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Microbial *β*-Galactosidases: Potential Industrial Applications

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A B S T R A C T

β-Galactosidase, (EC.3.2.1.23), most commonly known as lactase, which hydrolyses lactose into its glucose and galactose has potential applications in the dairy and food processing industries. This enzyme holds importance in formulating lactose-hydrolyzed products from both milk and whey for lactose-intolerant people. The enzyme is also crucial in biosynthesis of galacto-oligosaccharides as prebiotics for use in probiotics to restore gut health. Whey, the major dairy industry affluent can be used as a potent raw material for the growth of β -galactosidase producing microbes and the enzyme can further be used to hydrolyze whey thus reducing burden on soil and aquatic ecosystems. Free and immobilized preparations of β galactosidases have been exploited as biosensors in various applications such as industrial, biotechnological, medical, analytical areas. This review focuses on various sources of *β*-galactosidases, aspects of production, purification and immobilization of the enzyme and potential biotechnological applications.

Introduction

β-Galactosidase (EC 3.2.1.23, lactase, β-gal; systematic name β-D-galactosidegalactohydrolase) is the enzyme hydrolyzing the β-glycosidic bond between galactose and glucose, in lactose, a disaccharide.

One of the essential enzymes in human body, deficiency of β-galactosidase in intestine leads to lactose intolerance where people experience reduced ability to digest lactose in milk and dairy products termed as lactose intolerance. Hence β-galactosidase finds its application in dairy industry for the production of lactose-free dairy products (Champluvier *et al.,* 1988). Lactose induces crystallization in refrigerated dairy foods such as yogurts, creams cheeses etc, giving them gritty texture. Enzyme treated milk eliminates the issue of crystallization in frozen desserts and dairy products.

Whey, a byproduct of cheese making and dairy industry, is usually disposed as wastewater into surrounding lands which eventually affects the physical and chemical texture of soil gradually decreasing yield of crop (Brandão *et al.,* 1987). It mainly comprises of 55% of

milk nutrients-lactose, soluble proteins and lipids with a good proportion of minerals as well. Unwarranted disposal of whey not only results in a significant loss of nutrients but also poses pollution threat to soil.

Hence whey can be used as a potent raw material for the growth of β -galactosidase producing microbes and also can be bioconverted into value added products such as lactose hydrolyzed whey and GOS-GalactoOligoSachharides (Zhou and Chen, 2001). GOS is now widely used as a prebiotic food enriching beneficial gut bacteria like *Bifidobacteria* species.

β-galactosidase also plays an important role in genetics and molecular biology studies as a reporter/marker gene to monitor gene expression. The phenomenon is called α-complementation which forms the basis for the blue/white screening of recombinant clones.

The enzyme also has wide applications as biosensor to quantify lactoseas a quality control measure of milk and derivatives, in toxicology analysis of industrial affluents and discharges, forensics and drug screening.

This review focuses on various sources of β-galactosidases, provides an overview of significance of enzyme in food industries including whey and highlights new approaches and processes in sustainable utilisation of whey.

Sources of *β*-galactosidases

β-Galactosidases are found in plants like almonds, peaches, apples and apricots, but on commercial and industrial scale microbial sources such as bacteria, fungi, yeasts are used as they offer various advantages over plant sources such as easy handling, faster multiplication rate and high yield and ease of fermentation.

Bacterial sources

Bacterial β-Galactosidases occur mainly as intracellular enzymes (Picard *et al.,* 2005). Sources of enzyme producing bacteria include raw milk, curd, cheese, lassi, yoghurt and soil collected from dairy establishments. Amongst all bacteria studied so far, the enzyme from *E.coli* has been well explored but the industrial use of this β-galactosidase is limited to only analytical studies as enzyme from coliforms is not considered safe for food applications and also needs extensive purification steps that may increase cost of production. Therefore production of β-galactosidase from probiotic microorganisms which are safer for human is more preferred.

