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Interaction of Ruminal Bacteria in the Production and Utilization of Maltooligosaccharides from Starch

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The degradation and utilization of starch by three amylolytic and one nonamylolytic species of ruminal bacteria were studied. Pure cultures of *Streptococcus bovis* JB1, *Butyrivibrio fibrisolvens* 49, and *Bacteroides ruminicola* D31d rapidly hydrolyzed starch and maltooligosaccharides accumulated. The major starch hydrolytic products detected in *S. bovis* cultures were glucose, maltose, maltotriose, and maltotetraose. In addition to these oligosaccharides, *B. fibrisolvens* cultures produced maltopentaose. The products of starch hydrolysis by *B. ruminicola* were even more complex, yielding glucose through maltotetraose, maltohexaose, and maltoheptaose but little maltopentaose. *Selenomonas ruminantium* HD4 grew poorly on starch, digested only a small portion of the available substrate, and generated no detectable oligosaccharides as a result of cultivation in starch containing medium. *S. ruminantium* was able to grow on a mixture of maltooligosaccharides and utilize those of lower degree (<10) of polymerization. A coculture system containing *S. ruminantium* as a dextrin-utilizing species and each of the three amylolytic bacteria was developed to test whether the products of starch hydrolysis were available for crossfeeding to another ruminal bacterium. Cocultures of *S. ruminantium* and *S. bovis* contained large numbers of *S. bovis* but relatively few *S. ruminantium* and exhibited little change in the pattern of maltooligosaccharides observed for pure cultures of *S. bovis*. In contrast, *S. ruminantium* was able to compete with *B. fibrisolvens* and *B. ruminicola* for these growth substrates. When grown with *B. fibrisolvens*, *S. ruminantium* grew to high numbers and maltooligosaccharides accumulated to a much lesser degree than in cultures of *B. fibrisolvens* alone. *S. ruminantium-B. ruminicola* cultures contained large numbers of both species, and maltooligosaccharides never accumulated in these cocultures. Maximum growth rate appeared to be the major determinant of competitive fitness in these cocultures, but the influence of other important growth characteristics (e.g., substrate affinities) could not be ruled out.

Digestion in ruminant livestock is accomplished by anaerobic microorganisms that inhabit the forestomach or rumen of these animals. Because the diet of these animals is composed largely of plant polysaccharides, this population is composed primarily of saccharolytic organisms. This population is extremely complex, and over 200 species of bacteria, fungi, and protozoa have been isolated from the rumen (6, 11, 18, 19). The ruminal population is also metabolically diverse. Not all species are able to degrade the polymeric substrates as they are present in the diet and must rely on the hydrolytic capacity of other species to generate needed energy sources. In turn, these nonhydrolytic organisms may produce compounds that are needed for growth of other species. In this way considerable nutritional interdependence among ruminal bacteria exists. Examples of this phenomenon exist for cellulolytic and noncellulolytic species during the degradation of cellulose (2, 7, 10, 17). Similar interactions among other species are likely to occur during the degradation of other polysaccharides.

Starch is the major polysaccharide present in the diets of high-producing ruminant livestock such as dairy cattle and feedlot beef cattle. Starches are generally rapidly fermented in the rumen, and a variety of ruminal bacteria have the capacity to utilize this substrate. Previous work has shown that *Streptococcus bovis*, *Butyrivibrio fibrisolvens*, and *Bacteroides ruminicola* were among the most active starch-degrading species (3). Furthermore, it was demonstrated that the amylolytic activities of these organisms hydrolyzed starch with the production of maltooligosaccharides, which is consistent with the production of alpha-amylase-like enzymes. It was hypothesized that these oligosaccharide products of starch hydrolysis are important intermediates in starch degradation in the rumen and serve as important growth substrates for other nonamylolytic species. The objectives of the current study were to determine if maltooligosaccharides accumulate during the growth of amylolytic ruminal bacteria on starch and, if so, to determine if these substrates are available to other species of ruminal bacteria in a crossfeeding food chain that might mimic what occurs in the rumen.

MATERIALS AND METHODS

Organisms and growth conditions. *S. bovis* JB1, *B. ruminicola* D31d, *B. fibrisolvens* 49, and *Selenomonas ruminantium* HD4 were obtained from our culture collection (National Center for Agricultural Utilization Research, USDA Agricultural Research Service, Peoria, Ill.). All cultures were grown anaerobically in batch cultures at 39°C in a complex, Trypticase-yeast extract-containing medium (RGM; routine growth medium [5]) with glucose, starch, or corn syrup maltooligosaccharides as the growth substrates. The medium was also supplemented with hemin where needed for growth of *B. ruminicola*. Growth was monitored spectrophotometrically (Spectronic 70; Bausch & Lomb, Inc., Rochester, N.Y.) by determining the optical densities of cultures at 660 nm. In cocultures, growth was also monitored by direct microscopic counts of the species present. Samples were diluted as needed in RGM without added energy source, an aliquot was transferred to a bacterial counting chamber (Hausser Scientific, Horsham, Pa.), and the cells were counted immediately. Cultures were also sampled at

various times for chemical analyses and stored frozen until analyzed.

