"ISOLATION, CHARACTERIZATION & PRODUCTION OF β-GALACTOSIDASE"

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Abstract **:** β- galactosidase is an important enzyme for organisms as it is a key provider in energy production and a source of carbons through the breakdown of lactose to galactose and glucose. Many adult humans lack the lactase enzyme, which has the same function of β-galactosidase, so they are not able to properly digest dairy products. β-galactosidase is isolated from bacteria, fungi and plants. Any stress on the gut macrobiota due to biotic and abiotic factors lead to disorders resulting in to enhanced risk of disease development. Currently, use of probiotic as a therapeutic approach for the restoration of gut microbial community is on constant rise. From the economic viewpoint, permeabilization and immobilization technology will be the areas of great interest. This enzymatic hydrolysis of lactose will certainly remain a topic of considerable scientific and technological interest in coming times. This study was carried out to develop the optimum condition for β-galactosidase production. The optimum production of β-galactosidase was found to be at 48 h of incubation time period and at pH 7.5.

Keywords: β-galactosidase, *Streptococcus*, Folin Lowry Method, Enzyme Purification.

INTRODUCTION

β-galactosidase also called lactase, beta-gal or β-gal which belongs to enzyme family of glycoside hydrolysis and having E.C. Number 3.2.1.2. It is responsible for breaking down the disaccharide lactose into its monosaccharide components, glucose and galactose. β-galactosidase extracted from bacterial sources has been used for lactose hydrolysis due to several advantage including their high activity, ease of fermentation and the stability of the enzyme. β-galactosidase obtain from *Bifidobacterium* (a probiotic organism) is utilized in food and food flavorings.

β- Galactosidase is found in bacteria, fungi and yeast. In plants, it is mainly found in almonds, peaches, apple and apricots. However, on a commercial and an industrial scale, the most commonly used sources of β-galactosidase are *Aspergillus* and *Kluyveromyces.* The enzyme β-galactosidase can be obtained from a wide variety of sources such as microorganisms, plants, and animals. Enzymes of plants and animal origin have little commercial value but several microbial sources of β-galactosidase are of great technological interest.

β-galactosidase produced by many microorganisms but the major problems associated with the large scale production are microbial contamination, protein adherence and channelling. Periodic washing, pasteurization and flow direction of feed can solve these problems to a great extent and to increase the production.

Much of the research was carried out in the field of probiotics, insulin and fructo-oligosaccharides, but nowadays research is going on galactooligosaccharides (GOS), because of their health promoting effects and stability over a wide range of temperature and pH. GOS are well documented to be as effective prebiotic ingredients which modulate intestinal macrobiotic, barrier functions and provide other beneficial health effects such as stool improvement, mineral absorption, weight management and allergy alleviation. It is also used in genetics, molecular biology and other lifesciences especially as a reporter marker to monitor gene expression.

MATERIALS AND METHODS

ISOLATION

Microorganisms were isolated from fermented dairy milk of Amul Shakti. For isolation purpose Blood Agar plates which supplemented with 1 % peptone, 1% Beef Extract, 0.5 NaCl, 1.5 % Agar and 2.5 mL Human Blood were used. Plates were kept at 37^0C for 24 h in incubator.

CHARACTERISATION

After incubation period, well isolated colonies were selected and cultural characteristics were noted. Purity and morphology can be checked by Gram's staining. An array of physiological and biochemical tests were performed as per standard methods to identify the morphology of selected isolates.

INOCULUM PREPARATION

The colony of well isolated bacterium was transferred aseptically to 10 mL of pre-sterilized inoculums medium containing 2 g casein enzyme hydrolysates, 1 g yeast extract, 2 g sucrose and 4 g dipotassium phosphate having pH 7.0. Flask was kept on shaker at 40^0C for 24 h at 120 rpm.

FERMENTATION

Fermentation medium consist of 10 g lactose, 10 g peptone, 10 g yeast extract, 0.1 g NaH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 0.05 g $MgSO_4.7H_2O$ and final volume made to 100 mL by using distilled water. pH was adjusted to7.0 and sterilized in autoclave for 15 min at 121°C. After cooling, 5 mL of inoculum was inoculated to fermentation medium under aseptic condition. The flask was incubated at 40^0C for 24 h at 120 rpm.

ENZYME ASSAY

1 mL of crude enzyme & 1 mL of substrate solution with phosphate buffer were taken and incubated at 30°C for 30 min. 3 mL of DNSA reagent was added to it and was kept in boiling water bath for 15 minutes. Reaction mixture was cooled down by immersing the sample tube into cold water immediately. Mixture was mixed properly and absorbance was measured at 540 nm by using spectrophotometer.

