Degradation and Utilization of Hemicellulose from Intact Forages by Pure Cultures of Rumen Bacteria

JUDITH A. COEN AND B. A. DEHORITY

Department of Animal Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

Received for publication 30 April 1970

Several pure strains of rumen bacteria have previously been shown to degrade isolated hemicelluloses from a form insoluble in 80% acidified ethanol to a soluble form, regardless of the eventual ability of the organism to utilize the end products as energy sources. This study was undertaken to determine whether similar hemicellulose degradation or utilization, or both, occurs from intact forages. Fermentations by pure cultures were run to completion by using three maturity stages of alfalfa and two maturity stages of bromegrass as individual substrates. Organisms capable of utilizing xylan or isolated hemicelluloses could degrade and utilize intact forage hemicellulose, with the exception of two strains of Bacteroides ruminicola which were unable to degrade or utilize hemicellulose from grass hays. Intact forage hemicelluloses were extensively degraded by three cellulolytic strains that were unable to use the end products; in general, these strains degraded a considerably greater amount of hemicelluloses than the hemicellulolytic organisms. Hemicellulose degradation or utilization, or both, varied markedly with the different species and strains of bacteria, as well as with the type and maturity stage of the forage. Definite synergism was observed when a degrading nonutilizer was combined with either one of two hemicellulolytic strains on the bromegrass substrates. One hemicellulolytic strain, which could not degrade or utilize any of the intact bromegrass hemicellulose alone, almost completely utilized the end products solubilized by the nonutilizer. Similar synergism, although of lesser magnitude, was observed when alfalfa was used as a substrate.

Since the hemicelluloses can constitute a considerable portion of forage carbohydrate, their use as an energy source by the ruminant animal is of special interest. Early studies, with conventional digestion trials, indicated that a definite loss of pentose occurs in the ruminant digestive tract (18–20). Since then, the in vitro fermentation of various isolated and intact forage hemicelluloses has been demonstrated with mixed cultures of rumen organisms (11, 13–15), as well as with several pure cultures (6, 8, 12, 17). Dehurity (6), investigating whether the xylan-utilizing species of rumen bacteria were capable of digesting isolated hemicelluloses, found that from a total of eight strains of cellulolytic bacteria only those three capable of using xylan as an energy source were able to grow on isolated hemicelluloses. The five remaining strains of cellulolytic bacteria, although unable to utilize the isolated hemicelluloses as a source of energy, degraded these materials to a form soluble in 80% acidified ethanol. The extent of hemicellulose degradation or utilization, or both, varied between organisms and source of the hemicellulose. Degradation was defined as the conversion of ethanol-insoluble pentose to a soluble form and utilization as total pentose loss. Further studies with several additional strains isolated on a xylan medium revealed differences in the rate and extent of degradation, utilization, or both, between the isolated hemicellulose substrates and the various bacterial strains and species (8).

Dehurity and Scott (12) estimated hemicellulose digestion in two maturity stages each of bromegrass and alfalfa and found that digestion was appreciably decreased in the intact plant as compared with isolated hemicelluloses. In general, the extent of hemicellulose digestion varied with the maturity and type of forage, as well as with the species of bacteria. In limited synergism studies, it was observed that cellulose digestion was increased in all cases when the cellulolytic organisms were combined with Bacteroides ruminicola H8a, a noncellulolytic, hemicellulolytic organism. Kock and Kistner (17) confirmed

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1 Approved for publication as Journal Article no. 36-70 by the Ohio Agricultural Research and Development Center, Wooster, Ohio.
the results of Dehory and Scott (12) on the digestibility of forage hemicelluloses. Ten strains of Butyryrivibrio and two strains each of Ruminococcus flavefaciens, R. albus, and Clostridium sp., all capable of fermenting xylan, were able to solubilize the hemicellulose from ethanol-benzene-extracted low protein teff hay. Hemicellulose solubilization was based on the loss in weight of isolated holocellulose when treated with 24% KOH (w/v).

