Fermentation of Isolated Pectin and Pectin from Intact Forages by Pure Cultures of Rumen Bacteria

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Received for publication 28 September 1971

Studies on the rate and extent of galacturonic acid and isolated pectin digestion were carried out with nine strains of rumen bacteria (Butyri vibrio fibrisolvens H10b and D16f, Bacteroides ruminicola 23 and D31d, Lachnospira multipartus D15d, Peptostreptococcus sp. D43e, B. succinogenes A3c, Ruminococcus flavefaciens B34b, and R. albus 7). Only three strains, 23, D16f, and D31d, utilized galacturonic acid as a sole energy source, whereas all strains except A3c and H10b degraded (solubilized) and utilized purified pectin. Nutrient composition of the basal medium and separate sterilization of the substrate affected the rate and extent of fermentation for both substrates. Pectin degradation and utilization were measured with two maturity stages each of intact bromegrass and alfalfa. For bromegrass I, all strains tested (B34b, 23, D16f, D31d, D15d, and D43e) degraded a considerable amount of pectin and, with the exception of B34b, utilized most of what was degraded. Similar, but lower, results were obtained with bromegrass II, except for the two strains of B. ruminicola, 23 and D31d, which were unable to degrade and utilize pectin from this forage. All strains were able to degrade and utilize pectin from both maturity stages of alfalfa; however, values were considerably lower for strains 23 and D31d. Synergism studies, in which a limited utilizing strain, B34b, was combined with the limited degrading strain, D31d, resulted in a slight increase in degradation and a very marked increase in utilization of the pectin in all four forages. Similar results were obtained on both alfalfa substrates with a combination of strains B34b and D16f; however, no increases were observed with this combination on bromegrass.

Previous studies in this laboratory have investigated the digestion of cellulose and hemicellulose from intact forages by pure cultures of rumen bacteria (9, 17). The only other carbohydrate occurring in appreciable quantities in forage is pectin, which can constitute from 10 to 20% of the total carbohydrate complex in grasses and alfalfa, respectively (22, 25). Thus, the present study was undertaken to investigate the ability of several species of rumen bacteria to ferment both isolated and naturally occurring pectins.

In vivo trials with sheep have indicated that 75 to 90% of forage pectin is digested in the animal (23). The ability of both rumen protozoa and mixed cultures of rumen bacteria to ferment isolated pectin has been reported (1, 18, 27), and an earlier study from this laboratory revealed that a considerable portion of the pectin present in intact immature alfalfa is digested in vitro by mixed cultures (16). Both the rate and extent of pectin digestion were decreased with advancing maturity of the alfalfa.

Dehory (15) used a selective medium, containing purified pectin as the only added carbohydrate source, to isolate bacteria from the rumen contents of a steer fed alfalfa hay. Ten strains were selected for characterization, and subsequently identified as two strains of Lachnospira multipartus, four strains of Butyri vibrio fibrisolvens, three strains of Bacteroides ruminicola, and one strain of Peptostreptococcus...
sp. Bryant and co-workers have reported that these same species, except Peptostreptococcus, plus Succinivibrio dextrinosolvens and Bacteroides succinigenes, are all capable of fermenting pectin (3, 5–7). Their cultures were isolated on a nonselective glucose-cellobiose medium and subsequently tested with the pectin medium. The growth response to pectin by eight strains of B. ruminicola, nine strains of B. fibrisolvens, and two strains of L. multicus were measured in a medium containing pectin as the only added carbohydrate (15). Based on these results, two strains each of B. ruminicola and B. fibrisolvens, with widely differing growth responses, were chosen for the present study. One strain of L. multicus and the Peptostreptococcus sp. were also included, thus making a total of six pectin-fermenting strains. Several pure cultures of cellulolytic rumen bacteria have been shown to degrade isolated and intact forage 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 (9, 11). This ability appeared to be the result of a nonspecific, constitutive enzyme or enzymes (14), and, on this basis, one strain each of B. succinigenes, Ruminococcus flavefaciens, and R. albus were included in the present study to evaluate a possibly similar action on pectin.

