Biodegradation **9:** 343–357, 1998.

Microbial degradation of tannins – A current perspective

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Accepted 20 July 1998

Key words: biodegradation, condensed tannins, gallic acid, hydrolysable tannins, quercetin, rumen, tannase, tannins

Abstract

Tannins are water-soluble polyphenolic compounds having wide prevalence in plants. Hydrolysable and condensed tannins are the two major classes of tannins. These compounds have a range of effects on various organisms – from toxic effects on animals to growth inhibition of microorganisms. Some microbes are, however, resistant to tannins, and have developed various mechanisms and pathways for tannin degradation in their natural milieu. The microbial degradation of condensed tannins is, however, less than hydrolysable tannins in both aerobic and anaerobic environments. A number of microbes have also been isolated from the gastrointestinal tract of animals, which have the ability to break tannin-protein complexes and degrade tannins, especially hydrolysable tannins. Tannase, a key enzyme in the degradation of hydrolysable tannins, is present in a diverse group of microorganisms, including rumen bacteria. This enzyme is being increasingly used in a number of processes. Presently, there is a need for increased understanding of the biodegradation of condensed tannins, particularly in ruminants.

Introduction

Tannins are defined as naturally occurring water-soluble polyphenols of varying molecular weight, which differ from most other natural phenolic compounds in their ability to precipitate proteins from solutions (Spencer et al. 1988). This property is the basis for their past and present use in the tanning industry. They are widespread in the plant kingdom (pteridophytes, gymnosperms and angiosperms), are found in leaves, fruits, bark and wood, and can accumulate in large amounts in particular organs or tissues of the plant (Haslam 1989). Tannins are considered plant secondary substances as they are not involved in metabolic pathways. After lignins they are the second most abundant group of plant phenolics. The presence of a large number of phenolic hydroxyl groups enables them to form large complexes, mainly with proteins, and to a lesser extent with other macromolecules like cellulose and pectin (McLeod 1974; Mueller-Harvey & McAllan 1992).

Based on their structures and properties, they are distributed into two major groups – hydrolysable and condensed tannins (Figures 1 and 2). Hydrolysable tannins are composed of esters of gallic acid (gallotannins) or ellagic acid (ellagitannins) with a sugar core which is usually glucose, and are readily hydrolysed by acids or enzymes into monomeric products. The major commercial hydrolysable tannins are extracted from Chinese gall (*Rhus semialata*), sumac (*Rhus coriara*), Turkish gall (*Quercus infectoria*), tara (*Caesalpina spinosa*), myrobalan nuts (*Terminalia chebula*), and chestnut (*Castanea sativa*) (Table 1). Condensed tannins, also known as polymeric proanthocyanidins, are composed of flavonoid units, and are usually more abundant in tree barks and woods than their hydrolysable counterparts. The important commercial condensed tannins are extracted from wattle (*Acacia mollissima* and *A. mearnsii*), quebracho (*Schinopsis lorentzii* and *S. balansae*) and tree barks. A group which occupies an intermediate position in the tannin hierarchy is the family of catechin tannins combining elements of hydrolysable and condensed tannins. These tannins are quite common in tropical shrub legumes (Mueller-Harvey et al. 1987) and tea leaves (Graham 1992). Recently, gallic acid esters of

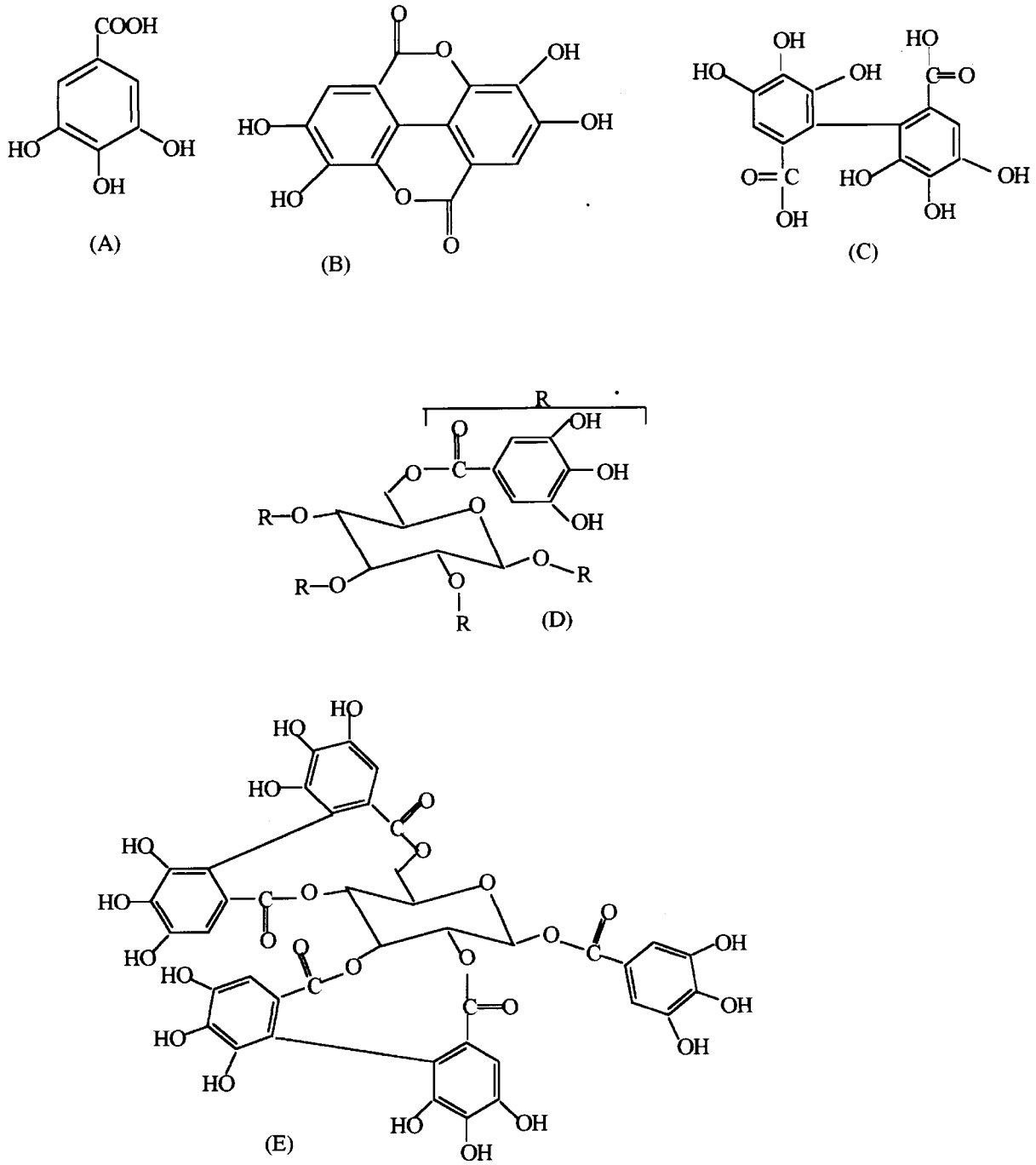

Figure 1. Structure of (A) gallic acid, (B) ellagic acid, (C) hexahydroxydiphenic acid, (D) gallotannin and (E) ellagitannin.

proanthocyanidins have also been discovered (Porter 1994).

