

Characterization of a Crude Thermostable β -galactosidase by the Bacterium PD1 Isolated from the Pong Dueat Hot Spring

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ABSTRACT

β -Galactosidase is an enzyme that hydrolyses lactose to glucose and galactose. The present study focused on the characterization of β -galactosidase from a thermophilic microorganism isolated from the Pong Dueat hot spring, Chaingmai Province, Thailand. *o*-Nitrophenyl-L-D-galactopyranoside (oNPG) was used as a substrate to determine the β -galactosidase activity from a strain PD1, which was found to be the active strain producing the highest β -galactosidase activity. The optimum pH and temperature for enzyme activity were 6.5 and 55°C, respectively. The enzyme was stable at pH range of 6 to 10. The half-life of enzyme at 80, 70, 60, 55, 50 and 45°C were 0.5, 2, 31, 62, 87 and 435 h, respectively. The K_m and V_{max} values for the synthetic substrate oNPG were 11 mM and 1.1×10^{-2} mmolL⁻¹min⁻¹, respectively.

Key words: β -galactosidase, lactose hydrolysis, thermostable

INTRODUCTION

Milk sugar, lactose, is a disaccharide that consists of monosaccharide (glucose and galactose) molecules bonded through a β 1-4 glycosidic linkage. It is the principal carbohydrate component in most mammal milk. Typically, lactose is commercially produced in food and pharmaceutical grades. It is mainly used as a filler or filler-binder in the manufacture of pharmaceutical tablets and capsules, allowing the drug to be evenly distributed through the lactose matrix and tableted (Holisinger and Kligerman, 1991). Lactose is further used in food technology.

These applications are based on some advantageous nutritional and technological properties of lactose. However, lactose may cause important problems within three main areas, health (lactose intolerance), food technology (lactose crystallization giving the products a sandy or gritty texture) and environment (a waste product from cheese whey manufacturing). Conversion of lactose by β -galactosidase reaction was employed to solve these problems. The enzyme β -galactosidase is widely distributed in nature including plants, bacteria, yeast, fungi or molds, actinomycetes, and archaebacteria. It is a commercially important enzyme due to its various

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applications in dairy and food industries. In the applications of β -galactosidase, it becomes evident that the enzyme not only catalyses the hydrolysis of β -D-galactopyranosidic linkage to glucose and galactose, but also the transgalactosylation reaction as well. For the hydrolysis reaction, the lactose-reduced ingredients in the food and dairy product are commercially produced for lactose intolerant persons. The catalysed transgalactosylation reaction is useful for the production of prebiotic galactooligosaccharide improving structural and functional modification of food materials, or pharmaceutical compounds (Mahoney, 1998). Nowadays, researchers show great interest in β -galactosidases from thermophilic micro-organisms since they have a number of advantages in industrial applications due to their ability to work at extreme conditions in which mesophilic enzymes are normally denatured. Thermostable β -galactosidases reduce microbial contamination, increase substrate (lactose) solubility, give higher product concentrations, and increase reaction rates (Zeikus *et al.*, 1998). Our experiments were aimed to characterize a thermostable β -galactosidase from a microorganism isolated from the Pong Dueat hot spring, Chaingmai Province.

MATERIALS AND METHODS

Microorganism

Strain PD1 was screened from soil from the Pong Dueat hot spring, Chaingmai Province, Thailand. For the screening of microorganisms producing thermophilic β -galactosidase, lactose medium (0.5% lactose, 0.5% peptone, 0.3% beef extract, 1.5% agar) was used. The ability to produce β -galactosidase was conducted on lactose medium plates containing 50 μ g/ml of 5-bromo-4-chloro-3-idolyl- β -D-galactopyranoside (X-gal).

Culture condition for enzyme production

The production of β -galactosidase was

carried out in 250 ml Erlenmeyer flask containing 100 ml culture medium (1% lactose, 1% peptone, 1% yeast extract, 0.5% $(\text{NH}_4)_2\text{SO}_4$, 0.3% K_2HPO_4 , 0.1% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$). After 15-h incubation at 50°C, cells were harvested by centrifugation and then lysed by three passages through a French Press (Aminco, USA) at 1200 bar and 4°C to obtain the intracellular fluid.

Enzyme activity assay

Activity of β -galactosidase was measured by incubating 20 μ l of suitably diluted enzyme with 480 μ l of 22 mM o-nitrophenyl- β -D-galactopyranoside (oNPG) in phosphate buffer pH 6.5 at 40°C. After 15 min, 0.4 M Na_2CO_3 was added to the reaction mixture to stop the reaction. The concentration of o-nitrophenol (oNP) released was determined by spectrophotometry at 420 nm. One unit of β -galactosidase activity (U) was defined as the amount of enzyme that released 1 μ mol of oNP from oNPG per minute under the experimental conditions described above.

