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Lactose hydrolysis approach: Isolation and production of β-galactosidase from newly isolated *Bacillus* strain B-2

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ABSTRACT

β-galactosidases are hydrolytic enzymes that are involved in catalytic process of lactose found in milk and make it as an outstanding industrial product for lactose intolerant population. These enzymes also regulate the transgalactosylation reactions in order to synthesize galactooligosaccharides. Considering their tremendous applications in different industries, various bacterial strains were isolated and screened for β-galactosidase production. The maximum enzyme producing strain was identified as *Bacillus* strain B-2 on the basis of morphological and biochemical characteristics. After selecting the appropriate growth medium, different fermentation parameters were optimized using submerged fermentation approach. It was observed that isolated bacterial strain produced maximum β-galactosidase with 1% lactose as a substrate after 48 h of incubation time. The current findings indicate that β-galactosidase from *Bacillus* strain B-2 can be a valuable candidate for different industrial applications.

1. Introduction

β-galactosidase [EC 3.2.1.23] catalyzes the hydrolytic process of β-1,4-D-galactosidic linkages found in lactose (β-D-galactopyranosyl-(1→4)-D-glucopyranose) and releases D-glucose and D-galactose as an end product. As a main milk sugar, lactose needs to be sufficiently metabolized to fulfill the energy demand of human beings and makes possible the consumption of milk and other dairy products by lactose intolerant people (Haider and Husain, 2009). β-galactosidase not only cleaves lactose but also involves in a transgalactosylation reaction to produce galactooligosaccharides (Gosling et al., 2010; Mussatto and Mancilha, 2007). Galactooligosaccharides (GOS) are non-digestible oligosaccharides that are consumed as prebiotics food ingredient to improve the growth of intestinal bifidobacteria. These bacteria generate positive effect on health by reducing the cholesterol level, produce different essential vitamins and also have anticarcinogenic properties (Grosová et al., 2008).

The enzyme β-galactosidase has significant applications in the dairy and pharmaceutical industries for reducing the effect of lactose crystallization in condensed milk and frozen dairy products. This enzyme can also be used to decrease the water pollution caused by whey released from cheese processing industry and to improve the milk digestibility for lactose intolerant people (Grosová et al., 2008; Elnashar and Yassin, 2009; Haider and Husain, 2008; Ansari and Husain, 2011). β-galactosidase has been isolated from different microbial species, plants and animals (Husain et al., 2011). Among them, bacterial isolates are widely employed for the high production of different enzymes at commercial level due to high metabolic diversity, fast growth and less time requirement (Torsvik et al., 1998; Bibi et al., 2014; Karim et al., 2015). β-galactosidase production differs with different strains and is regulated by the nutritional, physiological and biochemical nature of the microbial species used (Xia et al., 2010; Liu et al., 2011; Yu and O'Sullivan, 2014). Various environmental and fermentation factors such as growth temperature, pH, time, agitation, aeration, inoculum size, carbon and nitrogen sources, metal ions etc. directly affects the metabolism-mediated production yield (Prakasham et al., 2005, 2007; Rao et al., 2008). Hence, optimization of medium constituents is one of the crucial step that can be employed to increase the enzyme production and also helps to decrease the amount of unutilized components in media in order to attain a cost-effective high production yield.

The current study was designed to elaborate the best physicochemical conditions for the maximum production of extracellular β-galactosidase by newly isolated bacterial strain. Different bacterial species were isolated from indigenous source and identified on the basis of morphological and biochemical characteristics. Culture isolates were screened for β-galactosidase production and enzyme production was carried out by following a stepwise optimization strategy.

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2. Material and methods

2.1. Isolation and maintenance of bacterial strains

Several bacterial strains were isolated from soil sample collected from a dairy farm located in Karachi, Pakistan. Sample was collected in a sterile polythene bag and transferred to laboratory immediately. 1.0 g of soil was mixed in 100 ml of normal saline which was further serially diluted from 10^{-1} to 10^{-9} ratio with normal saline and dispensed 100 μl from last two dilution tubes on nutrient agar plates supplemented with 1.0 g dl^{-1} lactose and 50.0 μg ml^{-1} X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyrinoside) as substrate. Lawn was prepared by using sterile glass rod and plates were incubated at 37 °C for 48 h. The isolated colonies having different morphological characteristics were selected for pure culture study using streak plate method. Single isolated colonies were sub-streaked many times on nutrient agar plate (supplemented with 1.0 g dl^{-1} lactose) until pure cultures achieved. Purity of the isolated colonies was examined by Gram's staining method. Pure bacterial isolates were maintained on nutrient agar slant and stored at 4 °C for further studies.

2.2. Identification of β-galactosidase producing bacterial strains

Bacterial strains were identified on the basis of morphological and biochemical characteristics.

