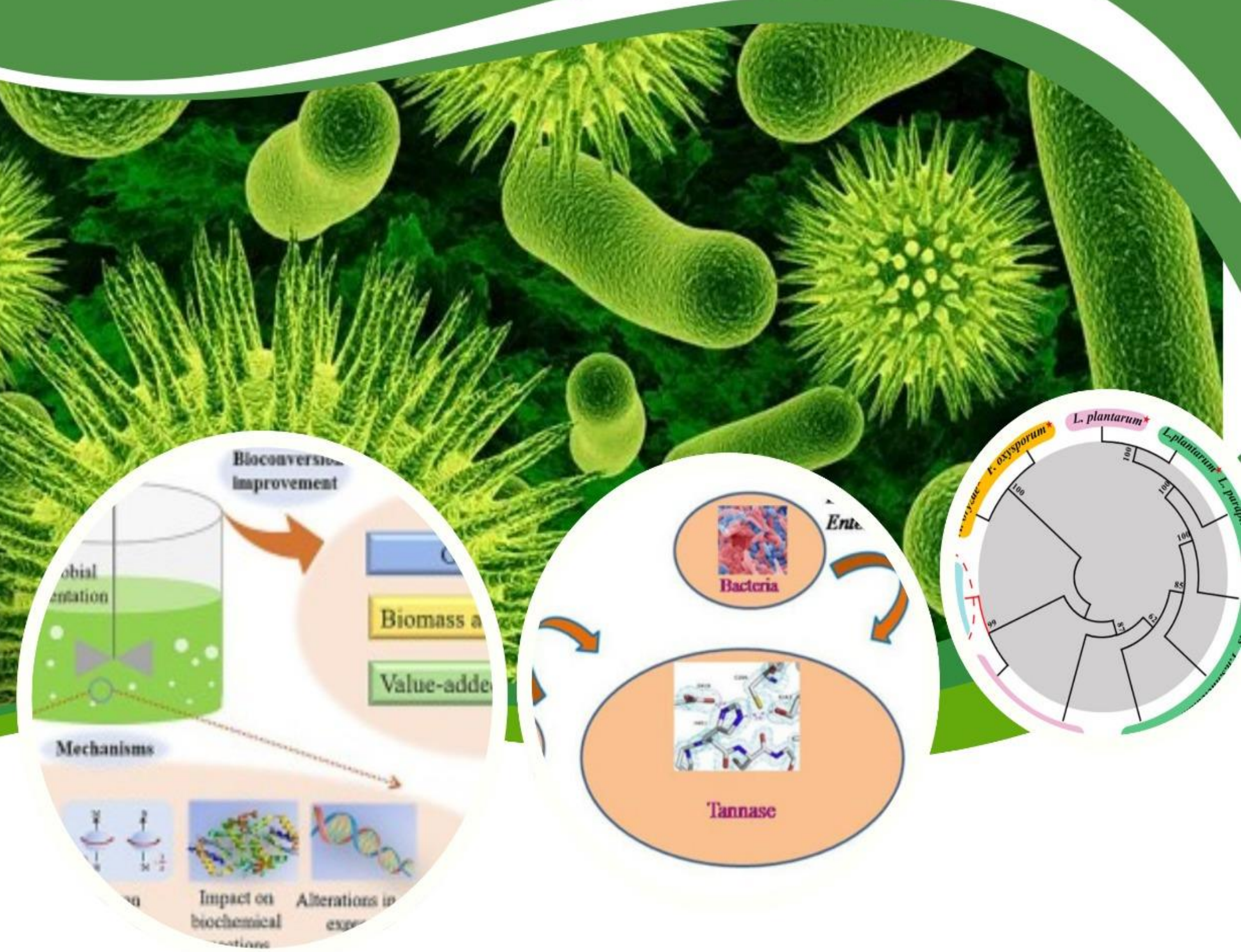

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A comprehensive review on tannase: microbial production and application

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A comprehensive review on tannase: microbial production and application

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ABSTRACT

Tannase (tannin acyl hydrolase, EC 3.1.1.20) is an important hydrolytic enzyme that catalyzes the breakdown of hydrolyzable tannins into gallic acid and glucose. Due to its wide range of industrial applications—in pharmaceuticals, food, beverages, animal feed, cosmetics, and bioremediation—the demand for tannase has significantly increased. Microbial sources, particularly fungi and bacteria, have emerged as the most efficient and sustainable means for large-scale tannase production. This review presents a comprehensive overview of tannase with an emphasis on its microbial production, including various strains used, fermentation techniques (submerged and solid-state), optimization strategies, and genetic advancements. Additionally, the review highlights the enzyme's diverse industrial applications, stability under process conditions, and prospects for future development using biotechnological innovations. This insight is aimed at fostering further research and industrial exploitation of tannase for sustainable and value-added bioprocesses.

