Chapter 4
Fungal Communities from Different Habitats for Tannins in Industry

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Contents

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4.1 Introduction

Tannin acyl hydrolase, commonly known as tannase, is a hydrolase enzyme that is induced in the presence of tannic acid (Belmares et al. 2004). It catalyzes the hydrolysis of ester and depside bonds in hydrolyzable tannins such as tannic acid and gallic acid esters releasing glucose and gallic acid (Sharma et al. 2000). This enzyme was accidentally discovered by Tieghem (1867) in an experiment of formation of gallic acid into an aqueous solution of tannins, where two fungal species grew later, identified as *Penicillium glaucum* and *Aspergillus niger* (Lekha and Lonsane 1997).

Tannase enzyme is known to display two different activities. The first one is an esterase activity; by which it can hydrolyze ester bonds of gallic acid esters with glucose (galloyl-glucose) or alcohols (e.g., methyl gallate). The second activity is called depsidase activity; by which it can hydrolyze depside bonds of digallic acid (Saxena and Saxena 2004). Tannase catalyzes the hydrolysis reaction of the ester bonds present in the gallotannins, complex tannins, and gallic acid esters (Vivas et al. 2004). Tannase can be obtained from plants, animals, and microbial sources. Microorganisms are considered the most important and commercial sources of tannase, that is because the produced tannases are more stable than similar ones obtained from the other sources. Moreover, microorganisms can produce tannase in high quantities. Microbial tannase is favored also because the microbes can be subjected to genetic manipulation more readily than plants and animals, resulting in an increase in tannase production (Aguilar and Gutierrez-Sanchez 2001; Purohit et al. 2006). As a result, enzymes of microbial origin are having important applications in many areas of bio-based industries (Barthomeuf et al. 1994). In microbial sources, the *Aspergillus* and *Penicillium* genus, and lactic acid bacteria mostly produce tannase (Zakipour-Molkabadi et al. 2013; Abdel-Azeem et al. 2021).

Tannase is an industrially important enzyme and has several applications in various industries such as foods, animal feeds, cosmetic, pharmaceutical, chemical, and leather industries (Aguilar et al. 2007; Jun et al. 2007; Kour et al. 2019). The major commercial applications of the tannases are elaboration of instantaneous tea or acorn liquor, hydrolyze tea cream in the processing of tea in the production of gallic acid (Lekha and Lonsane 1997), manufacturing of wine, beer, coffee, and fruit juices (Aguilar et al. 2001); cleaning up the leather industry effluents containing the pollutant tannin; and in the reduction of antinutritional effects of tannins in animal feed (Mukherjee and Banerjee 2006). Gallic acid (3,4,5-trihydroxybenzoic acid) and related compounds possess many potential therapeutic properties. The major application of gallic acid is the synthesis of a broad-spectrum antibacterial agent. It has always been a molecule of industrial importance because of its applications in different sectors from healthcare and food to dyes, inks, paints, and photography (Mukherjee and Banerjee 2003; Yadav et al. 2019). The present review shows relevant points related to the fungal tannase. The substrates for tannase production, the tannase producing fungi, production of tannase by fermentation using different methods, like submerged fermentation (SmF), solid-state fermentation (SSF) and

modified solid-state fermentation (MSSF), and ways to produce it, with the goal to contribute to the knowledge for potential applications of fungal tannase in food and other industrial products.

4.2 Tannins: Natural Substrate

Tannins are naturally occurring polyphenolic compounds with varying molecular weights that occur naturally in the plant kingdom (Aguilar et al. 2007). After lignin, tannins are the second most abundant group of plant phenolics (Aguilar et al. 2001). These phenolic compounds of high molecular weight are usually present in plants with molar masses extending from 500 to over 3000 Da and up to 20,000 Da (Rana et al. 2004). In the plant kingdom, these tannins are found in leaves, bark, and wood. Tannins are considered to be the plants' secondary metabolic products because they play no direct role in plant metabolism. Tannins are polymeric phenolic compounds with numerous hydroxyl groups and quite diverse in chemical structure (Hagerman 1992). It detected more than 8000 different tannins (Aguilar et al. 2007). They can be found in many different plants and plant residues such as *Anacardium occidentale* (cashew), *Vitis vinifera* (grape), *Malpighia glabra* (Barbados cherry), and *Hancornia speciosa* (mangaba fruit). One of the best sources of tannins is *Acacia* species, which belong to the family of Leguminosae in the plant kingdom. Such residues, rich in tannins, can be excellent substrates for the production of tannase (De Lima et al. 2014). Hydrolysis of some of the tannins yields the simple, seven-carbon gallic acid and others give ellagic acid or other phenolic acids (Okuda and Ito 2011).

