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MICROBIAL PRODUCTION OF BETA-GALACTOSIDASE ENZYME AND ITS APPLICATION IN DAIRY INDUSTRY

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ABSTRACT :

Lactose is a sugar found in milk and milk products. Lactose digestion requires an enzyme called lactase. Lactase breaks down lactose into simpler sugar glucose and galactose. The body then absorbs these simpler sugars into the bloodstream. Lactase deficiency and lactose malabsorption may lead to lactose intolerance resulting in digestive symptoms. In this context the present study emphasizes microbial production of beta-galactosidase enzyme and its application in manufacturing of lactose free milk and milk products. In present study production of beta-galactosidase enzyme was carried out, the extracted, purified enzyme was studied for effect of temperature and pH on catalytic activity. The extracted enzyme was used to treat raw milk to prepare lactose free milk.

KEYWORDS: *Beta-galactosidase, Lactose Intolerance, Lactose free Milk.*

INTRODUCTION:

Milk is popularly known to be as complete food as it furnishes all nutritive, metabolic requirements of an individual. Milk and its products are undeniably part of individual's life, from the birth of an individual till it continues with age. So there is necessity to investigate the physiological aspects associated with its digestion. As there is evidence of individuals showing clinical symptoms with digestion of lactose containing foods. The carbohydrate present in milk and milk products is Lactose, which is a disaccharide consisting of two monosaccharides moieties (glucose and galactose) joined via β1→4glycosidic bond. The Manufacturers also often add milk and milk products to boxed, canned, frozen, packaged, and prepared foods. Examples: Bread, waffles, pancakes, biscuits, cookies, doughnuts, toaster pastries, and sweet rolls, processed breakfast cereals, instant potatoes, soups, potato chips, corn chips, and other processed snacks, nondairy liquid and powdered coffee creamers, nondairy whipped toppings (Becker 1969, Inamdar 2016).

Lactose intolerance is the clinical syndrome, characterized by inability to digest lactose or impaired lactose digestion. The global investigation reveals that 70% of world's population is deficient in enzyme lactase among them Asian descents have higher ratio of lactase deficient's (60-100%) (Boyce 2010, Bury 2000, Bury 2001).

Lactase is located in the microvilli of small intestine. Lactase splits and hydrolyses dietary lactose into glucose and galactose, which then readily metabolized. The individual with deficiency of lactase enzyme shows elevated levels of unabsorbed lactose, which result into influx of fluid into bowel lumen, the unabsorbed lactose then enters the colon and is used as a substrate by intestinal bacteria, producing gas and short chain fatty acids via fermentation. The fatty acids cannot be absorbed by the colonic mucosa, Therefore more fluid is drawn into the bowel. A small proportion of lactose can be absorbed but the overall ingestion causes substantial rise of fluid and gas in the bowel causes bloating, diarrhea, and gas (Boyce 2010, Baran 1996). The problem with drinking milk and milk products is not only associated with deficiency of lactase,

other physiological and psychological factors can contribute to gastrointestinal symptoms that mimic lactose intolerance.

Sometimes individual confuse lactose intolerance with a milk allergy, While lactose intolerance is a digestive system disorder, a milk allergy is a reaction by the body's immune system to one or more milk proteins. An allergic reaction to milk can be life threatening even if the person eats or drinks only a small amount of milk or milk product. A milk allergy most commonly occurs in the first year of life, while lactose intolerance occurs more often during adolescence or adulthood (Baran 1996, Becker1969).

The abdominal bloating, a feeling of fullness or swelling in the abdomen, abdominal pain, diarrhea, gas and nausea (Vasiljevic 2001). The symptoms occur 30 minutes to 2 hours after consuming milk or milk products. Symptoms range from mild to severe based on the amount of lactose the person ate or drank and the amount a person can tolerate (Becker1969).

The present study focused on microbial production and extraction of lactase enzyme.

