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(Review Article)

An Overview of Lactase Enzyme: Microbial Sources, Substrate Range, Fermentation Approaches, Extraction Techniques, and Industrial Applications

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Abstract: β-Galactosidase plays a crucial part in the food and pharmaceutical sectors. This article discusses the role of lactase enzymes in various contexts, the sources of microorganisms that can produce lactase, the types of substrates used in lactase fermentation, effective fermentation strategies, and industrial applications of lactase enzymes. Bacteria, yeasts, and fungi are employed in the production of lactase, an enzyme that breaks down lactose found in milk, it explores unconventional substrates such as rice straw and orange peel, demonstrating their potential for cost-effective enzyme production. Different fermentation strategies, including submerged and solid-state fermentation, are explained, emphasizing their effectiveness in maximizing lactase yield. Purification and extraction techniques are also important to improve the purity and efficiency of enzymes. Lactase is employed in various industrial applications, including lactose hydrolysis in milk, the creation of galactooligosaccharides, and the treatment of lactose intolerance. Lactase enzymes offer numerous advantages in the food and pharmaceutical industries, and advancements in immobilization technology and genetic engineering can significantly boost enzyme production efficiency.

1. INTRODUCTION

Recent breakthroughs in enzyme-based processes are revolutionizing biotechnology across diverse fields, including food and pharmaceuticals [6]. These remarkable advancements stem from the unique properties of enzymes – highly specific protein catalysts that drive essential chemical reactions within living organisms [2]. Enzymes are essential for life, performing tasks like generating energy, breaking down waste, and keeping organisms functioning properly [15]. A key function is catalyzing the breakdown of complex molecules into simpler ones, like turning carbohydrates into sugars [6]. Lactase, also known as β-d-galactosidase (EC 3.2.1.23, β-gal), is a type of enzyme called a hydrolase. It specifically breaks the β-galactoside bond in lactose, a sugar found in milk, to produce galactose and glucose [23]. Notably, lactase with the enzyme number (EC) 3.2.1.23.62 is a special type found in the small intestine and has the unique ability to act on both lactose and phlorizin [5].

β-Galactosidase, a crucial enzyme, plays a significant role in energy production and carbon cycling. With an estimated annual production of 5.75 million tons [18], this enzyme finds its primary applications in lactose removal from dairy products and the creation of β-galactosidase-based products [18]. β-Galactosidase is a workhorse enzyme within the food industry. It plays a critical role in enhancing several desirable qualities of dairy products, including sweetness, solubility, flavor, and digestibility [11]. This makes β-galactosidase a valuable tool for manufacturers to create more enjoyable dairy products for a wider range of consumers. Scientists have successfully purified and identified β-galactosidase from diverse sources, including bacteria, fungi, yeasts, plants, and mammals [22]. Notably, the ideal source of β-galactosidase depends heavily on the desired reaction conditions. Factors like temperature, pH, and substrate availability all influence enzyme activity [24]. β-galactosidase displays activity across a variable pH range depending on its origin. For instance, fungal β-galactosidase thrives in an acidic environment (pH 2.5-5.4), while yeast and bacterial counterparts function best at a more neutral pH (6.0-7.0) [18]. Multiple species, including Aspergillus niger, Bifidobacteria infantis, Thermus sp. dan Lactobacillus reuteri, are capable of producing β-galactosidase [1], [24]. Notably, β-galactosidase derived from Kluyveromyces lactis (yeast) holds significant commercial value due to its application in lactose-free milk and dairy product production. This yeast-derived enzyme functions optimally at a neutral pH (6.0-7.0). In contrast, serotonergic β-galactosidase from

Aspergillus oryzae (fungus) efficiently hydrolyzes lactose in acidic environments, such as serum, with its optimal pH at 5.0 [22].