Growth substrates. The starch used in these experiments was a preparation of soluble potato starch (Sigma Chemical Company, St. Louis, Mo.; catalog no. S-2630) that contained no detectable glucose, maltose, or other oligosaccharides (as determined by high-pressure liquid chromatography [HPLC] and thin-layer chromatography [TLC] methods). Corn syrup maltooligosaccharides were prepared as follows: 60 ml (approximately 85 g) of corn syrup (Corn Products Corp., Argo, Ill.) was diluted with 540 ml of distilled water, and the mixture was combined with 2 volumes of ice-cold acetone and held on ice for 30 min. The precipitate formed was collected by centrifugation (10,000 × *g*, 30 min, 4°C), redissolved in 20 ml of distilled water, boiled under nitrogen, and sterilized (121°C, 15 min). The composition of this solution was (in milligrams per milliliter, as determined by HPLC) total carbohydrate, 291.5; glucose, 11.0; maltose, 11.9; maltotriose, 13.8; maltotetraose, 15.1; maltopentaose, 21.1; maltohexaose, 15.5; and maltoheptaose, 11.7. In addition, oligosaccharides up to at least degree of polymerization 12 could be detected, but these were not quantified by the HPLC method. The overall effect of this procedure was to prepare an oligosaccharides mixture more equal in individual components than the original corn syrup, which was approximately 50% (wt/vol) glucose and maltose. This maltooligosaccharide solution was added to medium at a level of either 0.5 or 1.0 ml/20 ml of medium.

HPLC determination of oligosaccharides. The oligosaccharide concentrations in cultures were determined by using an HPLC method (3). For simplicity (although technically inaccurate), the collective term oligosaccharides includes glucose and maltose as well as true oligosaccharides. Samples from cultures were centrifuged (10,000 × *g*, 5 min) to remove cells, and the supernatant fluids were desalted with a mixed-bed ion-exchange resin (90 mg/ml; Bio-Rad AG 501-X8D). The oligosaccharides in the samples were separated by HPLC on a Regis reversible amino column (Regis Chemical Co., Morton Grove, Ill.; 250 by 4.6 mm), with 70% acetonitrile as the eluent (2 ml/min). Peaks were detected by refractive index (Waters model 410 differential refractometer) and identified by comparison to retention times of authentic standards. The level of ion-exchange resin added was sufficient to reduce the salt content of samples below levels detectable by refractive index. At this level of addition, the resin did not result in a loss of oligosaccharides as determined by HPLC and TLC methods.

TLC of oligosaccharides. Identification of maltooligosaccharides was also accomplished by using a TLC method. Samples were applied to Whatman 150A K5 Silica Gel plates and developed three successive times in a solvent of nitroethane-ethanol-water (1:3:1). Plates were air dried, and spots were visualized by spraying plates with *N*-(1-naphthyl)ethylene-diamine hydrochloride (200 mg in 97 ml of methanol with 3 ml of concentrated sulfuric acid added) and heating to 100°C for 5 to 10 min.

Other analyses. Total carbohydrate concentrations were determined on whole, uncentrifuged samples by using the anthrone reagent (4). Lactic acid concentration was measured on culture supernatant samples by using an HPLC method. Lactic acid was resolved from other medium components on a HPX-87H organic acid analysis column (Bio-Rad Corp., Richmond, Calif.) and detected by refractive index (16).

