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Review

Production, characterization and applications of tannase

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ABSTRACT

Tannases, tannin acylhydrolases, are an important group of biotechnologically relevant enzymes which were utilized in a number of industrial applications, including the manufacture of instant tea, beer, fruit juices, some wines and gallic acid production. Tannases are by and large produced by microorganisms including Aspergillus, Paecilomyces, Lactobacillus and Bacillus. Tannases are generally produced on tannic carbon such as tannic acid, wheat bran, tea and coffee husk extract. Microbial tannases are mostly induced extracellular enzyme and produced by submerged fermentation and solid-state fermentation. The enzyme is most commonly purified by hydrophobic interaction chromatography in addition to reverse micelle. Most tannases can act in a wide range of temperature and pH, although tannases with acidic pH optima are more common. A sequence-based classification spreads tannases in many families thus reflecting the variety of molecules. Furthermore, tannase from *Lactobacillus plantarum* had been characterized by three-dimensional architecture. In recent years, a novel approach, metagenomic, was developed to exploring novel tannase from natural communities.

Contents

1. Introduction

Tannase (tannin acyl hydrolase, EC3.1.1.20) catalyzes the ester bonds present in gallotannins, complex tannins and gallic acid esters to release gallic acid. It is widely used as clarifying agent in the manufacturing of instant tea, beer, fruit juices and some wines, treating tannin-polluting industrial effluents and agricultural wastes. In addition, tannase plays an important role in production of gallic acid, the latter being an important intermediary compound in the synthesis of the antibacterial drug, trimethroprim, used in the pharmaceutical industry and also in the food industry; gallic acid is a substrate for the chemical or enzymatic synthesis

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of propyl gallate, a potent antioxidant. However, the practical use of this enzyme is at present limited due to insufficient knowledge about its properties, optimal expression and purification processes. This limitation is due to the complexity represented by tannase-like model of study and inductors [1].

Tannase was first discovered by Tieghem [2] in an experiment of formation of gallic acid into an aqueous solution of tannins, where grew two fungal species later identified as *Penicillium glaucum* and *Aspergillus niger* [3]. During the next one hundred years, certain filamentous fungi, mainly the following species: *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma* were found producing tannase [4–9]. Bacteria [10,11] and yeasts [12] also produced this enzyme. Beside this, in the last decade, there have been a number of efforts to improve the production, recovery, and purification processes of the enzyme. These efforts include the development of novel fermentation systems, optimization of culture conditions, production of the enzyme by recombinant microorganism and design of efficient protocols for tannase recovery and purification [13].

The growing importance of tannases within biotechnological perspectives can be easily envisaged by the number of recent articles covering aspects of this biocatalyst, such as biochemistry, assay protocols, molecular biology and biotechnological applications [7,14–17]. This review presents detailed information on various aspects of tannase, exploring scientific and technological facets, with emphasis on substrates, production, recovery and purification strategies, physicochemical properties, protein sequence characterization, molecular architecture and applications.

2. Tannase substrate: Tannins

After lignins, tannins are the second most abundant group of plant polyphenols. They are found worldwide in many different families of the higher plants, such as tara, gall, oak, myrobalam, sumach and so on. And high concentrations of tannins are found in nearly every part of the plant including root, bark, wood, leaf, fruit and seed. One of the major characteristics of tannins is their abilities to form strong complexes with protein, starch, cellulose, minerals and digestive enzymes including pectinase, amylase, lipase, protease, cellulase and β-galactosidase. These properties of tannin made them toxic or anti-nutritional to ruminant animals as they can reduce feed intake and lower nutrient digestibility and protein availability [18]. On the basis of their structural characteristics, tannins are divided into four groups: condensed tannins, completed tannins, gallotannins and ellagitannins (Table 1). Condensed tannins are polymers formed by the condensation of flavans, which were formed by linkage of *C*-4 of one catechin with *C*-8 or *C*-6 of the next monomeric catechin [19], and they are difficult to be hydrolyzed. Completed tannins are molecules with a polyol (generally D-glucose) as a central core, and the hydroxyl groups of these carbohydrates are partially or totally esterified with phenolic groups like gallic acid or ellagic acid. Gallotannins are polymers formed by organic acids, such as gallic, digallic and chebulic acids, esterified with the hydroxyl group of a polyol carbohydrate. Ellagitannins are polyphenol formed primarily from the oxidative linkage of galloyl groups in 1, 2, 3, 4, 6-pentagalloyl glucose [20]. Ellagitannins differ from gallotannins, in that their galloyl groups are linked through C—C bonds, whereas the galloyl groups in gallotannins are linked by depside bonds. The negative effect of tannins relates not only to taste but also directly to macromolecules, rendering them indigestible. Their ability to form stable complexes with enzymes and minerals affected the animal's feed intake, feed digestibility and efficiency of production. Beside this, the presence of tannins also can form tea cream in tea drinks and turbid phenomenon in alcohol and coffee. Tannins also inhibit the growth of a number of microorganisms, resist microbial attack and are recalcitrant to biodegradation [21]. However, some microorganisms are resistant to tannins and have developed various mechanisms and pathways for tannin degradation in their habitat. Many microorganisms which can produce tannase have been reported to use tannins as the sole source of carbon.