Keywords: Bacterial Tannase, Tanninacid, Galic acid, fermentation, Application

1. INTRODUCTION

A common biocatalyst in many industrial processes, enzymes have a number of benefits over chemical catalysts. The use of enzymes is promoted by green biotechnology for industrial-scale manufacturing of chemicals, fuels, secondary metabolites, and other materials, ideally from renewable resources. Tannin acyl hydrolase (E.C.3.1.1.20), which is a member of the class of industrial enzymes known as "hydrolases" (about 65%) according to catalyses the hydrolysis of ester and depside bonds in a variety of substrates, including gallo tannins, epigallocatechin-3-gallate, gallic acid esters, and epicatechin gallate, to release gallic glucose and acid. Tannase belongs to the esterase superfamily and is an adaptive, intracellular/extracellular, inducible hydrolase [1].

NATURAL SOURCES AUR INDUSTRIAL PRODUCTION

The production of gallic acid, instant tea, coffee-flavored refreshing drinks, and acron wine are among the main commercial uses of tannase in the food, feed, beverage, pharmaceutical, and chemical industries. Additionally, tannase is utilised in the production of animal feed, the clarification of fruit juices and beer, and the enhancement of grape wine flavour. Additionally, tannase is employed in the preparation of analytical probes to ascertain the structures cells, in the treatment of tannin-containing wastewater effluents from the leather and olive industries. Gallic acid is released when the ester bonds found in complex tannins, gallotannins, and gallic acid esters are catalysed by tannase (tannin acyl hydrolase, EC3.1.1.20). It is frequently used as a clarifying agent to treat tannin-polluting industrial effluents and agricultural wastes, as well as in the production of instant tea, beer, fruit juices, and some wines[2]. Furthermore, tannase is crucial for the synthesis of gallic acid, which is a substrate for chemical or enzymatic production and a significant intermediate compound in the synthesis of the antibacterial drug trimethroprim, which is used in the food and pharmaceutical industries. Tannase was first discovered by Tieghem. There are many applications for tannase in the food, beverage, pharmaceutical, and skin care industries. Tannases are becoming more and more popular due to their ability to synthesise and hydrolyse in the right solvent systems. The tannases' complex catalytic property has increased their commercial significance. Many factors, such as high production costs and a lack of understanding about some of the enzyme, have severely limited the large-scale application of tannase. attributes. Although tannase can be found in plants, animals, and bacteria, microorganisms are the most prevalent and significant source of this enzyme because of its superior Microbial tannase production, affordability, and stability [3]. Fungi are the most common source of tannse producers among microorganisms.

TANNASE SUBSTRATE: TANNINE

Tannase primarily breaks down hydrolyzable tannins such as tannic acid, gallotannins, and ellagitannins into simpler compounds like gallic acid and glucose.Gallic acid and glucose are released when the hydrolytic enzyme tannase catalyses the breakdown of ester and depside linkages in hydrolysable tannins like tannic acid [4]. Although tannase enzymes differ in structure depending on the microbial source, they usually have an α/β-hydrolase fold, which is a common characteristic of many serine hydrolases.

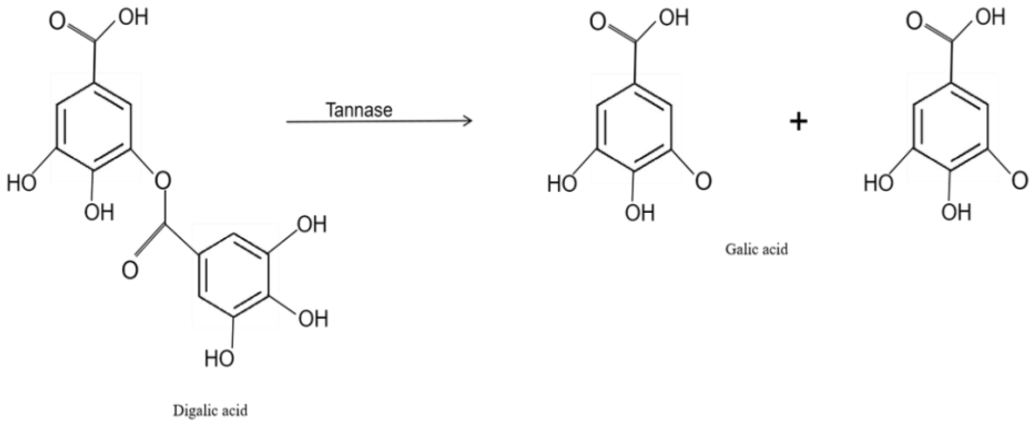

Fig : 1 Tannase substrate.