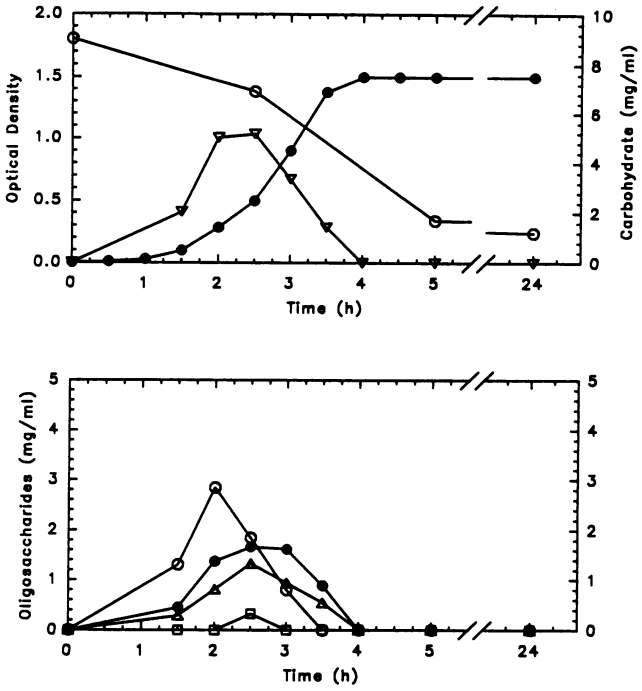

FIG. 1. Growth of *S. bovis* JB1 on 1% starch. Upper panel: ●, optical density at 660 nm; ○, total carbohydrate, and ▽, total oligosaccharides (sum of glucose through maltoheptaose). Lower panel (concentrations of individual products of starch hydrolysis): □, glucose; △, maltose; ●, maltotriose; ○, maltotetraose. Absence of a symbol at a particular time indicates that no oligosaccharide was detected (less than 0.05 mg/ml). Values are the means of duplicate cultures.

Chemicals. Oligosaccharide standards, amylose, and amylopectin were obtained from the Sigma Chemical Co.). All other chemicals were of reagent or biological grade.

RESULTS

Growth of amylolytic species on starch. The amylolytic ruminal bacteria *S. bovis*, *B. fibrisolvens*, and *B. ruminicola* were grown in RGM containing soluble potato starch, and the utilization of carbohydrate and distribution of maltooligosaccharides present (glucose through maltoheptaose) was monitored by HPLC. In all three cases the disappearance of starch was associated with an accumulation of hydrolytic products, but the patterns of intermediates produced and the extent of their ultimate utilization differed among the species. The presence of oligosaccharides of greater molecular weight but not quantifiable by the HPLC method was determined by TLC. Although sometimes present, these oligosaccharides were likely to be of minor quantitative importance on the basis of the intensity of the spots they produced upon TLC analysis.

S. bovis cultures rapidly converted the starch growth substrate into the small-molecular-weight oligomers maltose, maltotriose, and maltotetraose, which accumulated during growth (Fig. 1). A small amount of glucose was also detected. The appearance and disappearance of both maltose and maltotriose were parallel and constituted the greatest quantity of carbohydrate present late in growth, while maltotetraose concentration was the greatest early in growth. The overall utilization of carbohydrate was approx-

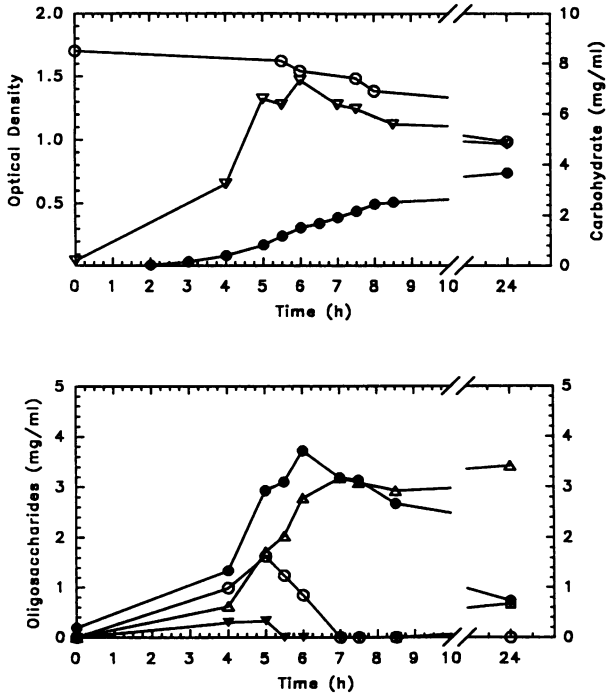

FIG. 2. Growth of *B. fibrisolvens* 49 on 1% starch. Upper panel: ●, optical density at 660 nm; ○, total carbohydrate, and ▽, total oligosaccharides (sum of glucose through maltoheptaose). Lower panel (concentrations of individual products of starch hydrolysis): □, glucose; △, maltose; ●, maltotriose; ○, maltotetraose; ▼, maltopentaose. Absence of a symbol at a particular time indicates that no oligosaccharide was detected (less than 0.05 mg/ml). Values are the means of duplicate cultures.