In view of these data, the present study was undertaken to (i) investigate whether hemicelluloses are degraded from an intact forage in a manner similar to that observed in the isolated materials by both the utilizing and nonutilizing strains; (ii) determine the influence of plant type and maturity as well as bacterial strain differences on the extent of hemicellulose degradation; (iii) study several previously untested species and strains of rumen bacteria for their ability to degrade intact forage hemicelluloses; and (iv) investigate possible synergistic effects on hemicellulose utilization or degradation, or both, by using an intact forage substrate. One additional objective in the present study was to measure the utilization or degradation, or both, of hemicellulose from an intact forage and compare these values with those obtained from a sample of hemicellulose previously isolated from this same forage.

**MATERIALS AND METHODS**

Forage substrates consisted of boot and bloom stages of bromegrass (Bromus inermis Lincoln) and prebloom, early bloom, and late bloom stages of alfalfa (Medicago sativa Vernal). All of the forages were harvested from pure stands, artificially dried on a wagon dryer, chopped, ground through a medium screen in a large Wiley mill, and finally ground through a 40-mesh screen in a small laboratory Wiley mill. Fescue grass and isolated fescue grass hemicellulose were supplied by the late Fred Smith of the University of Minnesota. The fescue grass was chopped and ground through a 40-mesh screen in a small Wiley mill for use as a substrate. Isolated hemicellulose was prepared from the same stand of fescue grass by the procedure of Myhre and Smith (21).

The anaerobic cultural techniques were similar to those described by Hungate (16), except for the modification proposed by Dehory (10) in the preparation of media. The fermentation medium contained 0.5% forage or 0.1% isolated hemicellulose, 40% clarified rumen fluid, 15% each of mineral solutions I and II of Bryant and Burkey (2), 0.0001% resazurin, 0.4% sodium carbonate, and 0.05% cysteine. The mixture was agitated with a magnetic stirrer, and 5-mL samples were pipetted anaerobically into pyrex culture tubes (16 by 150 mm), which were then closed with a size 0 rubber stopper. The individual tubes were autoclaved for 20 min at 121 C in a clamp-type rack to prevent the stoppers from blowing out.

The 10 pure cultures of rumen bacteria used for this study included strains A3c and S-85 of B. succinogenes, strains B1a and B34b of R. flavefaciens, strain 7 of R. albus, strains H10b and H17c of Butyryrivibrio fibrisolvens, strains H8a and D31d of B. ruminicola (subspecies brevis and ruminicola, respectively), and strain D15d of Lachnospira multiparas. The characteristics of these strains have previously been described (3−7, 10). Strains A3c, B1a, B34b, S-85, and 7, all cellulolytic, were isolated from rumen contents with a nonselective glucose-cellobiose medium; strains H8a, H10b, and H17c were isolated with a xylan medium; and strains D15d and D31d were isolated with a pectin medium. All cultures were carried in the medium in which they were originally isolated. Inoculum cultures were grown overnight in an optically clear 0.5% cellobiose broth containing 40% clarified rumen fluid and then diluted with anaerobic dilution solution until an optical density of 0.2 had been reached. A 0.2-ml amount of this suspension was used to inoculate each 5 mL of forage media. All turbidity measurements were made in Pyrex culture tubes (16 by 150 mm) on a Bausch & Lomb Spectronic-20 colorimeter, reading at 600 nm. The fermentations were allowed to incubate for 168 hr at 39 C. The inoculum level and the fermentation time had been previously determined (8) to allow for maximum rate and completion of forage cellulose digestion.

Estimation of forage hemicellulose concentration either by simple solubility methods or the more complex isolation procedures did not appear to be suitable for this study. Even though the constituent carbohydrates and per cent composition of plant hemicelluloses vary between species, approximately 80 to 90% of the hemicellulose is composed of pentoses. On this basis, it is believed that total pentose analyses would be indicative of changes in the hemicellulose fraction, and relatively simple analytical methods could be adapted for routine use. One possible source of error would be the contribution of ribose from plant and bacterial ribonucleic acid; however, this would be quite small in relation to overall pentose changes. Thus, hemicellulose concentration of the forages was estimated by hydrolysis in 1 N H2SO4 and measurement of total pentose present with the orcinol reaction (1). Hexose and uronic acid interference was removed by reading at two wavelengths and treatment with an anion exchange resin, respectively (12, 22).