On the basis of their structural characteristics, it is, therefore, possible to divide the tannins into four major groups: gallotannins, ellagitannins, complex tannins, and condensed tannins (Khanbabaee and van Ree 2001). The term "hydrolyzable tannins" includes both the gallotannins and the ellagitannins. Hydrolyzable tannins (HT) are involved in a monosaccharide core, usually, glucose, esterified with gallic acid, developing the gallotannins, or with hexahydrodiphenoic acid, the precursor of ellagic acid, and gallic acid, developing the ellagitannins (Serrano et al. 2009). Hydrolyzable tannins can be hydrolyzed into smaller compounds, for example, gallic and ellagic acid (Bele et al. 2010). Gallotannins are the simplest hydrolyzable tannins, containing polyphenolic and a polyol residue. The plants *Rhus semialata* (Chinese galls), *Quercus infectoria* (Turkish galls), *Caesalpinia spinosa* (Tara pods), *Aver ginnala* (Korean maple, *Acer tannin* leaves), *Rhus coriaria* (Sumac leaves), *Hammelis virginiana* (Hamammelis—hazelnuts) were documented to be rich in gallotannins (Bhat et al. 1998). Gallotannins yield gallic acid and glucose or quinic acid on hydrolysis (Khanbabaee and Van Ree 2001).

Ellagitannins yield ellagic acid, gallic acid, and glucose on hydrolysis. Plants such as *Caesalpinia coriaria* (Divi-Divi), *Caesalpinia brevifolia* (Algarobillatannin) *Terminalia chebula* (myrobalan seeds), *Castanea sativa* (Chestnut), *Eucalyptous sieberiana*, *Schinopsis* sp., *Quercus velonia*, *Q. aegilops* (Valonea tannin), *Quercus*

coccifera (Garouille) were reported to contain ellagitannins in their bark, nuts or leaves (Bhat et al. 1998; Mukherjee and Banerjee 2003). Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit forms acyl bond with pro anthocyanadines such as catechin, for example, tea polyphenols (Khanbabaee and Van Ree 2001). Condensed tannins are referred to proanthocyanidins. These are generally found in plants that possess a woody growth and also in plant gums and exudates (Haslam 1996). Common plants containing condensed tannins are *Acacia* sp. (Wattle tannins), *Schinopsis* spp. and *Loxopterygium* sp. (Querbacho wood), *Pinus* sp. (Pine bark), *Quercus* sp. (Oakwood), and *Aucoumea kleneana* (Gaboon wood) (Bhat et al. 1998). Condensed proanthocyanidins degrade in strong acid to give corresponding anthocyanidin (Falcão and Araújo 2018).

4.3 Different Source of Tannase

Tannase is known to be a ubiquitous enzyme of the microbial world (Murugan et al. 2007). Microorganisms such as bacteria, fungi, and yeasts are known as prominent producers, also, a few animals and plants have been found as a source of tannase. The enzymes are present in tannin-rich vegetables, fruits, leaves, and bark, and also a few of them are present in fruits of *Terminalia chebula*, pods of *Caesalpinia coriaria*, and bark of *Cassia fistula* (Madhavakrishna et al. 1960). Although novel researches showed that the colonizing microorganisms of these animals are the actual producers, not the animals (Belur and Mugeraya 2011).

4.3.1 Plant Source

Tannase is present in tannin-plants such as Turkish gall (*Quercus infectoria*), sumac (*Rhus coriaria*), tara (*Caesalpinia spinosa*), chestnut (*Castanea sativa*, Bhat et al. 1998), gum arabic tree (*Acacia nilotica*, Lal et al. 2012), red gram (*Cajanus cajan*, Kuppusamy et al. 2015) and waste testa (*Anacardium occidentales*, Lenin et al. 2015), English oak (*Quercus robur*), Pendunculate oak (*Quercus rubra*), Karee tree (*Rhus typhina*) leaves. Plants having condensed tannins are babul (*Acacia arabica*), konnam (*Cassia fistula*), avaram (*Cassia auriculata*), and others. Plants synthesize gallic acid, hexahydroxyphenic acid, and chebulinic acid in addition to the amount of sugar. These acids possibly undergo esterification with glucose molecules during the ripening process with the aid of tannase ultimately resulting in the synthesis of tannins.

4.3.2 Animal Source

Microorganisms that live in the gastrointestinal of the cow, goats, sheep, and other animals are known as effective tannase producers such as many species of bacteria isolated from fecal samples of native sheep and goats, which can hydrolyze acorn tannin in the rumen and reduce negative effects of tannin on animals (Mosleh et al. 2014). Examples of Tannase-producing bacteria are *Streptococcus pneumonia* and *Streptococcus*.

4.3.3 Bacterial and Fungal Sources

Microorganisms are producing tannase enzymes in large quantities than other organisms. They are the main source of commercial enzyme production. The microbial enzymes present several advantages over other sources since the microbial enzymes are more stable in comparison to similar enzymes from other sources (Jana et al. 2014). Microorganisms can ease of genetic manipulation of microbial enzymes thus; they are easy to manipulate genetically, which increases and improves the enzymatic activity (Aguilar and Gutierrez-Sanchez 2001; Purohit et al. 2006). The suitable tannase-producing microorganisms reported are fungi and few bacteria and yeast. Fungal tannase has high activity titer in the degradation of hydrolyzable tannins. However, yeast tannases relatively disintegrate tannic acid easily and flaunt a relatively lesser affinity on the other hand in the degradation of natural tannins (Kumar et al. 2019).

4.3.3.1 Yeast Tannase

There are few reports about yeast used as tannase production. Among yeast *Candida* species (Aoki et al. 1976) *Mycotorula japonica* (Belmares et al. 2004) *Pichia* sp. (Deschamps et al. 1983) *Debaryomyoes hansenii* (Deschamps et al. 1983).

