Role of Rumen Fungi in Fiber Degradation

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ABSTRACT

Anaerobic fungi inhabit the rumen and actively degrade plant cell walls. Rumen fungi produce high levels of cellulases and hemicellulases and are particularly proficient in producing xylanases. These enzymes are regulated by substrate (especially soluble sugars) available to the organisms. Fungi degrade unlignified (i.e., no histochemical reaction for phenolics) plant walls totally, indicating that enzymes are able to hydrolyze or solubilize the entire plant wall. These organisms are better able to colonize and degrade the lignin-containing tissues than are bacteria; phenolics are solubilized but not metabolized from the plant wall by fungi. Anaerobic fungi are unique among rumen microorganisms in that they penetrate the cuticle. Residues after incubation with fungi are physically weaker than those incubated with whole rumen fluid or with rumen bacteria, suggesting that fungi could alter the fibrous residue for easier mastication by the animal. Data indicate that cocultures of anaerobic fungi with methanogenic bacteria stimulate cellulose degradation; other data suggest that fungi are inhibited by certain rumen microorganisms. The interaction of rumen fungi with other organisms in relation to fiber degradation in the rumen requires additional study. Rumen fungi have the potential to degrade the more recalcitrant plant walls in forages, but this potential is not always reached in the rumen.

(Key words: rumen fungi, fiber, degradation)

INTRODUCTION

In the mid 1970s, Orpin (37) reported that organisms that previously had been identified as rumen zooflagellates actually were fungal zoospores. In a series of experiments, Orpin (37, 38, 39) reported on several aspects of the fungi, including their obligately anaerobic nature, the presence of chitin in the cell walls, and their colonization of plant fiber. Rumen fungi have been grown on rumen-simulating media, indicating that pH (about 6.5 to 6.7), temperature (39°C), and nutrient requirements are met in media designed for rumen bacteria. The semi-synthetic basal medium of Caldwell and Bryant (16), in which yeast extract, tryptone, hemin, and a mixture of VFA replaced rumen fluid, has supported a variety of anaerobic fungi. However, absolute requirements for growth have not been determined for most of the fungi. Orpin (42), in reviewing nutritional requirements for Neocallimastix patriciarum, reported requirements of heme, D-biotin, and thiamin or its precursors; sulfur was required in reduced form, and the nitrogen requirement was met with ammonium ions or amino acids.

Bauchop (11, 12), using microscopic techniques, reported that rumen fungi extensively colonized the lignin-containing tissues of forages and appeared to be active in fiber degradation. Bauchop (11) further showed that fungi were more prevalent in ruminants fed high fiber diets than in those fed less fibrous ones. Work in Australia (5) with sheep eating a fibrous, warm-season grass grown with or without sulfur showed that sulfur stimulated rumen fungi, which were the most active fiber-degrading organisms in this system. This major role for fungi has not been observed in other feeding systems and probably was an unusual one due to the feed and feeding conditions, but results did indicate the potential for extensive fiber degradation in vivo by anaerobic fungi.

Because of the unusual nature of the zoospores of some of the first isolates and the
obligately anaerobic nature of all isolates, the classification of these organisms has required some modifications by fungal taxonomists. A new family was created (Neocallimasticaceae) by Heath et al. (24) to accommodate these organisms. To date, the following list the genera and species of monocentric fungi: Neocallimastix frontalis, Neocallimastix parvicornis, Piromyces (formerly Piromonas) communis, Caecomyces (formerly Spheromonas) communis, and Caecomyces equi. The characterization of these rumen fungi has been reported in several recent reviews (17, 19, 23, 31). Other types of fungi with a polycentric growth pattern recently have been reported (8, 47). Barr et al. (10) established the genus Orpinomyces for organisms with polycentric growth and multiflagellated zoospores. Breton et al. (15) gave the name Neocallimastix joyonii to a polycentric isolate with characteristics similar to those described by Barr et al. (10). The systematics of the anaerobic fungi will require further refinement as information is collected on these unusual organisms.

