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Short Communication

Studies on Isolation and Characterization of Lactase Produced from Soil Bacteria

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Abstract

The enzyme β-galactosidase has many applications in biotechnology, pharmaceuticals and food processing industries. The present study was conducted to screen the soils from cattle shed near Kolkata for the isolation of lactase producing bacteria. The isolation was performed by plating on lactose agar medium containing the chromogenic substance X-gal. o-nitrophenyl-β-D-galactopyranoside was used as a substrate to determine the β-galactosidase activity of the two isolates BS1 and BS2 which were found to be the active strain producing a considerable amount of β-galactosidase. The bacterial isolate BS1 showed maximum β-galactosidase activity under shaking condition. Both BSI and BS2 produced extracellular and intracellular β-galactosidase activity. The optimum pH and temperature for enzyme activity were 7 and 40°C respectively.

Keywords: β-galactosidase, lactose intolerance, o-nitrophenyl-β-D-galactopyranoside, 5-bromo-4-chloro-3-idolyl-β-D-galactopyranoside, extracellular enzyme.

Introduction

Milk sugar lactose is commercially produced in food and pharmaceutical grades. The wide applications of lactose are based on some advantageous nutritional and technological properties of it. However, lactose may cause important problems mainly in three areas, health (lactose intolerance), food technology (excessive lactose crystallization resulting in the dairy products with a sandy or gritty texture) and environment (a waste product from cheese whey manufacturing). The enzymatic hydrolysis of lactose by β-galactosidase offers some solutions to solve these problems. The enzyme β-galactosidase is a commercially important enzyme because it not only catalyzes the hydrolysis of lactose into readily hydrolysable sugars glucose and galactose, but also the transglycosylation reaction as well. For the hydrolysis reaction, the lactose-reduced ingredients in the food and dairy products are commercially produced for lactose intolerant persons[1]. The catalyzed transglycosylation reaction is useful for the production of probiotic galactooligosaccharide improving structural and functional modification of food materials or pharmaceutical compounds[2]. During the last three decades, lactase has attracted attention of many researchers as it has a wide area of applications in dairy, confectionary, baking and soft drinks industries[3]. This enzyme occurs widely in nature and has been isolated from animals, plants as well as microorganisms. Compared to animal and plant sources, the microbial enzyme is produced at higher yields and is more technologically important. Despite the fact that many techniques have been developed based on different microorganisms[4-6], there is still a need to search new strains, which can display higher enzyme production. Keeping the above in view, the present study was performed to screen and isolate lactase producing bacteria from the soil of cattle shed.

Material and Methods

Chemicals: Analytical grade chemicals and reagents were obtained from SRL, India and E. Merck Germany. The substrate o-nitrophenyl-β-Dgalactopyranoside (oNPG) was purchased from Sigma Chemical Company, USA. Bacteriological media were obtained from HiMedia Laboratory Pvt. Ltd. India.

Screening of lactose producing microorganisms: Soil sample was collected from a cattle shed near Bally, Howrah, West Bengal. For the screening of microorganisms producing β-galactosidase, lactose medium (0.5% lactose, 0.5% peptone, 0.3% beef extract, agar 1.5%, pH 6.5) was used. Soil sample was serially diluted and plated on presterilised Petridishes containing lactose medium. The ability to produce β-galactosidase was conducted on lactose medium containing 50µg/ml of the chromogenic substance 5-bromo-4-chloro-3-idolyl-β-D-galactopyranoside (X-gal). Distinct colonies which exhibited blue coloration was subcultured and purified by streaking on lactose agar slants containing X-gal and incubated at 40°C for 24 hrs. The purity of the strain was verified by microscopic examination.

Preparation of inoculums: Pure culture of lactase producing bacteria isolated from soil was transferred from lactose agar slant into Erlenmeyer flask containing 100 ml presterilised lactose broth (10% lactose, 10% peptone, 10% yeast extract,

0.5% $(NH_4)_2SO_4$, 0.1% NaH_2PO_4, 0.05% $MgSO_47H_2O$, pH 7) and incubated for 24 hr at 35°C. One percent of the above cell suspension was used as inoculums.

Fermentation condition for β-galactosidase production: The production of β-galactosidase was carried out in 250 ml Erlenmeyer flask containing 100 ml culture medium (10% lactose, 10% peptone, 10% yeast extract, 0.5% $(NH_4)_2SO_4$, 0.1% NaH_2PO_4, 0.05% $MgSO_47H_2O$, pH7) and the flasks were kept under both static and shaking conditions for 24 hr at 40°C. After incubation, cells were harvested by centrifugation at 6000 rpm (REMI, R-24) for 10 mint, suspended in phosphate buffer (0.01 M, pH 7), washed 2 times by the same buffer, resuspended in the same buffer and then lyzed by sonicator (250 W, PIEZO-U-SONIC ULTRASONIC CLEANER). Cell debris was eliminated by centrifugation at 6000 rpm for 20 mint and the cell free extracts were obtained to get the intracellular materials. To get the extracellular β-galactosidase, after incubation, the culture filtrate was concentrated by lyophillzation. Both the cell free supernatants and concentrated culture filtrates were stored in freezer and used for enzymatic assay.

