

Rumen Microbiology

Allisonella histaminiformans and Its Potential Role in Ruminal Histamine Accumulation and Laminitis

J.B. Russell and M.R. Garner

Introduction

Dairy cattle are fed grain supplements to stimulate production, but it has long been recognized that grain can cause problems. When the rate of starch fermentation exceeds the buffering capacity of the rumen, acids accumulate, ruminal pH declines, and in severe cases the animal dies. In less severe cases, the animal survives, but the ruminal wall is ulcerated and often permanently scarred. Because the tissues above the hoof are also affected (laminitis), the animal can suffer temporary or even prolonged lameness (Takahashi and Young, 1981). In the 1940's, Dougherty and his colleagues (Dougherty, 1942) noted that grain feeding also promoted ruminal histamine production. Histamine is a powerful inflammatory agent, and they concluded that there was a direct correlation between "the histamine level of ingesta and the well being of the animal." Histamine is formed from the decarboxylation of the amino acid, histidine, and even a small conversion of histidine to histamine can be toxic (Suber et al. 1970). In the 1950's, Rodwell (1953) isolated histidine decarboxylating lactobacilli from sheep and horses fed grain-based rations. The potential involvement of lactobacilli was consistent with the observation that lactobacilli are highly pH-resistant bacteria that accumulate in the rumen when animals are fed an abundance of cereal grain. However, we were unable to isolate histamine-producing lactobacilli. Our histidine enrichments yielded a new bacterium, *Allisonella histaminiformans* (Garner et al. 2003).

Materials and Methods

Ruminal contents were obtained from dairy cows fed a standard dairy ration. The mixed ruminal bacteria were serially diluted (10-fold increments) into MRS, a medium "selective" for lactobacilli (deMan et al., 1960), that had been supplemented with 50 mM histidine. The histidine enrichments were then transferred successively in an anaerobic, carbonate-based medium (50 mM histidine) without glucose. The histidine enrichments were streaked onto the surface of agar plates (50 mM histidine) and colonies were picked and tested for histamine production. Histamine was assayed by a method that employed thin layer chromatography. Cellular fatty acids were extracted and assayed by gas chromatography. G+C analysis was performed using a spectroscopic DNA-DNA hybridization method. Amino acids were separated on a lithium cation exchange column and detected at 560 nm following ninhydrin post column derivation. Fermentation acids were analyzed by high-performance liquid chromatography. Chromosomal DNA from histamine producing isolates was purified using the FastDNA Spin Kit (Bio101, Vista, CA). Chromosomal DNA was mixed with primers (40 nmol 27F and 1492R), and the 16S rRNA gene region was amplified. By using the neighbor joining method, it was possible to calculate a distance matrix, and the precision of the relationships

improved by generating 1000 trees with a bootstrap method. Sequences from other bacteria were obtained from Genbank and aligned using Clustal X.

Results

When ruminal fluid from dairy cattle was serially diluted in anaerobic MRS medium containing histidine (50 mM), histamine was detected at dilutions as high as 10^{-7} . The histidine enrichments were then transferred successively in an anaerobic, carbonate-based medium (50 mM histidine) without glucose. All of the histamine producing isolates had the same ovoid morphology. The cells stained Gram-negative and were resistant to the ionophore, monensin (25 μ M). The doubling time was 110 min. The G+C content was 46.8%. Lysine was the only other amino acid used, but lysine did not allow growth if histidine was absent. Because carbohydrate and organic acid utilization was not detected, it appeared that the isolates used histidine decarboxylation as their sole mechanism of energy derivation. 16s rRNA gene sequencing indicated that the isolates were most closely related to low G+C Gram-positive bacteria, but similarities were $\leq 94\%$. Because the most closely related bacteria (*Dialister pneumosintes*, *Megasphaera elsdenii* and *Selenomonas ruminantium*) did not produce histamine from histidine, we propose that these histamine producing bacteria be assigned to a new genus, *Allisonella*, as *Allisonella histaminiformans* gen. nov., sp. nov. The genus name *Allisonella* honors Milton J. Allison, a prominent rumen microbiologist. Dr. Allison previously isolated *Oxalobacter formigenes*, a ruminal bacterium that decarboxylates oxalate (Allison et al., 1985). The species name is a Latin word that means “forming histamine.”