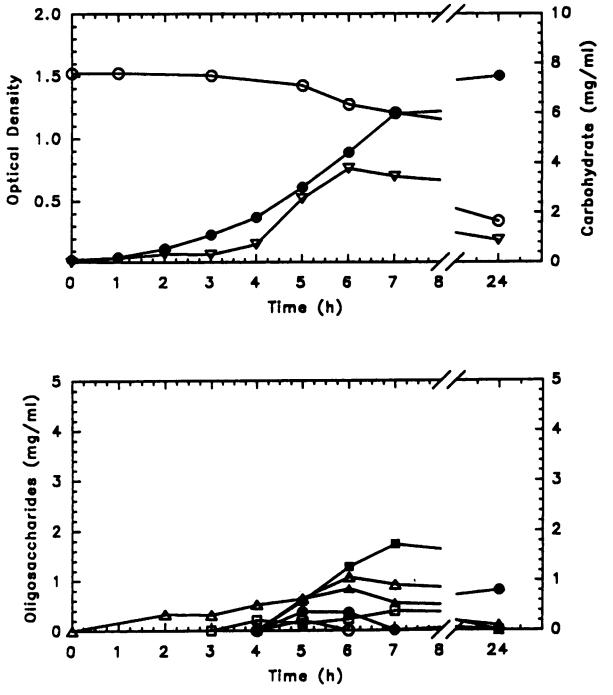

FIG. 3. Growth of *B. ruminicola* D31d on 1% starch. Upper panel: ●, optical density at 660 nm; ○, total carbohydrate; ▽, total oligosaccharides (sum of glucose through maltoheptaose). Lower panel (concentrations of individual products of starch hydrolysis): □, glucose; △, maltose; ●, maltotriose; ○, maltotetraose; ■, maltohexaose; ▲, maltoheptaose. Absence of a symbol at a particular time indicates that no oligosaccharide was detected (less than 0.05 mg/ml). Values are the means of duplicate cultures.

imately 90% complete after 24 h, and no maltooligosaccharides could be detected in culture supernatants after 4 h.

In *B. fibrisolvens* cultures (Fig. 2), maltopentaose was produced in addition to the oligosaccharides produced by *S. bovis*. The pattern of accumulation differed from that of *S. bovis* in that oligosaccharides initially accumulated in parallel but then the larger oligomers (G5 to G4) disappeared while maltose and maltotriose concentrations remained elevated. On prolonged incubation (e.g., 24 h), maltotriose levels declined and a small amount of glucose was detected. Total carbohydrate utilization was incomplete, with less than 55% of the available substrate depleted by 24 h, even though the conversion of starch to the measurable oligosaccharides (G1 through G7) was essentially complete by 6 h. The reason for the failure of *B. fibrisolvens* to use all the available carbohydrate is unclear. When provided with 1% glucose, strain 49 consumed 70% of the substrate and reduced the culture pH to 5.7 (not shown). In these starch cultures the pH was always greater than 6.0. Furthermore, incomplete utilization doesn't seem to be related to depletion of other required medium components (i.e., increased addition of ammonia, acetate, etc., do not result in increases in the extent of utilization).

The variety of oligosaccharide products detected in *B. ruminicola* cultures was the most complex of the organisms studied. In addition to the products found in the *S. bovis* and *B. fibrisolvens* experiments, *B. ruminicola* also produced significant quantities of maltohexaose and maltoheptaose (Fig. 3). Interestingly, no quantifiable maltopentaose was produced in these incubations, although small amounts were observed by the TLC detection method (data not shown). Compared with the *S. bovis* and *B. fibrisolvens* incubations, the accumulation of oligosaccharides was much less. The extent of total carbohydrate utilization by this strain was greater than 75% after 24 h.

Growth of *S. ruminantium* on starch and starch-derived oligosaccharides. The growth of *S. ruminantium* cultivated in media containing starch or glucose was compared (Fig. 4). When growth was on 0.2% starch, the extent of growth was slight and only 16% of the available carbohydrate was utilized. Increasing the starch concentration to 1% resulted in a marked increase in the growth of this strain, but utilization of available carbohydrate was still low (about 22%). When provided with glucose the substrate utilization was more complete with 100 and 84% of the substrate used by the 0.2% and 1.0% glucose cultures, respectively. In all cases, cultures provided with glucose grew more rapidly and to greater densities than cultures provided with comparable amounts of starch.

