Microbial Ecology

Bacterial Mechanisms to Overcome Inhibitory Effects of Dietary Tannins

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Abstract

High concentrations of tannins in fodder plants inhibit gastrointestinal bacteria and reduce ruminant performance. Increasing the proportion of tannin-resistant bacteria in the rumen protects ruminants from antinutritional effects. The reason for the protective effect is unclear, but could be elucidated if the mechanism(s) by which tannins inhibit bacteria and the mechanisms of tannin resistance were understood. A review of the literature indicates that the ability of tannins to complex with polymers and minerals is the basis of the inhibitory effect on gastrointestinal bacteria. Mechanisms by which bacteria can overcome inhibition include tannin modification/degradation, dissociation of tannin–substrate complexes, tannin inactivation by high-affinity binders, and membrane modification/repair and metal ion sequestration. Understanding the mechanism of action of tannins and the mechanism(s) bacteria use to overcome the inhibitory effects will allow better management of the rumen ecosystem to reduce the antinutritional effects of tannin-rich fodder plants and thereby improve ruminant production.

Introduction

Tannins are plant polyphenolic compounds present in both animal and human diets, which are subdivided into two groups based on chemical structure: hydrolyzable tannins and condensed tannins (proanthocyanidins). Both groups tend to have similar biological properties. Hydrolyzable tannins are present in plants as gallotannins or ellagitannins (Fig. 1). Gallotannins consist of a central molecule, such as glucose, surrounded by gallic acid units, e.g., pentagalloylglucose (tannic acid). Oxidative coupling of neighboring gallic acid units or oxidation of aromatic rings is responsible for structural variation and the production of large and complex hydrolyzable tannins [42]. Condensed tannins, the most common type of tannin found in forage and browse legumes, are polymers of flavonol units (Fig. 2). The concentration of *ortho*-phenolic hydroxyl groups is probably the key feature giving tannins their characteristic biological properties [64]. These are on gallic and ellagic acid in hydrolyzable tannins and on ring B phenolic hydroxyls and possibly esterified gallate in condensed tannins.

Based mainly on epidemiological studies, plant polyphenolics have health benefits in humans [17], and low to moderate levels of tannins (2–4%) prevent bloat in ruminants [29]. However, high concentrations of tannins in fodder plants (>5%) inhibit gastrointestinal bacteria and lower ruminant performance, mainly by reducing intake and nutrient digestibility. Although most of the efforts in this field have concentrated on the antinutritional effects in ruminants, tannins are common plant secondary compounds present not only in feed, but also in human diets. We hypothesize that some of the reported health benefits in humans may be due to their effect on the microbial population of the intestinal tract rather than a direct effect on the host.

Ruminal populations adapted to tannins can protect ruminants from the antinutritional effects of these compounds. Rumen inoculum from adapted animals enabled Ethiopian Highland sheep to use condensed tannin-containing *Acacia angustissima* without adverse effects [49]. Sheep generally lose weight on *A. anuera* (mulga), which contains 5–25% condensed tannin, but nitrogen digestion was improved by inoculation with feral goat rumen fluid or an *in vitro* cultured rumen inoculum [35, 37]. However, a pure culture inoculum of

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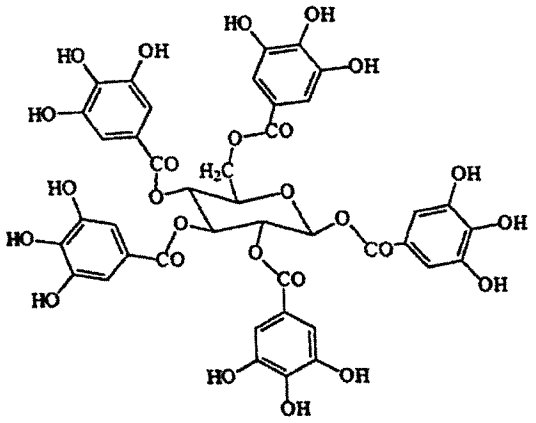