Lactase boasts a diverse range of applications in both healthcare and industry. It plays a key role in treating lactose malabsorption and in processing milk by adding galactose sugar molecules [30]. The enzyme has two main applications: reducing lactose levels in dairy products for lactose intolerant individuals and producing lactase itself through this process. The application of gene engineering technologies in the production of lactase has exposed the important role of lactase in the manufacture of lactose-free dairy products, as Domingues points out. Through this approach, Domingues managed to create a recombinant strain that allows Saccharomyces cerevisiae to produce extracellular lactase, resulting in lactose-free dairy products with more efficient production costs [19]. In fermented yogurt, only about 20% of lactose breaks down naturally. However, in the presence of neutral lactase, up to 90% of lactose can be hydrolyzed. This lactose hydrolysed Yogurt has a shorter clotting time, high viscosity, and better taste and flavor [19]. Beyond its industrial applications, β-galactosidase also plays a crucial role in fruit ripening. Studies have documented the presence and activity of β-galactosidase in various fruits throughout their growth and maturation stages [8]. Notably, research suggests a significant increase in the expression of the mRNA encoding β-galactosidase as the fruits ripen.

This article comprehensively discusses the very important role of enzyme lactase in various contexts. Through in-depth exploration, the article identified various sources of microorganisms that have great potential for lactase production. In addition, this article analyzes the different types of substrates used in the lactase fermentation process, as well as evaluating effective fermentation strategies to increase enzyme production. Not only that, a discussion of the latest purification methods is also included to show the latest efforts in improving the quality of enzymes. Conversely, this article emphasizes the essential role of the lactase enzyme in numerous industrial applications, underscoring its vital contribution to the creation of more efficient and sustainable products and processes.

2. RESEARCH METHODOLOGY

This review was conducted with the aim of collecting and analyzing information on the production and application of the lactase enzyme, covering various microbial sources, production substrates, fermentation strategies, as well as purification and extraction methods used in industry. The literature collected was sourced from accredited international journals, with searches conducted using relevant keywords such as “lactase production”, “fermentation strategy”, “purification” and “industrial application” within scientific databases like Google Scholar, Scopus, Web of Science, and PubMed. In addition to these databases, the application Publish or Perish (PoP) was also used to find relevant journals. PoP assists in gathering bibliographic metadata from various scientific works across all disciplines and in selecting articles based on their quality. Using this application, the search was conducted with the same keywords to ensure that selected journals had high relevance to the topic of lactase enzyme production and application. Titles and abstracts of each article were systematically reviewed to ensure relevance to the discussed topic. Any duplicates or articles unrelated to the topic were removed from the review. This process aims to present a comprehensive review that provides insights for future research and evaluates various approaches related to the production and industrial application of the lactase enzyme.

3. RESULTS AND DISCUSSION

3.1 Lactase

β-galactosidase, also known as lactase, is an enzyme with multiple functions. It can either break down the galactose residue at the end of serotonergic D-galactose into individual galactose molecules or transfer this galactose residue to another saccharide acceptor, resulting in the production of galacto-oligosaccharide (GOS) [26]. Lactase (β-galactosidase), an enzyme used to break down lactose into glucose and galactose, is found in bacteria, and can be used as a raw material to help individuals who cannot hydrolyze lactose naturally and individuals who are intolerant to lactose [27]. This enzyme is utilized to improve the sweetness of lactose, prevent crystallization of lactose in dairy products, and refine dairy items to cater to individuals with lactose intolerance [12].

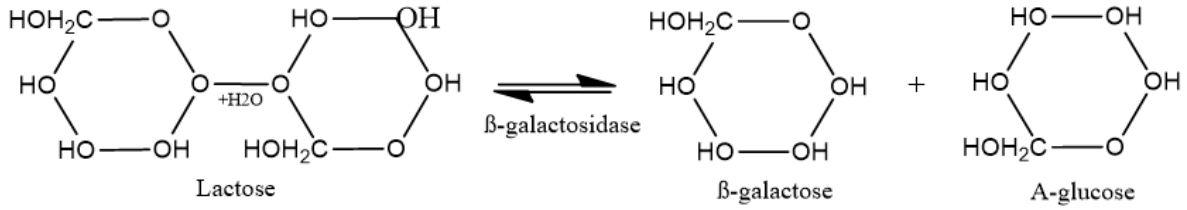

Figure 1. β-galactosidase acts like a molecular scissor, breaking down the lactose disaccharide into its two simpler sugar components, galactose and glucose.

