ISOLATION AND CHARACTERIZATION OF BETA-GALACTOSIDASE PRODUCING BACTERIA FROM SOIL

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Abstract: Study on the β-galactosidase production was carried out with different bacterial strains isolated from the soil sample near dairy processing plant. The production of extracellular β-galactosidase by isolates was optimized in a submerged fermentation. The effect of temperature, pH, carbon and nitrogen sources of the medium were optimized. The production of the enzyme was found to be high at the 48th hour after inoculation at 37°C and pH 11.Under optimal conditions; β-galactosidase was allowed to produce by the isolated strain in presence of different carbon and nitrogen sources. Among all Lactose and Peptone was found to be best for the maximum production of enzyme. The enzyme was purified using and the purified β-galactosidase was homogeneous with the molecular weight of 70 kDa as shown by SDS PAGE analysis.

Keywords: **Optimization, β-galactosidase, *Lactobacillus* sp, *Bacillus* Sp.**

INTRODUCTION

Inside living organisms various enzymes serve variety of functions. They are responsible for signal transduction and cell regulation, often via kinase and phosphatises. **β-Galactosidase**, also called **beta-gal** or **β-gal**, able to cleave β linked galactose residues from various compounds and cleave lactose into galactose and glucose [2, 3]. Substrates of enzyme β-Galactosidases include Ganglioside GM1, Lactosylceramides, lactose, and various glycoproteins.[1]

In Food and Dairy industry, lactose hydrolysis by use of enzymes of is one of the most important processes. For that, Beta-Galactosidases preparations are used [4].

For the hydrolysis reaction, the lactose-reduced ingredients in the food and dairy products are commercially produced for lactose intolerant persons [5]. The catalyzed transglycosylation reaction is useful for the production of probiotic galacto-oligosaccharide improving structural and functional modification of food materials or pharmaceutical compounds [6].

Also to avoid lactose crystallization in sweetened, condensed and frozen dairy products and solve problems associated with whey utilization and disposal the β-Galactosidase enzyme can be used [7].

Beta-Galactosidase can be obtained from various sources such as plants, animals and micro organisms. But micro organisms are considered as a suitable source for industrial applications. Among bacteria; yeast and fungi and a large number of bacteria are most suitable because they are Generally Regarded as Safe (GRAS).

So our work was focused on bacterial production of enzyme with high potentiality to produce galactosidase. The activity and stability of enzymes is influenced by the type of strain, cultivation conditions (temperature, pH, aeration, agitation, incubation time) and the growth medium composition (particularly carbon and nitrogen sources). Hence the culture conditions and media

components for the production of β-Galactosidase using native bacteria from the soil sample collected near milk processing area were optimized.

MATERIALS AND METHODS

Sample Collection: The soil sample near milk processing area of Dairy was collected. The samples were brought to the laboratory under aseptic conditions in a sterile container.

Strain isolation and screening: The strains showing blue colonies on Tryptic Soy Agar plates (g/l) (Tryptone-15, Soytone-5, NaCl-5, Agar-15) [8] with 0.5% lactose as the sole carbon source and 0.1% of 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-gal) were collected and streaked onto NA medium. Once the isolates were purified, the strain showing high enzyme activity was selected and used for further investigation.

Characterization of organism: The morphological and taxonomic characteristics of the strain like grams reaction, spore formation and motility test were examined [9, 10].

Enzyme Production

Inoculum Preparation for the Production of Beta- Galactosidase: One loopful of overnight grown bacterial culture was transferred to the LB broth as prescribed by Robert et al., 2006 [11].

The Production Medium:Lactose-10g/L, Peptone-1.5g/L, Yeast extract-1g/L, KH_2PO_4 -1g/L, NH_2 H_3PO_4 -7g/L, $MgSO_4$.$7H_2O$-1g/L and $CaCl_2$ -0.3g/L. For effective production, it was carried out in a shaker with an aeration rate of 200 rpm under the volume 50/200 v/v at 37°C for 24 hours to 48 hrs. The samples were centrifuged at 10,000 rpm for 10 min at 4°C and the culture filtrate was used for further assay procedures.