Tannins are found in many plant parts, including roots, bark, leaves, and seeds. They are the second most abundant plant polyphenol after lignins. Tannins, which are present in many plant families, including sumach and oak, are well-known for their ability to form potent complexes with minerals, proteins, enzymes, and starches. By decreasing feed intake, nutrient digestibility, and protein availability, these interactions can render tannins toxic or anti-nutritional to ruminants [5].

TANNASE PRODUCTION

Generally, bacterial tannase has been generated by two primary fermentation techniques: submerged fermentation (SmF), in which microorganisms flourish in a liquid nutrient medium, and solid-state fermentation (SSF), in which they grow on moist solid substrates with little water.

SUBMERGED FERMENTATION(SmF)

Generally, bacterial tannase has been generated by two primary fermentation techniques: submerged fermentation (SmF), in which microorganisms flourish in a liquid nutrient medium, and solid-state fermentation (SSF), in which they grow on moist solid substrates with little water[6].

SOLID-STATE FERMENTATION(SSF)

A more natural and non-toxic way, solid-state fermentation (SSF) using agro-industrial waste is an efficient technique for tannase production. It is well-suited for fungi and helps microbial growth on moist solids without free water. Although less often used than SmF for bacterial tannase, SSF has distinct benefits in sustainably developing tannin-rich the biomass. By using easy agricultural waste as substrates, SSF has attracted research interest because of its possibility for higher yields, improved product quality, and lower production costs relative to SmF [7][8].

MICROBIAL SOURCE

Tannic acid and its compounds are examples of hydrolysable tannins that are hydrolysed by tannase, also known as tannin acyl hydrolase (E.C.3.1.1.20). Because of their high enzyme yield and simple cultivation, microorganisms—particularly bacteria and fungi—frequently produce it. Aspergillus, Penicillium, Fusarium, and Trichoderma are among the well-known fungal genera that produce tannase in both submerged and solid-state fermentation. Bacillus and Staphylococcus species, which are frequently isolated from tannin-rich environments, are examples of bacterial sources [9]. Usually, tannins or similar substances like gallic acid can induce the production of tannase.

OPTIMIZATION PRODUCTION

pH is important for the growth of microorganisms and the function of enzymes in SSF. It has an impact on amino acid ions, which can change the structure and functionality of enzymes. (Pandey et al., 2001)11. While some enzymes continue to function at higher alkaline pH levels (8.0–8.9), tannase typically exhibits optimal activity between pH 4.5 and 7.0. When working on nutrient sources (carbon, nitrogen, phosphate, and metals) and environmental elements like pH, temperature, and aeration, it is possible to optimise both culture conditions and media composition for efficient tannase production [10].

PURIFICATION AND CHARACTERIZATION

A important and expensive stage in the synthesis of tannase is enzyme extraction. Compared to intracellular enzymes, tannase is simpler to extract because it is an extracellular enzyme that is produced by fungi and bacteria. Recovery techniques vary depending on the type of fermentation and frequently include buffer washing and centrifugation. After shaking and filtering, tannase can be effectively extracted from SSF using water or citrate buffer.Although there is little specific data available, tannase activity is strongly correlated with its protein structure. Bacillus subtilis tannase primarily has a globular shape, a β-sheet structure, and is affected by temperature and pH[11]. The gas diffusion and microseeding have been used to study its crystal structure, and it forms round to oval aggregates (~44 nm).The partial tannase purification is frequently necessary for commercial use, though total enzyme purity isn't always necessary. Standard methods like chromatography, salt precipitation, and ultrafiltration are popular but laborious and may cause enzyme loss. A quicker and more effective substitute that achieves high recovery and concentration in less time is reverse micellar extraction. The characteristics of tannases differ depending on the source; fungal and yeast tannases are glycoproteins, whereas bacterial tannases have few post-translational modifications [12]. The optimal pH, temperature, molecular weight, and reaction to metal ions have all been investigated. The cost of producing enzymes is greatly impacted by downstream processing, particularly for purified tannase that is required for research and some commercial applications. Purified forms are essential for structural and biochemical investigations, but high purity is not necessary for waste management applications. The majority of tannase purification techniques are multi-step, involving gel filtration, ion exchange, and concentration. These techniques are frequently expensive, time-consuming, ineffective, and have poor recovery rates.The tannase method was used to determine the protein content, and tannase activity was measured based on the release of 1 mmol of gallic acid per minute. 25.41% of the tannase was recovered following purification with 80% ammonium sulphate, and the specific activity doubled to 1.07 U/mg. Two peaks were visible in DEAE-cellulose chromatography, with the first peak exhibiting the highest tannase activity [13].