Figure 1. Structure of tannic acid (pentagolloyl glucose), a hydrolysable tannin.

tannin-resistant *Streptococcus gallolyticus*, formerly *S. caprinus* [67], was ineffective [36]. Other studies have shown positive results with pure cultures of tannin-resistant bacteria. An inoculum of 3×10^{11} CFU, of an uncharacterized tannin-resistant bacteria from goats, significantly increased body weight gain over 40 days in goats abruptly changed from a grass diet to a 100% *Calliandra calothyrsus* diet as compared to uninoculated goats [78]. A positive effect in crude protein balance was found in lambs fed a 7.1% peanut skin–tannin diet receiving a live inoculum of a tannin-resistant Gram-positive rod [41].

Increasing the proportion of tannin-resistant rumen microbial populations protects ruminants from antinutritional effects, but the reason for the protective effect is unclear. Populations adapted to tannins may reduce toxicity by degradation, modification, and/or complexation to bacterial polymers. Alternatively, tannin-adapted organisms may prevent sudden shifts in microbial populations and activity when animals are exposed to a tannin-containing diet. Sudden population shifts could result in harmful effects such as a reduction in nutrient digestion and uptake, and establishment and spread of pathogenic organisms. The effect of tannins on bacteria is species-specific as *Lotus corniculatus*-condensed tannins reduced the populations of four proteolytic bacteria, but total ruminal microbial protein remained unchanged [38]. Even low concentrations (0.7–2%) of tannins have a significant effect on the composition of the bacterial populations. Condensed tannins of *A. angustissima* resulted in a shift in predominant rat fecal bacteria toward tannin-resistant Enterobacteriaceae and *Bacteroides* species with a corresponding decrease in the Gram-positive *Clostridium leptum* group and others [69]. Molecular fingerprinting of bacterial ribosomal DNA visually demonstrates the dramatic effect dietary tannins have on bacterial populations of the gastrointestinal tract (Fig. 3).

Bacteria able to grow in the presence of tannins are generally referred to as tannin-tolerant. We feel that tannin-resistant is a better term to refer to bacteria able to withstand the inhibitory effect of tannins. Even strains

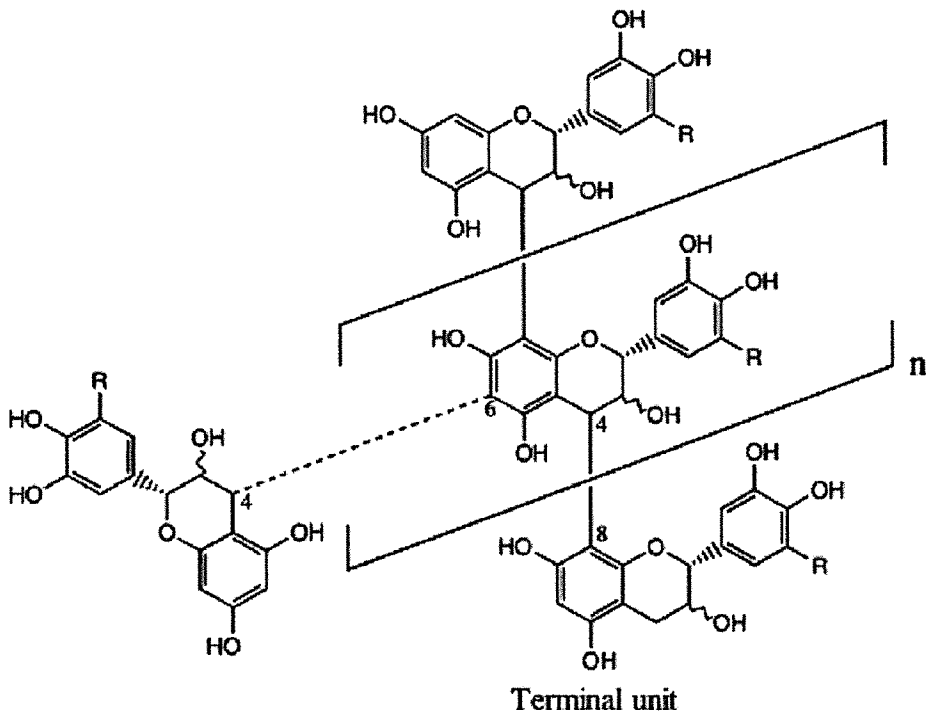

Figure 2. Pseudocatechin structure, the basic repeating unit in condensed tannins.