Enzyme Assay: The Beta-galactosidase activity was measured by the method of Gumgumjee and Danial [12]. The enzyme was assayed by using ONPG as a substrate prepared by dissolving 2.5 mg/ml of ONPG in 0.1M sodium acetate buffer (pH-5). 0.2 ml of the culture filtrate was added to 1 ml of the substrate solution and incubated at the temperature of 55°C for 20 min. The reaction was then stopped by adding 1 ml of 10% sodium carbonate. The absorbance was read at 420 nm and the amount of ONP was calculated using standard curve. One unit of enzyme activity was defined as the amount of enzyme that liberates 1 µM of ONP per min at the temperature of 55°C. The amount of protein was estimated by Bradford method using Bovine Serum Albumin (BSA) as a standard according to the instruction manual of Quick Start Bradford Protein Assay [13].

Optimization of Culture Conditions for Enzyme

Effects of temperature and pH: These were carried out by cultivating the isolate at different temperatures (20-60°C) [14] and different pH values (5-9) [15].

Role of Carbon and Nitrogen sources: Carbon sources such as glucose, xylose, maltose, starch, sucrose were tested at 1% (w/v) concentration. Nitrogen sources like peptone, beef extract, sodium carbonate, ammonium sulphate and ammonium chloride were also tested at 1% (w/v) concentration.

Purification of enzyme

The pH of the crude enzyme was adjusted to 5.0 and with the help of 1M $ZnCl_2$, the protein contents were precipitated and separated using centrifugation by Ammonium sulphate method. The precipitate was dissolved in 0.5 M EDTA and dialysed against 0.1 M Phosphate buffer (pH 7.5).

Characterization of purified β-galactosidase

The protein profile and the presence of purified β-galactosidase were confirmed by SDS PAGE analysis. Denatured Sodium Dodecyl Sulphate–polyacrylamide gel electrophoresis was performed with marker as described in Nakkharat and Haltrich [16]. Coomassie brilliant blue staining was used for the visualization of the protein bands.

RESULTS AND DISCUSSION

Because of various applications in various fields Beta-galactosidase has been produced. The enzyme β-galactosidase has two main biotechnological applications in milk and dairy industries, e.g. the removal of lactose from milk for lactose-intolerant people and the production of galacto-oligosaccharides (GOS) for use in probiotic food.

Isolation of Microbes from Soil

Total of 25 cultures were obtained by serial dilution method which were further screened for Beta galactosidase production on X Gal medium.

Screening of microbes for Beta galactosidase production

Out of 25 isolates 5 cultures were able to produce Beta Galactosidase which showed clear zone around the colonies

Identification of Microbes

Isolate No	Isolate Identified
1	*E.coli*
3	*Bacillus subtilis*
6	*Pseudomonas aeroginosa*
18	*Klebsiella pneumoniae*
23	*Lactobacillus sp*

Enzyme production and Assay

The 5 isolated cultures were subjected to the production of Enzyme and assayed by the standard protocol. Later they were subjected to the different parameters for the maximum production of the enzyme

Effect of pH:

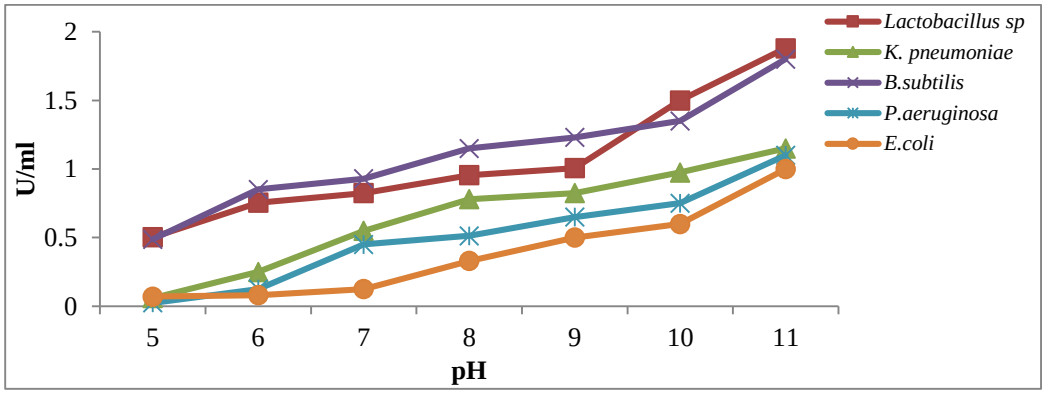

Fig.1: Effect of pH on Beta galactosidase enzyme production by Microbes

As the pH increased the production of Beta galactosidase increases by all the bacterial isolates. Because of the alkaline in nature, maximum concentration was obtained at pH 11. Controlling the pH of the culture during fermentation has been reported to enhance microorganism growth and enzyme production [17]. Results as shown in Figure 1 showed that the highest β-galactosidase activity was obtained when the pH of the medium was adjusted to 9 to 11 with all the bacterial isolates. The hydrogen ion concentration of on environment has the maximum influence of the microbial growth and enzyme production. pH 5.5 has been observed as optimum for the β-galactosidase production by Rajoka et al [18] and Hin [19].

Effect of Temperature:

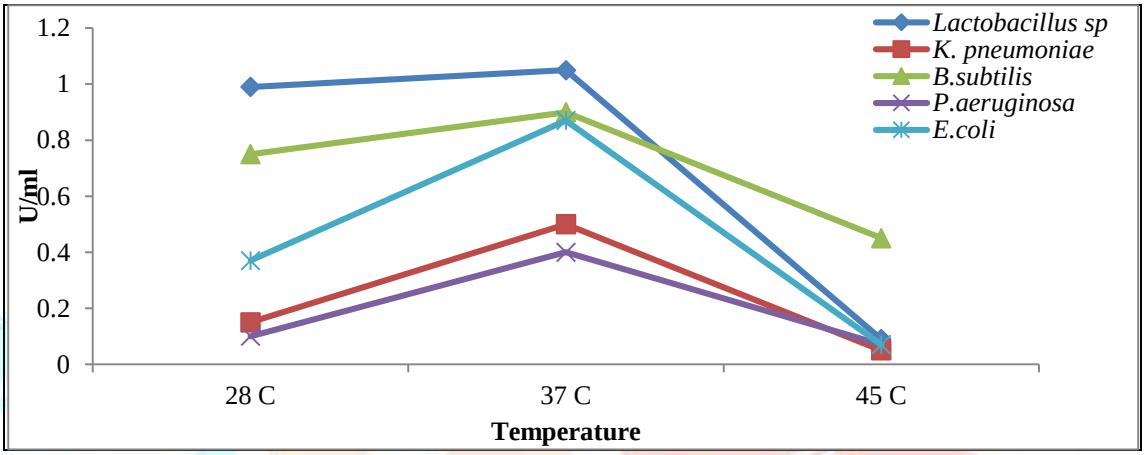

Fig 2: Effect of Temperature on Beta galactosidase enzyme production by Microbes

As the temperature is increased from 28 to 45 ^{0}C the production decreases in case of all the microbes isolated. The best production was found to be at 37 ^{0}C. Chakraborti *et al.*, 2003 [20] showed that Beta-galactosidase was produced at maximum level when maintained at temperatures of 37°C. Similarly, the temperature ranges of 28-37°C were found as optimum for the β-galactosidase production [21, 22].

Effect of Carbon Sources:

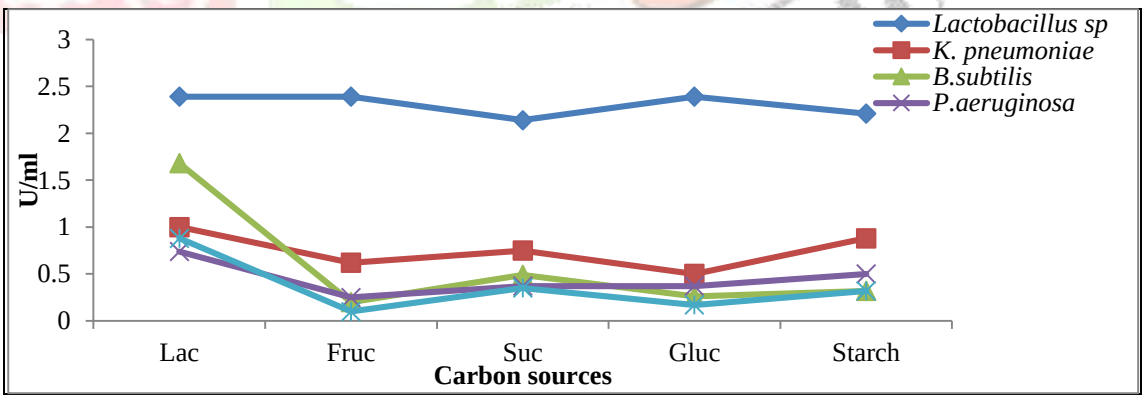

Fig 3: Effect of Different Carbon Sources on the Beta galactosidase production by Microbes