**MATERIALS AND METHODS**

Nine pure cultures of rumen bacteria were used in this study. They were strains D31d and 23 of B. ruminicola, strain A3c of B. succinigenes, strain B34b of R. flavefaciens, strains D16f and H10b of B. fibrisolvens, strain D15d of L. multicus, strain 7 of R. albus, and strain D43e, a Peptostreptococcus species. The characteristics of these strains have been described (7, 8, 10, 12, 15). Strains A3c, B34b, and 7, all cellulolytics, and strain 23, a hemicellulolytic, were isolated on a nonselective glucose-cellobiose medium; strain H10b, a hemicellulolytic, was isolated on a xylan medium; and strains D15d, D31d, D16f, and D43e, pectinolytics, were isolated on a pectin medium. All stock cultures were carried in the medium in which they were originally isolated.

Galacturonic acid (Exchange Lemon Products Co.) and purified pectin (Pectin NF, Calbiochem; Pectin NF, Sunkist Growers) were used as substrates. Intact forage substrates consisted of boot and bloom stages of bromegrass (Bromus inermis Lincoln) and prebloom and late bloom stages of alfalfa (Medicago sativa Vernal), which were harvested from pure stands, artificially dried, chopped, ground in a large Wiley mill, and finally ground through a 40-mesh screen of a laboratory Wiley mill. All substrates were added at a level of 0.5%.

The anaerobic culture techniques were similar to those described by Hungate (19), except for the modifications described by Dehority (15) in which the medium was autoclaved in individual culture tubes. In general, three different basal media were used. The first was the complete medium of Scott and Dehority (24) plus 0.0002% hemin, with the substrate added prior to sterilization. The second was a 40% clarified rumen fluid (CRF) medium (17), with the substrate added before sterilization. The third medium was the same as the first, except that the substrate was sterilized separately in water and then added aseptically to each tube. This latter medium will be designated as “combined” in subsequent sections. Recoveries for galacturonic acid and purified pectin were increased from 66 and 80% to 75 and 92%, respectively, when the substrate was sterilized separately.

For those studies with the intact forage substrates, a 40% CRF medium was used (9, 17). In this case, the 0.5% forage substrate was added to the medium prior to sterilization.

Optical density (OD) measurements were made in culture tubes (16 by 150 mm) at 600 nm with a Bausch & Lomb Spectronic-20 spectrophotometer. Growth was estimated turbidimetrically at 0 and 12 hr and every 2 hr until maximum OD was reached. All fermentations were made in duplicate and replicated.

Inoculum cultures were grown for approximately 16 hr in an optically clear broth identical to the fermentation media in all constituents except substrate. Strains A3c, B34b, and 7 were grown on cellulbiose; strains H10b and 23 were grown on glucose, and strains D15d, D31d, D16f, and D43e were grown on pectin. These inoculum cultures were diluted with anaerobic dilution solution until an OD of 0.2 was obtained. For all fermentations, 0.2 ml of the 0.2 OD inoculum was added to 5 ml of fermentation media. The fermentations were incubated for 168 hr at 39 C. Inoculum level and fermentation time were chosen to insure maximum rate and completion of the fermentation, as determined by Dehority and Scott (17).

Galacturonic acid concentration was determined colorimetrically by the orcinol reaction (2). Readings were taken at 670 nm, which was determined by an absorption curve as the wave length giving maximum absorption of known galacturonic acid.

Isolation of pectin from plant materials generally involves extraction and precipitation from an acidified ethanol solution (21, 26). Fractionation of fermentation mixtures with 80% acidified ethanol has been used for measuring degradation or solubilization of isolated and intact forage hemicellulose (9, 11); however, whether these were optimal conditions for estimating pectin solubilization had to be determined.

The heterogeneity of the pectin molecule itself, plus the variation associated with source, make quantitative estimations quite difficult. However, since most pectins contain 75% or more galacturonic acid (21, 26), it was believed that colorimetric analysis of this component would provide a reasonable estimate of pectin content. The only other source of uronic acids in the plant is from the hemicelluloses, and their concentration is low enough to be consid-
Based on preliminary studies, the following procedure was adopted for the analysis of isolated pectin. The entire contents of the fermentation tube (5 ml) were washed into a polypropylene centrifuge tube with 45 ml of a 5% acetic acid-95% ethanol solution. The contents were mixed and allowed to stand at room temperature for 60 min. The mixture was centrifuged for 25 min at 30,000 × g; the supernatant fluid was decanted into a 250-ml volumetric flask and brought to volume with distilled water. The precipitate was hydrolyzed by adding 10 ml of 0.5 N H₂SO₄ to each tube, covering the tube with a metal cap, and autoclaving for 30 min at 15 psi. After cooling, the hydrolysate was transferred to a 500-ml volumetric flask and brought to volume with distilled water. The supernatant fluid and hydrolysate were analyzed for galacturonic acid as described earlier, and pectin concentration was estimated from standard curves.