Anaerobic fungi have been found in the gut of ruminants other than sheep and cattle, including deer, reindeer, and impalas (12). Further, similar fungi are present in the horse cecum (41) and in the stomachs of marsupials, including kangaroos and wallabies (12). These types of anaerobic fungi also have been cultured from the feces of elephants (12). Early work in New Zealand (11, 12) and in Australia (5) suggested that the fungi in these locations were particularly active as fiber-degraders. Later work (6) comparing mixed fungal populations in one site in Australia and the US have indicated that those in Australia were more active in degrading fiber in incubations with or without bacteria. A common characteristic of rumen fungi related to ruminant nutrition is their ability to colonize extensively the lignin-containing plant cell walls of forages. Despite their ability to colonize lignocellulosic fiber and their apparent cosmopolitan nature, the role of rumen fungi in ruminant nutrition is not well understood. Aspects of fiber degradation by these rumen fungi are discussed herein.

FIBER-DEGRADING ENZYMES PRODUCED BY RUMEN FUNGI

Production of fiber-degrading enzymes and utilization of carbohydrates by rumen fungi have been investigated recently by several researchers using cultures isolated from various parts of the world, including Australia (20), Great Britain (29, 51, 52, 55), France (25), New Zealand (33, 34, 46), and the US (14). These various cultures are similar in fermentation products formed (most produce formate, acetate, lactate, ethanol, CO₂, and H₂ from soluble and fibrous substrates, and they produce high amounts of cellulases and xylanases. Wood et al. (55) reported that one unit of N. frontalis endoglucanase (evaluation of cell-free preparations of fungus and methanogen cocultures) was several times more active in solubilizing cotton fiber than one unit of endoglucanase from Trichoderma reesei C-30, which is one of the most active cellulases yet reported. Rumen fungi are even more active in

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Fungal isolate</th>
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<tr>
<td></td>
<td>MC1</td>
</tr>
<tr>
<td>Exoglucanase</td>
<td>.16</td>
</tr>
<tr>
<td>Endoglucanase</td>
<td>2.03</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>1.32</td>
</tr>
<tr>
<td>Xylanase</td>
<td>4.51</td>
</tr>
<tr>
<td>Xylosidase</td>
<td>1.32</td>
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</table>

1Maximum activity within 9 d of growth.
2From Borneman et al. (14).
3Substrates used to assay enzymes are as follows: avicel, carboxymethylcellulose, p-nitrophenyl β-D-glucopyranoside, xylan (larchwood), p-nitrophenyl β-D-xylopyranoside.
4The following names would apply to the isolates according to Gold et al. (19) and Barr et al. (10): MC-1 = Piromyces, MC-2 = Neocallimastix; PC-2, PC-3 = Orpinomyces spp.; PC-1 = unnamed polycentric fungus.

TABLE 1. Maximum specific activities (U/mg) of culture supernatants of rumen fungal isolates grown on bermudagrass.
producing xylanase, and Mountfort and Asher (34) reported that *N. frontalis* xylanase was the most active of all the endo-acting polysaccharides yet studied from the anaerobic fungi. Enzymes produced by several isolates are shown in Table 1.

The ability to produce high concentrations of cellulases and xylanases and to produce enzymes active against “crystalline” cellulose (highly ordered because of hydrogen bonding) substantiates the finding that fungi are potentially active fiber degraders. Although most of the studies have been carried out with the monocentric organisms, Borneman et al. (14) confirmed the high production of fiber-degrading enzymes from the unnamed polycentric fungi (Table 1). Studies to date, however, indicate that pectin is one cell wall polysaccharide that does not appear to be degraded or utilized by the fungi (20, 29, 46, 51). Although *Caecomyces* was not able to utilize purified cellulose, all three genera of rumen fungi were found to utilize a range of carbohydrates prevalent in forage fiber (20), indicating their ability to ferment and produce end products for the animal or for other microorganisms in the rumen ecosystem.