MATERIAL AND METHODS

Isolation and screening of bacterial culture:

The procured milk sample were serially diluted and plated on nutrient agar plates infused with 20 µl of X-Gal (20mg/ml in DMSO) and IPTG on its surface, and incubated at 37˚C for 24-48 hours. The plates were observed for formation of blue colour colonies, isolated blue colonies has been selected for further studies which indicates the presence of beta-galactosidase enzyme producing bacteria (Sreekumar 2010).

Preparation of Inoculums:

Loop full screened culture was transferred to nutrient broth and incubated in orbital shaker incubator at 37˚C for 24-48 hours (Robert 2006).

Production Medium:

The actively dividing bacterial culture was the then transferred to production medium, containing Lactose-10g/L, Peptone-1.5g/L, Yeast extract-1g/L, KH_2PO_4-1g/L, $NH_4H_2PO_4$-7g/L, $MgSO_4.7H_2O$-1g/L and $CaCl_2$-0.3g/L, the medium was autoclaved and inoculated with the prepared inoculum. The production flasks were incubated at 37˚C for 3-4 days on shaker incubator with an aeration rate 110 RPM (Jayashree 2012).

Enzyme extraction:

The extracellular enzyme was extracted by removing the cells through centrifuging the culture broth at 12,000 rpm in cooled condition for 10 minutes. The supernatant was used as crude enzyme and stored at 4°C until used. The pellets were washed repeatedly with 0.1 M phosphate buffer and mixture of 200µl 1% SDS-chloroform followed by vertex mixing for extracting intracellular enzyme through cell lysis.

Enzyme assay:

Enzyme activity was determined by measuring the release of O-nitrophenol from ONPG (o-nitrophenyl-a-D-galactopyranoside) at 420 nm. 100 µl of cell lysate was taken in a fresh tube and 900µl of buffer. A mercapto ethanol mixture was added and incubated in water bath at 37^0c for 10minutes. The reaction was started by the addition of ONPG and incubated at 37˚c in water bath. After 20min the reaction was stopped by adding 500 µl of Na_2Co_3.The O. D was recorded at 420 nm in UV spectrophotometer. One unit of enzyme activity was equal to the release of 1µ.mol. of p-nitro phenol/min (Noah 1997, Atlas 1995).

Quantitative estimation of protein:

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. All dilutions were done from the 0.1 mg/ml stock solution (Bradford 1976).

Immobilization of enzyme:

The partially purified enzyme was mixed with 3 % sodium alginate and the mixture was then suspended in chilled $CaCl_2$ solution for preparing immobilized beads.

Media and Parameters Optimizations

Optimization of Culture Conditions for Enzyme Production: The effects of time, temperature and pH on the production of the enzyme were studied. These were carried out by cultivating the isolate at different times (6- 96 hrs), different temperatures (20-60°C) and different pH values (5-9). The galactosidase activity and the protein content were assayed.

Production of lactose free milk-

Collection of milk sample:

The three different milk samples were collected from local market, and pasteurized prior to the treatment with extracted lactase.

Quantitative estimation of milk sugar:

The milk sample was examined after treatment with lactase enzyme for quantitative estimation of sugar to determine the change in lactose concentration of milk.

The lactose has one reducing end, whereas after lactase action produces two reducing end. So the increase in reducing sugar concentration in presence of DNS reagent is basis of sugar estimation.

Production of lactose free milk:

The immobilized enzyme beads were packed in glass column, and pasteurized milk sample was filled in column by closing the outlet of column for 10-15 min for the incubation at room temperature. After incubation treated milk sample was collected in fresh beaker and examined for quantitative analysis.

Characterization of Purified Enzyme

Effect of pH and Temperature variation enzyme activity:

The pH effect was determined by using ONPG as a substrate prepared in a buffers of selected pH values in the range 5-9. The different buffers used were 0.1 M sodium acetate buffer (pH 5-5.5), 0.1 M sodium phosphate buffer (pH 6-7.5) and 0.1M Tris-HCL buffer (pH 8-9) (Jayashree 2012). The activity of the enzyme was measured at different pH after 30 min. The effect of temperature was determined by incubating the reaction mixture at variable temperature range 35-95°C for 30 min and the activity of the enzyme was measured by standard ONPG conditions (Jayashree 2012, Nehad 2001).