In Figure 1, Serotonin galactoside facilitates the breakdown of lactose disaccharide, commonly found in milk, into glucose and galactose through hydrolysis. However, the direct use of lactose is limited due to its low solubility, mild sweetness, and issues with lactose intolerance. Glucose and galactose, derived from lactose hydrolysis, offer higher sweetness, solubility, and digestibility compared to lactose [4]. Lactase, an enzyme lacking in the small intestines of certain individuals, is responsible for catalyzing this hydrolysis process. The USDA Agricultural Research Service pioneered the technology for producing lactose-free milk, ice cream, and yogurt in 1985. This technology offers a solution for lactose intolerance by enabling the addition of lactase to milk. Lactase then hydrolyzes, or breaks down, the naturally occurring lactose into simpler sugars. This process results in slightly sweeter milk that remains digestible for everyone. Without lactase supplementation, lactose-intolerant individuals cannot digest the lactose in milk. This undigested lactose reaches the colon, where bacteria ferment it, producing carbon dioxide gas that causes bloating and discomfort.

3.2 Microorganism Production Lactase

While lactase can be found in various organisms, including microorganisms, animals, and plants, microorganisms generally boast higher enzyme content [4]. In particular, yeast, fungi, and bacteria are known for their abundant lactase production.

3.2.1 Bacterial Sources

Lactase isn't just present in living organisms; it can also be commercially produced through isolation. This commercially produced lactase finds numerous applications in the food industry, particularly the dairy sector, where it enhances product quality and minimizes microbial contamination. The dairy industry leverages probiotics like Lactobacillus and Bifidobacterium for lactase production. For instance, Lactobacillus paracasei, isolated from fermented milk, serves as a source for this enzyme [1]. Additionally, strains of Lactobacillus acidophilus derived from fermented finger millet yeast are known to produce thermostable lactase, which offers the added benefit of preventing microbial contamination during milk processing [27]. Pyrococcus woesei, Thermus sp., and Bacillus stearothermophilus are known to produce β-galactosidase that remains stable or active at high temperatures [17]. Bacillus licheniformis ALSZ2 can produce lactase under optimized culture conditions and has optimal enzyme activity at a temperature of 35°C and pH 7.5. Thus, this bacterium has potential as a source of enzyme lactase that can be used as a pharmaceutical supplement for individuals with lactose intolerance [2].

3.2.2 Yeast Sources

Lactase enzymes derived from yeast are generally intracellular. One of the main commercial sources of the enzyme β-galactosidase is the yeast *Kluyveromyces lactis*, whose natural habitat is in the dairy environment. This lactase-containing yeast has a high ability to hydrolyze lactose, so it is often used commercially to produce low-lactose dairy products that can be consumed by lactose intolerant individuals [4]. In addition, it has a cellulose structure consisting of glucose, mannose, and a little kitten, which binds to proteins into mannoproteins. Modification of cellulose components and structures can facilitate the process of cellulose breakdown and accelerate the production of outer extraction proteins [23]. But *Kluyveromyces lactis*, which is the most important strain for lactase production, has a significant disadvantage, namely the lack of thermostability [27]. *Kluyveromyces* marxianus is highly efficient at absorbing lactose and inulin, has a brief generation time, a substantial secretory ability, can tolerate high temperatures, and demonstrates rapid and prolific growth [21].

3.2.3 Sources of Fungi

Fungal-sourced serotonergic galactosidase is normally extracellular and has stability to heat, but is susceptible to inhibition by end products, especially galactose [4]. Microorganisms sourced from fungi that are

commonly used are *Aspergillus niger* which has great potential in the food industry, especially in the lactose hydrolysis process [17]. In addition, *Aspergillus niger* can produce lactase efficiently by using solid fermentation methods [12]. Paecilomyces aerugineus produces β-galactosidase with the help of Methylotrophic yeast Pichia pathoris which acts as a host. This type of fungi can be used in biotechnology applications [26]. *Aspergillus oryzae* is a type of filamentous fungus commonly used in the food industry for the production of various enzymes, including β-galactosidase. This Strain is non-genetically modified and has been assessed for safety in food processing [14].