that are inherently resistant must have some mechanism that blocks tannin action, or tannins are prevented from reaching or penetrating to the site of action. Resistance implies that some action is required on the part of the organism to withstand the inhibitory effect of tannins, whereas the word "tolerant" implies passive endurance. Tannin resistance may require adaptation, i.e., inducible resistance, and, as for other antimicrobials, resistance could be acquired through gene transfer. Tannin resistance is not limited by species or geographical barriers [55]. Phylogenetically diverse bacteria able to grow in the presence of tannins have been isolated from the gastrointestinal tract of animals by various groups around the world [7, 43, 45, 47, 48, 51–53, 69].

Although there is evidence that bacteria contribute to ruminant's ability to utilize high tannin-containing diets and tannin-resistant bacteria have been isolated, it is not clear how polyphenolic compounds exert an inhibitory effect on bacteria or how bacteria can overcome or withstand this effect. Studies in this field are complicated by the complexity and diversity of structures (Figs. 1 and 2) as well as the presence of mixtures of phenolic compounds in plants. The inhibitory effect of tannins is dependent on the type and concentration of tannin to which the bacteria are exposed. For instance, quebracho condensed tannins are less inhibitory to culturable gastrointestinal bacteria than *A. angustissima* tannins. This was determined by comparing the tannin-resistant fecal bacteria in rats (Table 1). The majority of the polyphenolics in *A. angustissima* consists of low molecular weight condensed tannins (monomers, dimers, and trimers) [70], whereas there is 50–75% polymeric tannin in commercial preparations of quebracho, with the remainder a mixture of nontannin low molecular weight phenols (A.E. Hagerman, pers. comm. [15]). Similarly condensed tannins from *Lotus pedunculatus* are more inhibitory to proteolytic rumen bacteria than those from *L. corniculatus* [39]. In addition to tannin complexity, there are a number of ways in which bacteria can overcome the inhibitory effects of tannins. A tannin-resistant bacterial strain could have a single resistance mechanism or multiple systems to reduce or avoid the action of tannins. In this article, we review the evidence for various mechanisms of tannin inhibition and bacterial resistance and discuss alternative possibilities in the hope of generating new research directions.

Modification and Degradation of Tannins

Tannin activity can be eliminated or reduced by bacteria capable of modifying or degrading polyphenolics. Some intestinal bacteria could even utilize tannins as an energy source, giving them a competitive advantage, without actual degradation. Reduced tannins can act as an electron donor for organisms growing on electron acceptors such as nitrate and fumarate. This was shown to be the case for humic substances in anaerobic soil environments [11].