Assay of β-galactosidase: The assay of both intra and extracellular β-galactosidase activity was performed with 0.5mM oNPG as the substrate in 0.2 mM phosphate buffer (pH 7). The activity of β-galactosidase was measured by incubating 20µl of suitably diluted enzyme with 480 µl oNPG at 40°C for 15 min. the reaction was stopped by adding 750 µl of 0.4 M Na_2CO_3 and the concentration of o-nitrophenol (oNP) released was determined by spectrophotometry at 420 nm. One unit of β-galactosidase activity was defined as the amount of enzyme that released 1 µmol of oNP from oNPG per minute under experimental conditions described above.

Characterization of crude β-galactosidase: The pH dependence of β-galactosidase activity was studied by incubating the crude enzyme in the respective buffer systems (0.1 M Glycine HCl buffer pH 3, Citric acid–dibasic sodium phosphate buffer pH 4-5, phosphate buffer pH 6-8, glycine–sodium hydroxide pH 9 and 10) for 1 hr at room temperature prior to the addition of substrate. The enzyme was then assayed for lactase activity under standard assay conditions.

In order to determine the temperature stability, lactase in the absence of substrate was kept at temperatures between 20 and 70°C for 1 hr. After adding the substrate, activity was determined by the standard method as described earlier.

Results and Discussion

Screening: In a screening of microorganisms for the production of *β-galactosidase* activity on X-Gal containing lactose agar 72 colonies were observed. Among them 13 colonies which were able to grow on lactose have the ability to produce *β-galactosidase* because they formed distinct blue colonies on lactose agar containing X-Gal (data not shown). Among different isolates, two bacterial strains were found to produce *β-galactosidase* in considerable amount. One of them was stained positively (BS1) and another one was stained negatively (BS2) in Gram stain. Further experiments were conducted using these two strains.

Assay of β-galactosidase: To determine whether the *β-galactosidase* activity was intracellular or extracellular, both the culture supernatant and the cell extract of the two isolates were assayed. For both the two strains enzyme activity was associated with the cells and was detected in the supernatant also. Thus BS1 and BS2 produced both intra and extracellular enzymes (table 1). Higher enzyme activity was observed when the fermentation was conducted under shaking conduction. Intracellular *β-galactosidase* showed a little higher enzyme activity than the extracellular one. Table 1 revealed that the isolated strain BS1was found to exhibit the highest *β-galactosidase* activity. All the enzyme assays were performed in triplicate and the mean values were reported.

Characterization of crude β-galactosidase: The intracellular β-galactosidase enzyme of BS1 was active in a broad range of temperature (10-90°C) and showed maximum activity at 40°C as shown in figure 1. A gradual decrease in the activity was observed at higher temperature. On the other hand the enzyme drastically lost its activity at lower temperature. The *β-galactosidase* was active at broad range of pH (3-10) and the maximum activity was observed at pH 7 (figure 2). Similar results have been reported for several *β-galactosidase* sources[7-8].

Table-1
β-galactosidase from isolates BS1 and BS2

Culture Type	β- Galactosidase Activity (U/mg)			
	Static		Shaking	
	Extracellular	*Intracellular*	*Extracellular*	*Intracellular*
BS1	30.34	39.17	33.82	41.52
BS2	29.52	36.7	32.01	38.65

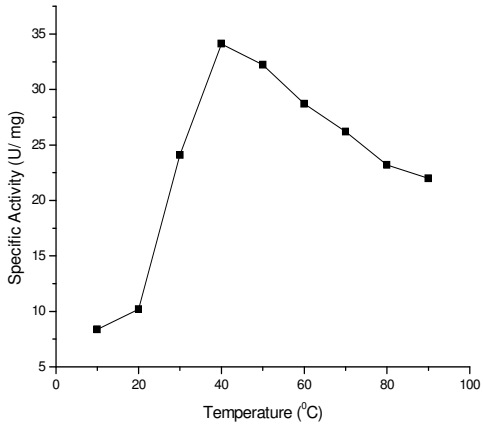

Figure-1
Effect of temperature on the β-galactosidase activity from isolate BS1

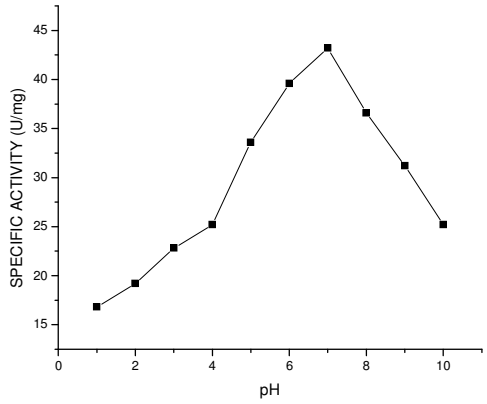

Figure-2
Effect of pH activity β-galactosidase from isolate BS1

Conclusion

In conclusion, we isolated two mesophilic *β-galactosidase* producing Gram-positive and Gram-negative bacteria BS1 and BS2 which can grow on lactose above 40°C. 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 the efficient hydrolysis of lactose in milk and whey and producing attractive products for food industry.

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