Tannins inhibit the growth of a number of microorganisms, resist microbial attack and are recalcitrant to biodegradation (Field & Lettinga 1992a). Condensed tannins are more resistant to microbial attack than hydrolysable tannins and are toxic to a variety of microorganisms. For this reason, tannins generally retard the rate of decomposition of soil organic matter via inhibition of biodegradative enzymes of the attacking organism (Scalbert 1991). Despite the antimicrobial properties of tannins, many fungi, bacteria and yeasts are quite resistant to tannins, and can grow and develop on them (Deschamps 1989). Certain moulds such as *Aspergillus* or *Penicillium* have been observed to grow on the surface of liquids of tannery pits and tannery wastes (Rajakumar & Nandy 1983). Some moulds develop easily on the surface of tannin-rich woods such as quebracho, European or American chestnut, and the phytopathogenicity of such type of microbes is determined to a large extent by their tannin-degrading activity (Farias et al. 1992, 1994).

A number of reviews on tannin biodegradation have appeared in the past which have provided a general idea of the biodegradation of these polyphenols (William et al. 1986; Deschamps 1989; Field & Lettinga 1992b; Saxena et al. 1995). Since the latest review of Saxena et al. (1995), a lot of work has been published on the industrial and agricultural applications of tannin biodegradation (Archambault et al. 1996; Hatamoto et al. 1996; Selinger et al. 1997; Lekha and Lonsane 1997; Lane et al. 1997) which warranted a fresh appraisal of the present scenario on tannin biodegradation. This review takes a holistic view of microbial degradation of tannins and the discussion includes the potential for manipulating the detannification property of certain microbial strains for beneficial effect on food, beverages, feed and fodder. Besides, it focuses on the emerging area of tannin degradation by gastrointestinal microbes of herbivores with particular emphasis on ruminal microorganisms. The current status of the work on microbial degradation of tannins is presented under the following main headings:

Bacterial degradation of tannins.
Fungal degradation of tannins.
Microbial tannase and tannin degradation.
Tannins and their interaction with gastrointestinal tract microflora.

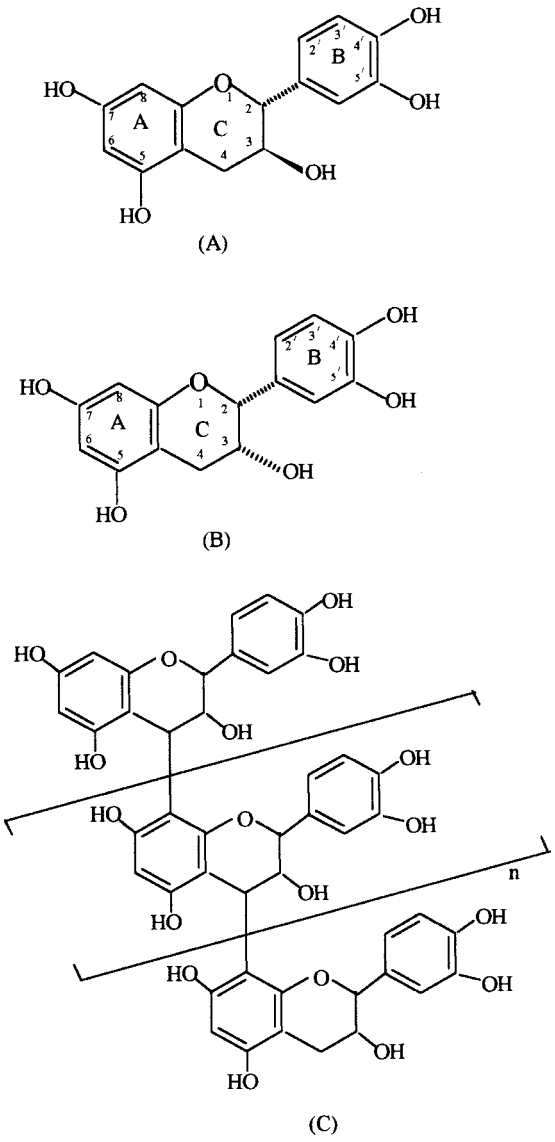

Figure 2. Structure of (A) catechin, (B) epicatechin, and (c) 4,8 linked procyanadin (condensed tannin).

Bacterial degradation of tannins

It is well known that tannins are toxic and bacteriostatic compounds making non-reversible reactions with proteins (Scalbert, 1991). Nevertheless, some bacteria may degrade many phenolic compounds including natural ones like catechol and protocatechuic acid (Deschamps 1989; Field & Lettinga 1992b). Lewis & Starkey (1969) reported for the first time the degradation of gallotannins by an aerobic bacterium, *Achromobacter* sp. Deschamps et al. (1980) made a detailed study on this phenomenon and isolated fifteen bacte-

Table 1. The major groups of tannins with their representative types and main sources

Hydrolysable Tannins	Catechin Tannins	Condensed Tannins
1. Gallotannins e.g. tannic acid (commercial name of Chinese gall tannins); yield gallic acid & glucose on hydrolysis	Catechin and epicatechin gallates; yield catechin/epicatechin and gallic acid on hydrolysis; have properties of hydrolysable & condensed tannins	Polymeric proanthocyanidins; yield monomeric flavonoids such as flavan-3,4-diols & flavan-3-ols on hydrolysis e.g., quebracho tannins from the wood of quebracho tree.
Sources: Tara pods (*Caesalpina spinosa*), gall nuts (pathological excrescences) from *Quercus infectoria* (Turkish gall) & *Rhus semialata* (Chinese gall), sumac leaves (*Rhus coriara*)	**Sources:** Tropical shrub legumes, tea leaves	**Sources:** Commonly found in fruits and seeds such as grapes, apple, olives, beans, sorghum grains, carob pods, cocoa & coffee, besides tree bark & heart wood.
2. Ellagitannins – yield ellagic acid & glucose on hydrolysis. **Sources:** Wood of oak (*Quercus* spp.), chestnut (*Castanea* spp.) and myrobalan (*Terminalia chebula*)		**Common types are** 1. Quebracho tannins from wood of *Schinopsis* spp., *Loxopterygium* spp. 2. Wattle tannins from *Acacia* spp. 3. Bark tannins from pine (*Pinus* spp.), oak (*Quercus* spp.) and gaboon wood (*Aucoumea kleneana*).