4.3.3.2 Bacteria Tannase

Lewis and Starkey (1969) reported first bacterium capable of hydrolyzing gallotannins as a sole energy source was *Achomobacter* sp., also, *Bacillus* and *Lactobacillus* genus represents a group of tannase producing bacteria (Mondal et al. 2001; Sabu et al. 2006; Nishitani et al. 2004). Lactic acid bacteria have an important role in tannin food degradation. Few other bacterial tannase producers comprise *Klebsiella* (Deschamps et al. 1983), *Lonepinella* (Goel et al. 2007), *Citrobacter* (Kumar et al. 1999), *Corynebacterium*, *Pantonea* (Pepi et al. 2010), *Serratia* (Belur et al. 2010),

Table 4.1 Bacterial tannases sources

Lactobacillus plantarum CECT 748 T	Rodríguez et al. (2008)
Lactobacillus plantarum ATCC 1491 T (recombinant)	Iwamoto et al. (2008)
Lactobacillus plantarum (recombinant)	Curiel et al. (2009)
Enterobacter sp.	Sharma and John (2011)
Bacillus licheniformis KBR 6	Mondal and Pati (2000)
Bacillus cereus KBR9	Mondal et al. (2001)
Bacillus sphaericus	Raghuwanshi et al. (2011)
Enterobacter cloacae	Beniwal et al. (2013)
Staphylococcus lugdunensis MTCC 3614 (recombinant)	Chaitanyakumar and Anbalagan (2016)
Enterococcus faecalis	Goel et al. (2011)

Pseudomonas (Selwal et al. 2010), *Selenomonas* (Skene and Brooker 1995), and *Streptococcus* (Jiménez et al. 2014) as shown in Table 4.1.

4.3.3.3 Fungal Tannase

Tannase is now known to be an ever-present enzyme of the microbial world and has widespread occurrence in various fungi. Most of the reported tannase-producing organisms are fungi (Bhat et al. 1998) and only a few are bacteria (Mondal and Pati 2000; Mondal et al. 2001). Fungi have the ability to degrade tannins as a sole carbon source (Aguilar and Gutierrez-Sanchez 2001) as shown in Table 4.2. The genus *Aspergillus* is considered as the best producer, followed by *Penicillium*, both standing out as great decomposers of tannins (Sabu et al. 2005). The main genera of fungi known as producers of tannase are *Aspergillus*, *Penicillium*, *Fusarium*, and *Trichoderma* (De Sena et al. 2014; Rastegari et al. 2020). Other fungi, including *Chaetomium*, *Rhizoctonia*, and *Cylindrocarpon* are capable of degrading tannery waste constituents (Cruz-Hernandez et al. 2005). The common genus used for tannase production either for research purposes or industrial production was *Aspergillus* and the common *Aspergillus* species used for tannase production was *Aspergillus niger* (Manjit et al. 2008). The tannase from *Aspergillus* has been used widely for the production of gallic acid from tannins (Purohit et al. 2006).

4.3.4 Rich Tannin Plants

Different studies have indicated the presence of hydrolyzable tannins in diverse plant species, especially in their leaves, fruits, bark, and branches. Endophytic microorganisms are fungi and bacteria living inside the aerial parts of the plants without, causing any seeming damage to the host (Abo Nouh 2019). According to Reges de Sena et al. (2014) isolated endophytic fungi isolated from Jamun (*Syzygium*

Table 4.2 Filamentous fungi capable of producing tannases and/or of using tannins as sole carbon source

Ascochyta boltshauseri	Lekha and Lonsane (1997)
Ascochyta pisi	Lekha and Lonsane (1997)
Ascochyta viciae	Lekha and Lonsane (1997)
Aspergillus aculeatus	Banerjee et al. (2001)
Aspergillus acidus	Prigione et al. (2018)
Aspergillus aureus	Bajpai and Patil (1997)
Aspergillus avenaceus	De Lima et al. (2014)
Aspergillus awamori	Bradoo et al. (1996)
Aspergillus caespitosum	Batra and Saxena (2005)
Aspergillus carbonarius	De Lima et al. (2014)
Aspergillus carneus	Aguilar et al. (2007)
Aspergillus clavatus	De Lima et al. (2014)
Aspergillus costaricaensis	Prigione et al. (2018)
Aspergillus fischeri	Bajpai and Patil (1997)
Aspergillus flavus	Aguilar et al. (2007)
Aspergillus flavipes	Aguilar et al. (2007)
Aspergillus foetidus	Banerjee et al. (2005)
Aspergillus fumigatus	Batra and Saxena (2005)
Aspergillus gallonyces	Belmares et al. (2004)
Aspergillus granulosus	De Lima et al. (2014)
Aspergillus japonicus	Bradoo et al. (1997)
Aspergillus niger	Rana and Bhat (2005)
Aspergillus ochraceus	De Lima et al. (2014)
Aspergillus oryzae	Bradoo et al. (1996)
Aspergillus parasiticus	De Lima et al. (2014)
Aspergillus ruber	Kumar et al. (2007)
Aspergillus rugulosus	Bradoo et al. (1996)
Aspergillus tamarii	Costa et al. (2008)
Aspergillus terreus	Bajpai and Patil (1997)
Aspergillus tubingensis	Prigione et al. (2018)
Aspergillus ustus	De Lima et al. (2014)
Aspergillus vadensis	Prigione et al. (2018)
Aspergillus versicolor	Batra and Saxena (2005)
Aspergillus viridinutans	De Lima et al. (2014)
Chaetomium globosum	Lekha and Lonsane (1997)
Cryphonectria parasitica	Farias et al. (1994)
Cunninghamella sp.	Bradoo et al. (1996)
Emericella nidulans	Prigione et al. (2018)
Fusarium oxysporium	Bradoo et al. (1996)
Fusarium solani	Bradoo et al. (1996)
Lentinus edodes	Vattem and Shetty (2003)
Lenzites elegans	Ordonez et al. (2011)