Lactobacillus in presence of Lactose, *Klebsiella* and *Bacillus* in presence of Glucose, *Pseudomonas* in presence of Starch and *E.coli* in presence of Lactose showed the enhanced production of Beta glalctosidase enzyme. Cheaper carbon and nitrogen sources are the key attraction for commercialization of the production processes and thus, ability of the microorganisms to grow and produce enzymes using these sources has been arguably a point of interest [23]. Selection of suitable carbon and nitrogen sources were the critical step during the enzyme optimization [24].

Effect of Nitrogen Sources:

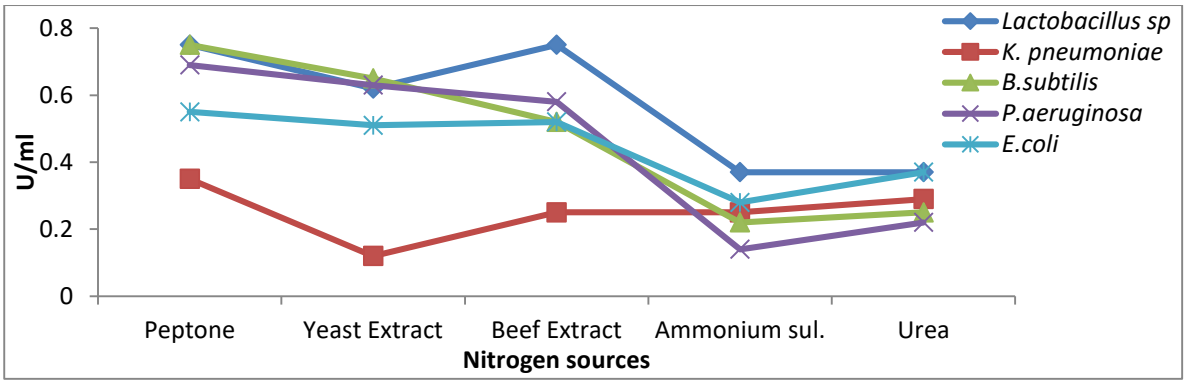

Fig 4: Effect of Different Nitrogen Sources on the Beta galactosidase production by Microbes

In most microorganisms, both inorganic and organic forms of nitrogen are metabolized to produce amino acids, nucleic acids, proteins, and cell wall components. Nitrogen sources may affect microbial biosynthesis of β-galactosidase [25]. Five different nitrogen sources (Three organic and two inorganic) were used to explore the best ones giving maximal enzymatic production by *Bacillus* isolate and *Lactobacillus* isolate.

While testing the β-galactosidase production in fermentation medium with different nitrogen sources, peptone (Fig. 4) were found to be the better nitrogen sources favoring maximum enzyme production. Next to them, beef extract also found to be increasing enzyme production in significant amount. Nurullah [26] has reported xylose and yeast extract as better carbon and nitrogen sources for β-galactosidase production for *Bacillus licheniformis*.

The presence of individual band near the molecular weight of 70 kDa indicates the presence of β-galactosidase. It has been previously reported that, β-galactosidases are also having molecular weight of 75 kDa [27] and 67.5 kDa [28].

CONCLUSION

The present study shows that there is appreciable production of extracellular β-galactosidase using the native Lactobacillus and Bacillus Sp. This suggests that these sp. can be a potential producer of extracellular β-galactosidase which may have applications in both industry and biotechnology. Due to the importance of these findings, further studies will be done for the enhanced enzyme production. These strain showed that it is an ideal candidate for hydrolysis of lactose in milk which can be used for lactose intolerant people.

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