rial strains belonging to the genera *Bacillus*, *Staphylococcus*, and *Klebsiella* by enrichment culture technique, using tannic acid as the sole source of carbon. Nine of the isolated strains grew both on tannic acid and gallic acid, whereas only four strains degraded catechol or catechin. These workers also isolated several bacterial strains by enrichment technique which were capable of degrading hydrolysable and condensed tannins, including chestnut, wattle, and quebracho commercial tannin extracts. Wattle tannin-degrading bacteria were identified as *Enterobacter aerogenes*, *E. agglomerans*, *Cellulomonas* and *Staphylococcus*, whereas quebracho tannin-degrading bacteria were *Cellulomonas*, *Arthrobacter*, *Bacillus*, *Micrococcus*, *Corynebacterium*, and *Pseudomonas.* The production of extracellular tannase (tannin acyl hydrolase; EC 3.1.1.20) by bacterial cultures with simultaneous release of gallic acid and glucose, was reported for the first time by Deschamps et al. (1983). Strains of *Bacillus pumilus*, *B. polymyxia*, and *Klebsiella planticola* produced tannase with chestnut bark as the sole source of carbon. Some of these strains degraded tannins to gallic acid, but one of the strains of *B. pumilus* yielded two intermediates considered to be di- and tri-gallic structures, probably bound to glucose (Deschamps & Lebeault 1984).

Mechanism of degradation

The gallic acid monomers are readily utilised as substrates by oxidative breakdown to simple aliphatic acids, which then enter the citric acid cycle (Field & Lettinga 1992b). Prior to ring cleavage, gallic acid is converted to pyrogallol by gallate decarboxylase (Figure 3). The aerobic breakdown of flavonoid compounds derived from condensed tannins occurs through two pathways (Figure 4). The first degradation pattern is marked by the cleavage of the heterocyclic ring of catechin, a flavan-3-ol, to phloroglucinol carboxylic acid and protocatechuic acid (Barz & Hosel 1975). Phloroglucinol carboxylic acid, by decarboxylation and scission of the aromatic rings by various oxygenases, finally forms β-ketoadipate, an aliphatic acid, through intermediates like phloroglucinol, resorcinol, hydroxyhydroquinone and maleyl acetate. Protocatechuic acid is also converted to β-ketoadipate through β-carboxy *cis*, *cis* muconate and catechol pathways. Quercetin, a flavonol, is broken into phloroglucinol and 3, 4-dihydroxyphenyl acetate through the second pathway of flavonoid degradation. The former ends up as β-ketoadipate, while the latter is not degraded further (Fewson 1981; Gibson & Subramanian 1984; William 1986).

The anaerobic degradation of gallotannins was first reported by Field & Lettinga (1987) who observed

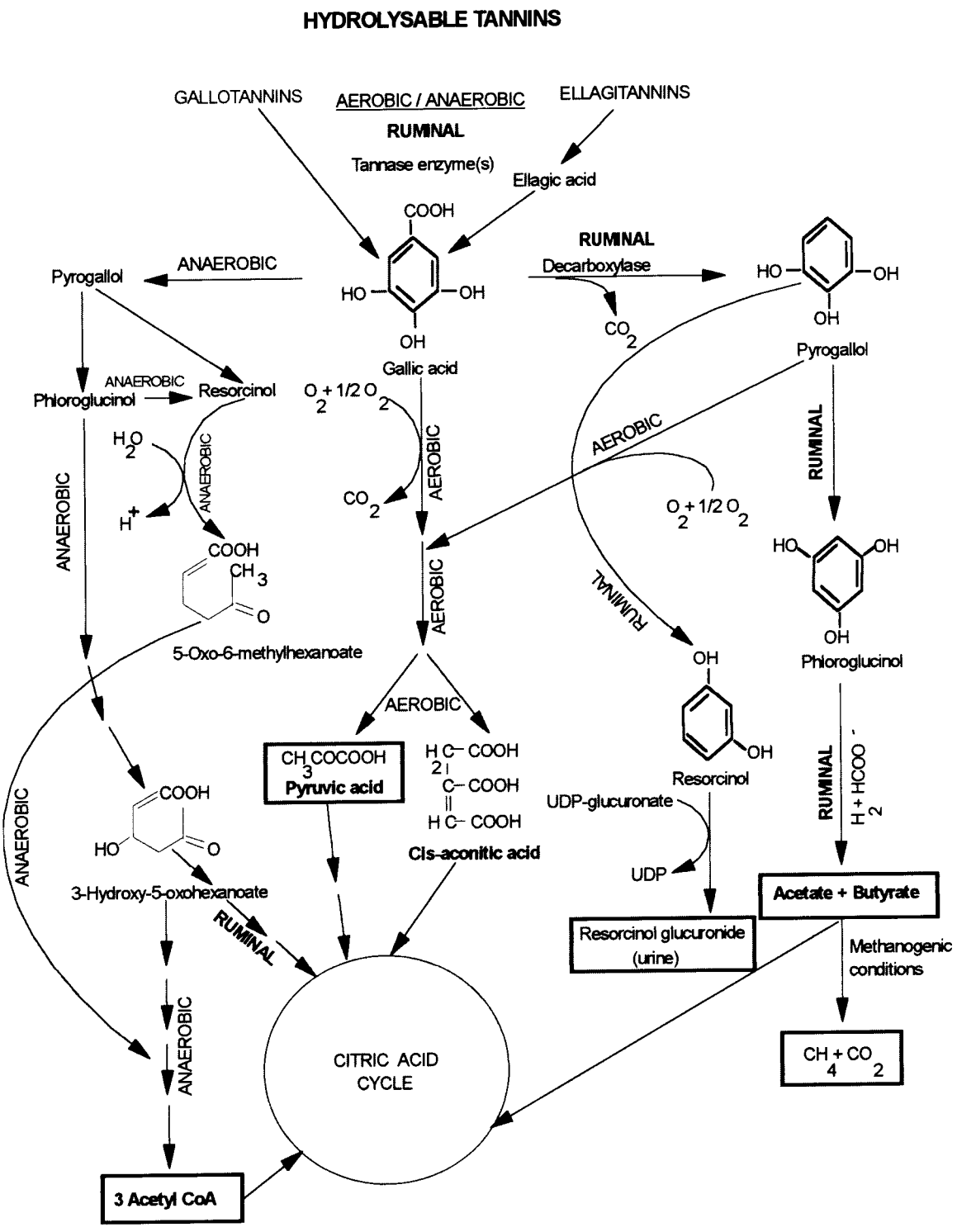

Figure 3. Pathways for biodegradation of hydrolysable tannins.

