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Exploring Milk as a Source: Studies on the Isolation and Characterization of β-Galactosidase

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Abstract: The enzyme β galactosidase has been used in the dairy industry for the hydrolysis of lactose and has many applications in pharmaceuticals and food processing industries. The aim of the study to screen β galactosidase producing bacterial isolate from raw milk. The isolation was performed by plating on nutrient agar medium containing X-gal and ONPG to determine the β galactosidase activity. Bacterial isolate MH2 showed highest activity of 186.2 (U/mg/ml) and were found to be active strain producing considerable amount of β galactosidase. So in future, the enzyme can be exploit at industrial level for the development of low lactose dairy products.

Keywords:β galactosidase, Lactose intolerance, X-gal, ONPG, raw milk, intracellular enzyme.

Research Paper

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INTRODUCTION

Lactose is a disaccharide that is found in milk and other dairy products. Lactose indigestion leads to dizziness, headache, excess gas, cramps and nausea after consuming significant amount of lactose (1). A lactose intolerant people does not have the ability to synthesize β galactosidase enzyme, so enzymatic hydrolysis of lactose is necessary to solve these problems. Nowadays, β galactosidase enzyme has been widely used for industrial as well as medical application. Therefore, products free of lactose can be consumed by lactose intolerant people and in dairy industries this creates a potential market for the application of β galactosidase (2). β galactosidase is widely distributed in nature and obtained from various sources such as plants, animals and microorganisms including yeast fungi and bacteria (3, 4). Among bacteria, yeast and fungi, bacteria are most suitable because they are regarded as safe, So work is carried by bacterial production of enzyme. (5) Despite the fact that many techniques have been developed based on different microorganisms, so there is still a need to search new strains, which display higher β galactosidase production. Keeping in view, the present study was performed to isolate and screen different bacterial strains from raw milk and to identify the most active bacterial strains for β galactosidase production.

MATERIALS AND METHODS

Sample collection

Raw milk samples were collected from different dairies of Punjab. Sample collection sites were Khurana sweets and dairy, Mohali; Sharma dairy, Ludhiana and Satpal dairy, Moga. The samples were brought to the laboratory under fully aseptic conditions in a sterile container.

Isolation of bacterial strains

Raw milk samples were subjected to serial dilutions, where 1 ml of each sample was added to 9 ml of sterile distilled water and the samples were serially diluted upto 10^{-5} dilution and were spreaded on nutrient agar medium. Incubation was done at 37° C for 24 h. Isolated colonies were further purified by streaking on medium and were preserved at 4° C.

Screening of bacterial isolates

The bacterial isolates to produce β galactosidase was plated on nutrient agar medium containing
5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) and Isopropyl β-D-1 thiogalactosidase (IPTG). The plates were incubated at 37° C for 48 h. Definite blue colored colonies were observed on the plates indicating the presence of β galactosidase producing bacteria (6).

Enzyme Assay

The bacterial cultures were grown at 37° C for 24 h in nutrient broth medium. The cultures were

centrifuged and cells were washed with 1 % NaCl by Z buffer (7) and cell pellet resuspended in 1 ml Z buffer which contains 0.1 % SDS and 2 drops of chloroform, followed by vortexing and incubation for 2 min at 37° C. The cell debris was separated by centrifugation at 4000 rpm at 4° C for 15 min. β galactosidase enzyme activity was quantitatively assayed by addition of 0.2 ml ONPG (o-nitrophenyl-β-D- galactopyranoside) and initiate the reaction for 15 min. When yellow color developed, the reaction was stopped by addition of 0.5 ml of 1 M Na_2CO_3(8).

Analysis of Protein Concentration

The protein concentration was determined by the Bradford's method [9] as a standard.

Morphological and Biochemical Characterization

The bacterial isolates were subjected to Gram's Staining (10) and and biochemical characterization according to Bergey's Manual of Systematic Bacteriology (11). Morphology of strains and various biochemical tests were performed for β galactosidase producing bacterial isolates.