Dairy cows fed a commercial dairy ration had large populations ($>10^6$ cells per ml) of *A. histaminiformans*, but this bacterium could not be isolated from cattle fed diets consisting only of hay. When stationary phase *A. histaminiformans* MR2 cultures were serially diluted into autoclaved ruminal fluid from cattle fed hay, histamine was not detected at dilutions greater than 10^{-2} even if histidine (50 mM) was added. In contrast, histamine was detected in the 10^{-9} dilution if the autoclaved ruminal fluid was obtained from cattle fed the commercial ration and supplemented with histidine (50 mM). The commercial ration contained large amounts of alfalfa and corn silage, and water-soluble silage extracts stimulated the growth of *A. histaminiformans* MR2 in vitro. Alfalfa silage extract was at least 8-fold more potent than the corn silage extract, and extract from only 5 mg of alfalfa dry matter promoted maximal histamine production in 1 ml of culture medium. Because non-ensiled alfalfa did not stimulate histamine production nearly as much as the alfalfa silage, the factor that stimulates the growth of *A. histaminiformans* appears to be a product of silage fermentation.

Discussion

Histidine decarboxylase, the enzyme that produces histamine, has been studied in great depth and is widely distributed in bacteria, but *A. histaminiformans* is the first histamine producing bacterium that can utilize histidine as its sole source of energy. It had generally been assumed that lactobacilli were responsible for ruminal histamine accumulation. Given the observation that our ruminal enrichments were not stimulated by glucose, it appeared that bacteria other than lactobacilli were responsible for histamine production. Previous workers noted that histamine did not accumulate in the rumen unless the diet had considerable amounts of grain and the pH was acidic. These results are consistent with the observation *A. histaminiformans* is acid resistant bacterium. *A. histaminiformans* could not be isolated from cattle fed hay, and we originally thought that this effect

might be due to pH or an antagonism. However, the observation that small amounts of yeast extract counteracted the “inhibition” in vitro suggested that another factor was involved. Based on the observation that the dairy ration had large amounts of silage, we tested the ability of silage extracts to stimulate growth. Results indicated that silages, and in particular alfalfa silage, stimulated the growth of *A. histaminiformans* in ruminal fluid from a cow fed hay.

Conclusions

Cattle fed dairy rations have *A. histaminiformans*, a bacterium that produces histamine, but its numbers in vivo are clearly diet-dependent. Further work will be needed to define more precisely the chemistry of the growth factor that stimulates *A. histaminiformans*, but the observation that the growth factor can be derived from alfalfa silage could have practical significance. Logue et al. noted that dairy cattle fed grass silage had a significantly greater incidence of laminitis and foot lesions than cattle that were fed non-fermented dry forage. Given the observation that foot problems are a primary reason why dairy cattle are eliminated from the milking herd, any practice that could reduce laminitis would have a positive impact on dairy cattle production.

Degradation of Alfalfa Cell Wall Polysaccharides by Pure Cultures of Five Rumen Bacterial Species

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Introduction

The rumen contains a diverse assemblage of bacterial species that interact both synergistically and competitively to degrade the cell wall matrix of forages. *Fibrobacter succinogenes*, *Ruminococcus albus*, and *R. flavefaciens* have generally been considered to be the dominant species responsible for cell wall degradation. While all three of these species have been reported to degrade both cellulose and hemicellulose, *F. succinogenes* is unable to utilize the pentose sugars released from hemicellulose. *Butyrivibrio fibrisolvens* is generally considered a poor cellulose degrader but good hemicellulose degrader, and *Lachnospira multiparus* has been suggested to be a specialist for pectin degradation. However, the data for cell wall polysaccharide degradation capabilities of these rumen bacteria are generally based on purified substrates rather than intact forage cell wall matrixes. Differences in affinity of these bacterial species for degradation of specific plant tissues is almost unknown. This study examined the ability of these five rumen species to degrade alfalfa stem tissues and alfalfa cell wall polysaccharides *in situ*.

Materials and Methods

Alfalfa stems were harvested after 21 and 32 d of re-growth from a single alfalfa genotype grown in the field. For quantitative measures of cell wall degradation, stems were freeze-dried and ball-milled. Tissue degradation was assessed using 100 mm thin sections made from 50% ethanol preserved stems. Both ground alfalfa and sections were inoculated with pure strains of *Butyrivibrio fibrisolvens* H17c, *Fibrobacter succinogenes* S85, *Lachnospira multiparus* 40, *Ruminococcus albus* 7, *R. flavefaciens* FD-1, both individually and as a five species mixture. Additional samples were inocu-

lated with rumen fluid. Incubation periods were 6, 24, and 96 h for the sections and 24 and 96 h for the ground alfalfa stems. Sections were evaluated by light microscopy for when degradation of individual tissues began and the extent of degradation achieved. The ground alfalfa stems were analyzed for residual cell wall polysaccharides and degradabilities were calculated. Cellulose was estimated as the cell wall glucose content; hemicellulose as the sum of xylose, mannose, and fucose residues; and pectin as the sum of uronic acids, galactose, arabinose, and rhamnose.