With a slight modification to remove hexose and pentose interference, the forage fermentations were analyzed by the same procedure. The modification involved treatment of the samples with an anion-exchange resin, Amberite IRA-400 in the acetate form, which removes galacturonic acid (17). Concentration of galacturonic acid was calculated as the difference in absorbance between resin-treated and untreated samples.

The overall procedure for forage media analysis included the precipitation with 90% acidified ethanol, centrifugation, and hydrolysis steps, as used with purified pectin, except that the supernatant fluid and hydrolysate were diluted only to 50 ml. Twenty milliliters of the supernatant fluid was placed in a 50-ml volumetric flask and brought to volume with distilled water; a second 20-ml sample was treated with 6 ml of resin. The sample plus resin was allowed to stand at room temperature with occasional swirling for 1 hr, after which the supernatant fluid was transferred to a second tube containing a similar quantity of fresh resin and treated for the same duration of time. The resin was removed by filtration and washed with distilled water, and the filtrate plus washings were diluted to 50 ml. Ten-milliliter samples of the hydrolysate were handled in a similar manner. The resin-treated and untreated samples were then analyzed by the orcinol procedure.

In this study, degradation is defined as the solubilization of 90% ethanol-insoluble pectin, whereas utilization is defined as a loss in total galacturonic acid. Degradation and utilization values were based on two replicate fermentations run in duplicate: a total of four tubes. When the coefficient of variation of the four values was larger than 15%, the fermentations were repeated. If the coefficient of variation remained above 15% after repeating the fermentations, a mean digestibility value was calculated from all eight fermentations, duplicates in quadruplicate. The coefficient of variation value of 15% was chosen as the upper limit to coincide with studies reported by Dehority and Scott (17) and Coen and Dehority (9) on the digestibility of forage cellulose and hemicellulose.

RESULTS

Only three strains of the original nine (B. ruminicola 23 and D31d and B. fibrisolvens D16f) used galacturonic acid as an energy source. Growth response and per cent utilization by these three strains is shown in Table 1. For both strains of B. ruminicola, 23 and D31d, the maximum OD was slightly higher in the combined than in the complete medium; however, the time to reach maximum OD was approximately the same. These results are reflected by the utilization data. Growth and utilization of galacturonic acid were both decreased in the CRF medium as compared to the complete medium. Growth curves revealed a much shorter lag phase with the CRF medium; however, rates of growth appeared to be similar on all three media. These data suggest that the galacturonic acid substrate became partially complexed or unavailable when autoclaved in the medium and that either this effect was more pronounced in the CRF medium or the CRF medium was limiting in a specific nutrient required by these strains.

On the other hand, growth of B. fibrisolvens D16f appeared to be much slower in the complete and combined media as compared to the CRF medium (Table 1). Extent of utilization was quite high in all three media and not closely associated with maximal OD. These data suggested the possibility of a limiting nutrient in the complete and combined media which slowed the rate of growth, and further investigations were made on this point. Suspensions of D16f were grown in CRF and complete media containing either galacturonic acid or cellobiose as the energy source. With the cellobiose substrate (Fig. 1), growing the inoculum in CRF broth reduced the lag phase approximately 12 hr, but the rate of growth did not appear to be influenced. Growth in the CRF medium was of the same magnitude for either inoculum. In the complete cellobiose medium, growth was reduced for the CRF grown inoculum and markedly reduced for the complete inoculum.