Most studies on the location of fiber-degrading enzymes produced by rumen fungi indicate that they are extracellular, being free in the culture fluid (34, 46); results to the contrary reported by Lowe et al. (29) perhaps were influenced by the shorter time of incubation (i.e., 72 h) at which time fungal wall autolysis was not extensive. Enzyme activity has been reported to be present in both the zoosporic and vegetative stages as well as the cell-free culture fluid (51, 52).

Some of the fiber-degrading enzymes are constitutive, and there is evidence that some of these enzymes are regulated by soluble sugars present (32, 33, 34). Glucose has been shown to repress cellulase production (33). Xylanase production was influenced by both soluble sugars and xylan concentration and was higher with crude wheat straw hemicellulose compared with xylan from different sources (34). The forces that regulate enzyme production and fiber degradation are complex in the rumen ecosystem and microniches in which the fungi participate. Therefore, the potential for enzyme production or activity may not be reached during in vitro or in vivo degradation of plant walls by the fungi.

Figure 1. Scanning electron micrograph of bermudagrass leaf blade incubated with rumen fluid plus streptomycin and penicillin to inhibit bacteria and select for fungi. Several different sporangial types are present with fungi colonizing the least digestible tissues such as the vascular bundle (B); the more digestible tissues like mesophyll (M) are degraded. Bar, 50 μm.

**COLONIZATION AND DEGRADATION OF PLANT CELL WALLS**

Ultrastructural studies have identified the type of plant cell walls that are colonized and the manner of degradation by the rumen fungi (5, 8, 11, 12). Zoospores produced from sporangia swim to acceptable sites of colonization (e.g., rigid, lignocellulosic plant walls), attach to the substrate, encyst, and develop a rhizoidal or rhizomycelial system that penetrates the plant wall and releases polysaccharidases against structural carbohydrates (12, 36). Fungi degrade the easily digestible, nonlignified (determined by histochemical methods) tissues such as mesophyll completely if colonization has occurred nearby (Figure 1). These results indicate that enzymes capable of degrading the total cell wall are produced by fungi. The physical relationship of rhizoid or hypha to the zone of degradation suggests that cell wall-degrading enzymes are released from the fungal structures and are, therefore, extracellular (Figure 2). These observations are in agreement with the previously discussed enzymatic studies, which indicated that cellulases and xylanases are released from the fungal walls (34, 46, 51).

*Journal of Dairy Science* Vol. 73, No. 10, 1990
The colonization of lignified tissues by rumen fungi is of interest in ruminant nutrition, because these tissues are the ones most limiting to digestibility (1). Electron microscopic investigations have shown that colony development is initiated on the lignin-containing tissues preferentially (Figure 3; (5)). Although chemotaxis has been shown with soluble sugars in these fungi (43) and chemotaxis is recognized for other chytrids (17), it is not known if chemotaxis to cell-wall components is important in colonization of specific plant walls. After colonization of the refractory lignocellulose, fungal rhizoids or hyphae partially or totally degrade plant walls. Lignin-containing tissues that are partially degraded by rumen bacteria, such as leaf blade sclerenchyma (2), are extensively and often completely utilized or solubilized by rumen fungi (5, 8). The breakdown of sclerenchyma cells by fungi is shown in Figure 2 and compared with degradation by the whole rumen population (Table 2). Other types of lignin-containing tissues that are totally resistant to bacterial degradation, such as xylem and sclerenchyma ring tissue in stems (2), are degraded partially by fungi (Figure 4).