3. Microbial sources of tannase

Tannin acyl hydrolade (E.C.3.1.1.20) is commonly referred as tannase, which can catalyze the hydrolysis of bonds present in the molecule of hydrolysable tannins such as tannic acid, methyl gallate, ethyl gallate, propylgallate and isoamyl gallate [1]. Tannase can be obtained from plant, animal and microbial sources. As for plant sources, tannase is present in tannin-rich vegetables mainly in their fruits, leaves, branches and barks of trees. For animal resources, the enzyme can extract from bovine intestine and the ruminal mucous [1]. The microbial origin, mainly bacterial and fugal, represents the most enzymes used in biotechnological applications. The production and application of tannase have been extensively studied, researches related strain isolation and improvement, process development, and application of tannases have resulted into a great number of scientific publications and patents [22]. And a list of the common microbial tannase producers is present in Table 2.

4. Fermentation conditions

Most microbial tannases are extracellular and are greatly influenced by nutritional and physico-chemical factors, such as pH, incubation temperature, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration. A list of several fermentation conditions used with different microbial is presented in Table 3.

The major factor for the expression of tannase activity has always been carbon since most tannases are induced enzymes and are thus generally produced in the presence of inducers, such as tannic acid [5,25,59,60]. However, their production is also significantly influenced by other carbon sources, such as arabinose, fructose, glucose, lactose, maltose, mannitol, sucrose and xylose. Tannase from *Paecilomyces variotii* was reported to be stimulated in the presence of glucose, while to be repressed in the presence of arabinose, raffinose, rhamnose, sorbose, starch and xylose [36]. Lagemaat and Pyle [61] reported that the glucose present in the media will be exhausted rapidly and this may lead to the partial induction of tannase. Banerjee and Pati [62] also reported the positive effect of glucose on tannase production by *Aureobasidium pullulans*. Seiji et al. [63] mentioned that tannase activity was expressed only when the organism is grown in presence of glucose. Selwal et al. [64] reported the production of a *Penicillium atramentosum* KM tannase in the presence of maltose, with a maximum production of about 31.1 U/ml when keekar leaves as the sole carbon sources.

Besides carbon sources, the different nitrogen sources in the medium also influence the tannase titers in production broth. A number of workers studied the presence of different nitrogen sources such as sodium nitrate, ammonium nitrate, ammonium sulfate, ammonium chloride and yeast extract [36,40,65,66]. Selwal et al. reported maximum tannase production by *Penicillium atramentosum* KM was obtained with ammonium chloride, i.e., 28.9 U/ml using amla and Di-ammonium hydrogen phosphate gave 31.9 U/ml using keekar leaves [64]. Sabu et al. reported an increase in the tannase production with ammonium nitrate in case of the medium containing tamarind seed powder (TSP) [52]. Banerjee and Pati reported maximum tannase production using Di-ammonium hydrogen phosphate [62]. However, a few workers reported inhibitory effects of nitrogen. Kumar et al. reported that supplementation of medium with ammonium sulfate resulted