

Characterization of partially purified β -galactosidase from *Bacillus* Sp MTCC-864

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Abstract

β -Galactosidase is an important enzyme for the hydrolysis of lactose in milk and other by-products of dairy industry such as whey and for the synthesis of galacto-oligosaccharides known for their prebiotic properties. In the present study we report the extraction, partial purification and characterization of intracellular β -galactosidase from *Bacillus* sp MTCC-864. The media containing tryptone, yeast extract and lactose was used for growth and enzyme production. The enzyme was extracted through ammonium sulphate fractionation and gel permeation chromatography technique using Sephacryl-200. Optimum enzyme activity was found at 50°C and pH 7.0. Further enzyme activity was enhanced in the presence of divalent metal ions such as Mg⁺⁺ and Mn⁺⁺, while it was inhibited by EDTA. The enzyme was thermostable and retained 70% of its original activity after 30min of incubation at 60°C. These properties of enzyme indicate its potential use for hydrolysis of lactose in milk and whey from the dairy industry.

Keywords: β -Galactosidase, Lactose intolerance, Thermostability, Oligosaccharides

INTRODUCTION

β -Galactosidase (EC.3.2.1.23) is an important enzyme in the food and pharmaceutical industry. The enzyme hydrolyzes the lactose into monosaccharides, glucose and galactose. It is used in the preparation of lactose hydrolyzed milk suitable for lactose intolerant people [1, 2]. Lactose hydrolysis is required for reasons related to health, food technology and environment. Concerning the health issue, lactose becomes a problem when there is insufficient intestinal lactase production. As a result of lactose indigestion it is converted into acids and CO₂ through fermentation by intestinal microflora causing giddiness, headache and nausea. Because of this problem the reduction of lactose content in the milk and dairy products is of prime importance and the enzyme is commercially used for this purpose. From the food technology view point, high lactose content in non-fermented milk products such as ice-cream and condensed milk, can lead to excessive lactose crystallization resulting in products with mealy, sandy or gritty texture. From the environmental perspective, lactose is associated with high biological and chemical oxygen demand for treatment on sewage disposal plants [3-5].

New applications for β -galactosidase such as in the recovery of biologically active oligosaccharides from milk have been described in the literature [6]. β -Galactosidase is known for its transgalactosylation activity. The hydrolysis of lactose occurs predominantly at low lactose concentrations, while oligosaccharide production by transgalactosylation increases with increasing concentration of lactose. Galacto-oligosaccharides (GOS) when

taken orally promote the growth of *Bifidobacterium* sp in the large intestine which leads to the improvement of microflora and suppression of putrefaction in the gut [7].

Earlier researchers have used β -galactosidase from various sources for the preparation of low lactose milk and dairy products [8]. β -Galactosidase from *E. coli* is one of the thoroughly studied enzymes since the *lac* operon played main role in elucidating the genetic control of gene regulation in *E. coli*. β -Galactosidase is also being used as reporter enzyme in the cloning and expression of proteins [9].

In the present study data on characterization of partially purified β -galactosidase with regard to effect of pH, temperature and metal ions are reported.

MATERIALS AND METHODS

All the chemicals used in this study were procured from Sisco Research Laboratory, India and Sigma, USA, while bacteriological media were procured from HiMedia Pvt. Ltd, Mumbai, India.

Bacterial cultures

The bacterium *Bacillus* sp MTCC-864 used in this study was obtained from Microbial Type Culture Collections (MTCC), Chandigarh, India. The culture was maintained at -20°C on nutrient agar slant overlaid with 50% glycerol. The culture was reviewed in TLY medium containing tryptone 8g, lactose 4g and yeast extract 2g per liter and the pH of the medium was adjusted to 6.8±0.2.

Extraction of β -galactosidase from *Bacillus* sp MTCC-864

Bacterial biomass was homogenized in a pre-chilled mortar and pestle using acid washed quartz sand in 0.05M sodium phosphate buffer. After sufficient cell lysis the cell suspension was centrifuged for 20min at 10,000rpm. Supernatant was concentrated and used as crude extract. Different concentrations of ammonium sulphate were added to the supernatant. After stirring on magnetic stirrer, it was kept undisturbed at 4°C overnight. Precipitate formed was collected

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by centrifugation at 10,000rpm for 20min. The pellet having β -galactosidase activity was redissolved in 0.05M sodium phosphate buffer with pH 6.5 and was dialyzed using 10kDa cut off dialysis membrane.