The ability of *S. ruminantium* to grow on starch hydrolytic products was examined by providing this strain with a mixture of oligosaccharides derived from corn syrup. Growth in this medium was rapid and comparable to that of glucose-grown cultures. Examination of the oligosaccharide composition of cultures showed that the utilization of glucose through maltoheptaose was extensive (Fig. 5). Maltotetraose through maltoheptaose disappeared early in growth, while glucose and maltotriose accumulated. After the larger oligomers were exhausted, the glucose disap-

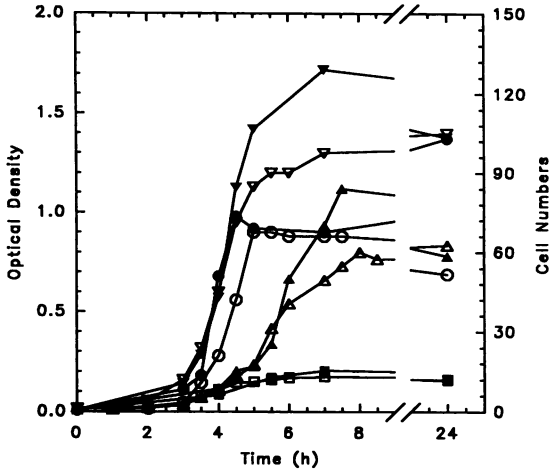

FIG. 4. Growth of *S. ruminantium* HD4 in glucose- or starch-containing media. ▽ and ▼, 1% glucose; ○ and ●, 0.2% glucose; △ and ▲, 1% starch; □ and ■, 0.2% starch. Open symbols represent optical density values, while closed symbols indicate cell number (10^7/ml) values. Total carbohydrate utilization by these cultures was 84, 100, 22, and 16% for 1% glucose, 0.2% glucose, 1% starch, and 0.2% starch cultures, respectively. All values are the average of duplicate cultures.

peared and was followed by maltotriose. The maltose present in these cultures only gradually decreased with time, and a small amount remained unutilized, even after 24 h.

Although the utilization of the oligosaccharides G1 through G7 was nearly complete, the utilization of total carbohydrate was poor and less than 40% of the available substrates were utilized. In an attempt to determine the nature of the unutilized carbohydrate, culture samples were analyzed for oligosaccharide composition by using TLC. These data confirmed the extensive utilization of lower-molecular-weight oligosaccharides as determined by HPLC and further showed that oligosaccharides of up to G10 were also metabolized by this organism. However, oligosaccharides greater than G10 were not utilized. This was also true of cultures provided with half this level of corn syrup dextrins. This suggests that strain HD4 has a limited capacity to utilize dextrins of large molecular weight.

Cocultures of amylolytic bacteria with *S. ruminantium*. Each of the amylolytic ruminal bacteria was coinoculated into starch-containing medium with *S. ruminantium* HD4, and the production and utilization of maltooligosaccharides were monitored over time. The growth of these cultures was determined both microscopically and spectrophotometrically. The amylolytic bacteria have markedly different morphologies from that of *S. ruminantium*, making microscopic measurement of the growth of individual species possible. Lactic acid concentration in these cultures was also measured. Both *S. bovis* and *B. fibrisolvens* produce lactate as a product of fermentation, while *S. ruminantium* HD4 can utilize lactate as well as produce it (15, 18). Thus, the utilization of lactate as well as maltooligosaccharides by *S. ruminantium* in these cocultures was possible. In all these cofermentations, lactic acid accumulated throughout the incubation period and the rate of accumulation paralleled the optical density increases of these cultures (data not shown). On the basis of this result, it seems unlikely that much utilization of lactic acid by *S. ruminantium* occurred in these