Although methods were described by Dehory (6) for estimating solubilization of total pentose in 80% ethanol with isolated hemicellulose and by Dehory and Scott (12) for the loss of total pentose when working with intact forages, procedures for measuring solubilization of pentose in 80% ethanol from the intact forage had to be developed. After a series of preliminary studies, the following procedure was adopted for routine use. The entire contents of the fermentation tube were transferred to a 40-ml glass centrifuge tube, 20 mL of a 5% acetic acid in 95% ethanol solution was added, and the contents were
mixed and allowed to stand at room temperature for 30 min. The mixture was then centrifuged 30 min at 2,000 × g, and the supernatant was decanted into a 50-ml volumetric flask. Acidified 80% ethanol solution (20 ml) was added to the residue, and the contents were mixed and recentrifuged for 20 min at 2,000 × g. The wash solution was decanted and added to the original supernatant, and the total supernatant was brought to volume with distilled water. To hydrolyze the hemicellulose in the residue, 5 ml of 1 N H$_2$SO$_4$ was added to each tube; the tube was covered with a metal cap and autoclaved for 1 hr at 15 psi. After cooling, the hydrolysate was transferred to a 50-ml volumetric flask and brought to volume with distilled water. The insoluble material was allowed to settle, and a sample was pipetted from the upper layer for further analysis. Portions (5 ml) of both fractions were treated with 2 g (wet weight) Amberlite IRA-400 anion exchange resin in the acetate form. The resin was removed by filtration and the filtrate plus washings, diluted to appropriate volumes, were then analyzed for total pentose by the orcinol method (1). Per cent transmission was read at 520 and 660 nm on an Evelyn colorimeter, which allowed correction for any color at 660 nm arising from hexose in the sample.

In this study, degradation is defined as the solubilization of 80% ethanol-insoluble pentose, whereas utilization is defined as a loss in total pentose (6).

Degradation and utilization values are based on duplicate fermentations in two replicates. A preliminary study involving two organisms and two different forage substrates (alfalfa and orchardgrass) was run to determine a feasible coefficient of variation for acceptance of duplicate analyses. Six replicates of each forage and organism were set up in duplicate, and coefficients of variation ranging between 5 and 10% were obtained. These were similar to the values obtained previously by Dehority (8), in a study on the digestibility of cellulose in intact forages in which a coefficient of variation of 15% was chosen as the upper limit for acceptance of the data. Thus, when a coefficient of variation larger than 15% was obtained between the four values in the present study, the fermentations were run twice again in duplicate.

**RESULTS**

Data on the utilization or degradation, or both, of hemicellulose from two maturity stages of bromegrass are presented in Table 1. It is apparent that all of the cellulolytic strains tested were capable of degrading a considerable amount of the 80% acidified ethyl alcohol-insoluble pentose to a soluble form without necessarily being able to utilize the resulting end products as energy sources. Values of 6% or less are considered to be within the range of experimental variation and are, therefore, of doubtful significance. Those cellulolytic strains which can ferment xylan, Bla and 7, were capable of utilizing the hemicellulose, although utilization by Bla was somewhat limited. Despite this utilization, neither of these