When galacturonic acid was the substrate (Fig. 2), inoculum from CRF broth again had a shorter lag phase as compared to the complete medium inoculum. However, in this case, lag phase, rate, and maximum growth were all markedly reduced in the complete medium. These data suggest that the complete medium is nutritionally limiting for strain D16f, and that nutrient carry-over in the inoculum could influence the various criteria. The large discrepancy observed between growth and utilization for strain D16f in the complete medium (Table 1) could have resulted from a slower
TABLE 1. Per cent utilization of galacturonic acid (GA) and growth response of several strains of rumen bacteria

| Mediuma | Growth response of B. ruminicola D23 | Growth response of B. ruminicola D31d | Growth response of B. fibrisolvens D16f | Per cent GA utilized by B. ruminicola D23 | Per cent GA utilized by B. ruminicola D31d | Per cent GA utilized by B. fibrisolvens D16f |
|---------|----------------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Complete | 1.18 (42)                         | 1.17 (51)                         | 0.16 (88)                         | 91.9 ± 1.1                       | 89.5 ± 1.0                       | 90.4 ± 2.5                       |
| CRF      | 0.53 (19)                         | 0.94 (31)                         | 0.79 (18)                         | 74.0 ± 1.8                       | 83.6 ± 0.6                       | 83.0 ± 0.3                       |
| Combined | 1.31 (42)                         | 1.34 (44)                         | 0.49 (119)                        | 96.2 ± 0.2                       | 96.5 ± 0.0                       | 94.0 ± 0.5                       |

a Complete (24): 0.5% GA substrate added; contained 0.33% GA by analysis. CRF: 40% clarified rumen fluid medium (17), 0.5% GA substrate added; contained 0.32% GA by analysis. Combined: 0.5% GA substrate added to the complete medium after sterilization; contained 0.38% GA by analysis.

Values expressed as maximum increase in optical density at 600 nm. Values in parentheses indicate hours of incubation required to reach maximum optical density.

Values express mean and standard error of the mean.

Fig. 1. Effect of inoculum on the growth response of Butyrivibrio fibrisolvens D16f in cellobiose medium.

Strains D16f, D15d, and D43e all exhibited an increased utilization in the CRF medium as compared to the complete medium, whereas 23 and D31d values were decreased. In essence, the pectin growth and utilization results for strains 23, D31d, and D16f are similar to those obtained with galacturonic acid. It appears that most of the differences observed for all strains could be the result of the pectin becoming partially unavailable when autoclaved in the medium, and nutritional limitations in the medium itself, or a combination of these factors.

Pectin degradation (solubilization of pectin in 90% acidified ethanol) ranged from 72.5 to 98.5% in all three media for the five pectin util-
TABLE 2. Growth response of several strains of rumen bacteria in purified pectin medium

| Organism                        | Maximum increase in optical density (600 nm) in medium* |
|---------------------------------|--------------------------------------------------------|
|                                 | Complete | CRF     | Combined |
| *Complete medium (24): 0.5% pectin substrate added, contained 0.41% pectin by analysis. CRF: 40% clarified rumen fluid medium (17), 0.5% pectin substrate added; contained 0.41% pectin by analysis. Combined: 0.5% pectin substrate added to the complete medium after sterilization; contained 0.47% pectin by analysis. |
|                                 |           |         |          |
| Bacteroides ruminicola 23       | 0.84 (34)*| 0.41 (12)| 0.85 (19)|
| B. ruminicola D31d D31d        | 0.80 (64) | 0.46 (19)| 1.25 (22)|
| Butyriovibrio fibrisolvens D16f| 0.45 (24) | 1.15 (19)| 0.95 (21)|
| Lachnospira multiparus D15d     | 0.49 (53) | 0.44 (16)| 0.88 (14)|
| Peptostreptococcus sp. D43e     | 0.06 (56) | 0.61 (82)| 1.15 (22)|

* Hours of incubation required to reach maximum optical density.