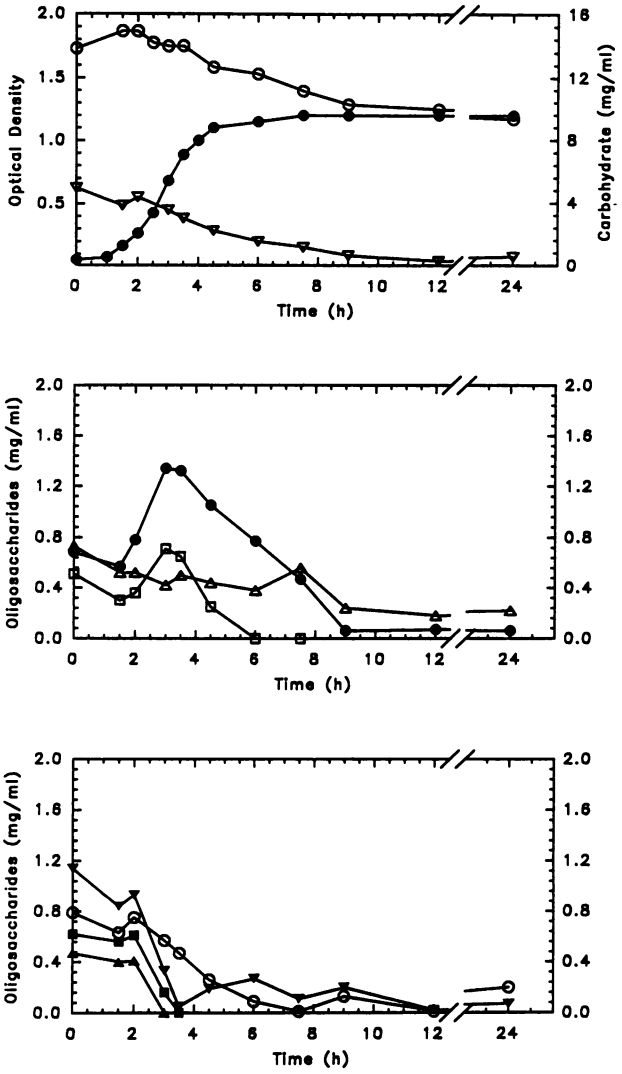

FIG. 5. Utilization of corn syrup maltooligosaccharides by *S. ruminantium* HD4. Upper panel: ●, optical density at 660 nm; ○, total carbohydrate; ▽, total oligosaccharides (sum of glucose through maltoheptaose). Center panel: □, glucose; △, maltose; ●, maltotriose. Lower panel: ○, maltotetraose; ▼, maltopentaose; ■, maltohexaose; ▲, maltoheptaose. Absence of a symbol at a particular time indicates that no oligosaccharide was detected (less than 0.05 mg/ml). Values are the means of duplicate cultures.

cultures. In fact it seems likely that this organism contributed to the lactic acid production of these cocultures.

Cocultures of *S. ruminantium* and *S. bovis* produced large numbers of *S. bovis* and comparatively few *S. ruminantium* (Fig. 6). As a result, the production and disappearance of maltooligosaccharides were essentially the same as for cultures with *S. bovis* alone (Fig. 1). In separate experiments, *S. ruminantium* was first inoculated into maltose-containing medium and grown to high density, and then starch and *S. bovis* were added (data not shown). Only when this was done was *S. ruminantium* able to compete for oligosaccharides with *S. bovis*. In these cultures the overall pattern of events was the same, but the level of accumulation was diminished.

In contrast to *S. ruminantium*-*S. bovis* cocultures, cultivation of *S. ruminantium* with *B. fibrisolvens* resulted in

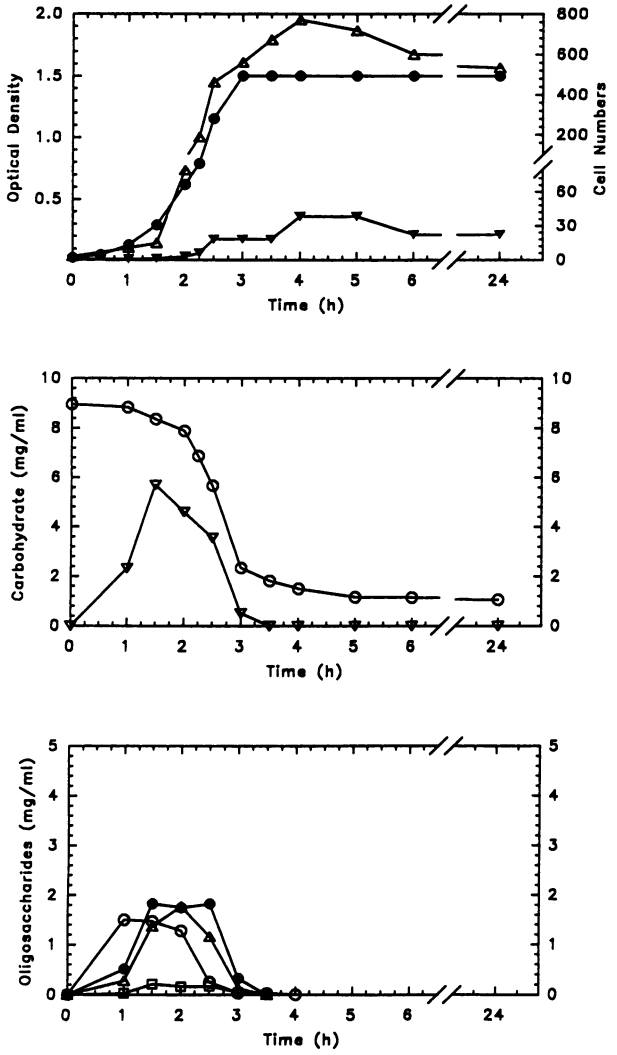

FIG. 6. Coculture of *S. bovis* JB1 and *S. ruminantium* HD4 on 1% starch. Upper panel: ●, optical density at 660 nm; △, number (10^7/ml) of *S. bovis*; ▼, number (10^7/ml) of *S. ruminantium*. Center panel: ○, total carbohydrate; ▽, total oligosaccharides (sum of glucose through maltoheptaose). Lower panel: □, glucose; △, maltose; ●, maltotriose; ○, maltotetraose. Absence of a symbol at a particular time indicates that no oligosaccharide was detected (less than 0.05 mg/ml). Values are the means of duplicate cultures.

