasives, ultrasonication) and chemical (ethanol or chloroform extraction) methods on released β -galactosidase activity were compared. As reagents for chemical permeabilization ethanol and chloroform were used, and in both experiments negligible β -galactosidase activity was observed (Fig. 4A). Use of ethanol and chloroform, as well as several other organic solvents has been widely applied in permeabilization of yeast and some bacterial cells for production of biomass with increased galactosidase activity [22,25]. Nevertheless, reports of LAB cell disruption and release of intracellular β -galactosidase activity are very scarce. Significant yield was achieved only in study by Choonia and Lele when ethanol (10%, w/v) and chloroform (7.5%, w/v) were used as plasmolysis solvents, but even in that case mechanical methods proved to be more efficient [24]. Generally, it is plausible that low extracellular yield observed in our study is due to the fact that Gram-positive bacteria, *Lb. acidophilus*, has high portion of peptidoglycan layer, which imparts rigidity to cell wall and obstructs the release of intracellular biomolecules, such as β -galactosidase [16,26].

Mechanical methods, on the other hand, widely investigated and used in bacterial β -galactosidase production, are proved to be more efficient than chemical methods in this study (Fig. 4A). Amongst mechanical methods the most efficient was cell disruption by abrasives. Two abrasive materials were used, and quartz sand was shown to be far more effective (2.1 IU mL⁻¹) comparing to glass beads (0.678 IU mL⁻¹). Also, different types of mechanical force were applied to

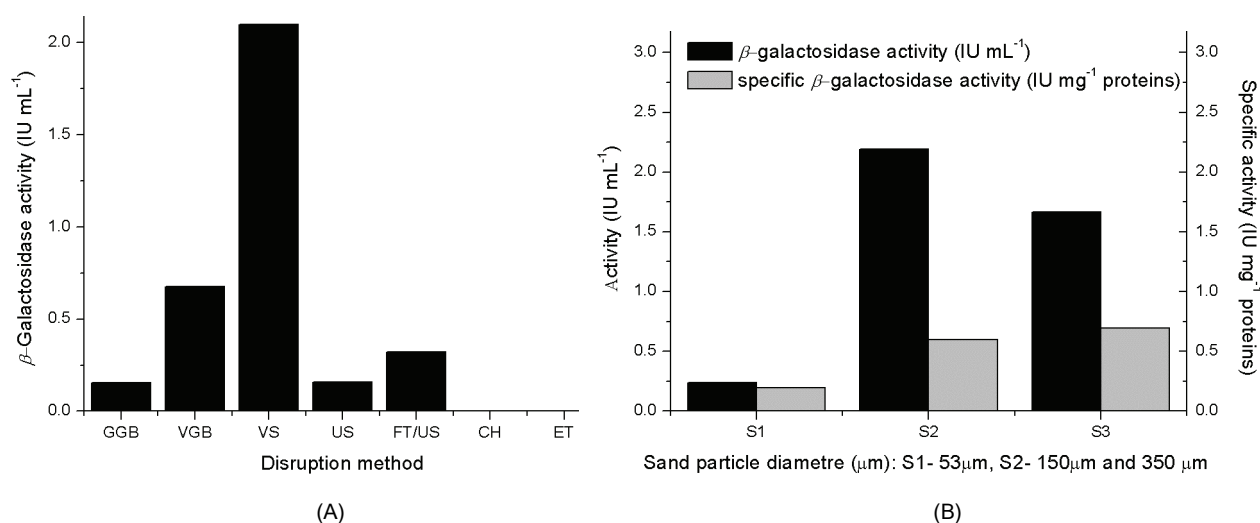


Figure 4. Effect of different cell disruption methods on released β -galactosidase activity (A): VGB – vortexing with glass beads, SO – sonication, GGB – grinding with glass beads, FT/US – freezing-thawing/ultrasonication, VS – vortexing with quartz sand, CH – treatment with chloroform, ET – treatment with ethanol. Effect of different sand particle diameter on β -galactosidase activity (IU mL⁻¹) and specific β -galactosidase activity (IU mg⁻¹ proteins) (B): S1 – 53 μm, S2 – 150 μm and S3 – 350 μm.

intensify friction between abrasives and biomass, and around 4 times higher β -galactosidase activity was obtained by means of vortexing cell suspension than by grinding.