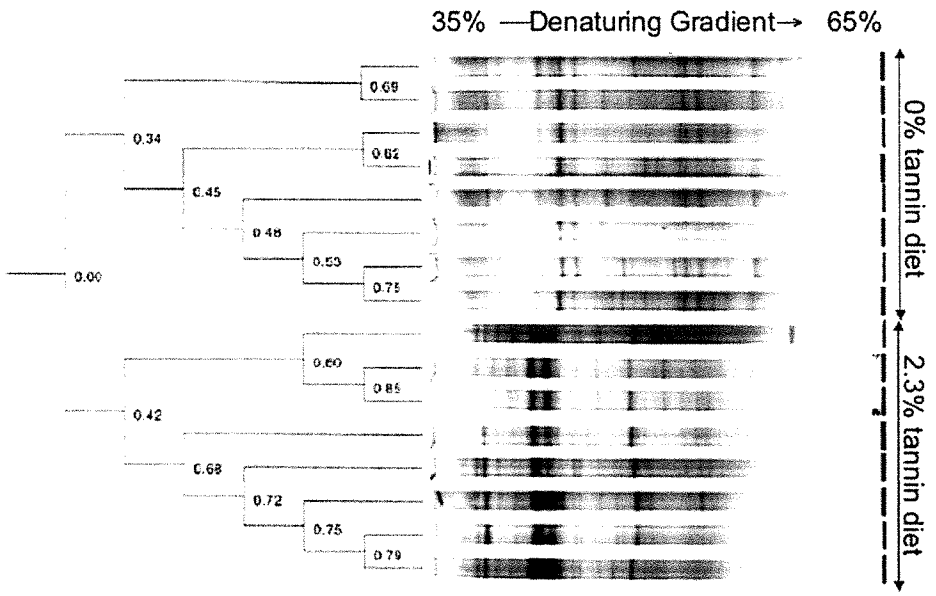

Figure 3. Dietary tannins have a significant effect on gastrointestinal bacterial populations. A visual representation of the differences in cecal bacterial populations from individual rats due to dietary condensed tannins is shown by denaturing gradient gel electrophoresis molecular fingerprint patterns of PCR amplified V3-16S ribosomal DNA. Samples cluster according to dietary treatment, i.e., control diet (0% tannins) or *Acacia angustissima* extract diet (2.3% condensed tannins). Cluster analysis was performed using Ward's algorithm and Dice coefficient similarity indices are indicated at the nodes of the tree. Further experimental details can be found in [69, 70].

Table 1. Studies on the biological effect of tannins are complicated by the complexity and diversity of structures as well as the presence of mixtures of phenolic compounds in plants

Extract (%)	*Quebracho (89% phenolics)*	*Acacia angustissima (90% phenolics)*
0.00	55,000	55,000
0.05	3500	3.2
0.10	2500	6.5
0.20	1600	0.2

This table indicates how the numbers (CFU/mL × 10^5) of tannin-resistant rat fecal bacteria depends on condensed tannin type and concentration in the culture medium. Further experimental details can be found in [69].

The various pathways and breakdown products of microbial tannin degradation in aerobic and anaerobic environments have been reviewed [5] and will not be detailed here. The reported activities of gastrointestinal bacteria on plant phenolic compounds, from simple phenolics to complex tannins, are summarized in Table 2. Although hydrolyzable tannins and some of the phenolic monomers can be degraded under anaerobic conditions in the rumen, the organisms capable of using phenolic compounds as energy sources do not predominate, as tannins are generally a minor component of the diet. Intoxication due to hydrolyzable tannins probably occurs because ruminants ingest large amounts of tannin-containing leaves without prior adaptation of the microbial populations.

There is a need for increased understanding of the fate of condensed tannins in ruminants. Flavonols such as quercetin can be degraded anaerobically in the intestinal tract [63], but intestinal bacteria that can degrade condensed tannins and their monomeric units, flavan-3-ols, have not been described to date. There have been attempts at isolating condensed tannin-degrading rumen bacteria but without success [25, 26]. *Selenomonas ruminantium* K2 is reported to grow with condensed tannins as sole carbon source [8], but no details were given as to the exact source and purity of condensed tannin or the growth rate achieved on tannin-containing medium. Studies with labeled condensed tannins indicate that there is some modification or disappearance from the gastrointestinal tract [56, 74]. However, there is no conclusive evidence of degradation or modification of condensed tannins by bacterial action in the digestive tract.