Lactic acid bacteria, adiverse group of *Lactococci, Streptococci, Lactobacillus and Bifidobacterium*, from GRAS group (Generally Recognized AsSafe) are regarded as good sources of β-galactosidase (Vasiljevic and Jelen, 2002). Among them yogurt bacteria like *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* so far stand as highest producers of the enzyme. The β-galactosidase of these cultures has been characterized, and is observed as stable and active at high temperatures (Kreft *et al.,* 2001). One of these probiotic organisms, *Bifidobacterium bifidum* plays major role in the synthesis of a galactooligosaccharide mixture that acts as a prebiotic encouraging the growth of beneficial colonic microflora (Depeint *et al.,* 2008). *Bifidobacterium infantis* (CCRC 14633), *Bifidobacterium longum* (CCRC 15708) and *Bifidobacterium longum* (CCRC15708) reported high enzyme activity (Hsu *et al.,* 2005). *B. longum* CCRC 15708showd highest production with Lactose and Yeast extract as C and N sources respectively at pH 6.5 and at 37 ^{0}Cafter 16 h of incubation (Hsu *et al.,* 2005). *Lactobacillus leichmannii* 313, a microaerophile, demonstrated ~ 5- 30-fold higher enzyme production when grown in static culture (i.e., those grown without shaking) than in the shaken cultures. β-galactosidase extracted from *S. thermophilus* is active at neutral pH and is more heat stable than the widely used fungal enzyme from *Kluyveromyces lactis* (Tari *et al.,* 2010).

Yeast sources

Yeast is one of the most commercial sources of the enzyme universally present at all dairy environments. Yeast β-galactosidases intracellular, are ideal choice to hydrolyze lactose in milk at slightly acidic to near neutral pH. β-galactosidase production by the yeast isolate *Kluyveromyces marxianus* also reported highest enzyme activity when grown on whey permeate fortified with 0.3% yeast extract, 0.05% magnesium sulphate,0.1% urea, at pH 5.5 and temperature 30°C after incubating for 28 h (Shweta Kumari *et al.,* 2011). Two strains of *Kluyveromyces fragilis* (145 and 276) and one of *Kluyveromyces lactis* also reported high β-galactosidase production at 35^{0} c and at pH 4.6 with deproteinized cheese whey added with 0.3% ammonium sulphate and 0.1% yeast extract (Maria De Fatima and Barbosa *et al.,* 1985).

Fungal sources

Fungal β-galactosidases are secreted as extracellular enzymes and show catalytic optimum at acidic pH ranging from 2.5 to 5.4and hence are more suitable for lactose hydrolysis in acidic whey and its permeates. Additionally, they display high activity 55-60°Cmaking them thermotolerant to withstand milk processing temperatures. Though they are acid stable and heat stable, fungal β-galactosidases are found to be more sensitive to galactose inhibition. β-galactosidase preparations from fungi may not be harvested in pure form as other enzymes namely amylase, lipase and protease also are co-secreted into the medium. This may subsequently restrict their usage to acidic range products and crude preparations and increase further purification steps. Extracellular β-galactosidase produced from *Aspergillus oryzae* is used widely on commercial scale. *A oryzae* derived β-galactosidase was reported to be active at pH 5 and at a temperature of 50^0Cand more suitable for treatment of whey while *Aniger*'s extracellular β-galactosidase reported to be more efficient to remove galactoses from oligosaccharides and polysaccharides that are of plant origin as compared to lactose hydrolysis (Kazemi *et al.,* 2016). Good yields of the enzyme with high activity were reported by both solid-state fermentation and submerged fermentation (Kennedey, 1987; Iwashita, 2002). A marine fungal isolate MF S4-11collected from Bay of Bengal near Vishakhapatnam showed maximum enzyme activity when incubated at 28^0C on a rotary shaker for 5 days (Anumukonda *et al.,* 2010).

Actinomycetes sources

This group of microbes has not been explored widely so far for β-galactosidase production and utilization. Crude β-galactosidase from *Streptomycessp* strain YB-10 showed maximal activity at pH 6.0 and 60^0Cwith p-NPG as substrate (Chang-JinKim *et al.,* 2003). β-galactosidase produced from another strain *Streptomyces thermocarboxydus* strain NBRC 16323 showed maximum activity at pH8 and 41^0C when incubated for 72hrs with whey and casein as C and N sources respectively (Neti *et al.,* 2025).

Recombinant β- galactosidases

Recombinant DNA technology when combined with optimization of process parameters and enzyme kinetics provides broadened potential to produce β-galactosidase with desired properties for further applications. β-galactosidasegene bgaB from thermostable *Bacillus stearothermophilus* when cloned and expressed in *B. subtilis* WB600 reported high activity of hydrolysis of lactose in milk. Enzyme kinetics and half life at 65°C and 70°C were reported to be 50h and 9h, respectively thus rendering it more suitable for both lactose hydrolysis and galactooligosaccharides synthesis in milk processing (Chen *et al.,* 2008). Recombinant β-galactosidasegene from thermoacidophilic *Alicyclobacillus acidocaldarius* was found to be stable with high activity at 65°C (Di Lauro *et al.,* 2008). Recombinant yeasts were designed with β-galactosidaseas reporter gene and combined with yeast estrogen screening assays. This innovation was successfully employed to monitor industrial waste water samples and the presence of bioactive effluents in them was confirmed (Bazin *et al.,* 2017). Recombinant β-galactosidase production from *Kluyveromyces sp* by tagging with cellulose binding domain (CBD) demonstrated one step purification and efficient immobilization on magnetic cellulose supports. This CBD tagged recombinant enzyme showed 7x higher thermal stability after immobilization and 1.2x high affinity towards lactose when compared to free enzyme (Gennari *et al.,* 2022).