Characterization of crude β -galactosidase

The pH dependence of β -galactosidase activity was determined in the pH range of 3.0–10.0. The stability of β -galactosidase at various pH was also studied by incubating the enzyme in the buffers (citrate phosphate buffer; phosphate buffer; Tris-HCl buffer and glycine-NaOH buffer) at 50 °C for 12 h. Subsequently, the residual activity was determined under standard assay conditions. The temperature optimum for oNPG hydrolysis was determined by incubating the reactions at 40–80 °C for 15 min. Thermal stability was studied by keeping an enzyme sample in the temperature range of 40–80°C and measuring the residual activity under standard assay condition. Kinetic parameters were determined using oNPG as a substrate, in 50 mM phosphate buffer (pH 6.5). Hydrolysis of oNPG was quantitatively determined by measuring the amount of released o-nitrophenol, similar to the standard activity

assay. Kinetic constants were calculated by using Lineweaver–Burk plots with non-linear regression (SigmaPlot).

RESULTS AND DISCUSSION

Screening

In a screening of microorganisms for the production of β -galactosidase activity on lactose agar, 14 colonies were isolated and 1 colony (PD1) was identified as potential β -galactosidase producers because they showed blue colonies on lactose agar containing X-gal. Strain PD1 was found to exhibit the highest of β -galactosidase activity (data not show).

Effect of pH on β -galactosidase

The enzyme activity of the β -galactosidase was examined at pH ranging from 3.0 to 10.0 (Figure 1). The optimum pH was 6.5. Similar results have been reported for several β -galactosidase sources such as *Thermotoga maritima* (Kim *et al.*, 2004), *Thermus* sp. T2 (Ladero *et al.*, 2002) and *Thermus* sp. A4 (Ohtsu

et al., 1998). At pH 6.0 and 7.0, the enzyme activities were more than 70% of the maximum activity. β -galactosidase was stable at high pH of 6–10 as it retained more than 80% of its activity for 12 h (Figure 2).

Effect of temperature on β -galactosidase

The effect of temperature on the enzyme activity was shown in Figure 3. Optimum temperature was 55°C. Our results agree with those of *Streptococcus thermophilus* and *Fusarium moniliforme* observed by Greenberg and Mahoney (1982) and Macris and Markakis (1981), respectively. At temperatures more than 70°C, the enzyme activity sharply declined. The thermostability of the enzyme was studied by incubating the enzyme at various temperatures in buffer pH 6.5 for 12 h. The β -galactosidase was stable at temperatures up to 60°C. However, the activities were decreased to only 3 and 49% at 70 and 80°C, respectively, after 1.5 h. The half-lives ($t_{1/2}$) of the enzyme at 80, 70, 60, 55, 50, 45 and 40°C were 0.5, 2, 31, 62, 87 and 435 h, respectively.

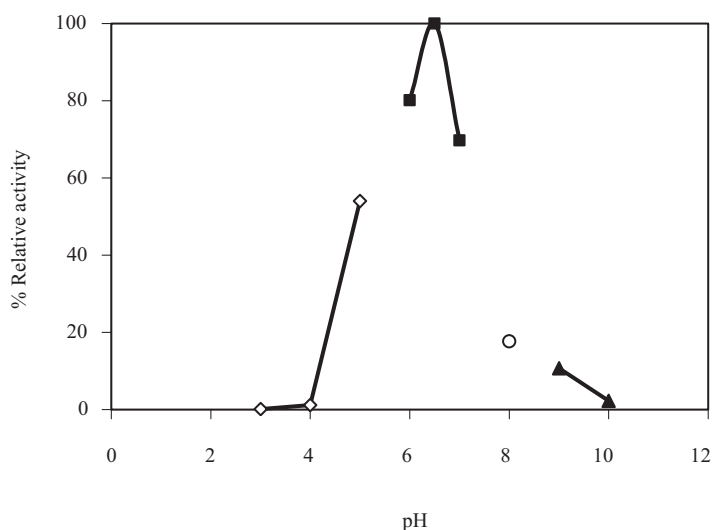


Figure 1 Effect of pH on the β -galactosidase activity from isolate PD 1. The buffer used for pH experiment was a citrate buffer (◇), sodium phosphate buffer (■), Tris buffer (○) and Glycine-NaOH buffer (▲).

Kinetic parameter

The K_m and V_{max} were calculated from a Lineweaver-Burk plot of activity as a function of oNPG concentration (Figure 4). The K_m was 11 mM and the V_{max} was 1.1×10^{-2} mmol L⁻¹ min⁻¹. The K_m indicates that the crude β -galactosidase has a weak affinity for oNG, compared to those of other reports for example, the K_m value of 5.9 mM for the thermostable β -galactosidase in *Thermus* sp. A4 (Ohtsu *et al.*, 1998).