RESULTS AND DISCUSSIONS:

Microbial enzymes are of high industrial importance; can be used as a food supplement/dietary product and are available commercially. Galactosidase from fungal and yeast sources has been used widely and has dominated application in the industrial sector. Traces of beta galactosidase were observed in five bacterial isolates. The bacterial strains present in dairy industry effluents have been proved to be probiotic and has a greater ability to produce galactosidase. Among five isolates isolate 1, 2 and isolate 5 were positive for beta-galactosidase enzyme, where as isolate 3 and 4 were negative (Table 1).

The serially milk samples cultivated into the screening medium incorporated with X-gal, which resulted in the formation of blue coloured colonies indicating the production of β-galactosidase by the respective bacterial strains. When the individual colonies were cultured in the sporulation media, organism exhibit spore forming ability. Among the isolates one best β-galactosidase producing organism having maximum enzyme activity was selected and preceded for further investigations.

Table 1- Screening of beta galactosidase producer

Sample	Gram staining	X-gal
Isolate 1	Positive	Positive
Isolate 2	Positive	Positive
Isolate 3	Positive	Negative
Isolate 4	Positive	Negative
Isolate 5	Negative	Positive

Table 2 - Effect of Incubation Time

Time of Incubation (Hours)	Enzyme Activity (U/ml)	Concentration of Protein (µg/ml)
6	0.15	20
12	0.20	25
24	0.25	30
48	0.30	54
72	0.23	27
96	0.22	23

Table 3- Effect of Incubation Temperature on enzyme production

Incubation Temperature	Enzyme Activity (U/ml)	Concentration of Protein (µg/ml)
20	0.1	22
25	0.27	29
30	0.29	33
35	0.39	40
40	0.35	30
45	0.20	29
50	0.10	25

Enzyme Characterization

Effect of pH and Temperature on beta-galactosidase activity

Table 4- Effect of pH on enzymatic activity

pH	Enzyme Activity (U/ml)	Concentration of Protein (µg/ml)
4	0.15	18
5	0.20	29
6	0.22	33
7	0.30	40
8	0.21	30
9	0.19	29

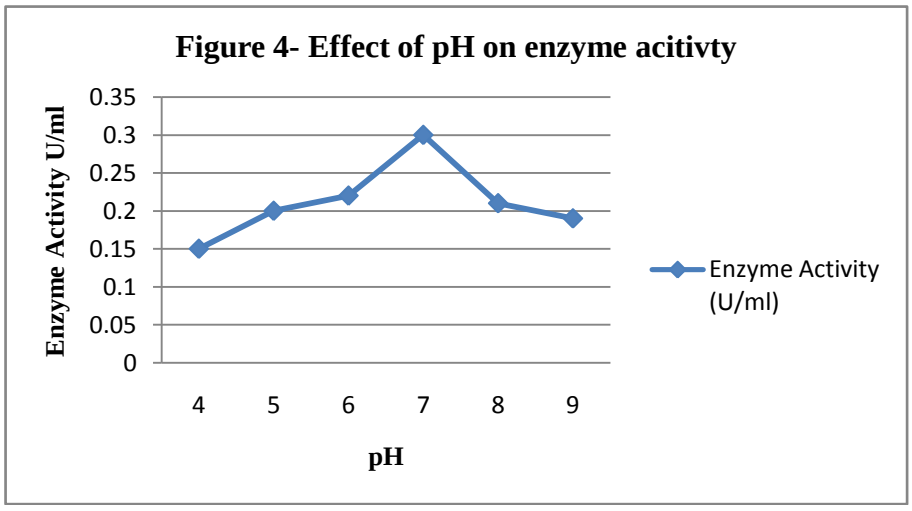

Table 5- Effect of temperature on enzymatic activity

Temperature	Enzyme Activity (U/ml)	Concentration of Protein (µg/ml)
20	0.10	10
30	0.25	17
40	0.38	34
50	0.30	30
60	0.21	13
70	0.19	11