PROTEIN ESTIMATION

Protein estimation was carried out by Folin Lowry method. 1 mL of test sample & 5 mL of alkaline solution were taken in test tube. It was properly mixed & left for 10 minutes. 0.5 mL of Folin –Ciocalteau regent was added to it and properly mixed. Tube was left for 30 minutes. Absorbance was taken at 750 nm with spectrophotometer.

PARAMETER OPTIMIZATION

Different parameters were studied to check the effective condition for the production of β-galactosidase.

INCUBATION PERIOD

To check the effective time period for the production of β-galactosidase enzyme, different time period Viz., 24 h, 48 h, 72 h and 96 h were selected.

INOCULUM SIZE

To check the effective inoculums size for the production of β-galactosidase different inoculum size Viz., 0.5 mL, 1.5 mL, 2.5 mL and 3.5 mL were selected.

pH

pH is the most important physical parameter which affect the overall production. So, to get the optimum pH, different pH values Viz., 6.0, 6.5, 7.0 and 7.5 were selected.

EXTRACTION OF ENZYME

Enzyme was extracted by rupturing the cells by using glass beads under the 15000 rpm at 4°C in cooling centrifuge. Extracted enzyme was collected and was concentrated using ammonium sulphate precipitation method.

AMMONIUM SULPHATE PRECIPITATION METHOD

In order to concentrate the crude extract of β-galactosidase enzyme and to remove possible contaminants, the ammonium sulphate was used. 80% saturation was found to be optimum for concentrating the enzyme.

Ammonium sulphate precipitation

DIALYSIS

After ammonium sulphate precipitation, the enzyme was dialyzed overnight against 10 mM phosphate buffer to obtain purified form of enzyme. In order to investigate the purity of the β-galactosidase, polyacrylamide gel electrophoresis under denaturing conditions was carried out.

RESULT & DISCUSSION

ISOLATION & CHARACTEIZATION OF MICROORGANISM

Very few colonies were observed on blood agar plate after incubation period of 48 h. Well developed colony was selected and was used to characterize the cultural and morphological features of producer organism which were shown in the table given below. Similarly to know the biochemical nature of organism various tests were performed which was also mentioned in the table 1.3.

Table 1.1 Cultural characteristics

Colony	Creamy white
Colony Size	Medium
Colony Shape	Small circular
Margin	Entire
Consistency	Mucoid
Elevation	Convex
Arrangement	Single
Pigmentation	Nil

Table 1.2 Morphological characteristic

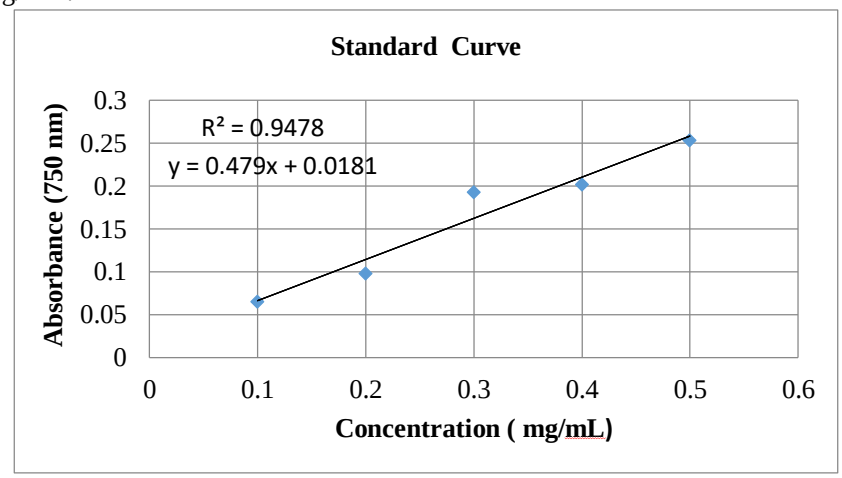
Size	Small
Shape	Round
Arrangement	Cluster
Gram reaction	Gram +ve

Table 1.3 Biochemical Analysis

Test	**Medium**	**Results**	
		Acid	Gas
Carbohydrate Fermentation Test	Glucose	+	-
	Sucrose	+	-
	Maltose	+	-
	Lactose	-	-
	Xylose	+	-
	Mannitol	+	-
Methyl Red Test	Glucose Phosphate Broth	-	
Citrate Utilization Test	Simmon Citrate Agar Slant	-	
Indole Utilization Test		-	
V-P Test		-	
Triple Sugar iron Test	Triple Sugar Iron Agar Slant	-	-

ENZYME ASSAY

Production of enzyme was measured by Standard assay of BSA. For standard graph, aliquots were prepared from the stock solution having concentration 1 mg/ mL.