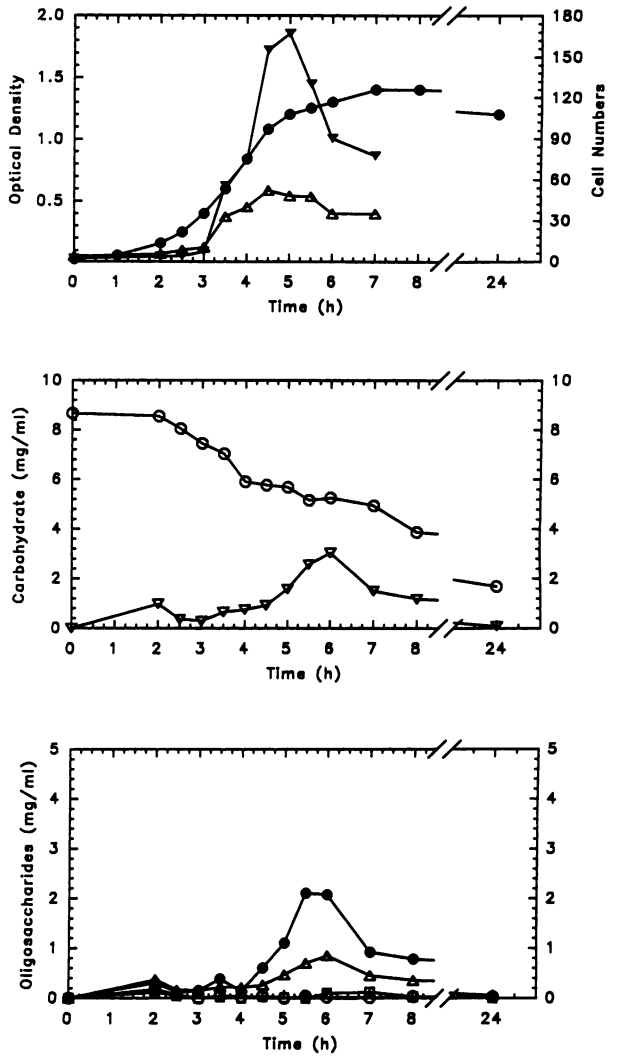

FIG. 7. Coculture of *B. fibrisolvens* 49 and *S. ruminantium* HD4 on 1% starch. Upper panel: ●, optical density at 660 nm; △, number (10^7/ml) of *B. fibrisolvens*; ▼, number (10^7/ml) of *S. ruminantium*. Center panel: ○, total carbohydrate; ▽, total oligosaccharides (sum of glucose through maltoheptaose). Lower panel: □, glucose; △, maltose; ●, maltotriose; ○, maltotetraose. Absence of a symbol at a particular time indicates that no oligosaccharide was detected (less than 0.05 mg/ml). Values are the means of duplicate cultures.

marked differences in the degradation and utilization of starch when compared with those in pure cultures of *B. fibrisolvens*. *B. fibrisolvens-S. ruminantium* cocultures degraded carbohydrate more completely than that observed for pure cultures and oligosaccharides accumulated to a much lesser degree (Fig. 7). The relative numbers of *S. ruminantium* in these cultures was great, and in fact this strain grew to higher numbers than the amylolytic *B. fibrisolvens*. The high numbers of *S. ruminantium* produced in these cultures was even greater than that observed for *S. ruminantium* grown on a comparable amount of glucose (Fig. 4). The pattern of oligosaccharide accumulation and disappearance in coculture was consistent with the patterns of production and utilization by these species when grown separately. That is to say, maltose and maltotriose were the major end products of starch degradation by *B. fibrisolvens* and the last to be depleted by both *B. fibrisolvens* and *S. ruminantium*. In contrast to pure cultures, cocultures completely utilized the carbohydrate provided.