Disruption methods which don't include use of abrasives were less effective, hence repeated freeze-thawing in ultrasonication bath released β -galactosidase activity of 0.322 IU mL^{-1} , while sole treatment in ultrasonication bath liberated activity of 0.157 IU mL^{-1} . Higher activity yields obtained using mechanical methods are in accordance with numerous studies indicating superiority of mechanical methods for intracellular enzyme isolation [16,24]. The main requirement for cell disruption methods is that they should be robust enough to disrupt cell envelopes efficiently, but at the same time gentle enough to preserve enzyme activity [15]. Therefore, poor results achieved by ultrasonication methods (Fig. 4A) can be explained by the fact that absorption of energy is intertwined with significant heat release and free radicals production, which can lead to lowering enzyme activity. Similar results were published by Bury *et al.* who investigated the effect of disruption methods on *Lb. delbrueckii ssp. bulgaricus* β -galactosidase production, finding that bead milling and high-pressure homogenization were comparable methods, while the sonication was the least effective [21]. Likewise, Dagbagli and Goksungur found sonication significantly less effective than glass beads treatment [15].

Since cell disruption with quartz sand gave the best results, further optimization was made by investigating the effect of abrasive particle size on activity yield. Three batches with different particle diameters (53, 150 and $350 \mu\text{m}$) were tested under same conditions with vortexing method. Even though the best choice, regarded the obtained specific β -galactosidase activity

(0.695 IU mg^{-1} proteins), would be the usage of $350 \mu\text{m}$ diameter quartz sand particles, differences in overall produced β -galactosidase activity were by far larger and favor usage of $150 \mu\text{m}$ diameter particles (Fig. 4B). Therefore, all further experiments were performed with $150 \mu\text{m}$ quartz sand particles.

Temperature and pH

β -Galactosidases characteristics can greatly vary depending on their source. Generally, they can be divided in two groups on the basis of their pH profiles: acidic β -galactosidases that originate from fungal sources and neutral β -galactosidases from yeasts and bacteria [1,2]. As expected, the optimum pH for obtained β -galactosidase from *Lb. acidophilus* is in the range between pH 6.5 and 7.5 (activity over 90% of maximum), with an optimum at pH 6.8 (Fig. 5). Similar neutral optimum pH ranges have been reported for several LAB species, including *Lb. acidophilus* [20].

These values correspond to ones of milk and sweet whey, making these enzymes highly applicable in the real industrial conditions. Yeast β -galactosidases exhibit rather low optimum operation temperatures and consequently are inappropriate for industrial usage due to the ease of contamination, hence LAB β -galactosidases seem to be more adequate choice. The optimum temperature of our obtained enzyme was found to be 45°C . Literature data, however, showed no consistency on this matter. Nguyen *et al.* claimed that the optimum temperature for both lactose and *o*-NPG hydrolysis was 55°C [27]. On the other hand, results published by Ismail *et al.* showed that temperature optimum is around 40°C [20]. Keeping in mind all presented results in this study, it is clear that our enzyme represents a valuable tool in milk and sweet whey processing and potential GOS production.