ENZYME ACTIVITY

APPLICATION OF TANNASE ENZYME

There are many applications for the enzyme tannase in the food, feed, leather, and pharmaceutical industries. It is mainly used to make gallic acid, acorn liquor, and instant tea from plant materials high in tannins. Tannins in beverages are less harmful when tannase is present [14]. By dissolving tannins and boosting colour and flavour, it helps winemakers preserve aromatic compounds and enhance wine quality. Tannase also creates gallic acid, a major pharmaceuticals. Because it can hydrolyse tannins, the enzyme tannase (tannin acyl hydrolase) is used extensively in the food and beverage industry, particularly in the tea,coffee
and juice processing sectors. Below is a summary of its uses:

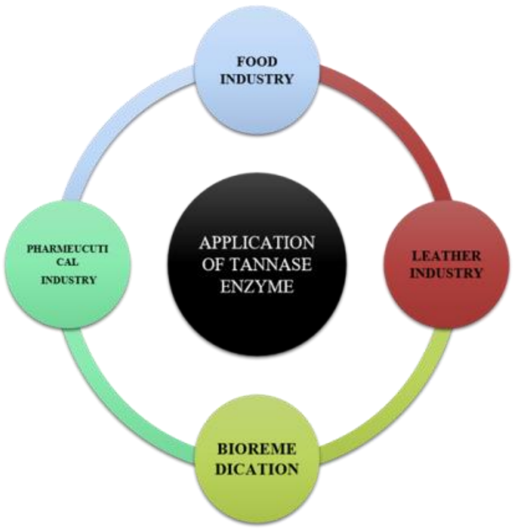

Fig 2 : Application of tannase enzyme

FOOD INDUSTRY: TEA AND COFFEE CLARIFICATION AND JUICE PROCESSING

TEA INDUSTRY

Gallic acid and glucose are released when the enzyme tannase catalyses the hydrolysis of ester and depside bonds in hydrolysable tannins, like gallotannins. this enzymatic process lessens the bitterness and astringency of tea. In addition, tannase treatment can improve antioxidant qualities, reduce tea sedimentation and turbidity, and enhance the overall flavour profile of tea beverages [15]. Tannase is a useful tool in the tea industry for creating smoother, more palatable tea products because of these advantages.

COFFEE INDUSTRY

Resolving the bitterness brought on by polyphenolic compounds is part of improving flavour in the coffee industry. Some substances have been found through research that reduce a feeling of bitterness in coffee brews, including 4-caffeoylquinic acid and 5-caffeoylquinic acid. In addition, roasting techniques can affect the production of bitter substances that add to coffee's overall bitterness, such as phenylindanes and chlorogenic acid lactic acid [16].

JUICE INDUSTRY

An enzyme called tannase efficiently solves common problems in fruit juice production, especially for berry, grape, and apple juices. Tannase improves juice quality in a number of ways by degrading tannins, which are polyphenolic compounds that cause bitterness, turbidity, and sedimentation. By the hydrolysing tannins, tannase reduces their ability to interact with proteins and polysaccharides, resulting in juices that are clearer and more looks good. Consistency The juice's shelf life is extended by the enzymatic reduction of tannins, which also lessens the possibility of sediment formation during storage [17]. Smoothing Flavours Tannase lessens bitterness and creates a smoother taste profile by converting harsh tannins into gallic acid. Because of these advantages, tannase is a useful tool for creating fruit juices that are stable, tasty, and of excellent quality.

PHARMACEUTICAL: GALLIC ACID PRODUCTION (USED IN DRUGS AND ANTIOXIDANTS).

Because of its many therapeutic uses, gallic acid (GA), a naturally occurring polyphenolic compound, is becoming more and more well-known in the pharmaceutical sector. It is a good candidate for drug development because of its strong anti-inflammatory, anti-cancer, anti-microbial, and antioxidant properties [18].