Tannin–Polymer Complexes. Tannins were initially named for their usage in tanning hides to produce leather, and their ability to form strong complexes with proteins and other polymers such as cellulose,

Table 2. Summary of cultivable gastrointestinal bacteria that can modify and/or degrade phenolic compounds under anaerobic conditions

Substrate	*Reaction/enzyme*	*Organisms*	*References*
Arabinoxylan side chains esterified to phenolic acids, e.g., ferulic acid	Esterases to release phenolic acids from plant material	*Butyrivibrio fibrisolvens*	[12]
		Clostridium LP1	[30]
Methoxylated aromatics, e.g., vanillic acid	Demethoxylation to aromatics, e.g., protocatechuate and acetate/ butyrate	Many acetogens, e.g., *Oxobacter (Clostridium) pfennigii*	[21] [23]
		Syntrophococcus sucromutans	
Ferulic acid	*O*-demethylation, dehydroxylation, side chain reduction, decarboxylation, ring reduction and cleavage	*Enterobacter* spp. *Escherichia coli* ATTC 15597	[13]
Phenolic monomers with 2 or 3 hydroxl groups in meta position, e.g., gallic acid	Ring cleavage	*Eubacterium oxidoreducens*	[24]
Sinapic, ferulic, *p*-coumaric acid	Sole carbon and energy sources. Degradation undetermined	Isolate 7-1	[1]
Tannic acid–protein complex	Clearing zones on tannin-treated agar	*Streptococcus gallolyticus*	[7, 54]
		Enterobacteria spp.	[53]
		Lonepinella koalarum	[52]
		Echerichia coli KN4	[44]
Tannic acid	Tannin hydrolysis to release gallic acid (tannin acyl hydrolyase E.C. 3.1.1.20 and esterases) and decarboxylation of gallic acid to pyrogallol (gallate decarboxylase)	*Streptococcus gallolyticus*	[54]
		Lonepinella koalarum	[52]
		Streptococcus KN1	[43]
		Selenomonas EAT2	[48]
		Selenomonas ES3 and EG19 (tannase only)	[48]
Flavonoids, e.g., quercetin, but not catechin and epicatechin	C-ring cleavage resulting in phenolic acids and nonaromatic fermentation products	*Clostridium* spp.	[77]
		Eubacterium oxidoreducens	[22]
		Eubacterium ramulus	[63]
Condensed tannin	Sole carbon and energy source. Degradation undetermined	*Selenomonas ruminantium* K2	[8]

hemicellulose, and pectin is still considered an essential characteristic of their biological activity. Tannins are multidentate ligands as they are able to bind via different phenolic groups to more than one point on the polymer surface. The reaction of tannins with proteins and other polymers depends on spatial configuration of molecules, availability of reactive phenolic groups, and pH. Protein affinity for tannin reactions are more effective at pH values close to their isoelectric points [27]. Size and structure of tannins is important as larger molecular weight allows more stable cross-linking of different protein molecules. Conformational mobility and flexibility also influences the effectiveness of complexation, and it has been observed that condensed tannins have a "relatively lower astringency" compared to hydrolyzable tannins possibly due to restricted rotation about the interflavan bond [71]. Strong complexes with proteins are formed by hydrophobic bonding between the aromatic ring structure of tannins and hydrophobic regions of the proteins and through hydrogen bonds with free phenolic hydroxyl groups and protein groups. There is evidence for selective protein interaction as tannins have a poorer affinity for compact globular proteins and a higher affinity for proteins with high proline content, possibly due to more open and flexible conformations [71].

Hydrogen bonds are also formed with carbohydrates, and *in vitro* and *in vivo* results of digestion of tannin-containing husks of *Cicer arietinum* (Bengal gram) suggested that carbohydrates are the main substrate bound by tannin in this system as measured by an increase in gas production or organic matter digestibility by the addition of PEG 6000 to inactivate tannins [72]. However, the authors do not take into account that carbohydrate fermentation could be decreased due to enzyme inhibition and their results are inconclusive as to which mechanism is involved.

The effect of tannin complexation with proteins and other compounds results in both extracellular enzyme inhibition and unavailability of substrates for digestion, which will lead to inactivation of microorganisms and ultimately death. Direct tannin interactions with bacterial cell membranes and extracellular structures such as fimbria will also occur.