Production and Purification

β-galactosidaseis an inducible enzyme and is produced when inducers like galactose, lactose, glycerol, thiogalactopyranoside, IPTG are added to the growth media. Enzyme is extracted from cell cultures by physical cell disruption methods like sonication and chemical methods like lysozyme-EDTA, sodium dodecyl sulphate (SDS)-chloroform treatments. Enzyme yield from *Lactobacillus* was highest with sonication method (Shrushti Makwana *et al.,* 2017) While permeabilization of *Kluyveromyces marxianus* cells with 50% (v/v) ethanol was effective at 25°C for 15 min and showed 90% hydrolysis of lactose when incubated for 150 min (Panesar *et al.,* 2007). The enzyme activity is found to be enhanced by the presence of various metal ions such as Mg^{++}, K^{+}, Mn^{++}, but is inhibited by Ca^{++}, Fe^{++} and other heavy metal ions (Panesar *et al.,* 2006).

Fungal β-galactosidases are efficient at slightly acidic range ie pH of 3- 5.4 while yeast and bacterial β-galactosidases are active at nearly neutral pH 6.0–7.0. pH range of natural lactose sources vary between 3.5 - 5.5 of acid whey and 6- 6.6 of sweet whey and milk. Enzyme assay is performed by determining reduction in lactose

concentration as well as increase in the resulting glucose and galactose (Nickerson *et al.,* 1976). Though lactose is the natural substrate for the enzyme, ONPG is ideal to monitor progress of the reaction efficiently by estimating the chromogen o-nitrophenol.

Crude enzyme is extracted and purified by a number of methods such as DEAE Cellulose Chromatography (Naoko *et al.,* 2010), Gel Permeation Chromatography and Ammonium Sulphate fractionation (Chanalia *et al.,* 2018; Alikkunju *et al.,* 2016) are attempted successfully.

Many microbes including *Streptococcus thermophilus* (Princely *et al.,* 2013), *Alteromonas* sp. (Li *et al.,* 2019), thermostable *Bacillus licheniformis* (Bekler *et al.,* 2015), *Streptomyces sps* (Lee *et al.,* 2003), *Kluyveromyces fragilis* (Maria De Fatima *et al.,* 1985), *Aspergillus oryzae* (Gargova *et al.,* 1995) are grown commercially for β-galactosidase production.

Immobilization of the enzyme

Immobilization of enzymes is a vital approach to achieve reusability of enzymes and reduce high costs of production and purification processes. Immobilization of whole cells eliminates further purification steps while immobilization of partially purified enzymes stabilizes its activity against denaturating parameters like pH, temperature, presence of additives and inhibitors.

Immobilized β galactosidase from *Aspergillus oryzae* on concanavalin A layered Caalginate-cellulose beads demonstrated an improved temperature-activity profile than free enzyme and 65% activity is reported even with 5% galactose which is otherwise an inhibitor to the enzyme (Shakeel Ahmed Ansari and Qayyum Husain, 2011). Adsorption and cross linking of β-galactosidase from *Lactobacillus plantarum* HF571129 onto ZnO nanoparticles was reported where the immobilized enzyme demonstrated an increase in Km- Vmax values from 6.64 to 10.22 mM and 147.5 to 192.4 µmol/min/mg respectively than that of free enzyme. The temperature and pH profile also was found to be widened from 50°C to 60 °C and pH 5 to 7.5. Moreover 90% enzyme activity was retained with immobilized β-galactosidase even after one month of refrigerated storage conditions while 74% activity was shown by the native enzyme under identical conditions (Selvarajan *et al.,* 2015). Similar results were reported with β-galactosidase from *Kluyveromyces lactis* immobilized on silicon dioxide nanoparticles by glutaraldehyde and the immobilized enzyme exhibited improved activity at high temperatures and also 50%activity observed even after eleventh reuse (Verma *et al.,* 2012).

Liposome based microencapsulation with lipid vesicles as carriers also was successfully attempted with β-galactosidase to enhance stability and reusability. 75% of initial activity of encapsulated enzyme in the presence of protease even 24 h exposure was reported showing high resistance to proteolytic degradation. (Rodriguez-Nogales and Delgadillo, 2005).