CONCLUSION

In conclusion, a thermostable β -galactosidase producing from the PD1 isolated from the Pong Dueat hot spring, Chaingmai Province, Thailand produces an intracellular enzyme with the high activity. Based on the relatively good pH and temperature stabilities which is comparable to those of other mesophilic β -galactosidases, the enzyme could be useful for

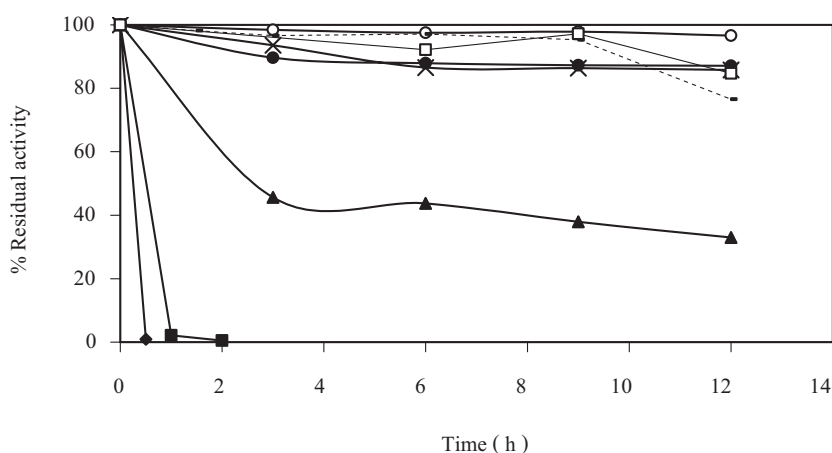


Figure 2 pH stability of β -galactosidase from the isolate PD 1. pH 3 (◆), pH 4 (■), pH 5 (▲), pH 6 (×), pH 6.5 (○), pH 7 (●), pH 9 (□) and pH 10 (- -)

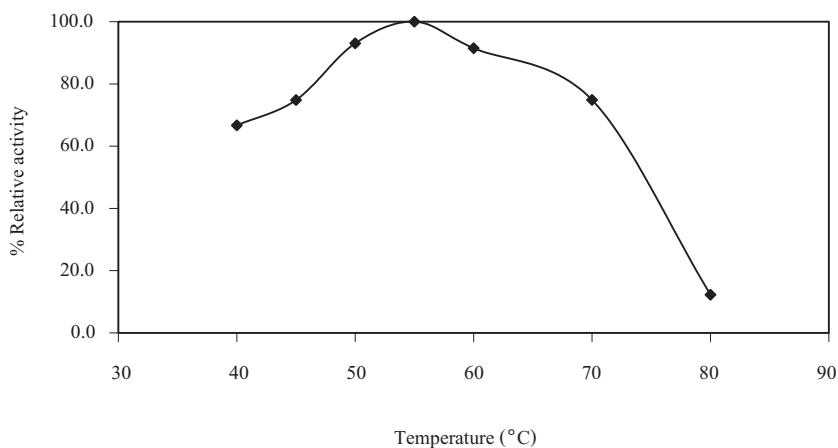


Figure 3 Effect of temperature on β -galactosidase activity from the isolate PD1. The buffer used for the experiment was a 50 mM sodium phosphate buffer, pH 6.5.

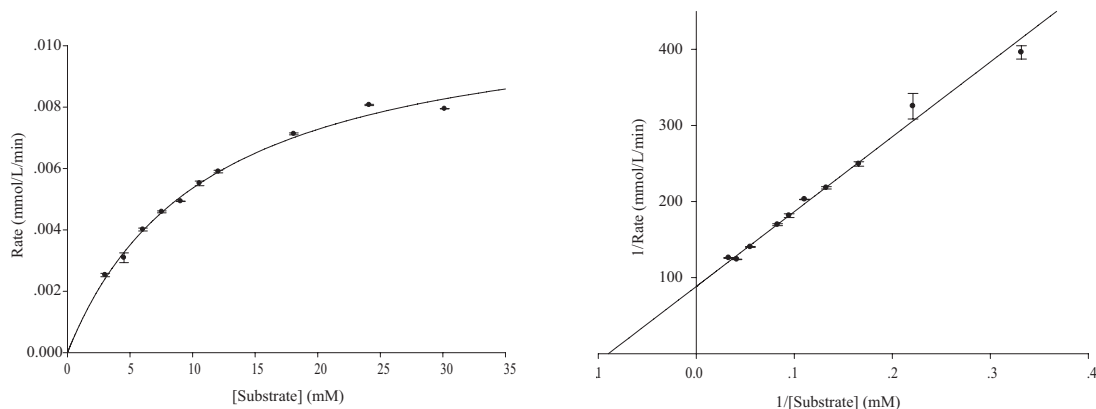


Figure 4 Kinetic parameter determination of the β -galactosidase by Lineweaver–Burk plot using oNPG as substrate.

the efficient hydrolysis of lactose in milk and whey and producing attractive products for food industry.

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