**Table 1. Degradation, utilization, or both, of hemicellulose from bromegrass by pure cultures of rumen bacteria**

| Strain | Forages$^a$ | Degradation | Utilization | Degradation | Utilization |
|--------|-------------|--------------|-------------|--------------|-------------|
|        | Brome I     |              |             | Brome II     |              |
|        |             | %            | %           | %            | %           |
| A3c    |             | 54.0 ± 0.7*  | 2.1 ± 1.2   | 31.8 ± 2.1   | 6.0 ± 2.2   |
| S-85   |             | 77.3 ± 1.2   | 3.0 ± 1.3   | 62.0 ± 1.7   | 2.4 ± 0.9   |
| 7      |             | 60.9 ± 0.4   | 46.0 ± 0.7  | 40.6 ± 0.6   | 29.4 ± 0.4  |
| Bla    |             | 56.6 ± 0.5   | 23.0 ± 0.9  | 34.7 ± 1.0   | 17.1 ± 0.9  |
| B34b   |             | 77.8 ± 0.9   | 0           | 61.1 ± 1.0   | 0           |
| H10b   |             | 51.9 ± 1.5   | 41.3 ± 1.8  | 32.5 ± 1.6   | 27.1 ± 1.3  |
| H17c   |             | 50.7 ± 0.7   | 44.8 ± 0.7  | 30.2 ± 0.1   | 26.8 ± 0.1  |
| H8a    |             | 4.7 ± 1.1    | 6.1 ± 1.1   | 5.0 ± 0.5    | 6.1 ± 0.5   |
| D31d   |             | 4.2 ± 1.7    | 4.1 ± 1.7   | 0.6 ± 0.4    | 0.6 ± 0.5   |
| D15d   |             | 2.2 ± 1.2    | 4.8 ± 0.7   | 3.1 ± 0.8    | 2.6 ± 1.0   |
| B34b + D15d |   | 78.3 ± 1.2   | 3.5 ± 0.8   | 62.9 ± 1.2   | 2.1 ± 1.3   |
| B34b + H8a |        | 84.1 ± 1.5   | 80.3 ± 2.0  | 70.3 ± 1.6   | 67.2 ± 2.0  |
| B34b + H10b |      | 81.3 ± 0.4   | 69.6 ± 1.2  | 65.8 ± 0.7   | 58.5 ± 0.7  |
| B34b + H8a + H10b + D15d | | 83.5 ± 0.2   | 78.7 ± 0.2  | 70.6 ± 0.6   | 67.3 ± 0.4  |

$^a$ Agronomic description: Brome I (boot stage) and Brome II (bloom stage). Values expressed as per cent.

$^b$ Cellulose digesting strains: Bacteroides succinogenes A3c, S-85; Ruminococcus albus 7; R. flavefaciens Bla, B34b; and Butyrivibrio fibrisolvens H10b, H17c. Noncellulose digesting strains: B. ruminicola H8a, D31d; and Lachnospira multiparus D15d.

$^c$ Mean and standard error of the mean.
strains could degrade as much hemicellulose as *B. succinogenes* S-85 or *R. flavefaciens* B34b, both nonutilizers. The second strain of *B. succinogenes*, A3c, had the lowest degradation values of the cellulolytics.

Of the hemicellulolytic organisms, only the two strains of *B. fibrisolvens*, H10b and H17c, were able to degrade and utilize the hemicellulose in intact bromegrass. Neither strain of *B. ruminicola*, H8a or D31d, was able to degrade any of the pentosan present. Both xylan isolate H8a and pectin isolate D31d can ferment xylan, and H8a can use several isolated hemicelluloses. The other pectin isolate, *L. multiparus* D15d, cannot utilize xylan and was unable to degrade any of the available pentosan.

Since a number of the cellulolytic strains could degrade but not utilize hemicellulose from the intact bromegrass, it seemed desirable to determine whether the xylan-digesting strains which could not degrade the hemicellulose could utilize the material solubilized by the cellulolytic species. *R. flavefaciens* B34b was used as the degrading nonutilizer in combination with either *B. ruminicola* H8a, *B. fibrisolvens* H10b, *L. multiparus* D15d, or all three organisms. These fermentations were run under the same conditions as described previously, by using 0.2 ml of a 0.2-optical density suspension as inoculum for each organism required (Table 1). In all cases, degradation was increased above that obtained with *R. flavefaciens* B34b alone. The most striking effect, however, was noted in the utilization of total pentose by the combination with *B. ruminicola* H8a, in which utilization increased from essentially zero for each organism alone to approximately 80 and 70%, respectively, for maturity stages I and II. A marked increase in utilization was also observed when B34b and *B. fibrisolvens* H10b were combined. For both maturity stages, the increase was approximately 30%. Combination of B34b with *L. multiparus* D15d was unsuccessful with regard to utilizing the degraded hemicellulose, which would be in agreement with the inability of D15d to use pentoses or xylan as energy sources (10). When all three organisms, H8a, H10b and D15d, were combined with B34b, degradation and utilization were essentially the same as obtained with the highest combination of two strains, i.e., B34b and H8a.

