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Microbial Degradation of Hydrolysable Tannins

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Abstract: Tannins are water-soluble polyphenolic secondary metabolites of higher plants with the molar mass from 300 to 3 000. Hydrolysable and condensed tannins are the two major classes of tannins. There are two groups of hydrolysable tannins: gallotannins and ellagitannins on the basis of structural characteristics. In nature, some microbes are resistant to tannins, even capable of degrading tannins into low-molar-mass tannins and lots of derivatives which have marked biological and pharmacological activities. Gallotannins can be degraded more easily than ellagitannins which have complicated structures with the further coupling C—C bond. However, some bacteria and fungi from the ellagitannin-rich soil, leaves and tannery liquors can hydrolyze ellagitannins. Because of the complicated structures of the further coupling C—C bonds, both complex tannins and condensed tannins are harder to be degraded than gallotannins and ellagitannins in both aerobic and anaerobic environments. This review could provide much references for the further researches on biodegradation of tannins.

Key words: hydrolysable tannins; degradation; microorganisms

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水 解 单 宁 的 微 生 物 降 解

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摘 要:单宁为高等植物的次生代谢产物,是相对分子质量为 300 到 3 000 的多酚类化合物,主要分为水解单宁和缩合单宁两大类。根据水解单宁结构特性的差异,又将其分为棓单宁和鞣花单宁。自然界中的某些微生物具有忍耐单宁的特性,甚至可以将单宁降解成具有一定生理或药理活性的小分子单宁或其衍生物。由于缩合单宁的分子结构复杂,对其微生物降解的研究远不及水解单宁深入。本文综述了近年来关于细菌和真菌降解棓单宁和鞣花单宁的研究情况,阐述了其降解机理,提出了微生物降解单宁需要解决的问题,可为单宁生物降解的进一步研究提供参考。

关键词:水解单宁;降解;微生物

In nature, tannins are found worldwide in many different families of the higher plants such as chestnut and oak wood. Depending on origins of tannins, their chemistry varies widely, and they have a molar mass of 300 to 3 000. Tannins of high concentration can be found in nearly every part of plants, such as in bark, wood, leaves, fruit, roots and seeds[1-2]. The occurrence of tannins in common foodstuffs is widely in fruits and nuts[3]. It is worth noting that some fruits and nuts produce either gallotannins or ellagitannins whilst others produce complex mixtures containing gallo-, ellagi- and condensed tannins.

In the early studies, it is defined that tannins are water soluble phenolic compounds with the molar mass between 300 and 3 000 [1], and they are either galloyl esters or their derivatives, in which galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores, or they are oligomeric and polymeric proanthocyanidins that can possess different interflavanyl coupling and substitution patterns[2].

On the basis of structural characteristics, hydrolysable tannins are classified into gallotannins and

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ellagitannins[2]. Gallotannins are those tannins in which galloyl units or their meta-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units. Upon hydrolysis by acids, bases or certain enzymes, gallotannins yield glucose and gallic acid. Ellagitanins are those tannins in which at least two galloyl units are C—C coupled to each other, and do not contain a glycosidically linked flavanol unit. Upon hydrolysis, the hydroxydiphenoyl residue undergoes lactonization to produce ellagic acid, which is not easily hydrolyzed because of the further C—C coupling of the polyphenolic residue with the polyol unit[4-5]. Complex tannins are those tannins in which a catechin or epicatechin unit is bound glycosidically to a gallotannin or an ellagitannin unit. Upon hydrolysis by certain enzymes, complex tannins yield catechin/epicatechin and gallic acid/ellagic acid[6]. Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C-4 of one flavanol moiety with C-8 or C-6 of the next monomeric flavanol, which are more difficult to be hydrolyzed[2-3,6-7].