Rumen fungi degraded the lignified sclerenchyma ring of bermudagrass stems by penetrating entirely through the cell wall, often transversing across two adjacent cells and causing erosion zones across primary and secondary walls and the middle lamella region. In this plant, the pit fields of the cell walls appeared to be a preferred site of attack. With some rumen fungi, appressorial-like structures that produced a penetration peg, a system similar to that in plant pathogenic phycomycete fungi, appeared to be involved in degrading plant walls (26). With other plants, such as alfalfa stems, the highly lignified and most refractory plant walls

Table 2. Area of sclerenchyma remaining in leaf blades incubated with rumen fungi (plus streptomycin and penicillin) or total rumen populations (no antibiotics) for 48 h.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Area (µm²) of sclerenchyma tissue remaining in</th>
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<tbody>
<tr>
<td></td>
<td>Midvein</td>
<td>Second-order vein</td>
<td></td>
</tr>
<tr>
<td>Whole rumen fluid</td>
<td>1542.7a</td>
<td>120.4a</td>
<td></td>
</tr>
<tr>
<td>Mixed rumen fungi</td>
<td>355.8b</td>
<td>15.8b</td>
<td></td>
</tr>
</tbody>
</table>

1From Akin and Rigsby (8).
2Area includes the total of abaxial and adaxial sclerenchyma from three to four leaf blades. Values within columns followed by different letters differ (P<0.05) using the t test.
3Inocula include whole rumen fluid without antibiotics in which bacterial degradation predominated. Streptomycin and penicillin were added to inhibit bacteria and to select for fungi.

Figure 3. Scanning electron micrograph of alfalfa stem incubated as in Figure 1 for 72 h. Fungi colonize the lignified ring cells (R), while the easily digestible parenchyma cells (P), which are not near the colonization site, are not degraded. Bar, 100 µm.
to degradation were not penetrated by fungal filaments but instead were degraded apparently by solubilization of the outer regions of the walls [Figure 5; (7)].

In addition to the greater degradation of lignified tissues by rumen fungi compared to bacteria, another unique attribute of fungi is their ability to penetrate the cuticle of grass leaf blades (8). Invasion of leaf stomata by hyphae has been observed in some studies (5, 12, 26, 38), and attack at these sites allows the fungi to have a greater penetration of the leaf substrate and not be limited to damaged sites. Colonization of the stomata was a prevalent means of penetration of the leaf in one study incorporating sulfur-fertilized warm season grass hay (5) and has been reported to be particularly evident in wheat straw leaf (12). Penetration of stem stomata does not appear to be particularly important (12). Other studies with mixed fungal populations (8) and pure culture isolates (4) have shown that rumen fungi appear to be able to penetrate the cuticle barrier at sites other than the stomata. The mechanism for this penetration has not been elucidated, but it appears that physical rupture of this plant barrier due to the penetrative nature of the rhizoids or hyphae is important.

DEGRADATION OF PHENOLICS AND LIGNIN

Rumen fungi degrade the lignin-containing plant walls and usually colonize such tissues preferentially. The ability to degrade and utilize lignin anaerobically would be an important attribute for rumen microorganisms. Therefore, studies have been undertaken to assess the ability of the fungi to attack the lignin component of plant walls. Windham and Akin (54) used antibiotics to manipulate procaryotic (i.e., bacterial) and eucaryotic (i.e., fungal and protozoal) groups in an in vitro system and then
assessed the digestibility of each group; rumen protozoa were not active as primary degraders of plant walls in this study. Results indicated that lignin as determined by the 72% H2SO4 method was not lost in the incubations. Similar techniques using antibiotics to select for microbial groups were used to evaluate the loss of lignin in [14C]lignin or [14C]carbohydrate in lignocellulose of highly lignified and poorly digested (in vitro digestibility = 36%) cordgrass (3). Results indicated that, in an in vitro incubation that stimulated a polycentric fungus, lignin was not converted to CO2 but was lost due to solubilization. Similarly, radiolabeled young oat plants were evaluated using 18 pure cultures of classified, monocentric fungi by Gordon and Phillips (20). Their results indicated that, even though 30 to 38% of the initial radioactivity was solubilized, negligible CO2 was produced. To date all data indicate that rumen fungal attack on lignocellulose results in solubilization but not metabolism of the phenolic component of lignocellulose.