In every case where applicable, degradation and utilization were reduced as the plant matured. In general, this reduction was in the magnitude of 15 to 20%, and the overall relationship of one species or strain to another was unchanged.

A similar series of experiments was set up with three maturity stages of alfalfa, and these results are presented in Table 2. All of the cellulolytic

### Table 2. Degradation, utilization, or both, of hemicellulose from alfalfa by pure cultures of rumen bacteria

| Strain | Forages<sup>a</sup> | Alfalfa I | Alfalfa II | Alfalfa III |
|--------|---------------------|-----------|------------|-------------|
|        | Degradation | Utilization | Degradation | Utilization | Degradation | Utilization |
| A3c    | 60.3 ± 0.5<sup>c</sup> | 5.1 ± 1.5 | 46.0 ± 0.7 | 2.4 ± 0.9 | 29.7 ± 0.6 | 0          |
| S-85   | 62.1 ± 0.9 | 0 | 46.7 ± 0.5 | 0 | 28.7 ± 0.8 | 0          |
| 7      | 50.1 ± 0.8 | 26.9 ± 0.5 | 37.9 ± 0.5 | 20.3 ± 1.0 | 31.6 ± 2.8 | 7.4 ± 3.1 |
| B1a    | 44.6 ± 0.8 | 10.1 ± 1.0 | 31.5 ± 1.1 | 6.5 ± 1.5 | 26.3 ± 1.2 | 0          |
| B34b   | 56.3 ± 1.3 | 2.1 ± 2.1 | 38.9 ± 2.0 | 4.3 ± 2.6 | 26.8 ± 0.7 | 0          |
| H10b   | 35.4 ± 0.8 | 34.1 ± 0.7 | 23.3 ± 1.5 | 23.7 ± 1.6 | 27.4 ± 1.2 | 27.0 ± 1.1 |
| H17c   | 28.1 ± 1.0 | 11.4 ± 0.7 | 17.6 ± 0.8 | 5.9 ± 1.4 | 16.3 ± 2.0 | 12.3 ± 2.7 |
| H8a    | 33.6 ± 3.1 | 33.9 ± 3.7 | 27.1 ± 4.0 | 26.3 ± 2.0 | 23.6 ± 1.9 | 20.6 ± 1.1 |
| D31d   | 43.4 ± 2.8 | 18.7 ± 2.6 | 38.7 ± 1.0 | 17.8 ± 0.9 | 35.6 ± 1.1 | 7.0 ± 1.4 |
| D15d   | 49.5 ± 1.0 | 23.2 ± 1.5 | 42.4 ± 1.0 | 21.5 ± 0.5 | 42.1 ± 1.0 | 14.4 ± 1.0 |
| B34b + H8a | 59.6 ± 1.1 | 54.8 ± 1.0 | 40.7 ± 2.0 | 38.2 ± 2.1 | 36.5 ± 0.9 | 33.9 ± 1.2 |
| B34b + H10b | 61.9 ± 1.1 | 43.2 ± 1.1 | 45.3 ± 0.6 | 31.0 ± 1.0 | 33.1 ± 1.5 | 19.6 ± 1.3 |
| B34b + D15d | 61.8 ± 1.4 | 14.9 ± 1.5 | 46.5 ± 0.5 | 13.3 ± 0.3 | 35.9 ± 1.6 | 0          |
| B34b + D15d + H8a + H10b | 61.8 ± 0.6 | 58.4 ± 0.7 | 43.6 ± 2.0 | 41.8 ± 1.5 | 36.4 ± 1.7 | 34.8 ± 1.6 |

<sup>a</sup> Agronomic description: I = prebloom; II = early bloom; III = late bloom. Values expressed as per cent.

<sup>b</sup> Cellulose digesting strains: *Bacteroides succinogenes* A3c, S-85; *Ruminococcus albus* 7; *R. flavefaciens* B1a, B34b; and *Butyribacter fibrisolvens* H10b, H17c. Noncellulose digesting strains: *B. ruminicola* H8a, D31d, and *Lachnospira multiparas* D15d.