RESULTS AND DISCUSSION

Screening

In a screening of microorganisms for the production of β galactosidase activity on X-gal containing lactose agar 17 colonies were observed (Figure 1). Among them five bacterial isolates were found to produce β galactosidase in considerable amount. Five bacterial isolates (SN1, MH2, SN3, PB1 and LD2) were further quantitatively screened to select the bacterial isolate, which produces maximum β galactosidase activity. β galactosidase activity of all five bacterial isolates is shown in [Table 1]. Except MH2, all other bacterial isolates showed very low level of the β galactosidase activity and MH2 bacterial isolate shows highest β galactosidase activity (186.2 U/mg/ml) as compared to other bacterial isolates. Hence, MH2 was selected for further characterization of bacterial isolate.

Assay of β galactosidase

To determine whether the β galactosidase activity was intracellular or extracellular, the culture supernatant and the cell extract of the bacterial isolate were assayed. The enzyme activity was associated with the cells and no activity was seen in the supernatant. Thus, the bacterial isolate produced intracellular enzyme. All the enzyme assays were performed in triplicate and the mean values were reported.

Morphological and Biochemical Characterization

Bacterial isolate MH2 appeared singly or in chains as straight rods, Gram negative organism under the microscope, creamish white, and circular colonies (Figure 2 a, b). Various biochemical tests were performed to identify bacterial isolate MH2 and was observed that MH2 was negative for VP test, Simmon's citrate test and urease test, and positive for MR and motility test.

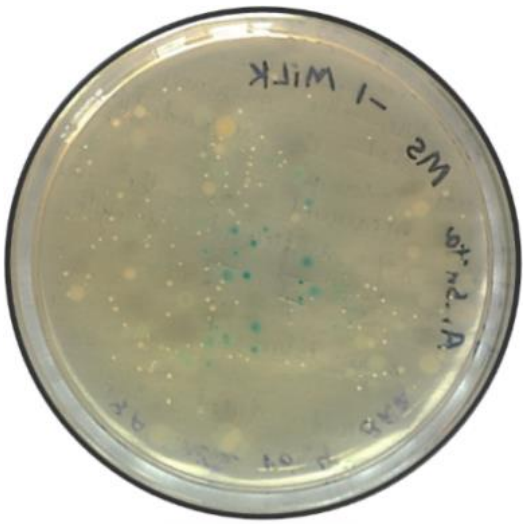
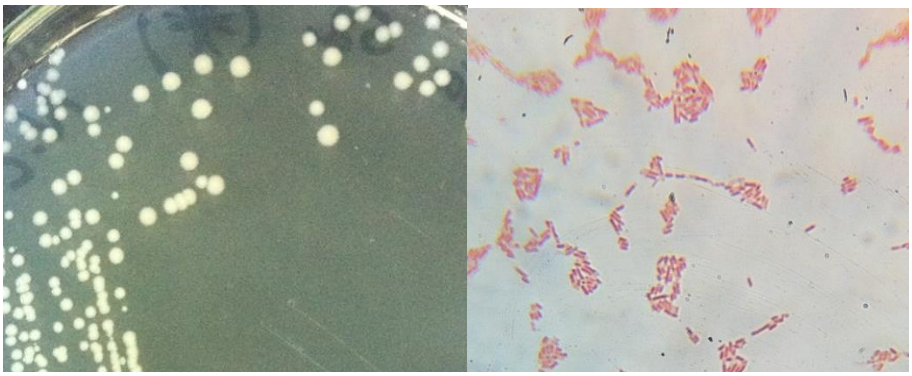


Figure 1: Green colonies of bacterial strain on X-gal plate.

Table 2: Comparison of bacterial isolates for β galactosidase activity

S.No	Bacterial isolate	β galactosidase activity (U/mg/ml)
1.	SN1	68.2
2.	SN3	27.31
3.	LD2	98.2
4.	MH2	186.2
5.	PB1	76.7



(a) (b)

Figure 2: (a) Colony morphology of bacterial strain on nutrient agar medium, (b) microscopic examination of Gram's staining at 40X

CONCLUSION

In conclusion, the isolation of bacterial isolate (MH2) from raw milk producing intracellular β galactosidase activity. Bacterial isolate MH2 showed highest activity at 186.2 (U/mg/ml). This suggests that MH2 bacterial isolate can be a potential producer of β galactosidase. The strain could be useful for the efficient hydrolysis of lactose in milk and producing attractive products for food industry.

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