The molar mass of molecules affects the characteristics of tannins directly. It is found that the higher molar mass of molecules tannins have, the lower biological activities they have[8]. So hydrolysable tannins are always degraded into gallic acid or ellagic acid which has marked biological and pharmacological activities[9]. Recently, it has been demonstrated that gallic acid and ellagic acid sulphate can inhibit the infection of human T-cell lymphotrophic virus type-1-carrying MT- 4 cells by human immunodeficiency virus (HIV)[10]. Thus the research on how to gain the highly biological small molecule tannins is on the way[11-12]. One efficient way to degrade the large molecule tannins into smaller molecule tannins with valuable bioactivities is microbial degradation on which a number of researches have appeared in the past which have provided a general idea of the degradation of these polyphenols by some microbes with a large extent of tannin-degrading activity. Many fungi, bacteria and yeasts are quite resistant to tannins and they have different mechanisms of tannin degradation. Besides, various microbes have different resistances to tannins[6]. The microbial degradation of condensed tannins is, however, less than hydrolysable tannins in both aerobic and anaerobic environments. As a result, a holistic view of microbial degradation of gallotannins and ellagitannins including their potentials for manipulating the detannification property of certain microbial strains for beneficial effects on food and fodder has been researched. The current status of the work on microbial degradation of tannins is presented under the microbial degradation of gallotannins and ellagitannins.

1 Microbial degradation of gallotannins

1.1 Bacterial degradation

It is well known that tannins are toxic and bacteriostatic compounds making non-reversible reactions with proteins[13]. However, some bacteria like *Achromobacter* sp., *Bacillus* sp., *Corynebacterium* sp., *Klebsiella* sp., *Citrobacter* sp., may degrade gallotannins and their monomers[14-16]. Deschamps et al. made a detailed study on the degradation of gallotannins by the aerobic bacteria, and isolated fifteen bacterial strains belonging to the genera *B.*, *Staphylococcus* and *K.* by enrichment culture technique, using gallotannins as a sole carbon source[15]. And it has been found that a few bacteria can produce an extracellular tannase to degrade gallotannins to gallic acid[15,17], even to yield two intermediates considered to be di-gallic and tri-gallic structures probably bound to glucose by *B. pumilus*[18]. Besides, certain kinetic characteristics of the extracellular tannase production by *B. licheniformis* are taken into consideration[19]. A bacterial strain capable of utilizing gallotannins as the sole carbon source is isolated from the effluent of a tannery and is identified as *C. freundii*, which can grow at concentrations as high as 50 g/L of gallotannins. *C. freundii* has more gallotannin tolerance than *B. pumilus*, *B. polymyxa*, *Corynebacterium* sp. and *K. pneumoniae* which are reported to degrade

10 g/L gallotannins[17] due to its production of extracellular tannase to hydrolyze them, and the proposed biochemical pathway for the degradation of gallotannins by *C. freundii has* been detected[14] (Fig. 1).