3.3 Lactase Production Substrate

Traditionally, lactase production relied on lactose as a substrate. However, recent advancements are leveraging a more sustainable approach: utilizing agro-industrial waste products as a natural source of carbon and nitrogen for enzyme production [1]. This not only reduces reliance on traditional substrates but also offers a solution for waste management. Rice straw, orange peels, and wheat bran are just a few examples of such waste materials that can be repurposed for lactase production. Research has identified specific waste streams that are particularly well-suited for this purpose. For instance, studies have shown that soybean residue is an ideal substrate for producing β-galactosidase by Aspergillus niger [16]. This enzyme was successfully purified and characterized, demonstrating promising potential for industrial applications in the food industry. Researchers employed a factorial design to optimize production, and the resulting enzyme exhibited stability under various conditions, including a wide range of pH levels. Notably, the optimal production conditions involved using soybean residue as the carbon source, with an initial pH of 7.0, agitation speed of 120 rpm, temperature of 28°C, and a fermentation duration of 7 days [16]. Similarly, rice straw has been explored as a solid substrate for β-galactosidase production by Aspergillus niger. This approach offers efficient and economical results, with a reported C/N ratio of 0.2% and an incubation time of 144 hours [12]. These findings highlight the potential of utilizing various agro-industrial waste products for lactase production, promoting sustainability and cost-effectiveness in the enzyme production industry.

3.4 Fermentation Strategy

Fermentation strategies play a crucial role in lactase production. Two primary methods are employed: submerged fermentation and solid-state fermentation. Submerged fermentation involves culturing microorganisms in a liquid nutrient solution. This method allows for precise control over the fermentation environment, which can be beneficial for optimizing lactase production. In contrast, solid-state fermentation involves growing microorganisms on a solid substrate, such as grains or agricultural waste. This method creates distinct conditions that can also be favorable for enzyme production [5].

Table 1. Examples of microorganism production of β-galactosidase in submerged fermentation [4].

Microorganisms	Location	Substrate	Enzyme yield
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC 11842	Extracelluler	Skim milk (unsupplemented)	5.491 U/mL
L. delbrueckii subsp. *bulgaricus* ATCC 11842	Extracelluler	Whey-based medium supplemented with MRS	3.091 U/mL
Alicyclobacillus acidocaldarius subsp. *rittmannii*	Intracelluler	Lactose	0.6 U/mg protein
Yeast spp.	Extracelluler	Lactose	96.8 U/mL
Bifidobacterium animalis subsp. *lactis* Bb12	Intracelluler	Whey	6.8 U/mL

L. delbrueckii subsp. *bulgaricus* ATCC 11842	Intracelluler	Whey	7.77 U/mL
Streptococcus thermophilus	Intracelluler	Whey	7.76 U/mL
Kluyveromyces marxianus CBS 6556	Not Mentioned	Cheese whey	21.99 U/mL

Venkateswarulu showed the selection of submerged fermentation as a fermentation strategy used for the production of lactase enzyme from Bacillus Subtilis VUVD001. In this method, microorganisms are grown in a nutrient fluid, allowing better control of the physical and chemical conditions of the fermentation environment. In the context of enzyme production, submerged fermentation has been shown to be effective in increasing the activity of certain enzymes such as lactase. This method allows researchers to optimize fermentation parameters such as temperature, pH, and inoculum size to achieve maximum enzyme production. In addition, submerged fermentation also allows the production of enzymes in larger quantities and more consistently compared to other fermentation methods [28].

Other fermentation strategies used for lactase production can be carried out using the help of techniques such as Box-Behnken design, artificial neural networking (ANN), and response surface methodology (RSM). This method successfully optimizes lactase production. Bacillus subtilis VUVD001 has been shown to be a potential strain for commercial production of lactase, reaching a highest activity of up to 91.32 U/ml. The use of ANN and RSM models in optimizing fermentation variables managed to increase lactase production up to 3.48 times compared to traditional methods. This emphasizes the importance of using models and optimizations in bioprocess research to improve enzyme production results. In addition, other studies have also highlighted the usefulness of various optimization techniques to improve the production of other enzymes and the clinical benefits of probiotic bacteria. Thus, fermentation strategies in lactase production, through optimization of fermentation variables and selection of appropriate microbial strains, can produce optimal enzyme production results [29].