Results and Discussion

Although the alfalfa stem internodes utilized in this study differed in age by 10 d of re-growth, cell wall composition was very similar. The two alfalfa maturities differed primarily in total cell wall concentration and the mature stems were slightly less degradable than the immature stems. For both the ball-milled alfalfa stems and the stem thin sections, increasing time of incubation resulted in increased cell wall degradation, although for the stem sections degradation was virtually complete by 24 h compared to greater degradation observed after 96 h for the ball-milled alfalfa. Averaged across both alfalfa maturity stages and incubation times, *Ruminococcus albus* degraded more cellulose than observed for rumen fluid (62.5 vs. 55.0%), with all other pure cultures and the mixture resulting in less than half the amount of cellulose degradation observed for rumen fluid. Contrary to expectations, *Fibrobacter succinogenes* degraded cellulose poorly (19.2%) and *Lachnospira multiparus* was able to degrade cellulose (19.2%).

Hemicellulose degradation was lower than observed for cellulose, although *R. albus* again exhibited the highest degradation (40.3%), followed by rumen fluid (26.7%). *Butyrivibrio fibrisolvens* was not an especially good hemicellulose degrader (15.6%) and *L. multiparus* was virtually incapable of degrading alfalfa hemicellulose (7.4%). All bacterial species and rumen fluid degraded pectin well. *L. multiparus* was not the best pectin degrader, consistent with reports that this species degrades pectin in intact cell walls much more poorly than it does purified citrus pectin. The microscopic examination of alfalfa stem tissue degradation matched the data from the ball-milled alfalfa (Fig. 1). *R. albus* was clearly able to degrade all tissues as well or better than rumen fluid. *F. succinogenes* and *R. flavefaciens* were able to degrade the non-lignified, thin-walled chlorenchyma and cambial tissues, but did not degrade the thick, non-lignified collenchyma tissue. *B. fibrisolvens* and *L. multiparus* attacked all the non-lignified tissues, but *L. multiparus* did not completely degrade the pectin-rich collenchyma tissue. While most of our results corresponded to literature reports for the degradation characteristics of these five rumen bacterial species, deviations from expectations were observed for several bacteria when grown on intact alfalfa cell wall matrices.

Conclusion

The quantitative measurements of ground alfalfa stem cell wall polysaccharide degradation by various rumen bacterial cultures matched the visual observations for degradation of thin sections from alfalfa stems reasonably well. Differences were noted among bacterial species for which alfalfa stem tissues each species degraded. The ability of bacterial species to degrade in situ cell wall polysaccharides did not always match previous literature for isolated substrates.