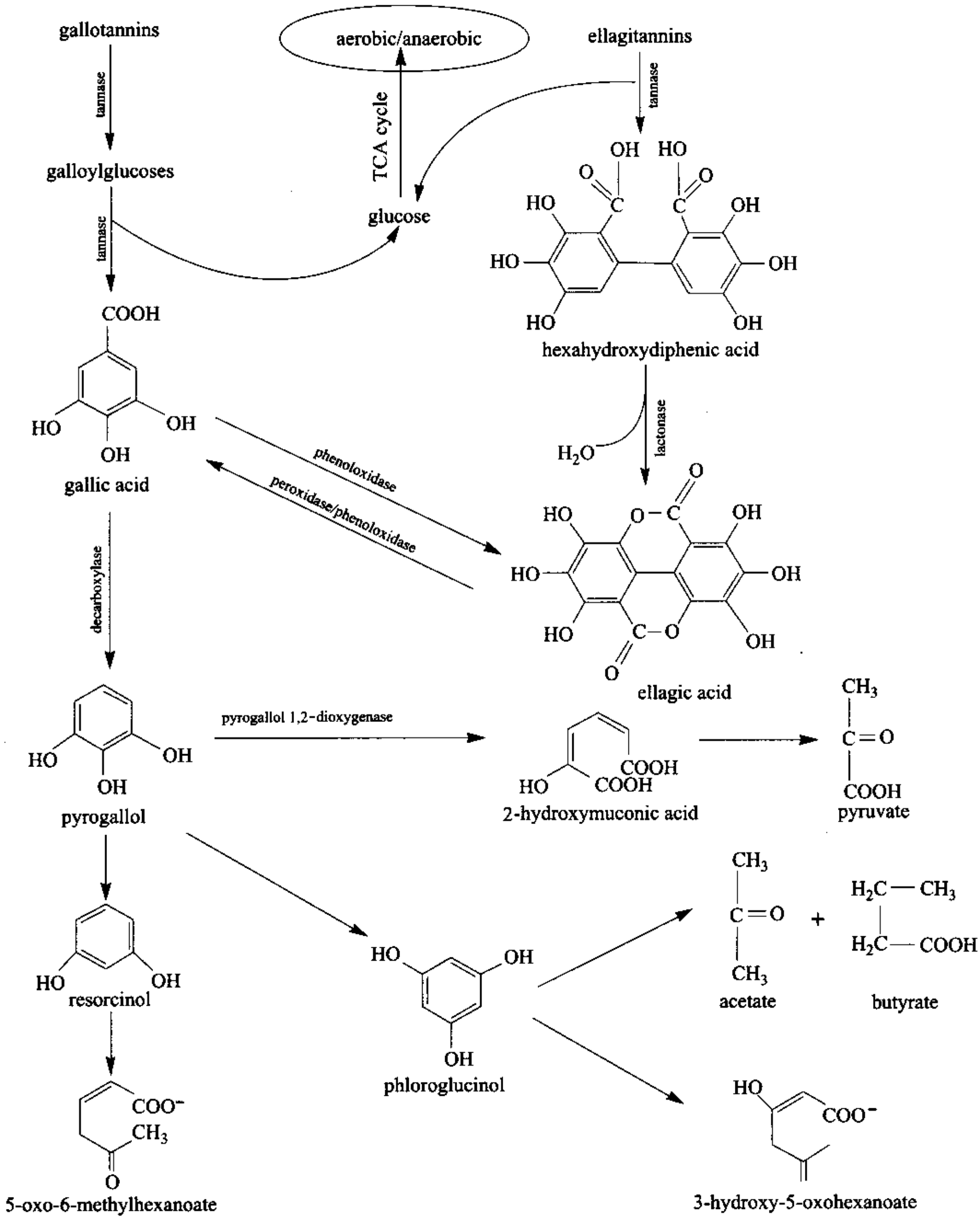

Fig. 1　Pathways for the biodegradation of gallotannins and ellagitannins with different enzymes

Animals that regularly browse tannin-containing plants have developed resistance to tannins, at least partly through the presence of tannin-resistant ruminal microorganisms[20]. Many tannin-tolerant bacterial isolates such as *Streptococcus gallolyticus*, *S. bovis*, *S. caprinus*, *Eubacterium oxidoreducens*, *Selenomonas ruminatium*, *Escherichia coli* as well as their phylogenetic ruminal strains are from the rumen[21-22]. A strain of *S. gallolyticus* has been isolated from feral goat rumen samples, which is resistant to gallotannins at concentration of up to 70 g/L[22]. In contrast, growth of the more common ruminal *S.* like *S. bovis*, is inhibited by gallotannins at concentrations lower than 5 g/L[15]. Resistance of *S. gallolyticus* to gallotannins was investigated by O′Donovant and Brooker and they described the mechanisms, expression of gallate decarboxylase activity and secretion of excracellular polysaccharide by which *S. gallolyticus* gains a growth advantage[21]. Extracellular polysaccharide matrix of *S. gallolyticus* can provide a protective barrier to the organism but it's not enough to provide protection against tannins, and possibly, that induction of gallate decarboxylase is a critical factor in tannin tolerance by *S. gallolyticus*. Gallate decarboxylase can decarboxylate gallic acid to pyrogallol, but *S. gallolyticus* does not transform pyrogallol further and the reason is not clear[22].

Various strains of *Selenomonas* have been shown to have the ability to hydrolyze the glycoside bonds in phenolic compounds and ferment the sugars, but are not able to degrade the heterocyclic ring[23]. *Selenomonas* sp. can produce tannin acylhydrolase to cleave the ester bonds between glucose and gallic acid in tannins, then utilize the glucose as a sole carbon source. It is reported that it is able to grow in concentration of gallotannins as high as 70 g/L[19]. Pyrogallol is the major product of tannin hydrolysis, which suggests that it is able to decarboxylate gallic acid further but not able to cleave the phenolic ring. Compared to *S.* sp., tannin acylhydrolase is a characteristic enzyme of *S.* sp. to hydrolyze gallotannins. While the *S.* sp. is also able to transform gallotannins to pyrogallol but is not tannin-acylhydrolase-positive. So tolerance to or hydrolysis of tannins is not only due to the production of tannin acylhydrolase but also to some other unknown mechanism[23].

