Mini review on role of β-galactosidase in lactose intolerance

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Mini review on role of β-galactosidase in lactose intolerance

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Abstract. This review mainly focuses on the role and properties of β-galactosidase in lactose intolerance and its industrial application. β-Galactosidase, hydrolyses the lactose into glucose and galactose and it is most commonly used in food based technology, particularly in the dairy manufacturing industry. This catalyst mainly focus for the improvement of new and novel products with hydrolyzed lactose, which can be appropriate for the lactose-intolerant persons, to improve the technological, texture and scientific properties of non-fermented dairy products. β-Galactosidase derived from the group of saccharides which is a converting enzymes in the family of hydrolases. They are broadly distributed in the several biological living systems. The enzymatic hydrolysis of lactose is also preferred in food based technology due to the low soluble range of lactose. The concentration lactose was found to be high in fermented dairy products such as ice cream, butter, cheese curd, yogurt, etc.., can prompt extreme lactose crystallization bringing about items through a coarse, abrasive surface. Lactose hydrolysis in dairy products enhances adaptability also, richness altogether. These products are extra edible. Also for this purpose, the utilization of β-galactosidase enzyme prior to the condensing operation can reduce the lactose content to a point where lactose was no longer a problem industrial application of β-galactosidase. In Industries, due to the positive and constructive effect on intestinal bacterial microflora, different types of applications are possible in β-galactosidase enzyme.

1. Introduction of β-galactosidase

β-D-galactosidase also known as lactase was an enzyme or protein which catalyzes the hydrolysis of lactose. This lactose is main and foremost carbohydrate present in most of the dairy products, to monosaccharide glucose and galactose [1] to get ingested above the intestinal epithelium and has a potential significance in the dairy industry. β-galactosidase also hydrolyzes the D-galactosyl residues in polymers, oligosaccharide and secondary metabolites. β-galactosidase belongs to sub-families 1, 2, 35 and 42 of GH-A superfamily of glycoside hydrolases [2]. β-galactosidase was a well known biocatalyst which catalyzes hydrolytic and transgalactosylation reactions. In some cases it takes part in the production of prebiotic galacto-oligosaccharide (GOS), which are synthesized due to its associative transglycosylase activity [3]. β-galactosidase has two enzymatic activities: on one hand, it cleaves or sep β-glycosidic bond amongst galactose and its organic residues and also cleaves cellobiose, calories, collaterals and cellulose. On the other hand, it catalyzes the transgalactosylation of lactose to allolactose [4]. There are of two types of lactases, neutral and acidic, based on their optimum pH for enzyme activity. β-galactosidase was an important enzyme in food processing and pharmaceutical industries. The nutritional value of lactose was limited because a large portion of population in the world lacks this

enzyme and cannot utilize lactose, as a result of which they develop lactose maldigestion or intolerance [5]. Lactose being hygroscopic which have the good and strong affinity to absorb odors and flavors. This leads to many imperfections in frozen condition, for example crystallization in milk products, improvement of sandy or gritty texture and deposit formation [6]. This, creates a possible advantages of β-galactosidase. Enzymatic hydrolysis of lactose could help in assimilation of food enriched with lactose sugars. Lactase enzyme is essential for the lactose intolerant person, and industrially important as well, because it is utilized to avoid lactose crystallization, to increase the solubility of milk products and also solve the issue created with usage and disposal of whey which would prompt to ecological or envronmental pollution [7]. Moreover, the application of β-galactosidase could solve the problem of crystallization during storage due to low dissolvability of lactose by producing hydrolyzed milk while manufacturing caramelized. In addition, the transgalactosylation property of β-galactosidasehas prominent therapeutic applications such as treatment of disorders and the improvement of digestive supplements. It also has potential applications in bioremediation, biosensor and diagnosis [8]. The enzyme is additionally utilized as a model for studying its activity in amorphous matrices [9].

β-galactosidase transgalactosylates lactose to allolactose which is the inducer for the lac operon. This makes a positive feedback loop. Allolactose binds to the lac repressor, which then has a reduced affinity for the lac operon. The activation of lac operon prompts for the β-galactosidase synthesis. In lac operon the *LacZ* gene is generally utilized as a part in molecular science as a reporter marker to screen the expression of gene. Lactose is β-galactosidase's natural substrate. However, it can also convert other substrates as it is specific only for the galactose residue of the substrate. Numerous aglycones,for example, X-gal, *o*NPG, pNPG can be converted by β-galactosidase. *o*NPG and pNPG are the most common used substrates for enzyme assays. X-gal is used in a screening method known as blue/white screening or α-complementation [4].