Many other novel and innovative entrapment and adsorption techniques were employed successfully to enhance the functionality and kinetics of β-galactosidase like entrapping *Aspergillus oryzae* derived enzyme with tosyl chloride activated cotton cloth (Albayrak and Yang, 2002), *Kluyveromyces lactis* β-galactosidase onto thiopropylagarose (Ovsejevi *et al.,* 2004), immobilizing *Kluyveromyces fragilis* β galactosidase with alginate-carrageenan gels beads (Mammarella and Rubiolo, 2005), adsorption onto polyclonal antibody bound cellulose for *A oryzae* derived β galactosidase (Haider and Husain, 2009). These immobilized enzyme systems offer promising potential to obtain high and significant yields and also sustainable affluent management in industrial scale-up.

Applications of *β*-galactosidase

Lactose hydrolyzed milk products

Absorption of Lactose, a disaccharide found in milk and other dairy products, requires the activity of *β*-galactosidase enzyme. While it is present abundantly in milk feeding infants, it is reported that 75% of adult population exhibits decrease in enzyme activity resulting in lactose intolerance (Sitanggang *et al.,* 2016). Such affected people experience abdominal pain and distention, diarrhoea, flatulence, nausea and cramps as undigested lactose is fermented by colon microbes releasing short chain fatty acids and build up of gases H_2, CO_2, and sometimes methane. Lactose intolerance can be treated broadly in two ways; one is oral intake of exogenous enzyme formulations just before consumption of milk products and the other is opting for lactose hydrolyzed dairy products for consumption. Oral formulations of exogenous enzyme are derived from acid tolerant Aspergillus strains to function in low gastric pH (Francesconi *et al.,* 2016; Panesar *et al.,* 2007). Lactose hydolyzed dairy products by microbial β-galactosidases

offers a more user friendly solution (Rao and Dutta, 1978). Yeast derived β-galactosidases are active at pH 6.5–7 to hydrolyze sweet whey while the enzyme from *A niger* with optimum pH 2–4 are more suited for hydrolyzing acid whey (O'Connell & Walsh, 2009) Lactose being hygroscopic tends to crystallize in ice cream, other condensed milk products causes gritty texture. Treating milk with β-galactosidase restores softness and creaminess and also helps in digestion (Nivetha & Mohanasrinivasan, 2017; Zadow, 1992; Stevenson *et al.,* 1983).

Whey Utilization -as growth mediumand Lactose hydrolsis

Whey a by-product in paneer and cheese making comprises of lactose (44–52 g/l), proteins (6–8 g/l) and minerals (4.3–9.5 g/l) approximately. Ultrafiltration of whey yields WPC-Whey Protein Concentrate and the resulting permeate still contains considerable amounts of lactose. Whey can be successfully used as substrate for cell cultivation (Prashar *et al.,* 2016) and also to produce several pharmaceutical intermediates (Domingues *et al.,* 2005). Hydrolyzed whey found its application as sweetener in canned fruit syrups, softdrinks, bakery and confectionary.

β-galactosidase production was reported with fungus *Trametes versicolor* J5at pH 6and at 32.5°C when incubated for 96 hours and with Actinomycetes isolate *Streptomyces thermocarboxydus* strain NBRC 16323 at pH 8and 41^{0}C when incubated for 72hrs with whey as C source (Elias *et al.,* 2019; Neti *et al.,* 2025).

Streptococcus thermophiles and *Lactobacillus delbrueckii* also reported high enzyme production enzyme in a growth medium enriched with whey and corn liquor (Cote *et al.,* 2004). Lactose hydrolysis of whey was found to be high with β-galactosidase from *Aspergillus oryzae* at 55∘C and from *Kluyveromyces lactis* at 10^{0}C (Dutra Rosolen *et al.,* 2015). With this application not only process costs are reduced but whey can be successfully eliminated.

Biosynthesis of Galacto-Oligosaccharides (GOS)

GOS are produced by the transglycosylation activity of β-galactosidase during the hydrolysis of lactose. They are the nondigestible oligosaccharides that are used as prebiotics to enhance health promoting gut bacteria like *Bifidobacterium sp.* and *Lactobacillussp.* and assist to bring down serum cholesterol levels, improve liver function, lower the risk of colon cancer both in adults and children. Galactooligosaccharides (GOS) synthesis from *porungo* cheese whey was reported with β-galactosidase from *Kluyveromyces lactis* with two immobilization strategies -one with calcium-alginate support and second with calcium-ConcanavalinA support.