Its fast metabolism and low bioavailability however, restrict its clinical use. Researchers are looking into the moment drug delivery methods like **bioavailability**. liposomes, dendrimers, and solid lipid nanoparticles as nanocarriers to get around these obstacles. By strengthening GA's stability, controlled release, and targeted delivery, these systems hope to increase the drug's therapeutic effectiveness [19]. Gallic acid has a lot of potential for use in pharmaceutical applications, and further developments in drug delivery systems should enable it to reach its full therapeutic potential.

LEATHER INDUSTRY: WASTE MANAGEMENT.

In an effort to reduce pollution and encourage circularity, the leather industry—which has historically had a major negative influence on the environment—is progressively implementing sustainable waste management techniques. With the goal of turning byproducts into useful resources. these programs concentrate on minimising, reusing, and recycling waste materials. The use of Zero Liquid Discharge (ZLD) systems, which allow tanneries to recycle up to 80% of their wastewater through sophisticated filtration processes, is one of the most effective methods. This method reduces the release of pollutants into nearby water bodies and drastically cuts down on the use of water. The industry is also investigating biological treatment techniques, like using particular bacterial species to compost animal hair waste [20]. In addition to solving the problem of disposing of solid waste, this process creates organic nutrients, which can increase the production of crops. Environmental efforts are further supported by the adoption of circular economy principles. Tanneries can lessen their dependence on virgin materials and the pollution they cause in the environment by recycling leather scraps into new products and recovering valuable materials like chromium from waste. Additionally, changing to enzymatic tanning methods from harmful substances lowers toxic emissions and enhances worker safety. For example, the risks to the environment and human health are reduced when enzymes are used in place of sodium sulphide during the liming process. If collected as ensemble, these sustainable practices improve the leather industry's environmental performance while also generating new revenue streams from byproducts and lowering waste disposal expenses. The long-term viability of the sector depends on going innovation and the execution of these methods [21].

BIOREMEDIATION AND ANIMAL FEED.

Bioremediation is being used more and more in the production of animal feed. This method lowers toxins and increases nutritional value by employing biological processes to break down substances in food components. Fungi with white rot are a useful tool for solid-state fermentation of biomass, such as straw from wheat. By breaking down cellulose and other anti-nutritional components, these fungi improve feed digestion and lower the amount of toxic substances like the aflatoxins. Furthermore, the use of biochar—a type of carbon dioxide made from organic materials—as a feed additive is being investigated. According to studies, adding biochar to animal diets can increase feed conversion ratios and growth rates in fish like Japanese flounder and the fish [22]. Using bioremediation techniques in the production of animal feed helps to create safer products while also addressing environmental concerns. These plants may add extra nutrients to animal feed and can be used to improve water quality in the aquaculture industry.

RECENT ADVANCES OF TANNASE ENZYME

Recent developments in the study of the tannase enzyme have greatly expanded its industrial uses in a number of fields, such as biotechnology, environmental management, and food processing. The development of a magnetic graphene oxide/polymer nanobiocatalyst for immobilising tannase is a noteworthy advancement. After ten consecutive uses, this nanobiocatalyst maintains 94.2% of its initial activity, suggesting exceptional stability and allowing for 100% recovery through magnetic separation. Because of these characteristics, it is ideal for large-scale processes such as the hydrolysis of tannins in the production of plant-based foods [23].

A new tannase that was isolated from Klebsiella pneumoniae has been used in the beverage industry to lessen the bitterness and haze in tea, beer, and fruit juices. By efficiently detannifying these drinks, this enzyme improves their flavour and clarity and raises the standard of the final product. Advance Development application has also been made in environmental applications, as tannase immobilised in alginate beads has shown excellent stability and reusability. Tannins in tannery effluents are effectively broken down by this immobilised enzyme, producing valuable gallic acid that can be used in a variety of industrial processes. Additionally, tannase production has been made more affordable by optimising the use of agro-residues like Indian gooseberry leaves. Enzyme activity has increased 1.26 times as a result of the establishment of ideal conditions through statistical techniques such as Taguchi and response surface methodology. This method encourages the use of agricultural waste while simultaneously lowering production costs [24]. All of these developments in the study of the tannase enzyme highlight its increasing importance in a variety of industrial settings, providing long-term answers to problems relating to tannins.

CHALLENGES AND LIMITATIONS OF TANNASE ENZYME

COST-EFFECTIVE PRODUCTION.