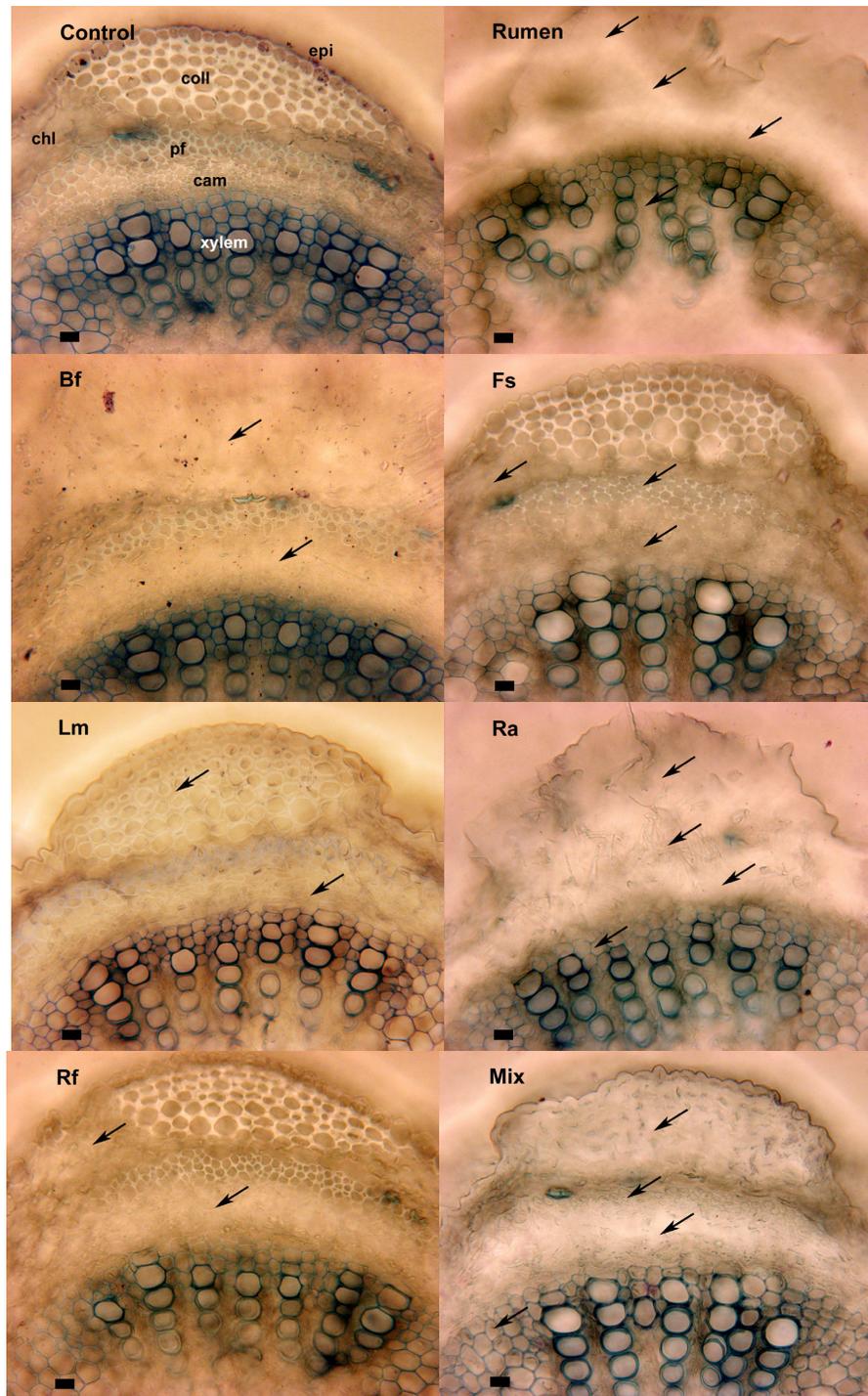


Figure 1. Degradation of immature alfalfa stem tissues after 24-h incubations with rumen fluid or *Butyrivibrio fibrisolvens* H17c (Bf), *Fibrobacter succinogenes* S85 (Fs), *Lachnospira multiparus* 40 (Lm), *Ruminococcus albus* 7 (Ra), *R. flavefaciens* FD-1 (Rf), or a mixture of the five pure cultures (Mix). The alfalfa stem tissues are identified in the Control micrograph (epi = epidermis; coll = collenchyma; chl = chlorenchyma; pf = phloem fibers, cam = cambium and secondary phloem; and xylem = xylem fibers and vessels). Tissues showing signs of degradation are indicated by the arrows. Bar is 20 μ m.

Albusin A, a Bacteriocin from *Ruminococcus albus* that Inhibits the Growth of *Ruminococcus Flavefaciens*

J. Chen, D.M. Stevenson, and P.J. Weimer

Introduction

In the rumen, the relative population size of one fiber-digesting bacterial species, *Ruminococcus albus*, has been shown to be considerably greater than the relative population size of two of the other major agents of fiber digestion, *R. flavefaciens* and *Fibrobacter succinogenes*. Several research groups have reported that the predominance of *R. albus* is due in part to its production of a bacteriocin (a protein produced by one bacterial strain that inhibits a closely related strain or species), but such an agent has not been purified from *R. albus*. Bacteriocins are of interest because they may represent a means to control populations of individual bacterial species without the use of antibiotics, which have a broader spectrum of activity and which may have undesirable effects via stimulation of the development of resistance by pathogenic microbes through horizontal gene transfer.

Methods

Ruminococcus albus strain 7 was grown in pure culture under CO₂ in 3.6 L of a modified Dehority medium that contained 3.5 g cellobiose and 1 g of yeast per liter. After 28 h of incubation at 39 °C, culture was passed through a 0.2 µm hollow fiber cartridge filter to remove cells. The resulting permeate was concentrated using a 10 kDa MWCO hollow fiber cartridge, and subjected to a purification scheme that included ammonium sulfate precipitation, Toyopearl HW50F size exclusion chromatography and DEAE 650M anion-exchange chromatography. Bacteriocin activity was assayed by spotting ~50 µL of sample from each stage of the purification onto Petri dishes containing a freshly-poured MDM agar that contained the *R. flavefaciens* test strain, followed by incubation at 39 °C in an anaerobic glovebag. Purification of the bacteriocin was assessed by SDS-PAGE. The purified bacteriocin fraction was recovered from the gel by electrotransfer to a PVDF membrane, and was subjected to N-terminal sequencing at the University of Texas Medical Center - Galveston. Sequence similarities were determined by BLAST search on the website of the National Center for Biotechnology Information.