(continued)

Table 4.2 (continued)

Neurospora crassa	Bradoo et al. (1996)
Paecilomyces variotii	Mahendran et al. (2005)
Penicillium acrellanum	Bradoo et al. (1996)
Penicillium aurantiogriseum	De Lima et al. (2014)
Penicillium canescens	De Lima et al. (2014)
Penicillium carylophilum	Bradoo et al. (1996)
Penicillium citrinum	Bradoo et al. (1996)
Penicillium charlessi	Batra and Saxena (2005)
Penicillium chrysogenum	Bradoo et al. (1996)
Penicillium crustosum	Batra and Saxena (2005)
Penicillium commune	De Lima et al. (2014)
Penicillium corylophilum	De Lima et al. (2014)
Penicillium digitatum	Bradoo et al. (1996)
Penicillium fellutanum	De Lima et al. (2014)
Penicillium frequentanse	De Lima et al. (2014)
Penicillium glabrum	Van de Lagemaat and Pyle (2005)
Penicillium glaucum	Lekha and Lonsane (1997)
Penicillium islandicum	Aguilar et al. (2007)
Penicillium lanosum	De Lima et al. (2014)
Penicillium lapidosum	De Lima et al. (2014)
Penicillium lividum	De Lima et al. (2014)
Penicillium montanense	De Lima et al. (2014)
Penicillium notatum	Aguilar et al. (2007)
Penicillium purpurogenum	De Lima et al. (2014)
Penicillium restrictum	Batra and Saxena (2005)
Penicillium simplicissimum	De Lima et al. (2014)
Penicillium spinulosum	De Lima et al. (2014)
Penicillium variable	Batra and Saxena (2005)
Penicillium verruculosum	De Lima et al. (2014)
Penicillium zacinthae	De Lima et al. (2014)
Rhizopus oligosporus	Vattem and Shetty (2002)
Rhizopus oryzae	Purohit et al. (2006)
Syncephalastrum racemosum	Bradoo et al. (1996)
Talaromyces subinflatus	Prigione et al. (2018)
Trichoderma hamatum	Bradoo et al. (1996)
Trichoderma harzianum	Bradoo et al. (1996)
Trichoderma viride	Bradoo et al. (1996)
Trichothecium roseum	Lekha and Lonsane (1997)

cumini (L.) Skeels) leaves, and identified as *Pestalotiopsis guepinii* can produce tannase enzymes. Mahapatra and Banerjee (2009) reported endophytic fungi *hyalopus* sp. isolated from medicinal plant *Ocimum sanctum* presented the high production of tannase enzyme. According to a study by Cavalcanti et al. (2017), endophytic

fungi from species *A. niger* and *A. fumigatus* isolated from barks of angico (*Anadenanthera colubrina*) and cajueiro (*Anacardium occidentale*) presented high production of tannase enzyme (Rana et al. 2020).

Cruz-Hernandez et al. (2005) isolated and characterized 11 fungal strains from species of (*Penicillium commune*, *Aspergillus niger*, *Aspergillus rugulosa*, *Aspergillus terricola*, *Aspergillus ornatus*, and *Aspergillus fumigatus*) isolated from soil and tannin-rich plants (damaged tissue from *Quercus* spp., *Carya illinoensis*, *Larrea tridentata* and *Pinus sembroides*) of the Mexican semidesert. These xerophilic fungi were able to produce tannase and degrade high tannin amounts in low humidity conditions. Zakipour-Molkabadi et al. (2013) reported strains of *Penicillium* sp. EZ-ZH190 from moldy tea leave samples can produce tannase enzymes.

4.3.5 Soil

Kasieczka-Burnecka et al. (2007) isolated an Antarctic filamentous fungus from the soil of King George Island (South Shetlands). This strain (identified as *Verticillium* sp.) produced two psychrophilic tannases with an optimal temperature of 20 and 25 °C, respectively. Marco et al. (2009) demonstrated that a novel extracellular tannase from the xerophilic fungus *A. niger* GHI was produced under solid-state conditions. Nalan and Merih (2009) isolates were selected for gallic acid production by tannase species were isolated from forest soil. Species producing tannase enzyme, for example, *A. niger*, *P. canescens*, *P. frequentans*, *P. spinulosum*, *P. purpurogenum*, and *P. zacinthae*. *Rhizopus oryzae* was isolated from soil samples of Indian Institute of Technology campus to show ability for tannase production (Abou-Bakr et al. 2013; Yadav 2021). Liu et al. (2016) reported amount of 35 strains of fungi species isolated from the soil of Caatinga were used for qualitative selection of strains with potential for production of tannase, the promising potential fungi *Aspergillus* spp. *UCP1284* is able to produce tannase using cashew bagasse as a substrate. In another study by Ire and Nwanguma (2020) 15 different soil samples were collected within Lagos (Oshodi), Nigeria for the potential of tannase production by *Lasiodiplodia plurivora* ACN-10 in SmF and SSF using *Terminalia cattapa* (almond leaves) and *Magnifera indica* (mango leaves) as substrates.