TABLE 3. Degradation and utilization of pectin by pure cultures of rumen bacteria

| Organism                        | Use of pectin in medium* (%) |
|---------------------------------|------------------------------|
|                                 | Complete | CRF     | Combined |
| Degradation Utilization         | Degradation Utilization      | Degradation Utilization |
| Ruminococcus flavefaciens B34b  | 33.4 ± 0.7a | 12.2 ± 1.8 | 30.1 ± 2.1 | 4.0 ± 2.3 | 28.4 ± 1.8c | 19.6 ± 2.5c |
| Bacteroides succinogenes A3c    | 0.5 ± 0.5 | 0.0 ± 0.0 | 14.6 ± 5.0c | 11.2 ± 5.0c | 4.4 ± 1.1 | 3.5 ± 0.8 |
| R. albus 7                      | 3.4 ± 2.0 | 0.5 ± 0.5 | 44.0 ± 1.4 | 22.6 ± 0.9 | 28.5 ± 3.0c | 19.1 ± 1.8c |
| B. ruminicola 23                | 81.3 ± 1.3 | 73.2 ± 1.8 | 72.5 ± 3.0c | 46.7 ± 6.1c | 84.9 ± 0.0 | 80.3 ± 0.0 |
| D31d                            | 86.0 ± 2.7 | 74.7 ± 1.0 | 74.9 ± 2.0c | 47.2 ± 6.8c | 85.3 ± 0.4 | 82.9 ± 0.8 |
| Butyriovibrio fibrisolvens H10b | 0.3 ± 0.3 | 0.3 ± 0.3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.9 ± 1.5 | 2.9 ± 1.5 |
| D16f                            | 81.1 ± 2.8c | 36.5 ± 6.3c | 91.8 ± 0.8 | 82.0 ± 0.0 | 98.5 ± 0.0 | 95.6 ± 0.8 |
| Lachnospira multiparus D15d     | 92.4 ± 1.0 | 36.9 ± 1.6 | 89.4 ± 0.7 | 72.1 ± 1.0 | 98.4 ± 0.0 | 87.8 ± 0.6 |
| Peptostreptococcus sp. D43e     | 84.7 ± 2.5 | 41.8 ± 2.6 | 87.8 ± 0.7 | 51.0 ± 1.0 | 96.8 ± 0.0 | 89.6 ± 0.0 |

* Complete medium (24): 0.5% pectin substrate added; contained 0.41% pectin by analysis. CRF: 40% clarified rumen fluid medium (17), 0.5% pectin substrate added; contained 0.41% pectin by analysis. Combined: 0.5% pectin substrate added to the complete medium after sterilization; contained 0.47% pectin by analysis.

* Mean and standard error of mean.

* Coefficients of variation greater than 15%; means calculated on all eight fermentation tubes (duplicates in quadruplicate).

Lyzing strains. Although no measurable growth response in either galacturonic acid or purified pectin medium was indicated for any of the three predominant cellulolytic species found in the rumen, *R. flavefaciens* B34b consistently degraded about 30% of the purified pectin in all three media and utilized between 4.0 and 19.6%. *B. succinogenes* A3c was essentially unable to degrade or utilize any pectin, whereas *R. albus* 7 degraded 44.0 and 28.5% in the CRF and combined media, respectively, and utilized 22.6 and 19.1%.

From the results on the degradation and utilization of purified pectin, six cultures were chosen for an investigation of pectin degradation and utilization with intact forages. These were *R. flavefaciens* B34b, a pectin degrader but limited utilizer; *B. ruminicola* 23 and D31d, *B. fibrisolvens* D16f, *L. multiparus* D15d, and *Peptostreptococcus* sp. D43e, all of which could extensively utilize purified pectin as an energy source.

Table 4 lists the data on the degradation and utilization of pectin from two maturity stages of intact alfalfa. Degradation and utilization of pectin from alfalfa decreased only slightly with increased plant maturity. Degradation of pectin by *R. flavefaciens* B34b and *B. fibrisolvens* D16f was diminished by 10 to 15% as the alfalfa plant matured, whereas de-
creases of less than 10% were noted for all other strains.

Each strain capable of utilizing purified pectin as a substrate also degraded and utilized intact alfalfa forage pectin. However, in comparison to the other species, both strains of *B. ruminicola* were extremely limited in their ability to degrade and utilize intact alfalfa pectin. All strains, except *R. flavefaciens* B34b, were able to utilize almost all of the pectin which they could degrade or solubilize, thus suggesting that degradation is limiting the utilization of pectin from intact alfalfa.