Results and Discussion

The purification scheme resulted in recovery of a protein fraction that could prevent the growth of all five strains of *R. flavefaciens* tested, but did not affect the growth of *F. succinogenes*, *Streptococcus bovis*, *Selenomonas ruminantium*, or *Escherichia coli*. Specific activity of the bacteriocin was 131-fold greater in the fraction than in the original culture supernatant. The fraction contained 36 kDa protein and small amounts of a 45 kDa protein (Fig. 1). Separate elution and testing of the two proteins from an SDS-PAGE gel revealed that the activity resided in the 36 kDa band. The eluted 36 kDa protein, named albusin A, had the N-terminal amino acid sequence SGLDAKGIVSQMKIGWNLGNTLDAXNXKV. This sequence displayed similarity to several endoglucanase enzymes from other *Ruminococcus* and *Clostridium* species (Fig. 2). However, these sequences are not considered to be active-site motifs for endoglucanase enzymes, and the chromatographic fraction containing albusin A had less than 1% of the endoglucanase specific activity of a typical *R. albus* culture supernatant. Moreover, the chromatographic fraction that eluted immediately afterward (peak 3, Fig. 1A), and which contained more of the 45 kDa protein and no 36 kDa

protein (Fig. 1B), displayed five-fold higher endoglucanase activity, suggesting that the endoglucanase activity resided in the 45 kDa protein. Attempts to clone the gene encoding albusin A into a pGEM-3Z vector, with detection by a degenerate probe based on the N-terminal sequence, were unsuccessful.

Based on its relatively large size (31 kDa), its relatively hydrophilic character, and its bacteriostatic (rather than bacteriocidal) activity, albusin A represents a class III bacteriocin, as defined by Klaenhammer. Unlike the more familiar, membrane-active class I and class II bacteriocins, the mode of action of class III bacteriocins is not known.

Conclusions

Albusin A is the first bacteriocin purified from a ruminal cellulolytic bacterium. Production of this bacteriocin apparently provides one mechanism by which *R. albus* can suppress the growth of *R. flavefaciens* in laboratory culture. Bacteriocin production by *R. albus* may be important in the dominance displayed by this species in the ruminal environment, although more definitive tests of this hypothesis must await purification of the agent in larger quantities that would permit its direct addition to the rumen of test animals.

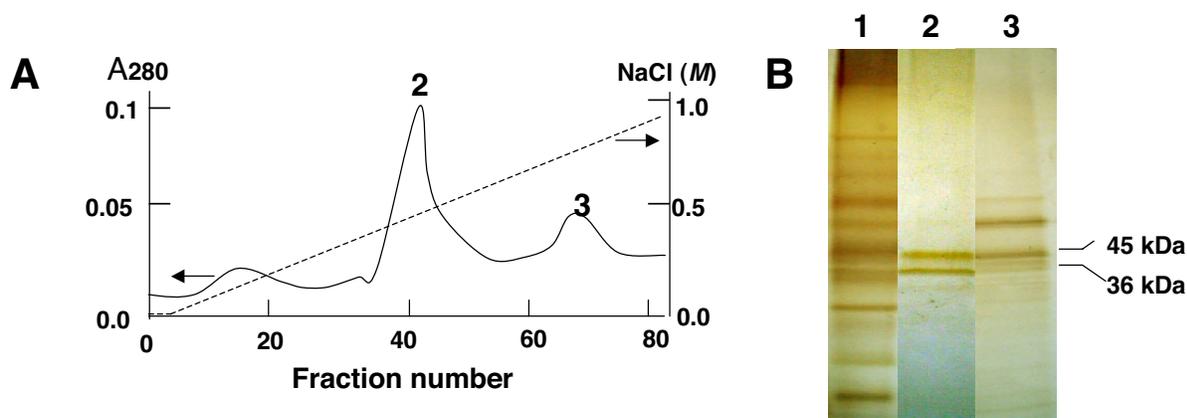


Figure 1. A) DEAE 650M column chromatography yielded a fraction (peak 2) that contained bacteriocin activity, and a fraction (peak 3) that did not contain activity. B) SDS-PAGE gels of peak 2 displayed protein bands of 36 kDa and 45 kDa, while peak 3 contained the 45 kDa band but not the 36 kDa band. The 36 kDa protein (albusin A) contained the inhibitory activity.