4.3.6 Mangroves and Caves

The mangrove ecosystem is very particular in that it harbors several tannins-rich plant species, such as *Rhizophora apiculata*, and *R. mucronata* (Georgei and Ong 2013). *Penicillium digitatum* FETLDS1, isolated from the dumping area of tannin-rich barks of *Rhizophora apiculata* in mangrove areas in Perak, Malaysia can produce extracellular tannase (Sandai et al. 2012). Georgei and Ong (2013) reported

Aspergillus niger FBT1, a local extracellular strain for tannase production, was isolated from soil collected from Mangrove Forest. Neethu and Pradeep (2018) reported *A. niger*, *A. japonicus*, and *A. aculeatus* species isolated from the soil and effluent samples were collected from different mangrove areas that have the ability to produce tannase enzymes.

Caves are an underexplored ecosystem that may reveal microorganisms of industrial and biotechnological application (Barton 2006). A study by De Melo et al. (2013) reported isolates belonging to seven different genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Epicoccum*, *Trichoderma*, and *Cladosporium*. Isolated from the soil of caves, they have the ability for tannase production. The most tannase-producing species are *Aspergillus japonicus*, *A. niger*, *A. tamarii*, *A. foetidus*, *A. tubingensis*, *A. ochraceus*, *Penicillium funiculosum*, *P. oxalicum*, *P. corylophilum*, and *P. citrinum.*

4.3.7 Marine Habitat

The deep-sea includes the deep-sea hypersaline anoxic basins (DHABs), which are depressions of the seafloor found at more than 2 km below sea level. *Aspergillus awamori*, *A. candidus*, *A. fumigatus* (Panno et al. 2013), *Penicillium notatum* (Gayen and Ghosh 2013), and several strains from *Posidonia oceanica* isolated from (DHABs). *Aspergillus awamori* BTMFW032, isolated from seawater, produced acidophilic tannase as an extracellular enzyme (Beena 2010; Verma et al. 2017). In a study by Panno et al. (2011), 88 fungal species are isolated from seagrass *Posidonia oceanica*, species identified by morphological and molecular methods, and the most important genera were *Penicillium*, *Cladosporium*, *and Acremonium*. Farag et al. (2018) reported *Aspergillus nomius* GWA5 was isolated from marine sediment to produce an active tannase for degradation of tannin.

4.3.8 Tannery or Industrial Effluents

Tannery effluent samples were used for isolating tannase-producing fungi and a promising fungus isolated from a tannery soil sample was identified as *Aspergillus niger* (Murugan et al. 2007). Manjit et al. (2008) reported a tannase yielding fungal culture identified as *A. fumigatus* MA, which was isolated from the effluent collected from a local small-scale tannery. The fungal culture produced high yields of extracellular tannase under SSF using different agroforest residues. *A. tamari* 1M138810 (B) a tannic acid degrading funguses was isolated from soil inundated by the effluent of a tannery at Oji River local (Enemuor and Odibo 2009). Hamada et al. (2013) reported tannase production from *Aspergillus niger* isolated from a tannery soil sample, with a maximum hydrolytic clear zone (53 mm ± 0). Brahmbhatt et al. (2014) reported tannase producing fungi belonging to genera *Aspergillus*,

Mucor, *Penicillium*, and *Rhizopus* species from various tea waste dump sites, agro-residue waste sites, and tannery effluents. These soil samples and tannery effluents are rich in tannins and their derivatives (Girdhari and Peshwe 2015). Twenty-nine fungal isolates were screened on the basis of their tannase-producing efficiency under stationary conditions isolated from soil samples of various tea waste dump-sites, agro-residue waste sites, and sites near local tannery industries.

The fungal isolates *Aspergillus fumigatus*, *Aspergillus carbonarius*, *Penicillium lividum*, and *Penicillium citrinum* exhibit maximum tannase activity. Shajitha and Nisha (2018) isolated a number of mycobiota like *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Rhizopus* spp., *Rhizopus stolonifer*, *Geotrichum* spp., *Penicillium* sp., *Trichoderma viride,* from tea industry waste disposal area soil for tannase production, only four fungal strains *Trichoderma viride*, *Aspergillus flavus*, *Rhizopus* spp., *and Aspergillus niger* showed the maximum hydrolyzing zone. A significantly highest hydrolyzing zone was shown by *Trichoderma viride* followed by *Aspergillus flavus*. Farhaan and Patil (2019) reported *Aspergillus* spp. and *Aspergillus flavus* respectively as tannase producers isolated from soil samples of nearby local tannery industries. A study by Al-Mraai et al. (2019) reported fungi of genus *Aspergillus* are active producers of extracellular tannase, *Aspergillus niger* isolated from soil and tea waste-producing tannase enzyme.