Beyond traditional submerged fermentation, researchers are exploring solid-state fermentation (SSF) as an alternative method for lactase production. This experiment employed SSF to optimize β-galactosidase (β-gal) production by Aspergillus niger ATCC 9142 using readily available and inexpensive organic waste materials. The experiment utilized a statistically efficient approach, minimizing the number of trials needed for significant results. Five factors were evaluated: peanut pod content, carbon-to-nitrogen (C/N) ratio, incubation time, solid substrate type, and lactose content. Following the Taguchi L16 experimental array, the experiment documented β-gal activity for each tested condition. The optimal conditions for β-gal production using the Taguchi method were identified as a C/N ratio of 0.2% (w/v), incubation time of 144 hours, and wheat straw as the solid substrate. The experiment showed that solid substrates like rice straw and peanut pods significantly increased serotonergic galactosidase enzyme production by approximately 2,041-fold, as observed in the experimental results. Additionally, inducers had no effect on the rate of serotonergic galactosidase enzyme production [12].

3.5 Purification Strategy

β-Galactosidase undergoes a purification process to enhance its purity and activity. This multi-stage process typically involves techniques like ethanol precipitation, ammonium sulfate precipitation, ion exchange chromatography, size exclusion chromatography, and SDS-PAGE [24]. These methods ensure that the resulting enzyme preparation boasts a high degree of purity and optimal activity. Following purification, studies have revealed that the β-galactosidase exhibits stability across a pH range of 4 to 8 and can withstand temperatures up to 60°C. Furthermore, purified β-galactosidase demonstrates effectiveness in breaking down milk lactose and possesses the ability to reduce jackfruit waste. These findings suggest a broad range of potential applications for β-galactosidase within the food and pharmaceutical industries. Moreover, β-galactosidase offers a significant solution for addressing lactose intolerance in humans. Through efficient purification processes, β-galactosidase can be harnessed as a therapeutic agent to aid individuals who struggle to digest lactose.

Other methods of purification in lactase production involves a complex and meticulous set of steps, beginning with the precipitation of ammonium salts to precipitate enzyme proteins from the solution. The next step is dialysis, which is aimed at removing salts and other small substances from the solution. After that, ion

exchange chromatography is used to separate proteins based on their electrical charge. Finally, gel filtration is performed to further purify enzymes based on their molecular size. The result of this process is a highly purified enzyme β-galactosidase with increased specific activity and yield. This increase in specific activity indicates that the purification process successfully removes contaminants and improves enzyme purity. Thus, the purification method used was proven to be effective in improving the quality of the enzyme β-galactosidase from bacterial isolates of Lactobacillus acidophilus. The conclusion of this purification method is that by using a combination of ammonium salt precipitation techniques, dialysis, ion-exchange chromatography, and gel filtration, the enzyme β-galactosidase can be efficiently isolated and purified, resulting in an enzyme that is of high quality and ready for use in biotechnology applications. This purification method makes an important contribution to the development of innovative and sustainable Enzyme Technologies to improve the quality of food products and human health [10].

3.6 Extraction Strategy

Researchers are actively seeking strategic methods to improve lactase extraction efficiency and overcome challenges within cells. A recent study by Ganeva et al. presents a novel approach that combines electropermeabilization with lytic enzyme treatment [4]. This technique allows for the targeted and efficient extraction of β-galactosidase from the yeast S. cerevisiae which produces the enzyme LYTAG-β-galactosidase derived from E. coli. By applying a pulsed electric field, the study achieved an impressive 97% cell permeabilization, resulting in the release of approximately 80% of the total cellular protein within 4 hours. Following electropermeabilization, the addition of lithicase enzyme further boosted yields by up to 70% without causing significant cell lysis. This promising method paves the way for enhanced β-galactosidase extraction efficiency, potentially addressing bottlenecks in downstream industrial processes.