Gene product	Total amino acids or MW (kDa)		
<i>R. flavefaciens</i> cellulase	455aa	24	hssa..rryadaadntmtafqitenmkvgwnlgnldryaqkanpkdpsk 71
<i>R. flavefaciens</i> EGA	759aa	25	fsfaplddtadaadnmtafqitenmkvgwnlgnldayaqkanpkdpsk 74
<i>C. cellulovorans</i> EG	515aa	25	aftgvrdrv.....p.aqqivnemkvgnlgnltmda..... 55
<i>C. cellulovorans</i> EGD	515aa	25	aftgvrdrv.....p.aqqivnemkvgnlgnltmda..... 55
<i>C. cellulovorans</i> EGB	441aa	22	aktgirdi.....t.sqqvkvemkvgnlgnltmda..... 52
<i>C. cellulovorans</i> EGB	440aa	22	aktgirdi.....t.sqqvkvemkvgnlgnltmda..... 52
<i>R. albus</i> EG	411aa	22	aktgirdi.....t.sqqvkvemkvgnlgnltmda..... 52
<i>C. acetobutylicum</i> EG5	370aa	21	atvnpdm.....t.sqqivndmkvgwnlgnltlda..... 51
<i>C. thermocellum</i> EGE	814aa	37	vmrgmrdi.....s.aidlvskeikigwnlgnltlda..... 67
<i>C. longisporum</i> EGA	517aa	21	lsisrnplevqaasmrs.aseivqemgvgwnlgnltldakitn..... 63
<i>R. albus</i> EGI	406aa	51	envpvsqthtndtmtvtsakdlvakmtngwnlgnltmdatagg..... 64
<i>R. albus</i> F-40 EGIV	50-kDa	1lsgl.....d.akgivsqmkiggnlg..... 20
Albusin A (~36-kDa band)	~36-kDa	1lsgl.....d.akgivsqmkigwnlgnltlda..... 25
Consensus sequence			-----V--MK-GWNLGNT-DA-----

Figure 2. Alignment of the N-terminal sequence of albusin A with sequences of several endoglucanases from Gram-positive bacteria.

Albusin B, a Second Bacteriocin from *Ruminococcus Albus* that Inhibits the Growth of *Ruminococcus flavefaciens*

J. Chen, D.M. Stevenson, and P.J. Weimer

Introduction

In the rumen, the relative population size of one species, *Ruminococcus albus*, has been shown to be considerably greater than the relative population size of two of the other major agents of fiber digestion, *R. flavefaciens* and *Fibrobacter succinogenes*. The predominance of *R. albus* in both laboratory culture and the rumen appears to be due in part to production of bacteriocins (proteins produced by one bacterial strain that inhibits a closely related strain or species). We have previously purified, from *R. albus* 7, a bacteriocin which we have designated albusin A. Here we report the purification of a second bacteriocin, albusin B, and the sequence of the gene encoding its production.

Methods

Ruminococcus albus strain 7 was grown in pure culture under CO₂ in 10 L of a modified Dehority medium that contained 3 g of cellobiose and 1 g of yeast extract per liter. After 28 h of incubation at 39 °C, cells were removed using a 0.2 µm hollow fiber filtration cartridge. The resulting permeate was passed through a 10 kDa MWCO hollow fiber cartridge, and subjected to a purification scheme that included ammonium sulfate precipitation and size-exclusion chromatography (BioGel P6 column).

Bacteriocin activity was assayed by spotting ~50 µL of sample from each stage of the purification onto Petri dishes containing a freshly-poured MDM agar that contained the *R. flavefaciens* test strain, followed by incubation at 39 °C in an anaerobic glovebag. Purification of the bacteriocin was assessed by SDS-PAGE. The purified bacteriocin fraction was recovered from the gel by electrotransfer to a nylon membrane, and was subjected to N-terminal sequencing at the University of Texas Medical Center - Galveston. *HincII* digests of *R. albus* DNA were cloned into a pGEM vector in *E. coli*, and recombinants selected by blue/white screening. A degenerate probe was used to identify the clone of interest by colony hybridization. DNA sequence upstream of the cloned segment was determined following two stages of PCR gene walking. Sequence similarities were determined by BLAST search on the website of the National Center for Biotechnology Information, and on the unfinished genome of *R. albus* 8 at the website of The Institute of Genomic Research.

Results and Discussion

The purification scheme took advantage of the fact that a substantial fraction of the inhibitory activity passed through a 10 kDa hollow fiber filter. The scheme resulted in recovery of a protein fraction that could prevent the growth of *R. flavefaciens* FD-1, but did not affect the growth of *F. succinogenes*, *Streptococcus bovis*, *Selenomonas ruminantium*, or *Escherichia coli*. This fraction had a specific activity against *R. flavefaciens* FD-1 that was 220-fold greater than that of the original culture supernatant. Silver staining of SDS-PAGE gels of this fraction revealed a single 31 kDa protein, designated AlbB. A degenerate oligonucleotide probe based on the N-terminal sequence of the purified AlbB was used to identify two putative clones in pGEM3Z vector in *E. coli*. Sequencing of these cloned segments revealed them to be identical but in opposite orientations. Because the clones did not encode for the first nine amino acids of the gene, gene-walking PCR was used to