4.4 Production of Tannase by Fermentation

Several fermentation systems have been developed for the production of tannase from fungi using various production media. These systems can be divided into liquid surface fermentation (LSF), submerged fermentation (SmF), solid-state fermentation (SSF), and modified solid-state fermentation (MSSF). Belmares et al. (2004) mentioned in a comprehensive review that tannase production has been carried out mainly under submerged (SmF) and solid-state fermentation (SSF) techniques, depending on the strain and culture conditions. Several studies have reported interesting advantages of tannase produced by SSF over that produced by SmF. All of these studies showed that the first advantage of SSF technique is the higher enzyme titers than in SmF, when comparing the production of the same strain and fermentation broth (Aguilar et al. 2002). Microorganisms can be utilized using SSF for conversion of plant materials and agro-industrial wastes that contain tannin-rich substrates while using SSF to produce economic products such as tannase and gallic acid production, while overcoming their accumulation problems in the environment (Paranthaman et al. 2009). Filamentous fungi are suitable for the SSF process compared to other fermentation processes (Aguilar et al. 2002). The fungal species of *Aspergillus* and *Penicillium* are the most active microorganisms known, capable of producing tannase through submerged and solid-state fermentation (Abdel-Nabey et al. 2011).

The conditions for obtaining the maximal production of the enzyme depend on two factors: the system utilized and the source of the enzyme (Rodriguez-Duran

et al. 2011). Medium optimization by single dimensional search is laborious and time-consuming, especially for a large number of variables and it does not ensure desirable conditions. Plackett-Burman design is widely used in screening experiments as the number of experiments run required is very few, leading to saving of time, chemicals, and manpower (Jamal et al. 2009). The powdered plant tannin substrates (leaves, fruits, pods, bark) were usually extracted either with water or with an organic solvent like acetone (Huang et al. 2005). Tannins were documented to be serving as dual purposes as a solid support in SSF and also as a carbon source such as *Larrea tridentates*, *Syzygium cumini*, *Ziziphus mauritiana* (Kumar et al. 2007). Among the various species, *A. niger*, *A. flavus*, *and A. oryzae* were found to be the best tannase producers using tannic acid as a sole source of carbon (Hassan et al. 2018).

4.5 Tannase Applications

Tannase has received a great deal of attention from the discovery, and it is used in a wide range of applications in many industries including in detannification of food (Boadi and Neufeld 2001), preparation of food preservatives, and pharmaceuticals (Belmares et al. 2004). Actually, the main applications of tannase are elaboration of instantaneous tea (Lekha and Lonsane 1997), acorn, and gallic acid production, which is used for the synthesis of trimethoprim (Yu et al. 2004). Also, tannase is used as clarifying agent in fruit juice (Shrivastava and Kar 2009) beer, wines (Bajpai and Patil 2008), and manufacture of coffee flavored (Anwar and Imartika 2007) beverages (Aguilar et al. 2001; Belmares et al. 2004), treatment of green tea to inhibit the carcinogenic and mutagenic effects of *N*-nitrosamines, stabilization of malt polyphenols (Lekha and Lonsane 1997), improved color stability, and additional organoleptic properties. In animal feeding, tannase is used to reduce the antinutritional effects of tannins and improve animal digestibility.

4.5.1 Food Industries

4.5.1.1 Instantaneous Tea Elaboration

After water, tea is the second most highly consumed beverage worldwide (Venditti et al. 2010). It is an infusion obtained from leaves of *Camellia sinensis* and is consumed by two-thirds of the world's population (Łuczaj and Skrzydlewska 2005). Tea drinking is associated with the reduction of serum cholesterol, prevention of low-density lipoprotein oxidation, and decreased risk of cardiovascular disease and cancer (Karak and Bhagat 2010). During the production of tea beverages, hot and clear tea infusions tend to form turbid precipitates after cooling. These precipitates, called tea creams, are formed by a complex mixture of polyphenols. Tea cream

formation is a quality problem and may have antinutritional effects (Lu et al. 2009). Tannase can hydrolyze the ester bonds of catechins to release free Gallic acid and water-soluble compounds with lower molecular weight, reducing turbidity, and increasing solubility of tea beverage in cold water at 4 °C (Aguilar-Zárate et al. 2014). Precipitates are formed by the interaction of phenolic compounds and caffeine called "tea cream." Tea cream formation is a feature that was effected on the storage quality of these products (Li et al. 2017). Thus, tannase has been widely used to hydrolyze tea cream in the processing of tea (Su et al. 2009).