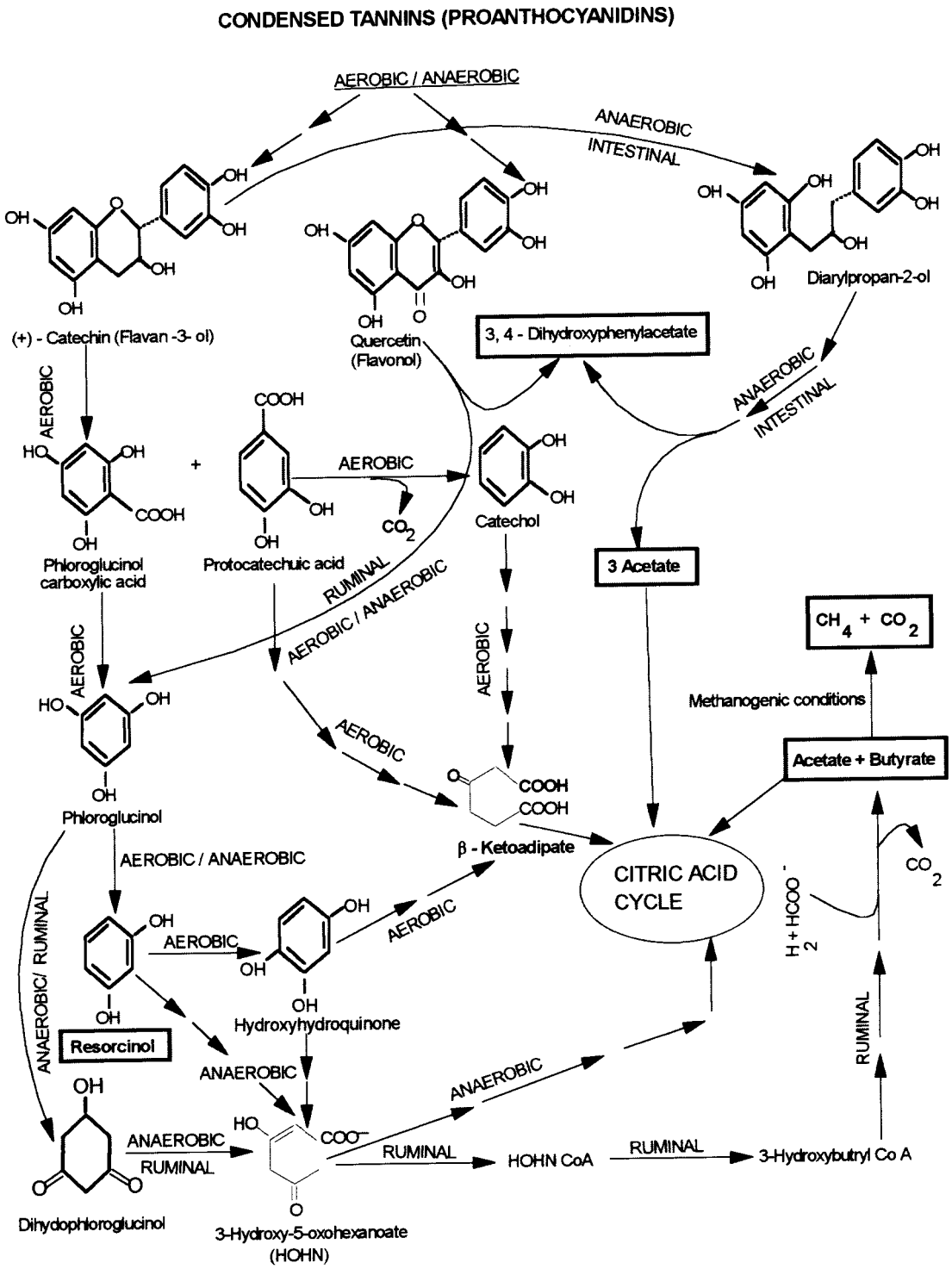

Figure 4. Pathways for biodegradation of condensed tannins.

the breakdown of tannic acid by a consortium of anaerobic sludge bacteria. Subsequently, a number of studies have been carried out on the biochemical mechanisms of anaerobic degradation of phenolic compounds, besides other aromatic compounds (Fuchs et al. 1994). Field & Lettinga (1987) observed that when gallotannins were present at subtoxic concentration, there was a high conversion of these hydrolysable tannins into methane. On the other hand, at concentrations which were toxic to methanogenic bacteria, they were degraded to acetate. The anaerobic decomposition of gallic acid, the monomer of hydrolysable tannins, occurs by different mechanisms (Figure 3). The first step is decarboxylation of gallic acid to form pyrogallol which is then isomerized to phloroglucinol by pyrogallol-phloroglucinol isomerase (Krumholz & Bryant 1988), and to dihydrophloroglucinol by phloroglucinol reductase (Brune & Schink 1990; Brune & Schink 1992). Dihydrophloroglucinol is then converted to 3-hydroxy-5-oxohexanoate (HOHN) by dihydrophloroglucinol hydrolase. HOHN is degraded by different pathways in anaerobic and in ruminal systems (Figures 3 and 4). In the anaerobic system, it is converted to 3,5-dioxohexanoate (triacetate) by HOHN dehydrogenase and ultimately to three molecules of acetyl-CoA via triacetyl-CoA by the sequential enzymatic action of triacetyl-CoA transferase, triacetate β-ketothiolase, acetoacetyl-CoA β-ketothiolase, phosphotransacetylase and acetate kinase (Brune & Schink 1992).