Residues of *Ruminococcus* Cellulose Fermentations as Components of Wood Adhesive Formulations

P.J. Weimer, A.H. Conner, L.F. Lorenz

Introduction

Ruminococcus bacteria are major agents of fiber digestion in the rumen. In pure culture, some ruminococci produce ethanol and other compounds, and thus may be useful for industrial-scale conversion of cellulosic biomass to fuels and chemicals. During the process of degrading cellulosic substrates, these bacteria produce a sticky glycocalyx that allows them to adhere to cellulose, and production of the glycocalyx appears to be a prerequisite of effective fiber degradation. The tenacity with which the bacteria adhere to fiber in an aqueous environment led us to examine the potential utility of these fermentation residues (which contain bacterial cells, their glycocalyx and the remaining cellulosic substrate) as adhesives that can add value to the ethanol production process. Such bio-based adhesives are of potential interest as partial or complete replacements for toxic, petroleum-based phenol-formaldehyde adhesives.

Methods

Ruminococcus albus strain 7, and *R. flavefaciens* strains FD-1 and B34b were grown in pure culture at scales up to 40 liters under CO₂ in a modified Dehority medium that contained 2 to 3.5 g of microcrystalline cellulose, 0.5 g of yeast extract and 25 μ moles of 3-phenylpropionic acid per liter. After 88-108 h of incubation at 39°C, the culture liquid was removed by aspiration, and the sticky fermentation residue was recovered by centrifugation. Protein content was determined by the Coomassie blue method following solubilization, and the carbohydrate composition of the glycocalyx was determined by boiling in neutral detergent solution to remove bacterial cells, followed by hydrolysis in 2 N TFA at 120 °C for 1.5 h and sugar quantitation by ion chromatography.

Adhesive formulations were prepared with wet fermentation residue (WFR) or lyophilized (freeze-dried) fermentation residue (LFR), either alone or in combination with different proportions of phenol-formaldehyde (PF). For comparison, control PF adhesives were prepared with 8% GLU-X, (a commercial adhesive extender derived from wheat flour) but without fermentation residue. The adhesives were used to prepare three-ply aspen panels (each panel 7" x 7" x 1/8") with the grain of the inner sheet oriented perpendicular to the outer two sheets. Panels were pressed for 8 to 10 min at 180 °C and 1.125 MPa (163 lb/in²), then cut into 1" x 3" lap shear specimens. The specimens notched with kerfs and subjected to shear strength and wood failure tests under both wet and dry conditions, according to standard testing protocols (American Society for Testing and Materials method PS 1-95).

Results and Discussion

Fermentation residues varied in protein content from 0.4 to 4.2%, depending on the completeness of the original fermentation. The neutral sugar composition of the residues displayed similar ratios (0.71 Glc, 0.18 Xylose, 0.08 Mannose, 0.02 Arabinose, molar basis) independent of bacterial species. The fermentation residues displayed no discernible odor before or after pressing, and the resulting bondlines in the panels were light in color, a desirable end-use property of adhesives.

Fermentation residues from *R. albus* 7 displayed superior adhesive properties to those of the two *R. flavefaciens* strains, most likely because the *R. albus* fermentation proceeded to a greater extent and thus would have had a higher ratio of (glycocalyx plus cells)/(unfermented residue). The LFR formulation displayed shear strength and wood failure values superior to the WFR formulations (Fig. 1). High wood failure rates are desirable, as they indicate that the adhesive is stronger than the wood itself.

Shear strengths of the LFR adhesives approached those of the control PF resins under dry conditions, but displayed undesirably low strength under wet conditions (Fig. 2). By incorporating the *R. albus* LFR into mixed adhesives with PF, acceptable shear strengths and wood failure values could be attained, even when 73% of the formulation was comprised of LFR. As expected, exposure of the panels to wet conditions decreased both shear strength and wood failure, but these values were better than most of those for soy-based protein adhesives..

Conclusions

These data suggest that fermentation residues containing the glycocalyxes and bacterial cells may have potential as components of wood adhesives. Additional experiments with more authentic biomass materials (e.g., alfalfa stems), and spanning a range of adhesive proportions and pressing conditions, should permit a more complete analysis of the practicality and economics of producing these residues as a value-added co-product of microbial fermentations.