The inhibitory effects of tannin–polymer complexes can be overcome as they are formed through noncovalent bonds and can therefore be disassociated. Increased concentrations of polymer(s) with a higher affinity for the polyphenols such as polyvinyl pyrrolidones and polyethylene glycol (PEG) can cause dissociation and this is effectively used to allow ruminants to feed on tannin-containing plants without adverse effects [66]. Therefore, excretion of polymers with a high affinity for tannins will bind and inactivate tannins, as is the case with proline-rich salivary gland proteins in some animals [40]. There is evidence for this in that some organisms produce extra glycocalyx in the presence of tannins. In the presence of condensed tannins, fungal spores of *Colletotrichum graminicola* produce glycoprotein mucilage with a high affinity for condensed tannins [62]. Scanning and transmission electron microscopy of leaf surfaces of an *in sacco* study comparing high and low tannin samples of *L. corniculatus* indicated that bacterial colonization of the surface of high tannin leaves tended to be by glycocalyx-enclosed microcolonies [9]. Extracellular polysaccharide is induced in the tannin-resistant *S. gallolyticus*, which completely encases the cells at tannic acid concentrations greater than 2% (w/v) as observed by field emission scanning electron microscopy [46]. Extracellular polysaccharide alone is not enough to provide resistance to tannins as increasing extracellular polysaccharide synthesis in a tannin sensitive bacteria, *S. bovis*, did not increase its tolerance to tannic acid [46]. Extracellular polysaccharide secretion is also not a universal mechanism as *Sel. ruminantium* K2 does not secrete large quantities of extracellular polysaccharide, but is resistant to both condensed and hydrolyzable tannins [6].

Tannin inhibition can be reduced by overproduction of enzymes or tannin-resistant enzymes, with few interactive sites for tannin binding. *Fibrobacter succinogenes* increases cell associated endoglucanase activity as extracellular activity is inhibited in the presence of tannins [2].

Bacterial degradation or dissociation of the tannin–substrate complex will allow utilization of the bound substrate. Gastrointestinal bacteria have been isolated that can degrade hydrolyzable tannin–protein complexes [45, 50, 52, 53]. This has not been found to be the case for condensed tannins. There was no significant degradation of calliandra tannin–protein complexes or fermentation of *in situ* complexed calliandra protein by proteolytic bacteria isolated from sheep and goats fed a tannin-containing diet [31].

Metal Ion Chelation

Dihydroxy phenolic groups can form stable complexes with many metal ions. Polyphenol structure affects metal precipitation and models have been proposed for metal/polyphenol precipitation [28]. Most studies concentrate on the polymer binding capacity of tannins, but metal ions can reach the functional groups of polyphenols more easily than the interacting groups of proteins. Metal chelation could therefore have a greater biological effect than polymer complexation. Metal chelators are known to have antimicrobial activities, and cause significant ecological changes [65]. Chelating of metal ions by polyphenols could result in iron deprivation, a decrease in activity of metalloenzymes, and inhibition of oxidative phosphorylation by inhibiting heme production necessary for cytochromes. Complexation with metals also results in acidification that is inhibitory to organisms.

Preventing growth of plant pathogens by metal complexation has been suggested to be an important plant defense mechanism mediated by phenolics [34]. There are indications that growth of *Escherichia coli* is inhibited by metal ion complexation as growth on low iron plates with tannin only occurred around wells containing an iron solution [10]. In another experiment, growth of *E. coli* on tannic acid-containing medium was restored after iron addition [10].

Condensed tannins from *A. angustissima* alter bacterial populations reducing diversity in rat fecal bacteria and resulting in a shift in predominant bacteria toward tannin-resistant Gram-negative Enterobacteriaceae and *Bacteroides* species. A corresponding decrease occurred in the Gram-positive *C. leptum* group [69]. Iron deficiency results in many of the same shifts in bacterial populations [3, 75]. An increase in iron has the opposite effect as repeated doses of ferrous sulfate over 4 weeks to rats resulted in a decrease in *Bacteroides* and an increase in clostridia [4].