In earlier studies on the ruminal degradation of tannins and their monomers, it was found that a number of rumen bacteria could degrade gallic acid, pyrogallol, phloroglucinol and quercetin to acetate and butyrate (Tsai & Jones 1975; Tsai et al. 1976; Patel et al. 1981; Krumholz & Bryant 1986b). In the ruminal system the HOHN degradation was found to follow a different pathway. HOHN-CoA which is derived by the enzymatic action of HOHN-CoA transferase, is transformed to acetate and butyrate by the rumen bacteria by the sequential enzymatic action of β-hydroxybutyryl-CoA dehydrogenase, butyryl-CoA dehydrogenase, acetyl-CoA acetyl transferase, enoyl-CoA hydrase, phosphate acetyltransferase and acetate kinase (Krumholz et al. 1987). Recently, Nelson et al. (1995) observed the breakdown of tannins and phenolic monomers like gallic acid, pyrogallol, ferulic acid and *p*-coumaric acid by a novel ruminal bacterial isolate and postulated a degradation route for these compounds similar to that found by earlier workers. Resorcinol, which is metabolized anaerobically to acetyl-CoA first by reduction and then hydrolysis to a six-carbon keto acid (Tshech & Schink 1985; Fuchs et al. 1994), is however not degraded further by ruminal bacteria and excreted as a urinary phenolic conjugate (Murdiati et al. 1992). We observed the sequential appearance of gallic acid, pyrogallol and resorcinol in unadapted cattle ruminal fluid when incubated *in vitro* with tannic acid (Singh, Bhat & Sharma; unpublished results).

The anaerobic degradation of condensed tannin monomers and related flavonoid compounds has been described in gastrointestinal tract and rumen (Barz & Hosel 1975; Brown 1977), and anaerobic sludges (Field & Lettinga 1989). In these different anaerobic environments, the reductive breakdown of catechin and quercetin proceeds through distinctly different pathways (Figure 4). The initial degradation product of catechin is diarylpropanol, which upon ring scission is transformed to acetate and phenylvalerolactone. The latter after conversion to phenylpropionate derivatives may accumulate as such, or be further converted to phenylacetate compounds and accumulate in the microbial system. On the other hand, quercetin is broken into phloroglucinol and phenylacetate derivatives (Field & Lettinga 1992b). Phloroglucinol is rapidly fermented in various anaerobic systems via the dihydrophloroglucinol and HOHN pathways to either acetate and butyrate (Krumholz & Bryant 1986b) or acetyl CoA (Brune & Schink 1992), whereas phenylacetate derivatives accumulate in the medium.

The eventual transformation of phenolic intermediates to cleaved products viz. aliphatic acids, hydrogen, carbon dioxide and methane requires a syntrophic association between the phenolic degraders and methanogenic bacteria (Field & Lettinga 1992b). In the absence of active methanogens, the conditions for cleavage of the aromatic ring becomes thermodynamically unfavourable. The thermodynamics of the degradation shifts to a favourable mode if these methanogens remove the organic acids and hydrogen. However, phloroglucinol and other trihydroxyphenols are readily converted to acetate due to favourable standard free energies, even in the absence of active methanogens (Kaiser & Hanselmann 1982; Schink & Pfennig 1982; Krumholz & Bryant 1986b). The other intermediates of flavonoid decomposition by anaerobic bacteria generally accumulate in the media as derivatives of phenylpropionic or phenylacetic acids, or other more simple phenolic intermediates such as catechol, phenol and *p*-cresol (Young 1984; Berry et al. 1987; Evans & Fuchs 1988).

Fungal degradation of tannins

Knudson (1913) was first to report that tannic acid could be degraded by a strain of *Aspergillus niger.* Filamentous fungi, especially species of *Penicillium* and *Aspergillus* have been implicated in tannin degradation. Lewis & Starkey (1969) reported that pure cultures of some soil fungi grew on media containing tannins as sole carbon source. Different sources of tannins were compared and both condensed and hydrolysable tannins were used as substrates. *Aspergillus*, *Penicillium*, *Fomes*, *Polyporus* and *Trametes* were shown to grow better on tannic acid (gallotannin) than on chestnut tannin (ellagitannin) or wattle tannin (condensed tannin).

Most of the fungal species that have been used for biodegradation of tannery effluent, belong to the genera *Aspergillus* and *Penicillium.* Other fungi, including *Chaetomium*, *Fusarium*, *Rhizoctonia*, *Cylindrocarpon*, and *Trichoderma*, are capable of degrading tannery waste constituents (Mahadevan & Muthukumar 1980). *Psalliata campestris* was found to oxidise catechin and *A. niger* could degrade gallic acid (Mahadevan & Sivaswamy 1985). *Cis*-aconitic, α-ketoglutaric and citric acids were the intermediates of this degradation. Gallotannins, besides catechin, were degraded by *A. fumigatus* to gallic acid in 6–8 days. Subsequently, other workers also reported that species of *Aspergillus* and *Penicillium* could utilise catechin, gallotannin and gallic acid as carbon sources (Saxena et al.1995). In a number of fungal systems, tannins have been found to be degraded rapidly in the presence of other metabolisable substances. Ganga et al. (1977) found that *A. niger* and *Penicillium* spp. grew profusely in a medium containing glucose and wood-apple tannin. With wattle tannin at 0.3% and glucose at 166.7 mm concentration, growth of *A. niger* improved. Additional carbon and nitrogen sources favoured rapid production of tannase which, in turn, cleaved tannins and provided a continuous supply of carbon source for growth. The effects of certain factors, such as temperature, pH and carbon sources on the decomposition of tannic acid and gallic acid by *Penicillium chrysogenum*, was studied by Suseela & Nandy (1985). However, their findings varied from the observations of earlier workers. The decomposition of tannic acid and gallic acid was maximum in shake cultures at 28 °C, and both these acids were found to be completely decomposed in 3 days, whereas sugars present as additional carbon source at 3% level retarded their degradation.

There are only a few reports on tannin-degrading yeasts. Initially, six strains of yeasts were isolated from tannery liquors and xylophagous insects, which showed growth and hydrolytic action on tannins in culture media containing various concentrations of gallotannins. The tannin degrading enzymatic system of *Candida* was found to utilise gallotannins as substrate (Aoki et al. 1976a, b). This yeast tannase hydrolysed the ester and depside linkages of tannic acid. Later, a number of yeasts were reported which could degrade condensed tannins (wattle tannins) (Otuk & Deschamps 1983; Vennat et al. 1986). The strains isolated and studied were of *Candida guilliermondii*, *C. tropicalis* and *Torulopsis candida.* The degradation was determined by the estimation of leucoanthocyanidin and flavan-3-ol groups after treatment with the yeasts. A strain of *C. guilliermondii* degraded the flavan-3-ol structures but did not affect the leucoanthocyanidin components. Most yeasts were efficient degraders of quebracho tannins and reduced the tannin content of pine and gaboon wood bark extracts by 70 to 80% in five days (Otuk & Deschamp 1983).