2. Sources of β-Galactosidase

The enzyme was ubiquitous in nature. β-galactosidasewas produced by various organisms including plants, animals and microorganisms. It is widely distributed in nature, in many plants like almonds, peaches, apricots, apples, tips of wild roses, seeds of soy bean, alfalfa and coffee. The enzyme was also found in animal organs like in intestine, brain, placenta and testis of dogs, rabbits, snails, calves, sheep, goats, rats [10]. Moreover, lectorswere also found in human saliva, in fetuses of primates and farm animals, in tissues of rats and mice and in the plasma serum and urine of dogs [11]. β-galactosidase is produced by a number of microorganisms [12].They can be produced from yeasts such as *Kluyveromyces marxianus, Kluyveromyces lactis* (*Saccharomyces fragilis* and *Kluyveromyces fragilis*) also *Candida pseudotropicalis,* from fungi such as *Neurospora croussa, Aspergillus foetidus, Aspergillus flavus, Aspergillus phoenicis, Aspergillus niger, Aspergillus oryzae, Mucor pusillus* and *Mucor meihei* [13], from bacterial cultures like *Bacillus megaterium, Bacillus coagulans, Bacillus stearco-thermophillus, Bacillus circulans, Escherichia coli, Thermus aquaticus, Streptococcus lactis, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus helveticus, Lactobacillus thermophila* [14]. Thermophilic lactic acid bacteria (LAB) also produce β-galactosidase. The β-galactosidase of these cultures shows high stability and activity at high temperatures. These microorganisms are used for scientific studies for the following reasons:

a) Fermented milk items have no adverse or confrontational effects on lactose maldigesters b) These bacteria are Generally Regarded as Safe (GRAS) so the galactosidase enzyme derived from them might be used without extensive purification. c) Selection of suitable strain might help in manufacturing enhance lactose absorption. [15].

3. Properties of β-Galactosidase

Properties of β-galactosidase from various sources differ with the organism. Applications of lactase rely on its operational pH range. According to the pH of the enzyme, it can be distributed into two types: acidic condition enzymes from fungi and neutral from bacteria and yeast [3].

Fungal lactases have an optimum pH in the range of 3.0-5.0. Enzymes from fungi are used on behalf of acid whey hydrolysis and permeate. Additionally, they have generally high optimum temperature between 55-60°C. Fungal β-galactosidase is not pure when compare to yeast enzyme because they may also contain other different enzymes for example amylase, lipase and Protease. Subsequently, applications of fungal β-galactosidase have been restricted to high acid products and pharmaceutical preparations. Fungal β-galactosidase are thermostable proteins but they are sensitive to product inhibition by galactose. Filamentous fungi use lactose at very low rates [16].

β-galactosidase from yeast require a best pH range from 6.0-7.0. Thus, they are utilized generally for lactose hydrolysis in milk and sweet whey with pH 6.6 and 6.2. Yeast β-galactosidase are created in high yields at relatively low prices due to its low heat stability and are also considered as safe for use in foods. If the temperature is increased above 55°C, the enzyme gets quickly inactivated. To stay away from the issue of microbial spoilage, hydrolysis wasfrequently carried out at 4-6°C for 16-24 h [3]. Lactose fermenting yeasts produce intracellular β-galactosidase. Some commercially available yeast β-galactosidaseunder the trade name of *Maxilact* and β-galactosidase are extracted from *K. lacteals* and *Lactozyme* from *K. fragilis*.

β-galactosidase from bacteria ensure an optimal unbiased pH range from 6.5-7.5. They have an best temperature from 50-60°C. The molecular weights of bacterial enzymes are in the range of 20,000-50,000kDa. Thermophilic bacteria also produce thermostable β-galactosidase.

β-galactosidase was an vital catalyst in the human living body, which is lack that can cause Morquio B syndrome or Galactosialidosis. The molecular weight of β-gal changes with different organisms. Divalent cations such as magnesium and manganese improve β-galactosidase activity, whereas positive or negative effects can be exposed from monovalent cations [5]. Ca^{+2} ions are inhibitors of β-galactosidase, however, the calcium present in milk is bound to casein. As it is not free in the milk, calcium does not inhibit the activity of β-galactosidase [3].

4. Lactose intolerance

Lactose is made up of disaccharide made up of two monosaccharides: glucose and galactose, the primary and essential carbohydrates present in milk. Absorption of lactose involves lactase which is present in the brush border of small intestinal tract. Lactose intolerance remains a state of inability to hydrolyze lactose, sugar present in dairy products such as milk, curd, butter, cheese, yoghurt. Reduced availability of β-galactosidase results in higher concentrations of indigested lactose. Lactose intolerance or lactose malabsorption was assessed as a major health problem for more than 70% of the world's health problems [17].

The β-galactosidase enzyme is located on the tips of the villi. It's activity originates from lactase-phlorizin hydrolase, within the intestinal mucosa. Lactose absorption takes place by lactose hydrolysis to glucose and galactose takes place. In intestinal enterocytes monosaccharides of glucose and galactose are assimilated into the blood stream. Glucose absorbed initially is used as most vital and energy basis, while galactose as a glycolipid and glycoprotein component.

However with low β-galactosidase activity, lactose remains unabsorbed which results in intestinal discomfort because of bacterial activity [18].