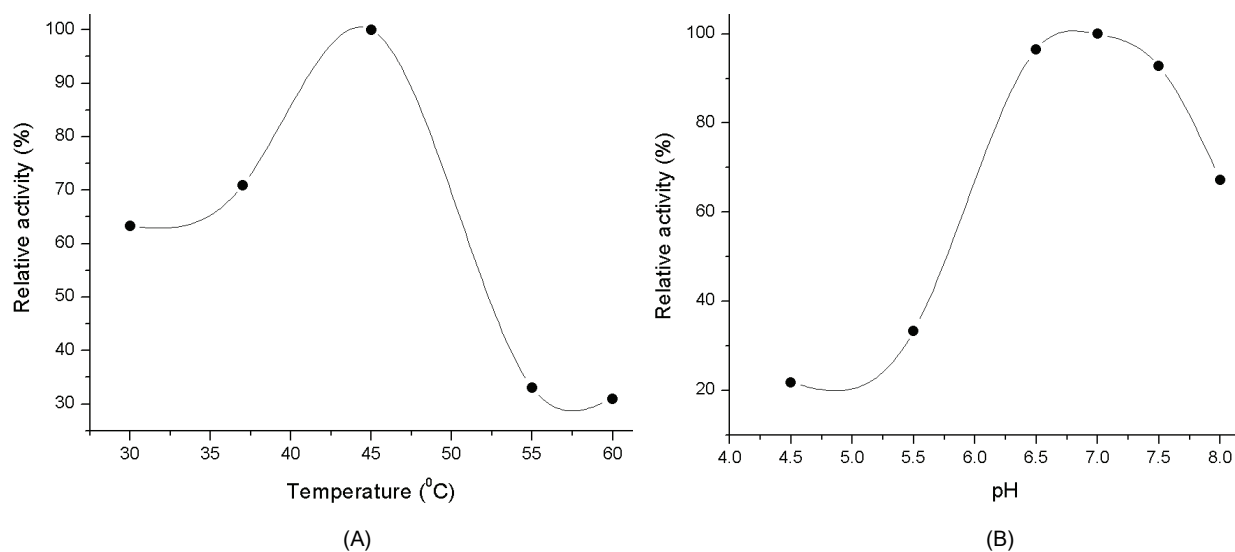


Figure 5. Effect of temperature (A) and pH (B) on relative β -galactosidase activity.

CONCLUSION

It was concluded that the highest biocatalytic potential was achieved by using *Lb. acidophilus*. In view of the greater efficiency of β -galactosidase exploitation, optimization of the relevant cultivation factors (fermentation time, carbon source and later lactose concentration) was conducted. Maximal enzyme activity was achieved by shake flask culture fermentation after 48 h. Culture medium yielding highest β -galactosidase activity comprised of modified MRS culture broth with 2.5% lactose. Accomplished β -galactosidase activities were significantly increased in continuance, by optimizing enzyme extraction method. Mechanical cell disruption, namely, vortexing with quartz sand of 150 μ m diameter particles proved to be an optimal choice. Based on the results reported in this paper, obtained crude cell-free extract β -galactosidase from *Lb. acidophilus* has potential application in the food industry especially in the field of milk and sweet whey hydrolysis, as well as in GOS production.