Mechanism of degradation

Degradation of hydrolysable tannins, particularly gallotannins, is best understood in fungal systems (Nishira 1961). The oxidative degradation of hydrolysable tannins has been studied in detail in *Aspergillus* spp. and the pathways of gallic acid degradation have been determined (Watanabe 1965; Mahadevan & Sivaswamy 1985), whereas the pathways leading to the degradation of hexahydroxydiphenoyl moiety, related biphenyl and biarylether structures, and the biodegradation of proanthocyanidins are slowly being unravelled. A lot of information is now available on the tannin biodegradation pathways used by fungi. In *A. niger*, gallic acid the phenolic monomer of hydrolysable tannins, is oxidatively cleaved by an oxygenase to form an unstable tricarboxylic acid intermediate. This is finally decarboxylated by an oxidative decarboxylase to form *cis*-aconitic acid (Figure 3), which then enters the citric acid cycle (Watanabe 1965). However, in *A. flavus* gallic acid is degraded to oxaloacetic acid and finally pyruvic acid through tricarboxylic acid intemediates (William et al. 1986). In *A. niger*, pyrogallol, the decarboxylated derivative of gallic acid is also oxidatively broken down into *cis*-aconitic acid by the same mechanism as has been observed for gallic acid in this organism (Figure 3).

The pathways of aerobic degradation of condensed tannins, phenolic monomers – catechin and quercetin and their intermediates like phloroglucinol have been well worked out in *Penicillium* spp. (Patel et al. 1990), *Aspergillus* spp. and soil fungi like *Chaetomium cupreum* (William et al. 1986). These are similar to the pathways found in aerobic bacterial degradation of such compounds. Initially catechin is degraded to phloroglucinol carboxylic acid and protocatechuic acid by catechin oxygenase. Further degradation of phloroglucinol carboxylic acid proceeds through the formation of phloroglucinol, resorcinol and hydroxyhydroquinone (Figure 4). Hydroxyhydroquinone, which is the last aromatic compound in the pathway, is cleaved by hydroxyquinol-1,2-dioxygenase to form maleyl acetate, which is then converted to β-ketoadipate. The metabolism of protocatechuic acid proceeds by two different pathways with both ending up in the formation of β-ketoadipate. In the first mechanism, it is converted by protocatechuate 3,4-dioxygenase to β-carboxy-*cis*, *cis*-muconate, which is then transformed to β-ketoadipate. In the second mechanism, protocatechuic acid is decarboxylated by protocatechuate decarboxylase to form catechol, which is cleaved by catechol 1,2-dioxygenase to *cis*, *cis* muconate and ultimately transformed to β-ketoadipate. The β-ketoadipate obtained from these pathways is used for the generation of acetyl CoA, with each molecule forming three molecules of acetyl-CoA.

Although numerous studies have been conducted on tannin degradation by various yeasts, not much is known about the pathways and the enzymes involved in breaking down the tannins and their intermediates to simple compounds in these microorganisms. It is possible that the mechanism(s) of their degradation may be similar to that observed in fungi.

Microbial tannase and tannin degradation

Some of the fungi such as *Aspergillus* or *Penicillium* have evolved tannin-degrading systems to withstand high concentrations of tannins (Yamada et al. 1968a, b). The enzyme tannase, which plays a prominent role in the degradation of gallotannins, was first reported in a *A. niger* strain by Knudson (1913), who found that it had a role in the degradation of tannic acid (the commercial name of Chinese gall tannin). Later, the enzyme was described and purified from various fungal strains and was found to be induced by methyl gallate and tannic acid but not by other simple phenols such as gallic acid, salicylic acid or methyl salicylate (Dhar & Bose 1964; Haslam & Stangroom 1966; Adachi et al. 1968, 1971; Otuk & Deschamps 1983). Among all known microbial producers of tannase, strains of some of the *Aspergillus* spp. are commercially the most efficient producers of this enzyme (Table 2). Tannase is now known to be an ubiquitous enzyme of the microbial world (Deschamps 1989; Field & Lettinga 1992b; Lekha & Lonsane 1997) and has widespread occurrence in various fungi, bacteria and yeasts. It is produced both as membrane bound and extracellular forms. A number of investigations on microbial tannase have shown that this enzyme is not equally active against all hydrolysable tannins. Fungal tannases are quite versatile and efficiently degrade different types of hydrolysable tannins (Lewis & Starkey 1969). However, yeast tannases are effective only in decomposing tannic acid and weakly degrade natural tannins (Deschamps 1989). Bacterial tannases can degrade tannic acid as well as natural tannins like chestnut, tara, oak and myrobalan tannins (Lewis & Starkey 1969; Deschamps et al. 1980, 1983; Deschamps & Lebeault 1984). Recently, tannase activity has been observed for the first time in an anaerobic ruminal bacterium isolated from goats browsing on tannin-rich forage (Skene & Brooker 1995). These workers have reported presence of this enzyme in *S. ruminatium* subsp. *ruminantium* and described its characteristics in this ruminal bacterium. This is the first report of tannase activity in a ruminal microorganism.

Tannase is active on galloyl residues of galloyl esters, as well as hexahydroxydiphenoyl and other residues of ellagitannins. Galloyl residues are usually more easily hydrolysed than the other groups (Scalbert 1991). The pH optimum of the enzyme is 5.0–6.0 and it is unstable above pH 6.0 (Iibuchi et al. 1968, 1972; Thomas & Murtagh 1985). Recent work on *A. niger* tannase has indicated that the enzyme is a glycoprotein with a molecular weight of about 186 kDa, and contains 43% sugar (Barthomeuf et al. 1994). The optimal temperature for activity was 35 °C, the esterase activity peaked at pH 5.0 while tannase activity was optimal at pH 6.0. This enzyme was stable between pH 3.5 and 8.0 at temperatures below 50 °C. Several attempts have been made to produce or isolate different tannases from the culture media of *Aspergillus*, and in particular to separate the esterase activity catalysing the hydrolysis of galloyl esters attached to glucose, from the depsidase activity catalysing the hydrolysis of depside linkages between two galloyl residues

Table 2. Microorganisms producing tannin acyl hydrolase (tannase)