Highest yields of GOS (63.2%) was reported with the former immobilization system with alactose turnover of 61.4% (Lais S. Bolognesi *et al.,* 2022). Yield of GOS depends on factors such as source of β-galactosidase, reaction conditions and nature of substrate. A maximum of 43.8% GOS production is reported with *B. angulatum*, while with *B. pseudolongum* yield is 26.8% (Rabiu *et al.,* 2001). Recombinant β-galactosidase is producedby expressing *B. infantis* genein *Pichiapastori* expression system (Mahdian *et al.,* 2016).

β-galactosidase as biosensor

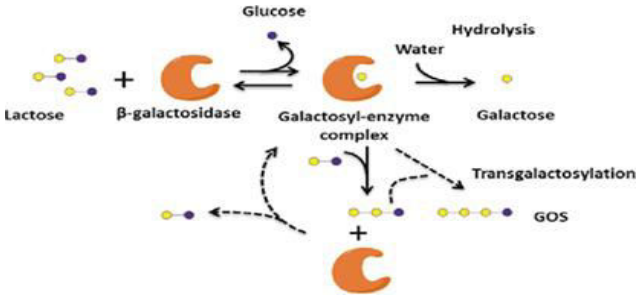
Biosensors are important tools in immunoassays, toxicology analysis, forensics, gene identification, high quality food processing and drug screening. Efficiency of biosensors depends on the choice of immobilization of enzymes on the transducer surface. Immobilization of the *E.coli* derived β-galactosidase on the silicon surface by glutaraldehyde binding showed to maintain 80% of activity at 24^{0}C till 10 days (Betancor *et al.,* 2008).

Berlin blue based biosensor methodfor β- Galactosidase and Glucose Oxidase exhibited high sensitivity to detect glucose and lactose in food products (Lukacheva *et al.,* 2007). β -galactosidase based biosensor found to identify mastitis affected cows and buffaloes based on the principle that lactose yield from the milk of these animals is very low (Pfeiffer *et al.,* 1990).

A hybrid biosensor by incorporating Glucose oxidase is evaluated in a FIA (Flow Injection Analysis) system that determined lactose concentrations ranging from 1 to 30 g/L in less than three minutes and found to be stable for four months (Ferreira, 2014).

Biosensor based wastewater screening for toxicity monitoring is reported by recombinant β -galactosidase activity in yeast combined with estrogen screening assays (Bazin and Seo, 2017).

Figure.1 GOS synthesis (courtesy- Huifang Yin and Bultema et al., 2017)



Cold Adapting *β*-Galactosidases-A Novel Approach

Cold active enzymes area vital survival mechanism for microbes in cold environments, as it is known that a drop of 10 °C in temperature causes a 2–3fold decrease in the reaction rate. Cold-active β-galactosidasesare produced from *Alteromonas*, *Alkalilactibacillus* and *Pseudoalteromonas* species that inhabit various deep lakes and glaciers of Antarctica (Skalova *et al.,* 2005; Li *et al.,* 2019; Hoyoux *et al.,* 2001). This novel finding finds its application in the production of lactose- free milk at 4 –8º C before pasteurization and packaging (Dekker *et al.,* 2019). Hydrolysis of cheese whey permeate by cold active β- galactosidase from *Pseudoalteromonas haloplanktis* yielded D-tagatose, which is used as natural low-calorie sweetener Van De Voorde (2014). This process was designed at 23º C, a temperature that requires no cooling or heating of the tank.

β-galactosidaseis one of the significant enzymes in food processing and dairy industries. The enzyme in both free and immobilized forms has been applied extensively in related domains like bioremediation and biosensor development for diagnostic and quality control of the processes. Production of novelgalacto-oligosaccharides by the enzyme redesigned the usage of prebiotics as nutritional supplements. Remediation of whey by β-galactosidase during downstream processing has potential to bring down pollution of water bodies in addition to yielding valuable by-products such as sweet syrup. Cold active β-galactosidases from psychrophilic microbes opened up new possibilities of milk and whey processing even at low temperatures.

Studies on molecular aspects of β-galactosidase kinetics, protein engineering to enhance pH and temperature tolerance to suit constantly evolving needs of dairy and food industries, regulation of secretion as extracellular enzyme by site directed mutagenesis, choice of nanoparticles to achieve maximum reusability and reproducibility can be explored to utilize the vast potential of the enzyme.

Conversion of these pilot scale technologies to potential scale up opens new opportunities in reutilization of the enzyme in downstream processes of commercial practices.

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Author Contributions

Kalyani Neti: Investigation, formal analysis, writing—original draft. Swati A. Peshwe: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict(s) of Interests The authors declare that there is no conflict of interests with anyone regarding the publication of this article.

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