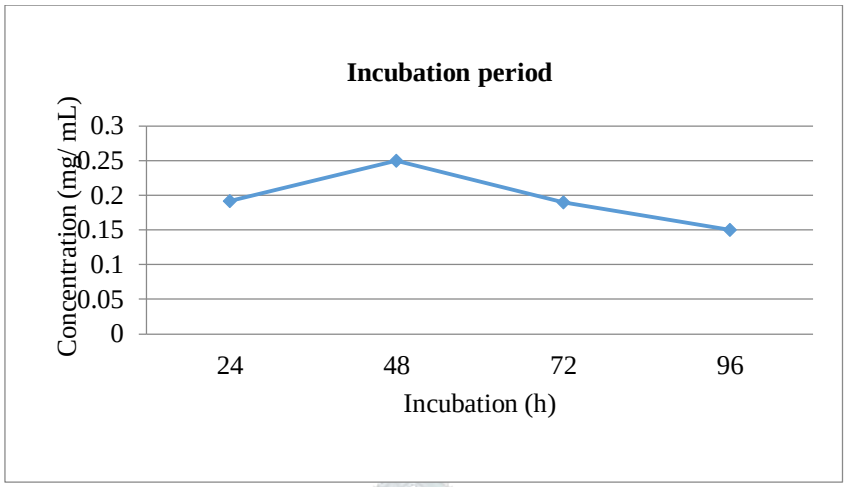


Standard Curve of BSA for protein estimation

PARAMETER OPTIMIZATION

INCUBATION PERIOD

In order to determine the optimum incubation period for β- Galactosidase production, the isolate was grown in the production media at various incubation times: 24 h, 48 h, 72 h and 96 h under shaking condition.

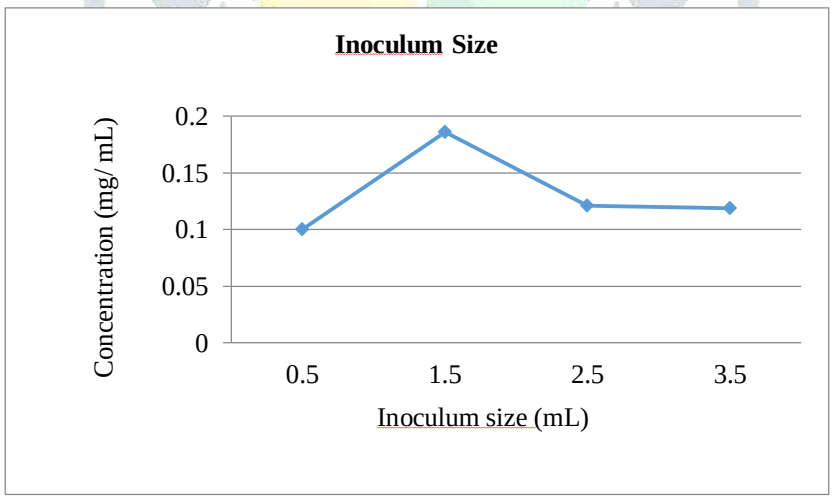


Effect of Incubation period on β- Galactosidase production

According to the results taken at regular intervals, the maximum enzyme production i.e. 0.25 mg/ mL was obtained at 48 h of incubation. Beyond this, the enzyme productivity was decrease which might be due to depletion of nutrient in the medium or catabolic repression of the enzyme.

INOCULUM SIZE

The amount of inoculum used is one of the important factors that influence the industrial fermentation, lag phase duration, specific growth rate, biomass yield and the quality of final product (Wanderley, *et. al.*, 2014). Little quantity of inoculums may take more time to reach high density to give large amount of product and if inoculums size is more it may associate with early depletion of nutrients and results into less production of enzyme.

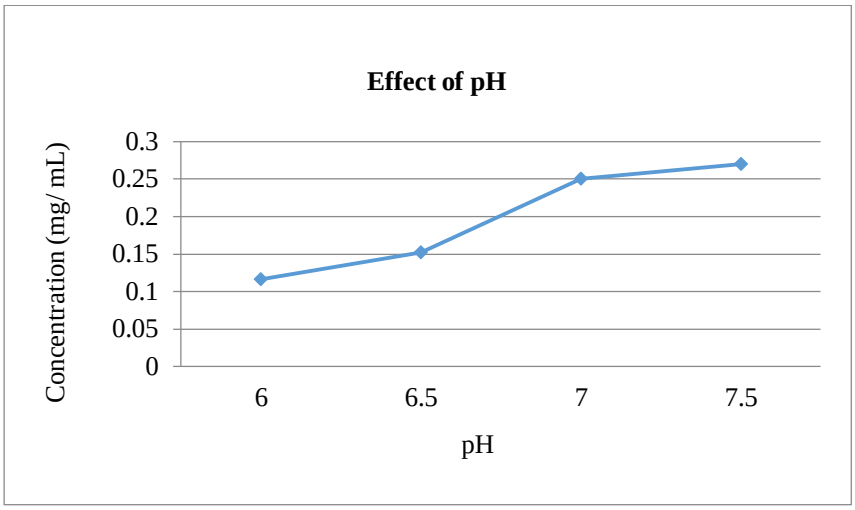


Effect of Inoculum Size on β- Galactosidase production

In this study, enzyme production i.e. 0.19 mg/ mL was found to be optimum at an inoculum size of 1.5 mL and beyond this productivity was observed to be decreased.