The inhibitory effect of metal ion chelation can be overcome if bacteria are not dependent on metal-containing enzymes or can use alternative enzymes. For example, *Bifidobacterium infantis* and *Lactobacillus acidophilus* are not inhibited by tannic acid [10]. Lactic acid bacteria do not require iron as they do not contain heme enzymes and iron-containing ribonucleotide reductase is replaced with an enzyme using adenosylcobalamine.

An enhanced ability to sequester metal ions can also reduce the negative effects of tannins. The importance of iron sequestration in the presence of tannins is indicated in that strains of the plant pathogen *Erwinia chrysanthemi* with detrimental alterations in the siderophore-mediated iron transport pathway were inhibited to a greater extent in the presence of chestnut polyphenols than the wild-type strain [34].

Cell Wall and Membrane Effects of Tannins

Tannin toxicity has been hypothesized to be a result of selective inhibition of cell wall synthesis. Condensed tannins from *Onobrychis viciifolia* (sainfoin) were found to bind to cells in four strains of ruminal bacteria: *Butyrivibrio fibrisolvens* A38, *Streptococcus bovis* 45S1, *Prevotella ruminicola* B_14, and *Ruminobacter amylophilus* WP225 [19]. Along with inhibition of growth and protease activity, morphological changes were observed in the strains with Gram-positive type cell walls. The results of an ecological study determined a shift in predominance of Gram-positive to Gram-negative cell walled bacteria [69]. These reports suggest that study of the membrane and cell wall effects of tannins would be a promising research area.

There is evidence from work performed on green tea catechins that polyphenolics disrupt membrane integrity, which will inhibit essential cell processes such as oxidative phosphorylation, and disrupt transport processes into and out of the cell. Epicatechin and epicatechin gallate were shown to cause leakage from liposomes [18]. *Staphylococcus aureus* cells were shown to absorb more epigallocatechin gallate than *E. coli* and cells aggregated, whereas aggregation of *E. coli* cells was not detected [18]. The authors hypothesized that this is attributable to membrane fusions. Other bacteria also form chains or filaments in the presence of tannins [62]. Work on green tea catechins by other authors confirm that membrane damage occurs and that flavonoids, catechins, and epigallocatechin gallate insert or interact in the outer polar zone of lipid bilayers in liposomes [20, 59, 73]. All eight green tea catechins tested were shown to reduce membrane fluidity in hydrophobic and hydrophilic regions of the lipid bilayers [76]. Other authors also presented possible evidence of a reduction in membrane fluidity as low concentrations of epigallocatechin gallate and epicatechin gallate prevented leakage from liposomes, presumably by sealing the gaps through which calcein leaked [16]. Research still needs to be carried out to clarify how these polyphenolics insert into the membrane and how they bind to lipoproteins.

The effect of tannins on the cell surface may not only be membrane-related, but there can be interactions with extracellular structures such as fimbria. *Porphyromonas gingivalis* is involved in periodontal disease, but growth and adherence onto human buccal epithelial cells is inhibited by green tea catechins [61]. The authors postulated that this is due to binding of the polyphenols to the fimbria of *P. gingivalis*.

There are various ways that bacteria can overcome membrane damage. From liposome data it was shown that negatively charged phospholipid head groups and negatively charged lipopolysaccharide at the exterior of the outer membrane have a protective effect against catechins [18]. Ikigai *et al.* showed that the Gram-positive *S. aureus* was more susceptible to epicatechin and epicatechin gallate than the Gram-negative *E. coli*, and attributed this to the negatively charged lipopolysaccharide at the exterior of the outer membrane.