2.3. Morphological characterization

Different characteristics of bacterial colony (color, shape, surface, margin and elevation) and cell morphology (cell type, shape and arrangement) were examined in order to identify the bacterial strains (Holt et al., 1994).

2.4. Biochemical characterization

Bacterial isolates were subjected to various biochemical tests including catalase test, Voges–Proskauer (VP) and methyl red (MR) test (Holt et al., 1994). Selective sugars such as glucose, fructose, maltose, lactose and sucrose were investigated for fermentation reaction. Gas and acid production were also examined to identify bacterial isolates.

2.5. Qualitative screening of bacterial isolates for β-galactosidase production

Pure bacterial cultures were grown at 37 °C for 48 h onto the lactose agar medium, supplemented with 50 μg ml^{-1} X-gal (chromogenic substrate) and 1.0 mM of isopropyl β-D-1-thiogalactopyrinoside (IPTG) to examine the capability of isolates for β-galactosidase production (Jaturapiree et al., 2012). Lactose agar medium contained (g L^{-1}): Lactose, 5.0; beef extract, 3.0; peptone, 5.0; Agar, 15.0 with pH-7.0. After 48 h of incubation period, blue colored colonies were examined. Selected isolates were stored on lactose medium slant and in 40% glycerol for short and long term preservation, respectively. Routinely used culture was maintained by regular sub-culturing on fresh media.

2.6. Quantitative screening of bacterial isolates for β-galactosidase production

Bacterial isolates were screened for β-galactosidase production using submerged fermentation approach in lactose medium without agar–agar. Inoculums (10.0 ml) were prepared by inoculating the cultures in growth medium and incubated at 37 °C in an orbital shaker (120 rpm) for 18 h. Inoculum tubes were transferred into production flasks (90.0 ml) and incubated at same conditions for 24 h. After 24 h of fermentation period, cell harvesting was performed at 40,248 × *g* for 10.0 min at 4 °C. The supernatant was passed through 0.22 μm filter membrane (Nitrocellulose, Millipore, Germany) to obtain a cell free filtrate (CFF). CFF was used to evaluate the catalytic activity of β-galactosidase. All the experimental work was carried out in triplicate and the results mentioned are the mean values of all the observations.

2.7. Enzyme assay for β-galactosidase activity

β-galactosidase activity was measured using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate (Onishi and Tanaka, 1995). For enzyme assay, the reaction mixture contained 0.2 ml cell free filtrate and 0.5 ml ONPG (6.0 mM) which was prepared in phosphate buffer (0.1 M; pH-7.0). The reaction mixture was incubated at 37 °C for 20.0 min under shaking condition. Afterwards, reaction was terminated by adding 1.0 ml of 1.0 M sodium carbonate. The absorbance of the *o*-nitrophenol (ONP) released, was measured spectrophotometrically at 420 nm. Enzyme units were calculated by a standard curve of ONP. One unit of β-galactosidase activity (*U*) was defined as the "*amount of enzyme that liberates 1.0 μmol of ONP per minute under standard assay conditions*".

2.8. Optimization of growth conditions for β-galactosidase production

One variable at a time approach was used to optimize the fermentation conditions for maximum production yield of β-galactosidase from selected bacterial isolate.

2.8.1. Selection of growth medium for maximum production of β-galactosidase

Bacterial isolate was cultivated into different previously reported media such as Medium-1 (Patil et al. 2011), Medium-2 (Kumar et al., 2012), Medium-3 (Batra et al., 2011), Medium-4 (Lee et al., 2012) and Medium-5 (Jaturapiree et al., 2012) under batch fermentation process to attain the maximum enzyme production. All the medium flasks were incubated at 37 °C for 24 h in an orbital shaker (120 rpm) and were autoclaved at 121 °C for 15.0 min prior to use.

2.8.2. Substrate concentration

The effect of lactose concentration was analyzed in the range of 0.0–3.0%. The fermentation flasks were incubated at 37 °C for 24 h under shaking conditions (120 rpm).

2.8.3. Fermentation time

The time course for β-galactosidase production was investigated for different time intervals (18–96 h). After each time interval, fermentation broths were centrifuged at 40,248 × *g* for 10.0 min at 4 °C. CFF was used to determine the catalytic activity of enzyme under standard assay conditions.

3. Results and discussion

β-galactosidase has remarkable potential to be used in different industrial processes such as in treatment of lactose, synthesis of galactooligosaccharides (GOS), bioremediation, development of biosensors and in medicinal field (Klein et al., 2013; Guimarães et al., 2010; Ammam and Fransaer, 2010; Nath et al., 2014). Considering the wide application of β-galactosidase, the present study was designed for its maximum production.