<sup>c</sup> Mean and standard error of the mean.
species were able to degrade the hemicellulose from intact alfalfa; however, the extent was generally lower than that observed with bromegrass. Marked reduction was obtained in the extent of utilization for strains 7 and Bla, especially in the more mature plant. Both strains of *B. succinogenes* degraded similar amounts of alfalfa hemicellulose, whereas a considerable difference was noted with bromegrass.

In contrast to the bromegrass data, all of the hemicellulolytic and pectinolytic species degraded and utilized the alfalfa pentosans. The results with *L. multiparus* D15d were particularly interesting since this strain cannot use pentoses, xylan, or degraded bromegrass hemicellulose as energy sources. An explanation of the degradation and utilization of alfalfa hemicellulose by strain D15d is not immediately obvious. Further studies with a second strain of *L. multiparus* have confirmed this difference in availability of grass and alfalfa hemicellulose.

Marked strain differences were observed between *B. ruminicola* H8a and D31d and *B. fibrisolvens* H10b and H17c with the alfalfa substrate. Both H8a and H10b utilized almost all of the hemicellulose they could degrade. On the other hand, H17c degraded considerably less hemicellulose and could utilize only a portion of the degraded material. Strain D31d degraded more hemicellulose than H8a but could not utilize even 50% of that degraded.

Results of the synergism studies with the alfalfa substrates were quite similar to those obtained with bromegrass although the extent of the increases was somewhat less. In contrast, utilization of alfalfa III by the combination of B34b and H10b, and all alfalfa stages with B34b and D15d, was considerably lower than utilization by H10b and D15d alone.

Table 3 presents the results on degradation or utilization, or both, of intact fescue grass hemicellulose and the hemicellulose after isolation from the forage. In general, the overall pattern of degradation and utilization is quite similar to that observed from bromegrass. Although these values correspond rather closely to those observed with the bloom stage of the bromegrass, the maturity stage of the fescue is unknown. In agreement with the degradation values observed on the bromegrass, strain H8a was totally unable to degrade any of the intact fescue grass hemicellulose (Table 3). However, this particular strain could almost completely degrade and utilize this hemicellulose when it had been separated from the intact plant. In all cases except strain D15d, the isolated hemicellulose was degraded to a considerable extent. These data are in close agreement with those reported by Dehority (6, 8) for several of these same strains on the isolated fescue grass hemicellulose substrate.

**DISCUSSION**

Dehority and Scott (12) estimated total pentose loss on the same forages with 7 of the 10 strains used in this study. The utilization values reported herein are in close agreement with their data, although a somewhat more complicated analytical procedure was used. Kock and Kistner (17) fermented ethanol-benzene-extracted intact teff hay with several strains of *R. albus*, *R. flavefaciens*, and *Butyrivibrio* species. Although not directly comparable because of differing analytical methods, the present results compare favorably

**TABLE 3. A comparison of hemicellulose degradation, utilization, or both, by pure cultures of rumen bacteria between intact fescue grass and isolated fescue grass hemicellulose**

| Strain* | Fescue grass | Isolated fescue hemicellulose |
|---------|--------------|--------------------------------|
|         | Degradation  | Utilization                    | Degradation | Utilization |
|         | %            | %                              | %           | %           |
| B34b    | 66.6 ± 1.1b  | 3.0 ± 1.7                      | 88.5 ± 0.9  | 0           |
| H10b    | 44.8 ± 1.8   | 38.0 ± 1.2                     | 87.5 ± 1.4  | 83.8 ± 1.3  |
| H8a     | 2.7 ± 0.8    | 2.0 ± 0.8                      | 82.0 ± 1.7  | 80.4 ± 1.7  |
| D15d    | 4.0 ± 1.1    | 1.3 ± 1.3                      | 1.7 ± 0.9   | 1.7 ± 1.1   |
| B34b + H8a | 69.0 ± 1.4  | 67.7 ± 1.4                     | 93.9 ± 1.4  | 87.0 ± 0.9  |
| B34b + H10b | 67.3 ± 0.9  | 64.8 ± 1.1                     | 91.3 ± 1.7  | 87.8 ± 2.0  |
| B34b + D15d | 67.9 ± 0.8  | 3.9 ± 0.5                      | 87.0 ± 1.1  | 3.8 ± 2.2   |
| B34b + D15d + H8a + H10b | 67.6 ± 0.7 | 65.9 ± 0.6                     | 87.4 ± 0.8  | 85.7 ± 1.1  |