Enzymatic treatment of tea beverages leads to a better color appearance, less cream formation, better taste, mouth feel, and overall acceptance (Lu et al. 2009). Also, the hydrolysis of the main tea phenols epigallocatechin gallate and epicatechin gallate to epigallocatechin and epicatechin, respectively, increases the antioxidant activity of tea beverage (Lu and Chen 2008). Tannase was documented to be used in deesterification of tea polyphenols in non-converted green tea leaves, which enhances the natural levels of gallic acid and epigallochatechin and favors the formation of epitheaflavic acids, which were responsible for bright reddish color and good cold-water solubility (Aguilar and Gutierrez-Sanchez 2001). *N*-nitrosodimethylamine (NDMA) inhibition by tannase-treated green tea is reported, owing to its antioxidant potential (Lu and Chen 2007). Tea infusions contain four types of catechins such as epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Chen et al. 2014). The taste of tea gets increased with increasing concentration of catechin and the taste palatability gets reduced (Zhang et al. 2016). Tea catechins have another disadvantage in that it has poor bioavailability (Fan et al. 2016). Safety evaluation of tannase enzyme that was from *A. oryzae* was conducted and found to be safe for its usage in tea preparation (Kumar et al. 2019).

4.5.1.2 Beverage Clarification

New fruit juices (pomegranate, cranberry, raspberry, etc.) have recently been acclaimed for their health benefits, in particular, for their antioxidant properties. However, the presence of high tannin content in those fruits is responsible for haze and sediment formation, as well as for color, bitterness, and astringency of the juice upon storage (Aguilar et al. 2007). Tannase is utilized to reduce and remove the undesirable bitterness present in fruits juices by enzymatic treatment possessing the advantage of lowering the haze and increasing the quality of the beverages (Rout and Banerjee 2006). When the concentration of tannins in fruits increase, such as in blueberry, pomegranate, and raspberry, leads to the formation of sediment, color, and bitter taste during the storage of the beverages. In these cases, an enzymatic treatment with tannase is recommended (Aguilar et al. 2007). Rout and Banerjee (2006) documented a reduction of 25% of tannin content in pomegranate juice by using a treatment with tannase, while by using tannase and gelatin the tannin content diminishes by 49%. Hydrolysis by immobilized tannase removed up to 73.6% of the tannin present in Indian gooseberry (*Phyllanthus emblica*) juice. This

enzymatic treatment reduced the content of tannin but increased the gallic acid concentration with a minimum reduction in vitamin C (only 2%) (Srivastava and Kar 2009, 2010).

Tannase is used as a clarifying agent in refreshing drinks with coffee flavor, and, recently, a process for the enhancement of the antioxidant properties of coffee by tannase and other enzymes has been patented (Bel-Rhlid et al. 2009). Tannase has also been utilized in grape musts and barley worts as a prefermentative treatment, coupled with conventional fining for stabilizing wine and beer (Cantarelli et al. 1989). Tannase is employed for the elaboration of acorn wine. Its use in this process favors the production of a better beverage with an alcoholic content of 10%, reducing sugars content of 7%, and a pH of 4.0. In this process, tannase produced by an *Aspergillus* strain helps in improving the flavor of the beverage (Aguilar and Gutierrez-Sanchez 2001). Tannase is used to prevent discoloration and haze development during beer storage. It was also used to make acorn wine from Korean acorns (*Quercus* spp.) and to treat grape must and juice along with lactase to remove phenolic substances for stabilizing and increasing the quality of the wine (Lekha and Lonsane 1997).

4.5.1.3 Nutritional Improvement of Legume Flours

Legumes are of major nutritional importance, especially in developing countries. Seed legumes have high protein contents, and this protein is of good biological value. However, they also have several anti-nutritional factors, affecting the digestibility of nutrients. Different thermal and biological processes have been used to reduce the anti-nutritional factors content, increasing their nutritional value. The flours obtained from the processed legumes can be used as ingredients in food preparations. Several studies mention the uses of tannase alone or in combination with other enzymes for the degradation of some antinutritional factors (tannins) present in legume flours. Duenas et al. (2007) studied the effect of the addition of tannase and other enzymes to a lentil (*Lens culinaris*) flour. They found the production of several phenolic compounds after the treatment with tannase, the decrease of other phenolics such as catechin, epicatechin, and catechin 3-*O*-glucose, and significant increment in the antioxidant activity. On the other hand, the application of tannase on pea (*Pisum sativum*) led to a decrease in all the phenolic compounds studied and a reduction in the antioxidant capacity (Duenas et al. 2009). But, in a different experiment, the hydrolysis of pea flour by tannase led to a significant improvement in the daily weight gain of rats (Urbano et al. 2007).

4.5.2 Animal Feeds Industries

It is well known that high levels of dietary tannins have negative effects on animal nutrition. Tannins form a strong complex with enzymes, minerals, and other nutrients. They are also responsible for a bitter taste, which considerably reduces the feed intake (Belmares et al. 2004). The presence of tannins in tannin-rich plants and agro-industrial waste, which are used as animal feed, make them unusable. Tannins are known as anti-nutritional factors because of their interaction with macromolecules such as proteins makes them indigestible (Frutos et al. 2004). Anti-nutritional effect of tannin could be reduced by a treatment with tannase or tannase-producing microorganisms. For example, there are some cultivars of sorghum with high content of tannins. Tannin content could be decreased by enzymatic treatment, and this material could be used as a complement in animal diet (Aguilar and Gutierrez-Sanchez 2001). Nuero and Reyes (2002) reported the production of an enzymatic extract containing tannase from mycelial wastes of *penicillin manufacture*. This preparation was applied to several flours used as animal feed (barley, bran, maize, oat, rye, soya, and wheat flour). The enzymatic extract from mycelia waste released similar amounts of reducing sugars from all flours when compared with a commercial enzymatic additive used in animal feeding. Tannase is applied for the treatment of tannin-rich plants in the production of animal feed. If they are first treated with tannase, tannin content is decreased and this can then be used as a complement in animal diet. Tannase utilization can be carried out in two ways: direct contact of enzymatic extracts with the material to hydrolyze the polyphenols and avoid their unpleasant polymerization, or growing tannase-producing fungal strains on tannin-rich materials, which are degraded to simpler compounds (De Sena et al. 2014).