It seemed desirable to determine whether those strains which could utilize isolated pectin, but were limited in their ability to degrade and utilize plant pectin, could utilize the material solubilized by the cellulyotic strain B34b. Thus, *R. flavefaciens* B34b was combined in the same fermentation with either *B. ruminicola* D31d or *B. fibrisolvens* D16f. For these studies, 0.2 ml of a 0.2 OD suspension of each organism was used as inoculum. These results are presented in the bottom two lines of Table 4. In both cases, degradation was increased above the extent obtained with either organism alone. The most striking effect, however, was on the utilization of plant pectin by both combinations. Utilization increased from approximately 30 and 25% for either B34b or D31d alone to 82 and 74% for maturity stages I and III, respectively. The combination of B34b and D16f also increased pectin utilization; however, the magnitude of the increase was considerably less.

The results from a similar series of forage fermentations using two maturity stages of bromegrass are presented in Table 5. With the possible exception of *B. fibrisolvens* D16f, both degradation and utilization of pectin from intact bromegrass were found to decrease markedly with increased plant maturity, which is in contrast to the earlier observations with alfalfa. This maturity effect was especially severe with both strains of *B. ruminicola*. When considering the first maturity stage of bromegrass, pectin degradation and utilization values were similar to those observed with alfalfa I for *R. flavefaciens* and *Peptostreptococcus* sp. Values for *B. ruminicola* were somewhat higher, whereas degradation and utilization values for *L. multiformis* and *B. fibrisolvens* were lower than those found with alfalfa.

Results of the synergism studies on bromegrass I indicated that degradation was not improved with either combination of organisms above that of *R. flavefaciens* B34b alone. The B34b and D31d combination resulted in an increased utilization of approximately 30%, whereas only a 5% increase in utilization was observed with the B34b and D16f combination. The combination of B34b and D31d on the bromegrass II substrate resulted in a marked synergistic effect on both degradation and utilization. In contrast, degradation and utilization with the B34b and D16f combination were reduced below that of D16f alone.

**DISCUSSION**

The fermentation of galacturonic acid by those strains isolated in a selective pectin
medium agreed with the results of Dehority (15), who reported that of the strains used in this study only D16f and D31d were able to grow with galacturonic acid as the sole energy source. These data suggest that different types of pectinolytic enzymes are present in the various species. The possible relationship between types of pectinolytic enzymes and utilization of galacturonic acid has been discussed previously (15).

The ability to degrade and utilize the isolated and naturally occurring pectins varied between the pectinolytic strains (D16f, D15d, D31d, and D43e). In only one instance was degradation below 75% on the isolated pectin substrates. However, utilization data varied between 36 and 95% depending on the organism, culture medium, and method of medium preparation. Intact forage pectin was degraded and utilized to some extent by all pectinolytic organisms, with the exception of the two B. ruminicola strains and the mature bromegrass substrate. This ability varied both between organisms and between forages; for example, B. ruminicola D31d degraded and utilized more pectin from bromegrass I than alfalfa I, yet the decreases in pectin degradation and utilization attributed to increased maturity with the alfalfa were minimal, whereas the bromegrass II values dropped to essentially zero. At the same time, Peptostreptococcus sp. D43e degraded considerably more pectin than D31d from both alfalfa I and bromegrass I.

The two strains of B. ruminicola, 23 and D31d, were somewhat similar in their ability to degrade and utilize galacturonic acid, isolated pectin, and intact forage pectin. Of particular interest was their almost complete inability to degrade and utilize pectin from the mature bromegrass. In contrast, a marked difference was observed between the two strains of B. fibrisolvens. Strain D16f was able to degrade and utilize galacturonic acid, as well as both isolated and naturally occurring pectins, whereas strain H10b was unable to degrade or utilize any of these substrates. In general, the differences noted between strains and species in the present study tend to parallel results on hemicellulose degradation and utilization (9, 11, 13). On this basis, it would be desirable to investigate a number of strains of a given species, isolated on various selective and non-selective media, before concluding whether this was a definite species characteristic.