The three types of lactase deficiencies are congenital, primary and secondary. The most severe among the three is congenital lactase deficiency (CLD) or alactasia. This is rare and infrequent abnormality condition in genetic level where β-galactosidase activities are completely reduced or else absent. Primary lactase deficiency (PLD) or adult-type hypolactasia or hereditary lactase deficiency is one of the common categories having a regular physiological, biological and functional process when the β-

galactosidase production is decreased in the small intestine. The genetic defect is due to inheritance of autosomal recessive trait and also decrease in β-galactosidaseactivity with age. It happens in early childhood and progress throughout the life.

Secondary lactase deficiency (SLD) can occur at any age. This condition is transient with reduced level of lactase due to uncontrolled coeliac disease, malnutrition, gasterroenteritis, inflammatory bowel disease, diarrhoea, cancer. [19].

Lactose when remains unabsorbed in large intestine leads to loose stools as this increases the osmotic load drawing in the fluids and electrolytes into lumen. The intestinal bacteria can use lactose as a substrate for its growth, the bacterial metabolism would produce flatulence releasing volatile fatty acids, methane, hydrogen and carbon dioxide .When excessive gas is produced in the intestine it leads to intestinal distension and abdominal cramps [20]. Lactose load in large intestine would cause poor absorption of calcium as it lowers the pH of the digested food and fermentation of lactose would take place with the help of colonic microflora. Symptoms include diarrhea, bloating, abdominal pain, nausea, bloating, flatulence, blanching and cramps with production of H_2, CH_4, CO_2 and short-chain organic acids are caused after the absorption of lactose or lactose containing food substances which can lead to decrease quality of daily life activities [21]. For every individual, virus-related or pathogenic infection, allergic conditions, cancer chemotherapy can cause damage in intestinal region due to β-galactosidase insufficiency.

Lactose intolerant people can endure fermented dairy products when compare to fresh milk with the same quantity of lactose. The enhanced lactose digestion appears due to auto digestion by microbial β-galactosidase [22]. Additional approach to digest lactose by drinking of unfermented milk product containing live lactic acid bacteria (For example: *Bifidobacterium longum* and *Lactobacillus acidophilus*). The β-galactosidase activity can be increased when the bile is present in *L.acidophilus* because bile increases the absorbency of cell membranes and allows lot of substrate to go in and stay hydrolyzed [23]. Pre-hydrolyzed dairy products through β-galactosidase can get enriched nutrition milk items with low quantity of lactose for lactose intolerant people. The fungal or yeast β-galactosidase is commercially available in the market. The β-galactosidase is also available in the tablet form extracted from *Aspergillus* strain (low pH) which can be immediately taken before and after having the milk products.

5. Applications of β-Galactosidase

5.1. Health

About 75% of the world's adult population are unable to consume milk and other milk products due to lactose intolerance. This problem can be solved, if lactose present in the products was hydrolyzed by β-galactosidaseto readily utilizable sugars like glucose and galactose [22]. Another application of enzymatic hydrolysis of lactose is the formation of GOS. These are indigestible compounds which act as dietary fibre. They promote the growth of intestinal bifidobacteria that are essential for healthy functioning of intestine and liver.

5.2. Food technology

High content and low solubility of lactose in dairy products for example frozen milks, ice-cream condensed milk and whey spreads, leads to lactose crystallization causing grimy and gritty texture. β-galactosidase usage in food industry can decrease the concentrations of lactose and develop the quality or value of dairy items by increasing the creaminess, softness, digestibility and sweetness of the product. Lactase also improves the utilization of high protein supplements containing milk.

5.3. Environment

In cheese industry, production of lactose as waste causes many commercial and environmental issues. Lactose hydrolysis by β-galactosidase found in whey can be used to make sweet syrup. The sweet syrup can be used as a source of sugar in confectionary, feedstuffs, soft drinks, sweets, baking, ice-cream, dairy desserts, molasses [24].

5.4. Biotechnological applications

Enzyme hydrolysis of lactose is a main biotechnological method as the hydrolysed products can be consumed by lactose maldigesters. Immobilization of β-galactosidase in liposomes is done to improve the taste of lactose-hydrolyzed milk. Cold-active β-galactosidase is used in food industries for producing lactose-free dairy products. The natural whey waste can be utilized as a low-priced, easy availability substrate for bacterial cell cultivation after the lactose hydrolysis by β-galactosidase. Whey proteins recovered through ultrafiltration and hydrolyzed to deliver numerous valuable pharmaceutical products.

5.5.Biosensor applications

Biosensors are essential tools in the field of agro diagnostics, immunoassays, screening of drug, forensics, and analysis of gene expression, gene toxicology analysis, and Pharmacogenomics. They have many advantages such as sensitivity, accuracy, reliability andlow cost. It combines the biomolecule recognition and sensitivity of signal transducers. Various biosensors are established by different bio recognition elements such as whole cell, antibiotics, nucleic acids and peptides [25].

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