Partial purification of β -galactosidase by gel permeation chromatography

A glass column (100X2cm) was packed with the matrix (Sephacryl-200) swollen in distilled water overnight at room temperature and equilibrated with 0.05M sodium phosphate buffer pH 6.5 and the same was used to elute the sample at a flow rate of 10ml per hr. The void volume was determined by passing Blue dextran through the column. The crude enzyme sample (2ml) obtained by ammonium sulphate precipitation was loaded. Fractions were collected about two times of the bed volume and the O.D was taken at 280nm and analysed for enzyme activity. The fractions showing activity were pooled, concentrated at low temperature and stored at 4°C for further studies.

β -Galactosidase assay

The enzyme assay was done using Guarante (1983) method [10] with slight modifications. O-nitro phenyl-D-galactopyranoside (ONPG) 7mM in 0.05M sodium phosphate buffer pH 6.5 was used as substrate at 37°C. The enzyme sample was added to 0.5ml of substrate and the reaction was stopped by adding 0.5ml of 1M Na_2CO_3 after 5min. Liberated ONP (o-nitro phenol) was measured spectrophotometrically at 420nm and activity was expressed in μM solution of ONP produced/min/ml of enzyme solution.

Effect of substrate concentration, pH, temperature and metal ions on enzyme

Effect of substrate concentration on enzyme activity was studied by using ONPG solution in the range of 1 to 10mM. The pH optimum was determined at 37°C by using 0.1M acetate buffer (pH 3.7-5.5), 0.1M phosphate buffer (pH 5.5-7.0) and 0.1M Tris-HCl buffer (pH 7-9) in the reaction mixture. The effect of temperature on enzyme activity at pH 6.5 was determined by incubating reaction mixture at different temperatures (20-65°C). Thermostability of enzyme was studied by incubating the aliquots of enzyme at temperatures of 37-80°C in a water bath and checking residual activity after 5, 10, 20 and 30min. The activity of heat treated enzyme along with untreated (control) was measured at 37°C. The effect of different metal ions was determined by adding them to reaction mixture and measuring the enzyme activity.

RESULTS AND DISCUSSION

Extraction of β -galactosidase

The culture supernatant and cell lysate of *Bacillus* MTCC-864 were analysed for β -galactosidase activity to know whether the enzyme was intracellular or extra cellular. All the enzyme activity was found associated with cell extract indicating its intracellular nature and no enzyme activity was detected in supernatant. The enzyme was extracted by conventional ammonium sulphate precipitation. The 40-60% fraction revealed maximum enzyme activity and was selected for further purification by gel permeation chromatography (GPC). The elution profile displayed only one protein peak, associated with β -galactosidase as shown in Fig. 1.

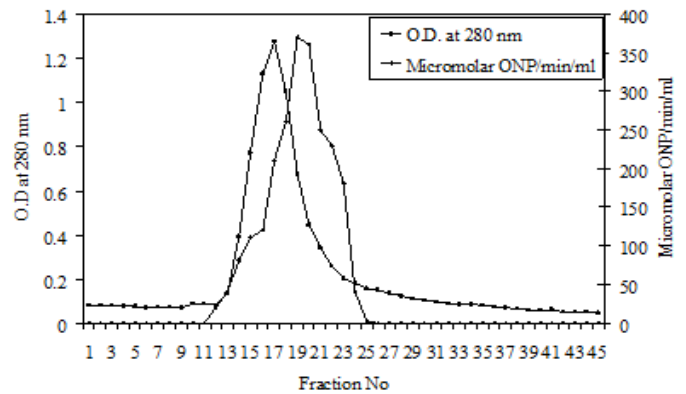


Figure 1: Elution profile of enzyme on Sephacryl-200.