4.5.3 Gallic Acid Production

Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic compound and the monomeric unit of the gallotannins and complex tannins. Gallic acid and related compounds possess many potential therapeutic properties including anticancer and antimicrobial properties (Ow and Stupans 2003). One of the most important applications of tannase is gallic acid production from hydrolyzable tannins (Kar et al. 2002). Gallic acid is commercially important for its applications in several industries for the synthesis of a variety of chemicals used in the food and pharmaceutical industries. Gallic acid is utilized as a precursor for the synthesis of trimethoprim (2,4, diamino 3,4,5 trimethoxy benzyl pyrimidine) is an antibacterial drug (Sittig 1988). A broad-spectrum antioxidant and antibacterial agent, which is bacteriostatic, since it inhibits folic acid metabolism in pathogenic bacteria (Mukherjee and Banerjee 2003).

In combination with sulfonamide, trimethoprim exerts antibacterial effect at low concentrations against *Streptococci* and *Staphylococci* bacteria, *Shigella* sp.,

Corynebacterium diphtheriae, *E. coli*, *Vibrio cholerae*, *Bacillus pertussis*, and *Clostridium welchii*. Choi et al. (2010) reported the potential of gallic acid as an antiviral agent. They have also cytotoxicity against cancer cells (Beniwal et al. 2013). Curiel et al. (2010) reported a process for the enzymatic production of gallic acid. They immobilized a recombinant tannase from *L. plantarum* expressed then utilized the immobilized enzyme for the hydrolysis of commercial tannic acid. At least 95% of tannic acid was transformed into gallic acid, obtaining an almost pure compound.

Tannase is an enzyme characterized for catalyzing the hydrolysis of gallic acid esters. Weetall (1985) reported the enzymatic synthesis of a variety of gallic acid esters. He applied an immobilized tannase from *Aspergillus niger* to a solution of gallic acid in different alcohols (C1–C12) and diols (C3–C6). In the chemical industry, gallic acid is used for forming pyrogallol, and gallate esters find their applications in food industries, cosmetic industries, and so on. Pyrogallol has also been used as a photographic film developer (Banerjee et al. 2007; Yu and Li 2008; Beniwal et al. 2013). It is also used in the leather industry, in manufacturing gallic acid esters, such as propyl gallate, a potent antioxidant utilized as an antioxidant in fats and oils, in the manufacture of pyrogallol, and as a photosensitive resin in semiconductor production (Sariozlu and Kivanc 2009).

4.5.4 Bioremediation of Tannin-Contaminated Wastewaters

Tannins occur commonly in the effluents derived from several agro-industries. The treatment of this kind of wastewaters is usually difficult because tannins are highly soluble and inhibit the growth of many microorganisms (He et al. 2007). Industrial tannins when used in the tanning industry can represent a serious environmental problem on a global level, although vegetable tanning agents are natural materials, they are poorly biodegradable and act as growth inhibitors toward many microorganisms, ultimately affecting the receiving ecosystem (Prigione et al. 2018). The tannase enzyme has potential uses in the treatment of tannin-containing effluents and pre-treatment of tannin-containing animal feed (Belur and Mugeraya 2011). Fungal strains capable of performing the biotransformation of polyphenolic substances contained in tannins could have a certain environmental impact as bioremediation agents. Moreover, biotransformed tannins could have several applications in agriculture, in the animal feed and wine industries, and the tanning process, for example, improving tanning yields or leather quality (Prigione et al. 2018).

Several authors have reported the biodegradation of tannin-containing wastewaters using model systems. Kachouri et al. (2005) studied the biodegradation and decolorization of olive-mill wastewater by *Aspergillus flavus*. The fungi removed 58% of color and 46% of the chemical demand of oxygen of the wastewater after six days of cultivation. More recently, Murugan and Al-Sohaibani (2010) reported the use of immobilized tannase from *Aspergillus candidus* for the removal of tannin and the associated color from tannery effluent. Enzymatic treatment removed about

42% of the tannin content and 20% of the color of tannery wastewater. These findings suggest that tannase or tannase-producing microorganisms could be utilized for pretreatment of tannin-rich wastewaters.

4.6 Conclusion

Tannin acyl hydrolase (tannase) is one of the important hydrolytic microbial enzymes. Fungi are the most producing tannase in other microorganisms with special to genus *Aspergillus*, which is considered as the best producer of tannase. Microbial tannase is more stable than others sources of tannase and can be genetically modified so that the enzyme is considered industrially important and has several applications in various industries such as foods, animal feeds, cosmetics, pharmaceutical, chemical, industries, and so on. The most important application of the tannase enzyme is producing gallic acid and also degradation of industrial tannins because it causes a serious problem in the environment.

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