Acknowledgements

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REFERENCES

- [1] P.S. Panesar, S. Kumari, R. Panesar, Potential Applications of Immobilized β -Galactosidase in Food Processing Industries, *Enzyme Res.* **2010** (2010), doi: 10.4061/2010/473137.
- [2] P.S. Panesar, R. Panesar, R.S. Singh, J.F. Kennedy, H. Kumar, Microbial production, immobilization and applications of β -D-galactosidase, *J. Chem. Technol. Biot.* **81** (2006) 530–543.
- [3] D.C. Vieira, L.N. Lima, A.A. Mendes, W.S. Adriano, R.C. Giordano, R.L.C. Giordano, P.W. Tardioli, Hydrolysis of lactose in whole milk catalyzed by β -galactosidase from *Kluyveromyces fragilis* immobilized on chitosan-based matrix, *Biochem. Eng. J.* **81** (2013) 54–64.
- [4] M. Ghosh, K.K. Pulicherla, V.P.B. Rekha, A. Vijayanand, K.R.S. Sambasiva Rao, Optimisation of process conditions for lactose hydrolysis in paneer whey with cold-active β -galactosidase from psychrophilic *Thalassospira frigidophilosprofundus*, *Int. J. Dairy Technol.* **66** (2013) 256–263.
- [5] Q. Husain, β Galactosidases and their potential applications: a review, *Crit. Rev. Biotechnol.* **30** (2010) 41–62.
- [6] M.R. Kosseva, P.S. Panesar, G. Kaur, J.F. Kennedy, Use of immobilised biocatalysts in the processing of cheese whey, *Int. J. Biol. Macromol.* **45** (2009) 437–447.
- [7] S. Sen, L. Ray, P. Chattopadhyay, Production, Purification, Immobilization, and Characterization of a Thermostable β -Galactosidase from *Aspergillus alliaceus*, *Appl. Biochem. Biotech.* **167** (2012) 1938–1953.
- [8] D.P.M. Torres, M.P.F. Goncalves, J.A. Teixeira, L.R. Rodrigues, Galacto-Oligosaccharides: Production, Properties, Applications, and Significance as Prebiotics, *Compr. Rev. Food Sci. F.* **9** (2010) 438–454.
- [9] R. Jovanovic-Malinovska, P. Fernandes, E. Winkelhausen, L. Fonseca, Galacto-oligosaccharides Synthesis from Lactose and Whey by β -Galactosidase Immobilized in PVA, *Appl. Biochem. Biotech.* **168** (2012) 1197–1211.
- [10] B. Splechtna, T.H. Nguyen, M. Steinböck, K.D. Kulbe, W. Lorenz, D. Haltrich, Production of Prebiotic Galacto-Oligosaccharides from Lactose Using β -Galactosidases from *Lactobacillus reuteri*, *J. Agric. Food Chem.* **54** (2006) 4999–5006.
- [11] C. Schwab, V. Lee, K.I. Sørensen, M.G. Gänzle, Production of galactooligosaccharides and heterooligosaccharides with disrupted cell extracts and whole cells of lactic acid bacteria and bifidobacteria, *Int. Dairy J.* **21** (2011) 748–754.
- [12] T. Vasiljevic, P. Jelen, Oligosaccharide production and proteolysis during lactose hydrolysis using crude cellular extracts from lactic acid bacteria, *Lait* **8** (2003) 453–467.
- [13] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding, *Anal. Biochem.* **72** (1976) 248–254.
- [14] F. Alves, F. Filho, J. Medeiros Burkert, S. Kalil, Maximization of β -Galactosidase Production: A Simultaneous Investigation of Agitation and Aeration Effects, *Appl. Biochem. Biotech.* **160** (2010) 1528–1539.
- [15] S. Dagbagli, Y. Goksungur, Optimization of β -galactosidase production using *Kluyveromyces lactis* NRRL Y-8279 by response surface methodology, *Electron. J. Biotechnol.* **11** (2008).
- [16] J. Geciova, D. Bury, P. Jelen, Methods for disruption of microbial cells for potential use in the dairy industry—a review, *Int. Dairy J.* **12** (2002) 541–553.
- [17] H.A. Murad, R.I. Refaea, E.M. Aly, Utilization of UF-Permeate for Production of β -galactosidase by Lactic Acid Bacteria, *Pol. J. Microbiol.* **60** (2011) 139–144.
- [18] G. Montanari, C. Zambonelli, L. Grazia, M. Benevelli, C. Chiavari, Release of β -galactosidase from *Lactobacilli*, *Food. Technol. Biotechnol.* **38** (2000) 129–133.
- [19] C.A. Hsu, R.C. Yu, C.C. Chou, Production of β -galactosidase by Bifidobacteria as influenced by various culture conditions, *Int. J. Food Microbiol.* **104** (2005) 197–206.
- [20] S.A.A. Ismail, Y. El-Mohamady, W.A. Helmy, R. Abou-Romia, A.M. Hashem, Cultural condition affecting the growth and production of a β -galactosidase by *Lactobacillus acidophilus* NRRL 4495, *Aust. J. Basic Appl. Sci.* **4** (2010) 5051–5058.
- [21] D. Bury, P. Jelen, M. Kalab, Disruption of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 cells for lactose hydrolysis in dairy products: a comparison of sonication, high-pressure homogenization and bead milling, *Innov. Food Sci. Emerg. Technol.* **2** (2001) 23–29.
- [22] G.A. Somkuti, M.E. Dominiacki, D.H. Steinberg, Permeabilization of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* with ethanol, *Curr. Microbiol.* **36** (1992) 202–206.