During wastewater treatment, performed in continuous anaerobic reactor fed with shea cake, high removal rates of tannins and production of organic acids and methane are consistently observed. Strains of *S. gallolyticus* and *Escherichia coli* are isolated from this anaerobic digester. *E. coli can* tolerate gallotannins and decarboxylate only *p-hydroxybenzoic* and vanillic acids to their corresponding phenol and guaiacol under anaerobic and aerobic conditions without further degradation[24].

1.2 Fungal degradation

Many fungal species like *Aspergillus*, *Fusarium*, *Penicillum*, *Sporotrichum*, *Rhizoctonia*, *Cylindrocarpon* and *Trichoderma* can degrade hydrolysable tannins, particularly gallotannins[15]. Most of these fungal species have been used for biodegradation of tannery effluent[6]. Some ericoid and ectomycorrhizal fungi can also degrade soluble polyphenolic materials like gallotannins and ellagitannins to reduce their antinutritional effects. It is reported that the presence of gallotannins induces *Hymenoscyphus ericae* of ericoid endophyte to produce extracellular polyphenol oxidase. Mycelial yields and the extent of gallotannin degradation by *Hymenoscyphus ericae* are both enhanced by exogenous ammonium[25].

A tannin-degrading strain of *A. niger* is grown at pH value 5.0 and 30℃ in a defined medium where tannins are the sole carbon source and energy. The fungus has variable growth in gallotannins and quebracho tannin-medium and can tolerate these tannins even up to 150 g/L without showing any growth inhibition[26]. Its tolerate concentration is much higher than usual tolerate concentrations as high as 70 g/L[22] and 100 g/L[13] of this polyphenolic compound. *Aspergillus* and *Penicillium* have been reported to grow at high concentrations of gallotannins as a sole carbon source[13]. Several factors affecting the growth and enzyme activities of *A. niger* such as nitrogen sources, pH value, temperature, oxygen diffusion and the combined parameters on the tannin degradation have been reported. *A. niger* can resist to 50 g/L gallotannins at pH value 3.5 and 28℃, with agitation speed of 150 r/min and urea of 1.0 g/L[27].

There are only a few reports on tannin-degrading yeasts. Initially, six strains of yeasts are isolated from tannery liquors and xylophagous insects, which show the growth and hydrolytic action on tannins in culture media containing various concentrations of gallotannins. Yeasts like *Candida nitrativorans*, *Debaromyces hansenii*, *Pichia adzetti*, *P. monospora*, *P. polymorpha* and *P. strasburgensis* can degrade tannins[19]. The tannin-degrading enzymatic system of *Candida* is found to utilize gallotannins as substrate[28].

2 Microbial degradation of ellagitannins

Compared to gallotannins, ellagitannins are much more difficult to be degraded by microbes because of its complex structures with the further coupling C—C[14]. Some bacteria, fungi and yeasts can only hydrolyze the galloyl residues of galloyl eaters in ellagitannins, but some other bacteria and fungi from the ellagitannin-rich soil, leaves and tannery liquors can produce highly active tannase to hydrolyze hexahydroxydiphenoyl and other residues of ellagitannins[29].

2.1 Bacterial degradation

Ellagitannins are common in wines aged in oak barrels, because the wood of some varieties may contain up to 10% ellagitannins by weight, contributing to the sensory properties of wine[30]. Ellagic acid is a dimeric derivative of gallic acid and is generally recognized as the hydrolytic byproduct following the release of a hexahydroxydiphenoyl easter group from ellagitannins[31].

A range of oenological lactic acid bacterial species and reference strains for their potentical to degrade ellagitannins in grape must and wine have been researched. It is reported that none of the strains belonging to the oenological species of the genus *Lactobacillus*, *Leuconostoc*, *Oenococcus* or *Pediococcus* are tannase producers, with the exception of *Lactobacillus plantarum* which is positive for tannase activity[29].