* Cellulose digesting strains: *Ruminococcus flavefaciens* B34b and *Butyrivibrio fibrisolvens* H10b.
Noncellulose digesting strains: *Bacteroides ruminicola brevis* H8a and *Lachnospira multiparus* D15d.

* Mean and standard error of the mean. Values expressed as per cent.
with their 25 to 67% range of hemicellulose solubilization.

The complete inability of B. ruminicola H8a to degrade and subsequently utilize the brome hemicellulose was quite surprising, since this organism, a xylan isolate, was capable of degrading and utilizing hemicellulose isolated from several grasses (8). This observation led to a study of possible synergistic effects of combining a degrading nonutilizer, R. flavaefaciens B34b, with the hemicellulolytic strains H8a or H10b, the pectinolytic strain D15d, or all three together. The most striking results were with the combination of strains H8a and B34b, in which utilization increased from zero for each strain alone to 80% for the organisms together. Except in the case of D15d, some increase in degradation and a marked increase in utilization were observed for all combinations. These data suggest that the hemicellulolytic organisms were probably fermenting the oligosaccharides and other products solubilized by strain B34b. Synergism was also studied with the alfalfa substrates; however, the magnitude of the increases observed was lower. Undoubtedly, this was because those strains that could not degrade brome hemicellulose both degraded and utilized alfalfa hemicellulose to a reasonable extent.

Dehority and Scott (12) previously observed a small but significant increase in the extent of cellulose digestion when they combined strain H8a, a noncellulolytic, with several cellulolytic strains. Combined with the present results, these data suggest the possibility that a physical masking effect can occur in the intact plant between the cellulose and hemicellulose components. The variation observed between bromegrass and alfalfa, particularly with strains H8a, D15d, and D31d, would further indicate that there are definite differences in structure (or orientation) of hemicelluloses between intact legumes and grasses.

The ability of L. murinus D15d to degrade and utilize alfalfa hemicellulose was quite surprising, since it was unable to ferment xylan, brome hemicellulose, or isolated fescue grass hemicellulose. The organism may be producing some type of soluble oligosaccharides which are permeable to its cell membrane. No explanation is readily available for the marked decrease in utilization when strain D15d was combined with strain B34b. The degradative end products produced by B34b would appear to differ in some way from those produced by strain D15d. Consequently, this competitive degradation would result in less useful substrate for D15d.

For all strains and combinations of strains, hemicellulose degradation or utilization, or both, in the fescue grass appeared quite similar to that previously obtained with brome grass. Both hemicellulolytic organisms, H8a or H10b, were capable of increased degradation and utilization when the hemicellulose had been previously separated from other plant constituents, either chemically prior to fermentation in the case of fescue grass or by a nonutilizing cellulolytic strain, B34b, with both fescue and brome grass. The complete inability of strain H8a to degrade any of the fescue grass hemicellulose substantiates the observation that this hemicellulolytic strain can utilize grass hemicellulose only after it has been removed from the plant in some way.

Where applicable, the hemicellulose degradation patterns for each organism across the two types of forage correspond rather closely to the data obtained by Dehority and Scott (12) for cellulose digestion from these same forages. This point, the high degradation values observed for the cellulolytic isolates, and the previously reported evidence that the enzymes involved are constitutive would possibly tend to support the suggestion that hemicellulose degradation by the nonutilizing cellulose digestors is a nonspecific function closely associated with cellulase activity (9). In this case, the cellulolytic organisms could quite easily be contributing to the over all rumen fermentation of forage hemicellulose by supplying the utilizers with more soluble substrate than they alone could degrade.

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