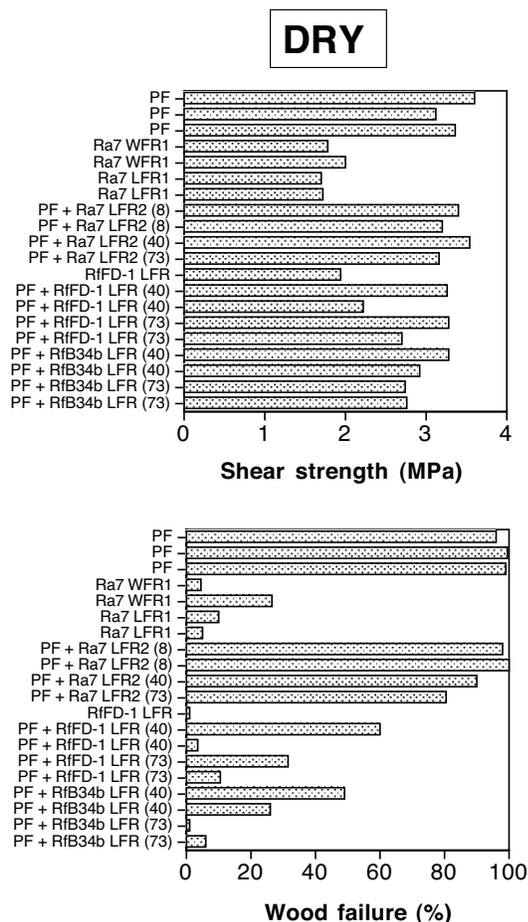


Figure 1. Shear strength (A) and wood failure percentage (B) for 3-ply aspen plywood panels prepared with adhesives described in Table 2, tested under dry conditions. Numbers in parentheses indicate percentage of fermentation residue by weight in the adhesive formulation. Samples having different lower-case letters within treatments differ ($P < 0.05$). Pooled standard error for shear strength = 0.58 MPa. Pooled standard error for wood failure = 16.9%.

WET

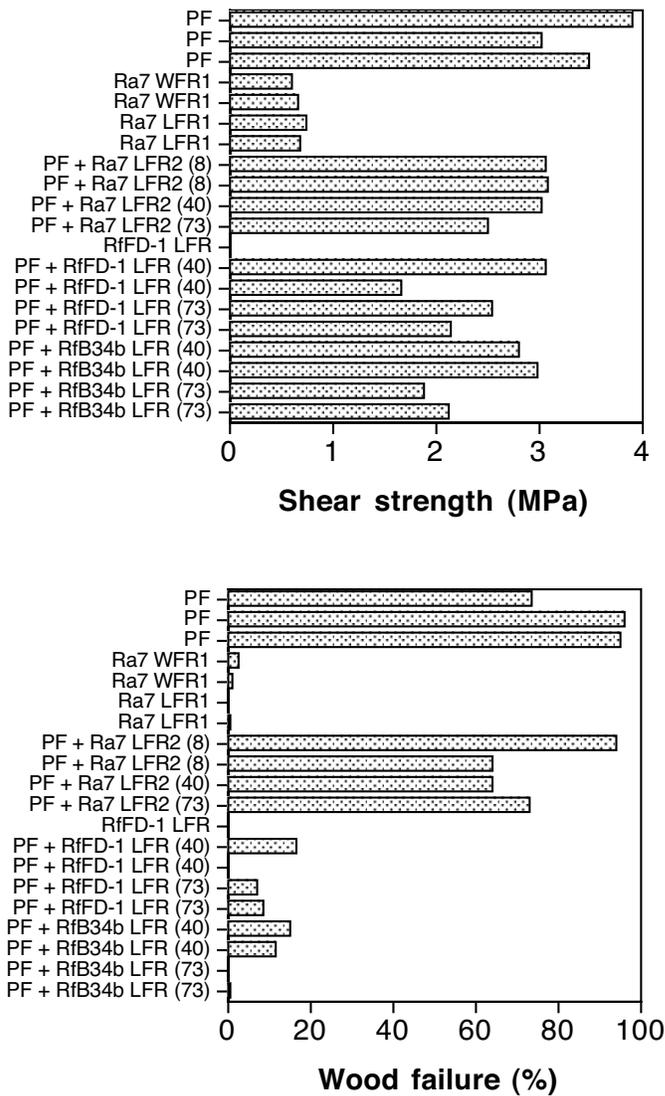


Figure 2. Shear strength (A) and wood failure percentage (B) for 3-ply aspen panels prepared with different adhesives, tested after vacuum/pressure/soak/drying [VPSD] treatment. Numbers in parentheses indicate percentage of fermentation residue by weight in the adhesive formulation. Samples having different lower-case letters within treatments differ ($P < 0.05$). Pooled standard error for shear strength = 0.46 MPa. Pooled standard error for wood failure = 16.4%.