Characterization of β -galactosidase

Substrate dependence

The *Bacillus* MTCC-864 β -galactosidase exhibited a typical hyperbolic response with increasing concentration of ONPG as the assay substrate as shown in Fig. 2. Higher values of substrate were inhibitory to the enzyme indicating substrate inhibition.

Effect of temperature and pH

Temperature and pH are the most important factors which markedly influence enzyme activity. The enzyme was found active in a broad range of temperature 35-65°C. However it showed maximum activity at 50°C as shown in Fig.3. A gradual reduction in enzyme activity was observed at either side of temperature optima. The data indicates that the enzyme can work effectively at high temperature as compared to low temperature.

Similarly the enzyme also showed a broad pH optima exhibiting maximum activity at pH 7.0 Fig. 4 and lost the activity at extreme pH values. Since the enzyme is active at milk pH it can be effectively used for lactose hydrolysis. Similar results were found by earlier workers [11].

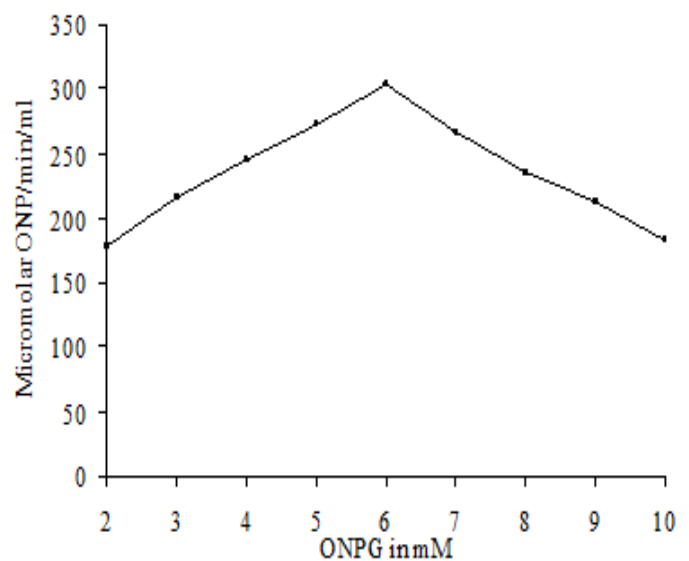


Figure 2: Effect of substrate concentration on *Bacillus* MTCC-864 β -galactosidase activity

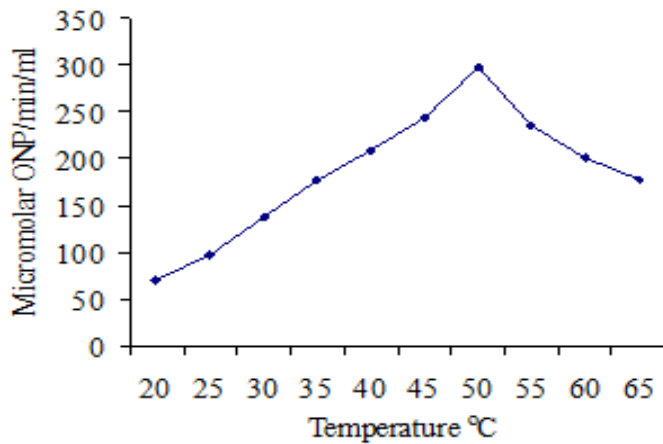


Figure 3: Effect of temperature on enzyme activity

Thermostability

The temperature effect on enzyme revealed general decrease in the stability of the enzyme with time (5-30min) at temperature 50°C as shown by decreased enzyme activity. The enzyme retained 75% of its original activity even at 60°C for 30min indicating its thermostability. It showed highest stability at 50°C and least stability at 80°C (Fig. 4). The high temperature inactivation may be due to incorrect conformation due to hydrolysis of the peptide chain, destruction of amino acid or aggregation [12].