Rumen fungi have recently been shown to produce esterases that release p-coumaric and ferolic acids from their methyl esters and from intact plant cell walls (Borneman et al., 1989, Appl. Microbiol. Biotechnol., in press). It is possible that these enzymes might play a significant role in degrading plant walls high in phenolics. Phenolic monomers (but not dimers) are toxic to rumen fungi (8, 22); therefore, the influence of esterases and release of high concentrations of p-coumaric and ferulic acids on digestibility require additional study.

**PHYSICAL DEGRADATION OF FIBER BY FUNGI**

Abilities to degrade extensively certain lignified tissues, to partially degrade and weaken the more resistant tissues, and to penetrate the cuticle barrier in forages are characteristics that indicate rumen fungi are better able than rumen bacteria to degrade the structural barriers in plants. Therefore, fungi might be considered to have an ability to modify particle size or other fiber characteristics (18, 50) that relate to intake or passage of fiber through the intestinal tract. In a feeding study with sulfur-fertilized warm-season grass, which stimulated the fungal population, the major result was an increase in feed intake considered to be influenced by the ability of fungi to attack and weaken anatomical barriers to fiber breakdown (5). Wilson and Engels (53), in reviewing the results of experiments related to breakdown of particles by rumen fungi, indicated that mastication and rumination were responsible for most of the reduction in particle size, but they also concluded that microorganisms could weaken plant structures for easier rumination. In a study (7) to quantitatively evaluate the potential of mixed bacterial and mixed fungal populations to weaken structural barriers in vitro, stems incubated with fungi were significantly (P<.05) weaker than those incubated with bacteria (Table 3). In this study, particle size was not altered by any microbial group, but fungal filaments degraded tissues most refractory to degradation in both grass and legume stems. Joblin (27) reported that *Caecomyces* isolates were able to “fibrillate” plant fragments due to the expansion of a bulbous rhizoid, whereas *Neocallimastix* and *Piromyces* isolates did not alter the plant’s external integrity in this study. Therefore, rumen fungi appear to have the ability to alter the characteristics of fibrous residue thus potentially aiding in mastication and fiber flow in the digestive tract and subsequently increasing feed consumption. However, a direct relationship of fungal degradation of fiber to feed intake has not been established and requires additional research.

**INTERACTION OF RUMEN FUNGI WITH OTHER MICROORGANISMS**

Rumen fungi obviously must interact with a variety of protozoal and bacterial species in their ecosystem. However, information describing such interactions is relatively scarce. Early work (13) showed a synergistic influence of methanogenic rumen bacteria on the cellulolytic activity of fungi, increasing the extent of digestion by 55% and altering the fermentation products towards higher amounts of acetate, methane, and CO2 at the expense of formate and H2. Similar results occurred with a fungus grown in triculture with two methanogens (35). Recent studies by Stewart and Richardson (49) indicated that cocultures of rumen fungi and methanogenic bacteria enhanced the resistance of fungi to growth-promoting antibiotics (i.e., monensin and lasalocid) for animals. However, other studies have indicated that rumen bacteria
TABLE 3. Characteristics of bermudagrass and alfalfa stems incubated with rumen microbial groups.1,2

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>% Dry weight loss</th>
<th>Strength of residue (Newton)4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alfalfa</td>
<td>Bermudagrass</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>19.8a</td>
<td>21.1b</td>
</tr>
<tr>
<td>Whole rumen fluid</td>
<td>38.4bc</td>
<td>47.2b</td>
</tr>
<tr>
<td>Bacteria</td>
<td>35.7c</td>
<td>46.4b</td>
</tr>
<tr>
<td>Fungi</td>
<td>40.9bc</td>
<td>53.8c</td>
</tr>
</tbody>
</table>