Polyphenols decrease membrane fluidity, thereby increasing membrane fluidity by increasing unsaturated fatty acids; *trans* to *cis* isomerization and a change in phospholipid polar head groups allowing looser packaging will reduce inhibitory effects. Similar mechanisms are present in solvent-resistant *Pseudomonas putida*, although in this case the objective is to make the membranes more rigid as solvents such as toluene increase membrane fluidity [57, 58]. *Lactobacillus plantarum* increases its membrane fluidity by a higher incorporation of unsaturated fatty acids ($C_{16:1}$) in the presence of the phenolic monomers caffeic acid and ferulic acid [60]. However, the opposite occurred in the presence of tannic acid. The

authors hypothesize that the changes in the presence of tannic acid are due to a decreased oxygen level in the medium caused by "oxygen-trapping" by tannic acid as similar membrane changes have been noted when available oxygen levels were varied. An alternative method for which no evidence was found would be to increase membrane biosynthesis allowing rapid repair of damaged membranes.

Conclusions

The ability of plant polyphenols to complex with polymers and minerals appears to be the basis of their inhibitory effect on bacteria. Bacteria can be directly inhibited by tannins interacting with membranes, cell walls, and/or extracellular proteins, but tannins can also have an indirect effect by making nutrients unavailable. Bacteria that are predominant in tannin-rich systems may not be resistant *per se*, but are less affected by nutrient limitations or are better able to access limiting nutrients. The major strategies that gastrointestinal bacteria use to overcome inhibitory effects of tannins are summarized in Table 3.

Studies should be undertaken on pure cultures of bacteria to determine the antimicrobial effect of tannins and the mechanism(s) bacteria use to overcome toxic effects. Resistant strains can be mutated to become sensitive or *vice versa*; in this way, the genes involved in conferring resistance can be identified. The last strategy was used successfully in our laboratory to determine the effect of *Acacia mearnsii* (Black wattle) condensed tannins on *E. coli* [68]. In aerobic medium the tannins were found to be toxic because they generated hydrogen peroxide. The inhibitory effect was overcome by increasing the oxidative stress response thereby inactivating the hydrogen peroxide. Pure culture studies will have to be coupled with *in vivo* studies as confounding factors, such as the formation of biofilms; effective tannin concentration and tannin interactions with other species will affect *in vivo* susceptibility to tannins.

Alternatively, gene expression studies of tannin-resistant bacteria in medium with and without tannins can be compared. Preliminary results of this strategy being used in our laboratory indicate that the whole genome response of *E. coli* to tannins includes a complex set of transcriptional responses for maintaining the integrity of the cell membrane [79, 80].

Studies generally focus on the bacteria in the gastrointestinal tract, but archaeal methanogens, protozoa, and fungi also play important functional roles, especially in the rumen. There have been very few studies looking at the impact of tannins on these groups. Fungal numbers were affected by *C. calothyrsus* in sheep diets, but total protozoal counts were not affected [32]. Some studies focusing on the effect of tannin on cellulolytic fungi are reported in a recent review [33]. More work is necessary to determine the effect of tannins on these groups and the impact on ruminant physiology and digestion.

A greater understanding of the inhibitory effect of tannins and the mechanism(s) that bacteria use to overcome these effects will allow better management of the rumen ecosystem. As mentioned in the Introduction, the addition of uncharacterized mixed, or pure cultures, of tannin-resistant bacteria improved production on tannin-containing feeds, but a pure culture of *S. gallolyticus* was ineffective. Understanding the mechanism of resistance will allow selection of tannin-resistant organisms that can inactivate the tannin, thereby protecting not only themselves, but also tannin-sensitive bacteria. Engineering rumen bacteria with the fluoroacetate gene from a soil bacterium has been shown to be effective in protecting sheep from fluoroacetate poisoning [14]. Cellulolytic bacteria, which are essential for fiber degradation, appear to be tannin-sensitive. Knowledge of tannin resistance mechanisms may make it possible to genetically engineer tannin-resistant strains of these essential organisms.

Table 3. Bacterial strategies to resist antimicrobial agents and the mechanisms that confer resistance to tannins

Strategy	*Tannin resistance mechanism*
Drug inactivation	Cleavage of hydrolyzable tannins Excretion of high affinity tannin binders
Circumvention	Metal ion sequestration Altered enzymes Dissociation of tannin–substrate complexes
Target modification	Membrane modifications/repair
Prevent access to target	Extracellular polysaccharide formation

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