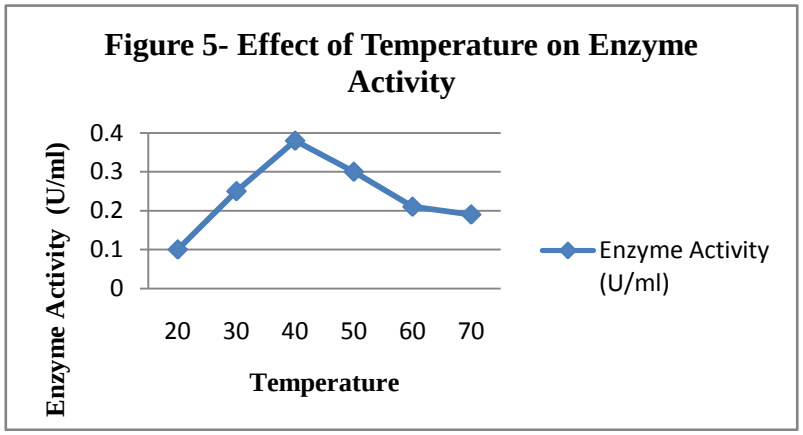

Table 6- Lactose free milk

S. No.	O.D at 540 nm Untreated milk	O.D at 540 nm Lactase Treated milk
Sample 1	0.4	0.8
Sample 2	0.45	0.9
Sample 3	0.35	0.7

Incubation time depicts the characteristics of the culture and is also based on the growth rate and enzyme production. There was a profound influence on the activity of enzyme (0.30 U/ml) at 48 hrs (Table. 2). The decrease in the enzyme activity after 48 hrs might be due to the decrease in the amount of nutrients in the medium or due to denaturation of the enzyme. β-galactosidase was produced at maximum level when maintained at temperatures of 35°C (Table. 3). The hydrogen ion concentration affects microbial productions of enzyme by affecting its nutrient uptake, the maximum production was observed at pH 7 (0.30 U/ml).
The purified enzyme was characterized based on the temperature and pH and its activity was found to be maximum at the temperature of 40°C (0.38 U/ml) (Table-5, Fig-5) and pH 7 (0.30 U/ml) (Table-4 Fig-4). The stability of the purified enzyme was determined between the temperature ranges of 35 - 45°C where it retained 100% of its activity at the temperature of 45°C and at pH 7 (90%) (Fig.4,5). The thermo stability of the enzyme was between the temperature range of 20-37°C when maintained at pH 7. A slight variation in the thermo stability range was observed at 27- 37°C. The selected milk samples were treated with microbially synthesized lactase enzyme. In all three milk samples the concentration of lactose decreases and enzyme treated sample exhibits double O.D. which is sign of increased monosaccharides concentration (Table-6).

CONCLUSION:

The manufacturing of lactose free milk and similar products have significant commercial importance as well as they are serving as a life boon for individuals suffering from Lactase deficiency. Yet there is huge scope for increasing the production rate per day and necessity to focus on cost cutting of such products by either use of immobilized enzyme in production process. Lactose is a sugar found in milk and milk products. Lactose digestion requires an enzyme called lactase. Lactase breaks down lactose into simpler sugar glucose

and galactose. The body then absorbs these simpler sugars into the bloodstream. Lactase deficiency and lactose malabsorption may lead to lactose intolerance resulting in digestive symptoms. In this context the present article emphasizes on problem of lactose intolerance and the technique for manufacturing lactose free milk and milk products.

Microbial enzymes are of high industrial importance; can be used as a food supplement/dietary product and are available commercially. Galactosidase from fungal and yeast sources has been used widely and has dominated application in the industrial sector.

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