A number of challenges prevent the tannase enzyme from being produced economically, which prevents its extensive industrial use. The use of costly and high-purity substrates, like pure tannic acid, is a major problem that raises production costs. Researchers are investigating the use of agro-industrial waste products, such as Indian gooseberry leaves, as substitute inexpensive substrates in order to address this. These tannin-rich materials can be used to produce tannase at a lower cost through microbial fermentation processes.Tannase recovery and purification from fermentation broths can be challenging and costly[25]. To increase the economic viability of tannase production, effective and economical purification techniques must be developed. In addition, there remains issues with the scalability of tannase production methods. Although studies conducted in laboratories have produced positive results, regulatory compliance, cost control, and process consistency issues must be resolved before these findings can be applied to industrial-scale production [26].

In conclusion, although cost-effective tannase production is possible, resolving problems with substrate prices, fermentation optimisation, purification procedures, and scalability is essential to turning tannase into a commercially viable enzyme for a range of industrial uses.

ENZYME STABILITY AND STORAGE

Although tannase enzymes are crucial for many industrial processes, their practical use may be limited by serious stability and storage issues. Environmental elements like pH and temperature can affect these enzymes. For example, the ideal pH range for activity is 4.5 to 6.5 [27].

REGULATORY CONCERNS.

The main causes of these difficulties are complicated approval procedures, dissimilar international regulations, and safety concerns. Global regulatory agencies hold differing opinions regarding the use of tannase enzymes in food, and the Code of Federal Regulations makes no mention of their approval as a food additive. Tannase enzyme approval can be a drawn-out and difficult procedure that frequently calls for a large amount of safety and efficacy data [28]. The market launch of tannase-based products may be delayed due to this variation.

FUTURE PROSPECTS IN TANNASE ENZYME AND APPLICATION

Tannase enzymes have a bright future in advance of them thanks to developments in biotechnology, sustainability, and industrial applications. By using agricultural and industrial byproducts such as black tea waste and Indian gooseberry leaves, which are inexpensive substrates for microbial fermentation, researchers are concentrating on streamlining production processes. This strategy encourages waste valorisation in addition to lowering production costs [29].

Applications-wise, tannase is being used more and more in a variety of industries. By eliminating substances that cause haze, it helps the food and beverage industry clarify fruit juices, beers, and teas, improving the quality of the final product [30]. The ability of tannase to produce bioactive substances like gallic acid, which have antioxidant qualities advantageous for skincare and health applications, benefits the pharmaceutical and cosmetic industries. In order to reduce environmental pollution, tannase is used in the leather industry to bioremediate tannery effluents, changing toxic tannins into less dangerous compounds. Additionally, technological advancements are improving the stability and effectiveness of tannase enzymes. For example, recombinant DNA technology has made thermostable tannases possible, increasing their industrial utility by enabling their use in procedures that call for higher temperatures. Additionally, bioimprinting methods have been investigated to enhance tannase's catalytic capabilities, resulting in increased yields in the synthesis of esters such as propyl gallate, a substance with uses in cosmetics and food preservation [31]. The use of tannase enzymes is consistent with the ideas of green chemistry. by making it possible to turn agro-industrial wastes that are high in tannin into useful products [32].

SCOPE IN BIOTECHNOLOGY AND INDUSTRIAL ENZYME APPLICATIONS.

Industrial enzymes are essential to contemporary biotechnology because they provide effective and sustainable solutions for a range of industries [33]. Their versatility and significance are highlighted by the wide range of applications they find in industries such as food processing, pharmaceuticals, textiles, biofuels, and environmental remediation [34]. Enzymes like lipases, amylases, and proteases are used in the food and beverage industry to improve procedures like baking, brewing, and dairy production. For instance, lactases help create lactose-free products, cellulases enhance the texture of baked goods, and amylases convert starches into fermentable sugars. In addition to increasing production efficiency and lowering energy consumption, these enzymatic processes also improve product quality. Enzymes such as cellulases and pectinases, which are employed in denim finishing and bio-polishing, are advantageous to the textile industry. Enzymes like cellulases and ligninases help the biofuel industry convert biomass into fermentable sugars, which can subsequently be utilised to create biofuels like ethanol and biodiesel. Enzymes are used in the pharmaceutical industry to synthesise antibiotics and therapeutic proteins. For example, lipases are used in the formulation of some medications, and chymotrypsin and trypsin are involved in the production of insulin [35].

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