Of the three normally predominant cellulolytic species found in the rumen, none showed a growth response in either galacturonic acid or pectin medium. However, R. flavefaciens strain B34b and R. albus strain 7 degraded (solubilized) approximately 30% of the purified pectin and results suggested a slight amount of utilization. B. succinogenes A3c was almost completely inactive in this respect. Although R. flavefaciens B34b was the only cellulolytic species tested on the intact forage substrates, it was capable of degrading as much or more of the pectin from intact forages than the pectinolytic strains. The limited utilization of pectin observed for this strain with the forage substrates might be attributed to a permeability of the cell wall to some of the specific oligosaccharides produced. It would be of considerable

| Organism | Use of pectin in forages* (%) |
|----------|------------------------------|
|          | Brome I | Brome II |
|          | Degradation | Utilization | Degradation | Utilization |
| Ruminococcus flavefaciens B34b | 71.3 ± 2.2 | 29.8 ± 2.9 | 35.5 ± 3.5 | 8.1 ± 4.5 |
| Bacteroides ruminicola | 55.0 ± 2.9 | 52.6 ± 2.6 | 5.7 ± 3.2 | 4.9 ± 1.9 |
| D31d | 43.3 ± 2.9 | 49.7 ± 2.0 | 1.0 ± 1.0 | 2.6 ± 1.6 |
| Butyrivibrio fibrisolvens D16f | 55.3 ± 3.1 | 49.7 ± 2.9 | 46.7 ± 2.8 | 45.3 ± 2.7 |
| Lachnospira multiparus D15d | 45.6 ± 3.1 | 43.2 ± 3.3 | 28.3 ± 2.3 | 23.9 ± 3.4 |
| Peptostreptococcus sp. D43e | 65.5 ± 2.4 | 51.9 ± 1.6 | 29.0 ± 2.2 | 21.2 ± 2.6 |
| R. flavefaciens B34b + | 72.6 ± 3.2 | 70.1 ± 2.9 | 52.5 ± 4.0 | 53.0 ± 4.6 |
| B. ruminicola D31d | 68.8 ± 2.7 | 54.3 ± 4.2 | 43.7 ± 2.9 | 34.8 ± 3.7 |

* Agronomic description: I = boot stage, II = bloom stage.
* Mean and standard error of mean.
* Coefficient of variation greater than 15%; mean calculated on all eight fermentation tubes (duplicates in quadruplicate).
interest to test this strain on isolated alfalfa pectins.

The degradation of isolated pectin by *R. flavefaciens* B34b and *R. albus* 7 plus the extensive degradation of intact forage pectin by B34b, accompanied by a very limited utilization, might suggest that this ability is similar to the activity previously observed with hemicellulose substrates. Dehority (11) and Coen and Dehority (9) reported that many of the rumen celluloxytic organisms were capable of partially degrading isolated and naturally occurring hemicelluloses from an ethyl alcohol-insoluble form to soluble products. Utilization of the hemicelluloses as an energy source was not associated with degradation, and subsequent studies with resting cell and cell-free systems suggested that the degradation of the hemicellulose occurred as a result of the action of a nonspecific constitutive enzyme (14).

Definite synergism was observed by combining a pectin-degrading, but limited utilizing strain (B34b), with the pectinolytic strains D16f and D31d. The most striking synergistic results were observed with the combination of strains B34b and D31d, where utilization of alfalfa I increased from 30% for either organism alone to 82% for the organisms together, and on bromegrass II, where utilization increased from less than 10% for each organism alone to 53% for the combination. Except for the combination of strains B34b and D16f on the bromegrass II substrate, increases in degradation and utilization were observed for each of the other combinations. In general, synergism in the utilization of forage pectin paralleled earlier results on synergism with respect to intact forage hemicellulose utilization (9).

The above results strongly suggest that, although the different pectinolytic species vary markedly in the amount of pectin they can degrade or solubilize from a specific intact forage, if the pectin can be freed from the forage either chemically or by another organism, it is then almost completely available for utilization. On the basis of the extensive degradation and marked synergism observed, it appears that the celluloxytic bacteria could contribute to the overall rumen fermentation of forage pectin by supplying the pectin-utilizing bacteria with more soluble substrate than they could degrade alone.

No explanation is readily available for the slight decrease in degradation and utilization of bromegrass pectin obtained with the combination of strains B34b and D16f. A somewhat similar pattern was observed in the synergism studies on hemicellulose utilization from alfalfa by using a combination of *R. flavefaciens* B34b and *L. multiparus* D15d (9). The degradative end products produced by B34b may differ markedly from those of D16f on this particular forage and, subsequently, cannot be utilized by D16f. Competitive degradation, possibly influenced by rate phenomena, could then result in less available substrate for strain D16f.

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