Effect of metal ions

The effect of different metal ions along with EDTA was studied at 1, 2, 5 and 10mM concentrations (Fig. 5). It was observed that Na⁺ and K⁺ had very little influence on enzyme activity. However Mn²⁺ and Mg²⁺ enzyme exhibited hyperbolic response and at concentration of 10mM around 3 fold increase in activity was observed. Requirement of bivalent metal ions has also been shown earlier for β -galactosidase from Antarctic *Bacillus* sp., *K. lactis* and *E. coli* [9, 11, 14]. EDTA being a metal chelating agent decrease enzyme activity as a function of its concentration. β -Galactosidase from *Pseudoalteromonas haloplanktis* [13] and *E. coli* and *K. lactis* have earlier been found inhibited by EDTA [14, 15].

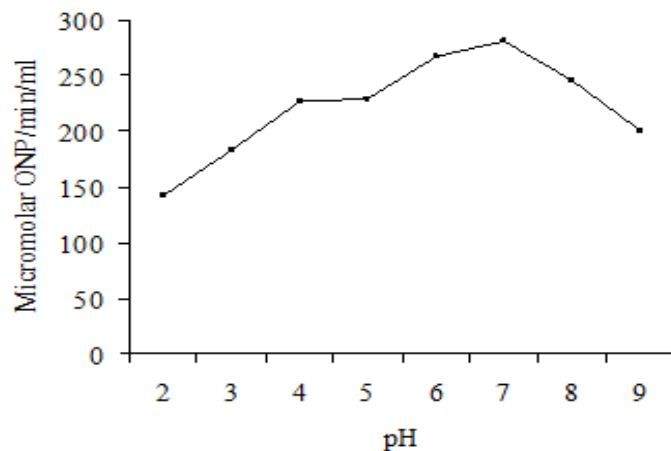


Figure 4: Effect of pH on enzyme activity.

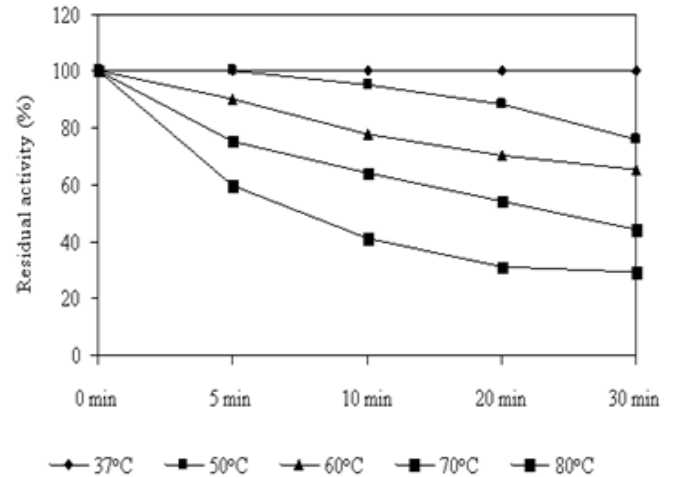


Figure 5: Thermostability of enzyme.

CONCLUSION

The present study revealed that the intracellular β -galactosidase from *Bacillus* MTCC-864 is a metalloenzyme with strict requirement of divalent metal ions to enhance its activity. The enzyme is active at wide range of pH and temperature and it can be used for hydrolysis of lactose in milk around neutral pH and other dairy industry operations.

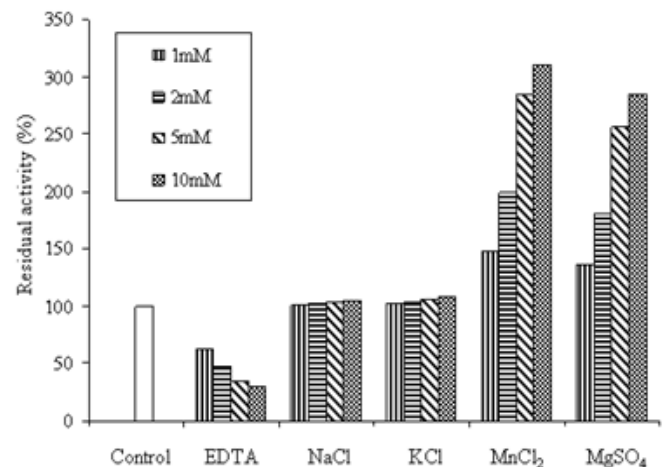


Figure 6: Effect of metal ions on enzyme activity.

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