pH

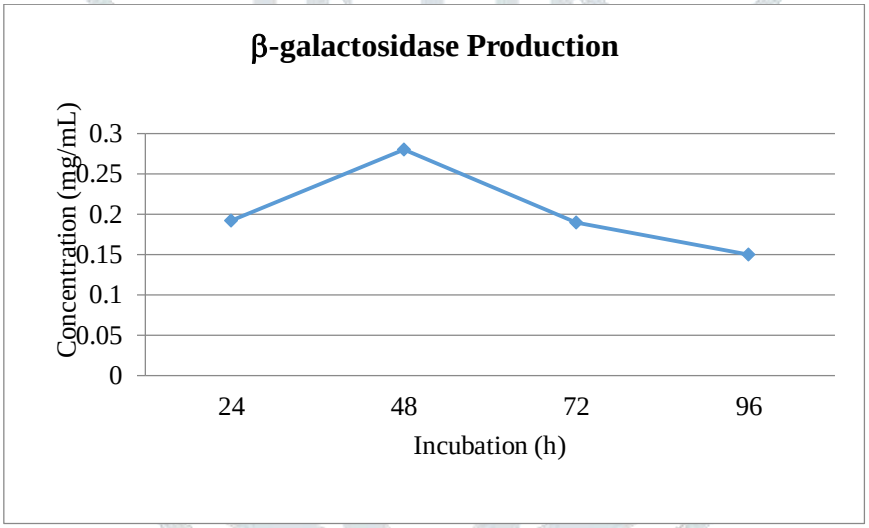
Among all the process parameters studied for enzyme production, pH seems to be of very important because the metabolic activity of bacteria were sensitive to slight change in the pH. All enzymes are functions optimally at particular pH and show the optimum activity. It is the important parameter which affects the final concentration of product also.



Effect of pH on β- Galactosidase production

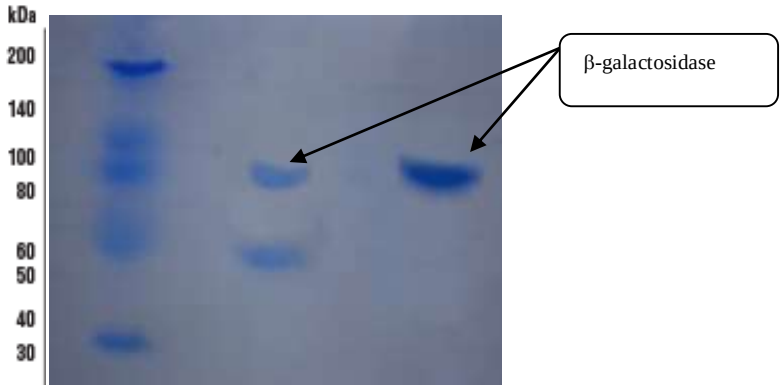
For ß galactosidase production lower pH was found to be less effective. Highest β-galactosidase production i.e. 0.27 mg/ mL was observed at pH 7.5.

After optimizing all the process parameters, one set was performed under optimized parameters to check the β-galactosidase production. The obtained results were represented in the graph given below. The highest production was observed to be 0.28 mg/ mL.



Standard Set

After ammonium sulphate precipitation and dialysis, purified enzyme sample was run through SDS PAGE and obtained bands were compared with the ladder sequence which indicates the presence of β-galactosidase.



SDS PAGE Gel Electrophoresis

CONCLUSION

Lactose intolerance is a physiological state in human beings where organism lack the ability to produce an enzyme named lactase/ β-galactosidase. Individuals lacking lactase will not be able to digest milk and is often possess a problem in new born infants. One way is to produce β-galactosidase in large quantities to solve this problem. Many organisms are known to produce β-galactosidase. In present study isolated organism *Streptococcus* was found to be producer of β-galactosidase. So parameters were standardized for improved production through the isolate. Isolated *Steptococcus* can use inexpensive and easily available material such as deproteinized milk whey as a substrate for the production of β-galactosidase and make process economically viable.
Isolated biocatalyst showed activity over a broad range of temperature and pH, which makes it an interesting candidate for various industrial as well as biotechnological applications. Optimum β-galactosidase production was noticed to be 0.28 mg/ mL under optimum condition i.e. 15 mL of inoclum size, 48 h of incubation period and at pH value of 7.5.

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