Bacteria		
∗	*Achromobacter* sp.	(Lewis & Starkey 1969)
□	*Bacillus pumilis*	(Deschamps et al. 1983)
□	*Bacillus polymyxa*	(Deschamps et al. 1983)
□	*Corynebacterium* sp.	(Deschamps et al. 1983)
□	*Klebsiella planticola*	(Deschamps et al. 1983)
∗	*Pseudomonas solanacearum*	(Deschamps 1989)
∗	*Selenomonas ruminantium*	(Skene & Brooker 1995)
Fungi		
♦	*Aspergillus niger*	(Knudson 1913; Haslam & Stangroom 1966; Barthomeuf et al. 1994; Lekha & Lonsane 1994; Bajpai & Patil 1996; Bradoo et al. 1996)
♦	*Aspergillus oryzae*	(Iibuchi et al. 1967; Doi et al. 1973; Beverini & Metche 1990; Bajpai & Patil 1996; Bradoo et al. 1996)
∗	*Aspergillus flavus*	(Yamada et al. 1968a)
♦	*Aspergillus japonicus*	(Ganga et al. 1977; Bradoo et al. 1996)
∗	*Aspergillus aureus*	(Bajpai & Patil 1996)
♦	*Aspergillus awamori*	(Bradoo et al. 1996)
∗	*Aspergillus fischerii*	(Bajpai & Patil 1996)
∗	*Aspergillus rugulosus*	(Bradoo et al. 1996)
∗	*Aspergillus parasiticus*	(Bajpai & Patil 1996)
∗	*Aspergillus terreus*	(Bajpai & Patil 1996)
∗	*Penicillium chrysogenum*	(Rajakumar & Nandy 1983; Bajpai & Patil 1996; Bradoo et al. 1996)
∗	*Penicillium notatum*	(Ganga et al. 1977)
∗	*Penicillium islandicum*	(Ganga et al. 1977)
∗	*Penicillium digitatum*	(Bradoo et al. 1996)
∗	*Penicillium acrellanum*	(Bradoo et al. 1996)
∗	*Penicillium carylophilum*	(Bradoo et al. 1996)
∗	*Penicillium charlesii*	(Bradoo et al. 1996)
∗	*Penicillium citrinium*	(Bradoo et al. 1996)
□	*Cryphonectria parasitica*	(Farias et al. 1992)
□	*Fusarium solani*	(Bajpai & Patil 1996; Bradoo et al. 1996)
∗	*Fusarium oxysporium*	(Bradoo et al. 1996)
□	*Rhizopus oryzae*	(Hadi et al. 1994)
□	*Trichoderma viride*	(Bajpai & Patil 1996; Bradoo et al. 1996)
∗	*Trichoderma hamatum*	(Bradoo et al. 1996)
∗	*Trichoderma harzianum*	(Bradoo et al. 1996)
∗	*Helicostylum* sp.	(Bradoo et al. 1996)
∗	*Cunnighamella* sp.	(Bradoo et al. 1996)
∗	*Syncephalastrum racemosum*	(Bradoo et al. 1996)
★	*Neurospora crassa*	(Bradoo et al. 1996)
Yeasts		
□	*Candida* sp.	(Aoki et al. 1976a)
□	*Pichia* spp.	(Deschamps 1989)
∗	*Debaryomyces hansenii*	(Deschamps 1989)

★ Poor producer. ∗ Moderate producer. □ Good producer. ♦ Best producer.

(Haslam & Stangroom, 1966; Beverini & Metche, 1990). These studies showed that this fungus produces several tannase isoenzymes, but failed to provide fractions with exclusive esterase or depsidase activity. The ratio of the two activities did vary in the different fractions but the relative specificity of each enzyme was low. Barthomeuf et al. (1994) could, however, separately assay tannase and esterase activities in *A. niger* using tannic acid and methyl gallate as substrates, respectively.

Bradoo et al. (1997) has reported maximum production of extracellular tannase activity in *A. japonicus* after 24 h of incubation and Gupta et al. (1997) have developed a method based on tannic acid binding, for the purification of the extracellular form of this enzyme. The enzyme was produced constitutively on simple and complex sugar substrates but activity was doubled in the presence of tannic acid as sole carbon source. It showed strong end-product inhibition with gallic acid which was of the competitive type. Parametric optimisation of the enzyme yielded 1.13 fold increase in enzyme production at 30 °C and pH 6.6 with 0.2% glucose and 2% tannic acid in Czapek-Dox minimal medium. A number of protocols have also been developed for production of tannase by various fermentation procedures (Yamada 1967). These are the surface culture of *A. niger* (Doi et al. 1973; Barthomeuf et al. 1994), solid-state process for economic production of tannase by *A. niger* (Lekha & Lonsane 1994), solid-state fermentation of *Rhizopus oryzae* (Hadi et al. 1994; Chatterjee et al. 1996) and liquid-surface fermentation of *A. japonicus* (Bradoo et al. 1997). The present state of knowledge about the production and extensive utilisation of tannase has been reviewed by Lekha & Lonsane (1997).

Petri plate techniques have been used for determining the tannin-protein complex degrading activity of bacteria (Osawa & Mitsuoka 1990) and fungi (Bhat et al. 1996; Bradoo et al. 1996), beside screening of microorganisms producing extracellular tannase (Bradoo et al. 1996). Different studies have been carried out on the development of assay systems for estimation of microbial tannase activity in order to monitor their ability to degrade hydrolysable tannins. Dhar & Bose (1964) did pioneering work on developing a new method using methyl gallate as a substrate. This was followed by methods based on artificial substrates such as *p*-nitrophenyl gallic acid (Haslam & Tanner,1970). Methods using changes in absorbance at 310 nm (Iibuchi et al. 1967) and 260 nm after the protein precipitation of residual gallotannin (Deschamps et al. 1983), were also used. Skene & Brooker (1995) have also developed a method based on the colorimetric determination of gallic acid released during the enzymatic reaction. Recently, a spectrophotometric method has been described to determine tannase activity, gallic acid and residual gallotannin during fungal hydrolysis of gallotannins (Bajpai & Patil 1996).

Tannins and their interaction with gastrointestinal tract microflora

Tannins are present in large number of feeds and forages, e.g., tree leaves, agro-industrial byproducts, agricultural wastes, and are one of the most common antinutritional factors. They characteristically bind protein; the strength and nature of the binding depends on the chemical nature of the reactive phenolic groups (Van Buren & Robinson 1969). Formation of complexes of tannins with nutrients, especially proteins, has both negative and positive effects on their utilisation (Reed 1995). In small quantities, condensed tannins are useful as they prevent bloat and protect proteins but when present in large quantities, reduce forage quality.