During quantitative and qualitative studies of the tannase-producing bacteria in the intestinal microflora of various mammalian species[32], another novel type of tannin-degrading bacteria from human fecal samples and fermented food is isolated[33]. A brief report is presented on the ecological prevalence, phenotypic characteristics, and identities of these ellagitannin-degrading bacteria. *Lactobacilli* sp. with tannase activity is isolated from human feces and fermented food. A PCR-based taxonomic assay reveals that the isolates belong to *L. plantarum*, *L. paraplantarum* and *L. pentosus*. Additional studies on a range of *L.* sp. from established culture collections confirm that this enzymatic activity is a phenotypic property common to these three species[33].

2.2 Fungal degradation

Although *Aspergillus*, *Penicillum*, *Fomes*, *Polyporus* and *Tremetes* can grow better on gallotannins than on ellagitannins[6], they still have the chance to produce highly active tannase to degrade ellagitannins into ellagic acid, gallic acid even pyrogallol. Besides, some of them also produce peroxidase like laccase to cleave the aromatic rings[34]. Different fungal strains of *Rhizopus* sp., *Phanerochaete* sp., and *A.* sp. are introduced to the detoxification of coffee husk in solid state fermentation. *R.* sp. and *A.* sp. show good detoxification of ellagitannins. Using *R. arrizus*, the best degrading rate of ellagitannins is 65 % which is obtained with pH value 6.0 and moisture 60 % in 6 d. Also, the best detoxification rate achieved by the *A.* sp. isolated from the coffee husk is 65 % for ellagitannins[35]. A strain resisting valonia tannin (ellagitannin from *Quercus aegilops*) is isolated from nature and proved to be quite biodegradation of valonia tannin and is identified as *Endomyces* sp. The optimum conditions for the degradation are pH value 4.5 for 8 d, which leads to 64 % COD removal rate[36].

The ability of several fungal strains to degrade and to detoxify cork (the outer bark of the cork oak tree, *Quercus suber* L.) boiling wastewater is investigated[37]. The fungal strains used involve *Sporothris* sp., *Trichoderma koningii*, *Chrysonilia sitophila* and *Penicillum glabrum* isolated from cork bark as well as *Fusarium flocciferum* and *Phanerochaete chrysosporium*. The results obtained in the degradation experiments carried out with each fungus show that most fungi display similar abilities, with a COD reduction of 54.2 % attained within 5 d of incubation except for *F. flocciferum* which presents the highest value for the COD reduction of 62 %[38]. In addition, a rise in pH values is detected with all the strains, except for *P. glabrum*. Toxicity tests performed on *Vivro fischeri* reveal that fungal treatment of the wastewaters causes the complete loss of toxicity in the cases of *Sporothris* sp., *T. koningii*, *P. chrysosporium* and *F. flocciferum*. The other two tested strains are also able to detoxify the raw wastewaters, causing a ten-fold decrease in toxicity. The results obtained in sequential biodegradation experiments with different pairs of fungi show that although the COD reduction dose not exceed 10 %, an important reduction in toxicity and a pH value rise are obtained[37].

3 Future trends

Nowadays, many researches have been made on gallotannin biodegradation and have gained great success in further utilization. Some of the industrial applications of these findings are in the production of tannase or the biotransformation of tannic acid to gallic acid and pyrogallol besides detannification of foods and fodders. Although ellagitannins have the typical C—C bound which is more difficult to be degraded than gallotannins, concerted efforts are still in progress to improve ellagitannin utilization. Currently, more attention is mainly focused on intestinal microflora biodegradation of tannins especially ellagitannins which can contribute to the definition of their bioavailability for both human beings and ruminants. Recently there also have been endeavours to utilize the tannin-degrading activity of different fungi for ellagitannin-rich biomass. Due to the complicated structures of complex tannins and condensed tannins, like existence of the C—C bounds among catechin, epicatechin, or proanthocyanidin units, the biodegradation of them is much more difficult and recently there is fewer researches on them. Therefore, the work on the mechanisms of gallotannin and ellagitannin biodegradation can result in the overall understanding to the biodegradation of complex tannins and condensed tannins. Also it is much easier for us to see out the structure-activity relationships of these tannins with low molar mass of molecules and their derivatives, which can lead us to the understanding of marked biological and pharmacological activities.

In view of the evidence for beneficial effects of tannin biodegradation, there is a need to clarify: 1) screening and tameness of high tannin-tolerant microbes; 2) some other unknown factors on microbe tolerance to tannins or degradation of tannins; 3) acquisition of degraded products with high bioactivities; 4) utilization of tannin degrading biotechnology.

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