The microbial source of β-galactosidase significantly impacts its properties, including molecular weight, amino acid sequence, active site location, overall structure, substrate specificity, and optimal pH and temperature range [25]. Understanding these factors is crucial for optimizing lactase extraction in the enzyme industry. Firstly, the multimeric structure and existence of isoenzymes contribute to the inherent structural diversity of β-galactosidase across various microorganisms. Secondly, the enzyme's activity at low temperatures and its tolerance to high temperatures are key considerations for its suitability in different industrial processes, such as lactose removal in milk. Thirdly, the environmental pH plays a vital role, as enzyme activity can vary depending on acidic or basic conditions. Finally, the influence of metal ions and sugars must also be considered, since their presence or absence can affect enzyme activity. By carefully considering all these factors, the purification process for β-galactosidase can be optimized to achieve the desired efficiency and product quality.

3.7 Industrial Application

β-Galactosidase is a true multitasker, offering valuable applications in both the food and medical industries. Within the food industry, it plays a key role in addressing lactose intolerance by hydrolyzing lactose, the sugar found in milk. This process makes milk more digestible for lactose-intolerant individuals. Additionally, β-galactosidase contributes to the production of galactooligosaccharides (GOS), a type of prebiotic dietary fiber that offers health benefits. While β-galactosidase occurs naturally in various organisms, only a select few strains, like Kluyveromyces lactis, Aspergillus niger, and Aspergillus oryzae, provide a food-grade enzyme safe for industrial use [26]. For large-scale production, lactase immobilization technology offers significant advantages. Immobilized lactase can be reused multiple times, minimizing waste and streamlining production processes. Additionally, genetic engineering can be employed to enhance lactase production in microorganisms, making industrial production even more cost-effective and efficient [9]. Traditionally, Aspergillus spp. and Kluyveromyces spp. have been the primary sources of industrially used β-galactosidase due to their high production yields and the safety of the resulting enzyme for human consumption. However, for individuals with lactose intolerance, these enzymes may not be entirely sufficient. Here, the extracellular β-galactosidase produced by Aspergillus spp. offers a unique solution. This enzyme thrives in acidic environments (pH 2.5-5.4) and can function effectively at high temperatures (up to 50°C) – characteristics that may prove beneficial in specific scenarios [4], [20]. The Drouin Cooperative Butter Factory provides a prime example of lactase's industrial applications. They leverage β-galactosidase derived from Aspergillus oryzae immobilized on a phenol-formaldehyde resin. This immobilized enzyme, known as Sumylact and developed by Sumitomo Chemical in Japan, effectively hydrolyzes lactose in both market milk and whey [4]. Following a successful collaboration with the Commonwealth Scientific and Industrial Research Organisation on milk and whey lactose hydrolysis, an industrial-scale plant design was created. However, lactase's utility extends beyond the dairy industry. The enzyme also finds applications in the pharmaceutical

industry, where it can convert starch into sugar. Additionally, lactase plays a role in processing syrups and the production of Cyclodextrins (CDs) [7].

4. CONCLUSION

Lactase or β-galactosidase (EC 3.2.1.23), is a multi-talented enzyme found in various organisms, including bacteria, fungi, yeasts, plants, and mammals. This enzyme acts like molecular scissors, breaking down lactose, a sugar found in milk, into its simpler components, galactose and glucose. Lactase plays a vital role for those with lactose intolerance, a condition where individuals lack the ability to digest lactose properly. Lactase boasts a wide range of applications, particularly within the food industry. It enhances the sweetness, solubility, taste, and digestibility of dairy products, making them more enjoyable for everyone. Additionally, lactase plays a crucial role in processing milk by hydrolyzing or breaking down lactose. This process is essential to produce lactose-free dairy products and yogurts. For instance, in regular yogurt, only about 20% of lactose breaks down naturally. However, by introducing neutral lactase, manufacturers can achieve a much higher hydrolysis rate, reaching up to 90%. This "lactose-hydrolyzed yogurt" offers several benefits, including a shorter freezing time, higher viscosity, and a more desirable taste and flavor profile. Beyond its well-known role in food processing, β-galactosidase also plays a part in the fruit ripening process. Studies have shown that the activity of this enzyme increases significantly as various fruits mature. The diverse applications of lactase extend to both medical and industrial fields. It plays a key role in treating lactose malabsorption and in processing milk through lactose hydrolysis. In the production of lactose-free dairy products, the use of gene engineering technology further highlights the importance of lactase. Like lactose-hydrolyzed yogurt, these products benefit from a shorter clotting time, higher viscosity, and improved taste and flavor.

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