- [23] M. Puri, S. Gupta, P. Pahuja, A. Kaur, J.R. Kanwar, J.F. Kennedy, Cell Disruption Optimization and Covalent Immobilization of β -D-Galactosidase from *Kluyveromyces marxianus* YW-1 for Lactose Hydrolysis in Milk, *Appl. Biochem. Biotech.* **160** (2010) 98–108.
- [24] H.S. Choonia, S.S. Lele, Release of β -galactosidase from Indigenous *Lactobacillus acidophilus* by Ultrasonication: Process Optimization, *Chem. Eng. Commun.* **198** (2011) 668–677.
- [25] Y. Numanoglu, S. Sungur, β -Galactosidase from *Kluyveromyces lactis* cell disruption and enzyme immobilization using a cellulose-gelatin carrier system, *Process. Biochem.* **39** (2004) 705–711.
- [26] P. Jafarei, M.T. Ebrahimi, *Lactobacillus acidophilus* cell structure and application, *Afr. J. Microbiol. Res.* **5** (2011) 4033–4042.
- [27] T.H. Nguyen, B. Splechtna, S. Krasteva, W. Kneifel, K.D. Kulbe, C. Divne, D. Haltrich, Characterization and molecular cloning of a heterodimeric β -galactosidase from the probiotic strain *Lactobacillus acidophilus* R22, *FEMS Microbiol. Lett.* **269** (2007) 136–144.

IZVOD

OPTIMIZACIJA PROIZVODNJE β -GALAKTOZIDAZE POMOĆU BAKTERIJA MLEČNE KISELINE

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Enzim β -galaktozidaza, poznatija kao laktaza, predstavlja industrijski izuzetno važan enzim, koji ima primarnu ulogu u hidrolizi disaharida laktoze. Upotrebom ovog enzima u industriji mleka i mlečnih proizvoda dolazi do poboljšanja fizičkih i senzornih karakteristika proizvoda, kao i do povećanja svarljivosti proizvoda, a samim tim i prevazilaženja problema netolerancije na laktozu. Takođe, hidrolizom laktoze surutke, rešava se pitanje njenog ekološki prihvatljivog odlaganja. Sa druge strane, enzim pod kontrolisanim reakcionim uslovima može katalizovati proces transgalaktozilacije, odnosno prenošenja galaktozil jedinica na druge šećere prisutne u sistemu (najčešće laktozu), pri čemu nastaju izuzetno važna funkcionalna jedinjenja galaktooligosaharidi. Enzim β -galaktozidaza može biti različitog porekla – biljnog, životinjskog ili mikrobnog. Međutim, najznačajniji među njima su mikrobnog porekla, zbog lake fermentacije, velike brzine rasta i razmnožavanja ćelija, visokih prinosa, visoke aktivnosti, kao i stabilnosti enzima. Kao posledica velikog komercijalnog interesa za ovaj enzim, opisane su različite metode dobijanja i prečišćavanja dobijenih enzima iz različitih mikroorganizama. Poslednjih godina bakterije mlečne kiseline privlače sve više pažnje kao potencijalni izvori β -galaktozidaza najviše zahvaljujući svom statusu bezbednih za upotrebu u prehrambenoj i farmaceutskoj industriji, čime se omogućava neometano korišćenje enzima bez primene komplikovanih metoda prečišćavanja. U ovom radu ispitana je mogućnost proizvodnje β -galaktozidaza pomoću nekoliko vrsta bakterija mlečne kiseline. Kao najbolji producent među ispitanim bakterijama pokazala se bakterija *Lactobacillus acidophilus*. Najveća aktivnost β -galaktozidaze, dobijena je mikroaerofilnom fermentacijom u modifikovanoj komercijalnoj MRS podlozi, sa 2,5% laktoze kao jedinim izvorom ugljenika. Fermentacija je vršena na tresilici (150 rpm) u trajanju od 48 h i na temperaturi od 37 °C. Kako je enzim intracelularan, u cilju razaranja ćelija i oslobađanja enzima, primenjeno je više različitih fizičkih i hemijskih metoda, a daleko najboljom pokazala se metoda vorteksiranja sa kvarcnim peskom (150 μ m) kao abrazivnim sredstvom. Ovako dobijen enzim pokazao je maksimalnu aktivnost pri temperaturi od 45 °C i pH u opsegu 6.5–7.5.

Ključne reči: *Lactobacillus acidophilus* • β -Galaktozidaza • Proizvodnja • Razaranje • Optimizacija