1One-cm stem sections incubated 72 h with: total mixed populated (whole rumen fluid), fluid plus cycloheximide to inhibit eucaryotes and select for bacteria (bacteria), or fluid plus streptomycin and penicillin to inhibit fiber-digesting bacteria and select for eucaryotes (fungi). Note: protozoa were not active as primary fiber-degraders as observed by electron microscopy.
2From Akin et al. (7).
3Values within columns with different superscripts differ at P≤0.05.
4Newton = Force that gives to a mass of 1 kg an acceleration of 1 m/s².

may reduce fiber degradation by rumen fungi. Colonization of forages by fungi (as determined by sporangial counts on leaf blades) was substantially greater when antibacterial antibiotics were included in rumen fluid media (Table 4). Other unpublished work (48) indicated that solubilization of straw by N. frontalis was markedly reduced when incubated in coculture with ruminococci. Further, mixed fungal populations were unable to colonize or degrade grass leaves in the presence of a growing culture of an unidentified coc cocobacillus (9). Although information on fungal-bacterial interactions is scarce, even less information seems to be available concerning the interaction of rumen protozoa and fungi. Some evidence has been presented by Orpin (42) that removal of ciliates by defaunation increases the fungal population and that fungal zoospores may be ingested by rumen protozoa. Certainly the interaction of fungi with bacteria and protozoa needs to be clarified if understanding of the role of rumen microorganisms in ruminant nutrition is to be expanded.

TABLE 4. Rumen sporangia colonizing the leaf surface of bermudagrass incubated with rumen fluid with or without antibiotics.1

<table>
<thead>
<tr>
<th>Incubation</th>
<th>+ S and P</th>
<th>- S and P</th>
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</thead>
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<tr>
<td>(h)</td>
<td>Ï</td>
<td>Ï</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>1.2</td>
<td>.3</td>
</tr>
<tr>
<td>48</td>
<td>10.0</td>
<td>1.6</td>
</tr>
<tr>
<td>72</td>
<td>8.2</td>
<td>4.4</td>
</tr>
<tr>
<td>96</td>
<td>4.8</td>
<td>3.4</td>
</tr>
</tbody>
</table>

1From Akin and Rigsby (8).
2Five sites for each of 12 leaf blades for two cows examined. + S and P = inclusion of streptomycin and penicillin to rumen fluid.
3Not determined.

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ble carbohydrates resulted in decreased fungal populations; in contrast, fungal populations were high with fibrous diets such as grass silage, mature ryegrass, and straw (E. Grenet, 1989, personal communication). Research on the influence of microbial groups on associative feed effects showed that fungal populations were stimulated with corn and soybean hulls, but this increase did not result in higher microbial digestibilities (Windham, W. R., and D. E. Akin, 1989, unpublished data). The influence of diet on rumen fungi might occur because of direct effects such as supplying a growth factor (40) or because of indirect effects by influencing competing organisms (9, 42).

**IMPORTANCE OF ANAEROBIC FUNGI IN THE RUMEN**

Research from several laboratories has shown the ability of rumen fungi to attack, weaken, and partially or fully degrade recalcitrant tissues in in vitro samples and from digesta removed from the rumen. Amino acid compositions of a few isolates of rumen fungi have been evaluated, and initial indications are that absorption by the animal could be extremely high (21, 28). Despite these positive attributes of the fungi in regard to fiber digestion and their apparent cosmopolitan nature, a definitive role for them in the rumen ecosystem has not been established. For example, increases in fungal populations or the in vitro digestion of plant material by the fungi was not reflected in substantial or consistent increases in fiber digestion by rumen inocula (54). The interaction with rumen bacteria and protozoa requires further study, for the apparent potential of the fungi (as determined in vitro) is not always realized in the rumen ecosystem (6). The unique attributes of the rumen fungi in degrading fiber, the high amounts and activities of cellulases and xylanases, and the potential for improving the efficiency of forage utilization warrant further evaluation of these new and unusual organisms.

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SYMPOSIUM: RUMEN MICROBIAL ECOLOGY AND NUTRITION

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