Tannins inhibit the activity of enzymes of rumen microbes (McLeod 1974; Martin & Akin 1988; Makkar et al. 1988; Bae et al. 1993). Condensed tannins from *Lotus corniculatus* have been shown to inhibit extracellular endoglucanase activity of *Fibrobacter succinogenes* (Bae et al. 1993) and extracts of condensed tannins from *Onobrychis viciifolia* reduced growth and proteolytic activity of *Butyrivibrio fibrisolvens*, *Ruminobacter amylophilus* and *Streptococcus bovis* (Jones et al. 1994). In contrast, *Prevotella ruminicola* appears to produce extracellular material which may protect the organism from the effects of tannins (Jones et al. 1994). For grazing herbivores, tannins present in plants can, in general, adversely affect their nutrition by reducing intake, protein digestibility, inhibiting digestive enzymes or by direct systemic toxicity (Kumar and Singh, 1984). This leads to a reduction in their feed intake, adversely affects rumen fermentation and significantly depresses digestibility of almost all the nutrients. Hydrolysable tannins are toxic and cause poisoning in animals if sufficiently large amounts of tannin-containing plant material, such as leaves of oak (*Quercus* spp.) and yellow-wood (*Terminalia oblongata*) are consumed. (Garg et al. 1992; Fillipich et al. 1991). They are apparently metabolised by the ruminal microflora to

phenolic compounds such as gallic acid, which is neither hepatotoxic nor nephrotoxic to animals. However, pyrogallol the decarboxylated product of gallic acid, is produced in high concentration in the rumen of sheep and causes methaemoglobinaemia in this animal (Zhu et al. 1995). In monogastric animals, or in ruminants if the rumen is by-passed, hydrolysable tannin such as tannic acid can be absorbed through intact or injured gastrointestinal tract and ultimately cause kidney and liver necrosis (Zhu et al. 1995).

Normally, rumen microbes have capability of degrading and detoxifying many incriminating and antinutritional factors into simpler and non-toxic constituents (Selinger et al. 1996). Some herbivores have developed mechanisms for overcoming adverse effects of tannins. One of the mechanism may be the harbouring of gastrointestinal microflora which have developed the ability to degrade tannins to innocuous compounds, utilise them for their growth, and may thus contribute to the overall growth of the animal. Earlier, these microbes were known to have the ability to degrade only phenolic monomers (Tsai & Jones 1975; Tsai et al. 1976; Patel et al. 1981; Krumholz & Bryant 1986a, b), and it was thought that they do not have this capability against tannins. However, some recent reports indicate the presence of tannin-tolerating and degrading microorganisms in both wild and domesticated herbivores.

Recent studies have focused on the possible degradation of tannin-protein complexes by microorganisms of digestive system of such animals who largely feed on tannin-rich forages. Brooker et al. (1994) isolated *Streptococcus caprinus* from the ruminal contents of feral goats browsing on tannin-rich *Acacia* species, which gave zones of clearing on tannic acid-protein agar medium. This novel rumen microbe was found to be tolerant up to a 3% concentration of hydrolysable or condensed tannins, but did not utilise them as an energy source. Nelson et al. (1995) have also reported isolation and characterisation of a ruminal bacterium from a goat fed on a diet containing desmodium (*Desmodium ovalifolium*), a tropical legume which contains up to 17% condensed tannins. This anaerobic bacterium was capable of degrading hydrolysable tannins upto 3% and did not degrade condensed tannins. In contrast to these *in vitro* studies, rumen bacterial species that enzymatically cleave structural bonds in tannins and utilise the degradation products for their own growth, had not been described till Skene and Brooker (1995) isolated a ruminal bacterium from feral goats browsing on tannin-rich *Acacia* species and identified it as a strain of *Selenomonas ruminatium* subsp. *ruminantium.* Recently, Perez-Maldonado & Norton (1996) have reported substantial degradation and disappearance of labelled condensed tannins from *Desmodium intortum* in the gastrointestinal tract in sheep and goats.

Earlier, the presence of tannin-protein degrading microbes (*Streptococcus* sp.) in faeces of koalas, which mostly browse on eucalyptus leaves, had been demonstrated (Osawa 1990; Osawa & Mitsuoka 1990). The enterobacteria capable of degrading tannic acid-protein complexes were also isolated from the caecum of these animals and identified as *Streptococcus bovis* Biotype I (Osawa 1992; Osawa & Sly 1992). These microbes play an important role in koalas' ability to obtain dietary protein from tannin-rich eucalyptus leaves However, till date, no fungal or protozoan strain has been isolated from the gastrointestinal tract of wild or domesticated herbivores, which has tannin-tolerating and degrading activity. The only report of a tannin-protein complex degrading fungus is from the faeces of hill cattle largely consuming oak leaves (Bhat et al. 1996). This fungal strain identified as *Aspergillus niger* van Tieghem, has an optimal growth at 30 °C and pH 5.0 in an aerobic or microaerophillic environment. It has a high tannin tolerance and is a prolific degrader of gallotannins (Bhat et al. 1997).

Concluding remarks

The work on the mechanisms of tannin degradation, especially hydrolysable tannins, by different microorganisms has resulted in our understanding of their biodegradation in natural environments like tannery effluents (Saxena et al. 1995) and waste waters (Field & Lettinga 1992b). 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 (Lekha & Lonsane 1994), besides detannification of foods (Archambault et al. 1996) and upgradation of beverages meant for human consumption (Cantarelli et al. 1989; Lane et al. 1997). There is also a need for increasing our knowledge about the biodegradation of condensed tannins, which can lead to the overall understanding and commercial use of these tannins. This will facilitate application of tannin-degrading enzymes or the genes encoding them in strategies for improved industrial (Hatamoto et al. 1996) and livestock production (Selinger et al. 1996),

besides selection of better microbial strains for the bioremediation of tannin-rich wastes (Field & Lettinga 1992b).

Concerted efforts are in progress world wide to improve tannin-rich feeds and fodders by biodegradation of their tannins by microbial strains which are known to be strong tannin degraders. Currently attention is mainly focused on fungal detannification and recently there have been endeavours to utilise the tannin-degrading activity of different fungi for detannification of tannin-rich biomass. These are detannification of oak leaves by the fungus *Sporotrichum pulverulentum* (Makkar et al. 1994), biodegradation of tannins in sericea lespedeza (*Lespedeza cuneata*) by the white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus* (Gamble et al. 1996) and reduction of tannins in canola meal by an enzyme preparation from a white rot fungus *Trametes versicolor* (Lorusso *et al.* 1996). This work is in an incipient stage and further studies have to be carried out to exploit the potential of various fungal strains for pilot and large scale applications.

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