S. ruminantium had an even more profound effect when cocultured with *B. ruminicola* rather than with *B. fibrisolvens*. As with *B. fibrisolvens* cocultures, *S. ruminantium* was able to grow to high numbers (Fig. 8). The effect on the concentration of accumulating oligosaccharides was dramatic. At no time during these incubations were any maltooligosaccharides detected in these cultures. This indicated that the compounds produced during the degradation of starch by *B. ruminicola* were assimilated as rapidly as they were produced. The overall extent of carbohydrate utilization by these cocultures was comparable to that for cultures of *B. ruminicola* alone (Fig. 3).

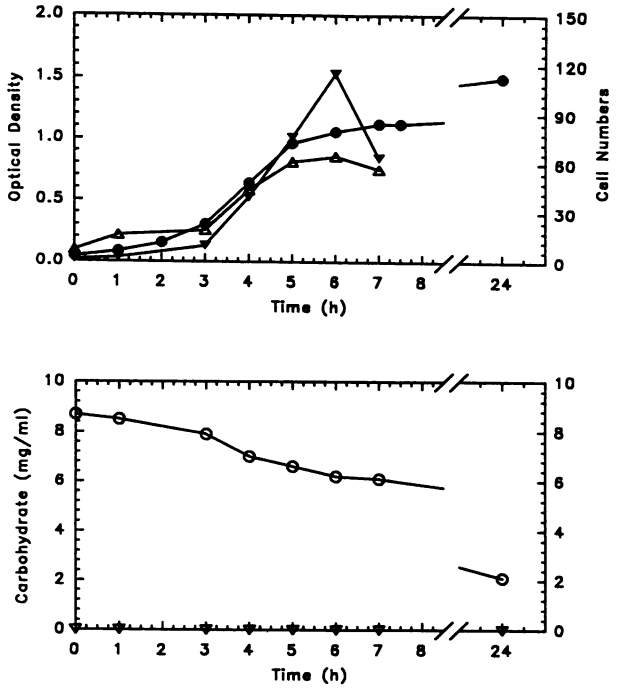

FIG. 8. Coculture of *B. ruminicola* D31d and *S. ruminantium* HD4 on 1% starch. Upper panel: ●, optical density at 660 nm; △, number (10^7/ml) of *B. ruminicola*; ▼, number (10^7/ml) of *S. ruminantium*. Lower panel: ○, total carbohydrate; ▽, total oligosaccharides (sum of glucose through maltoheptaose). Values are the means of duplicate cultures.

DISCUSSION

When the amylolytic ruminal bacteria *S. bovis* and *B. fibrisolvens* were grown in a starch-containing medium, maltooligosaccharides accumulated. The pattern of products observed in these incubations were consistent with those previously found for the extracellular amylolytic activities produced by these strains (3). The products of starch degradation by *B. ruminicola* D31d had not been determined, but on the basis of those produced during the digestion of starch by strain B_14 of *B. ruminicola*, these were expected to be more complex than those observed for the other starch-degrading bacteria (3). Indeed, strain D31d generated oligomeric products of greater variety than the other species during growth in starch-containing medium. The accumulated oligosaccharides in these starch-degrading cultures could serve as potential energy sources for nonamylolytic species. A parallel phenomenon has been demonstrated for the use of cellulodextrins by noncellulolytic ruminal bacteria (13).

A coculture system that contained an amylolytic bacterium and a dextrin-utilizing, nonamylolytic bacterium was constructed. *S. ruminantium* HD4 was tested as a possible dextrin-utilizing species, since previous work showed that this strain grew poorly in starch-containing media and produced little if any amylase (1, 3). The current study confirmed that this strain grows poorly on starch when added at 0.2%. However, when provided with starch at high levels (1%), it was able to grow and utilize about 20% of the starch. The possibility that the starch used contained some oligosaccharides was considered, but analysis of the starch by both HPLC and TLC methods showed that this preparation of starch contained no low-molecular-weight dextrins. It is possible that *S. ruminantium* is able to attack starch at exposed ends by an exoclipping mechanism but is unable to degrade starch internal to alpha 1,6 branches. Experiments comparing the utilization of amylose versus that of amylopectin (each at 1.0%) indicated that this might be true, since this strain was able to utilize approximately 40% of provided amylose but only 7% of amylopectin (not shown).

The suitability of *S. ruminantium* HD4 as a dextrin-utilizing species was demonstrated by its ability to grow on corn syrup dextrins. Growth of strain HD4 on maltooligosaccharides was rapid and was accompanied by near complete disappearance of low-degree of polymerization oligosaccharides (i.e., glucose through maltoheptaose). This range of substrates encompassed the major products accumulated by the three species of amylolytic bacteria. Only *B. ruminicola* degraded starch with the generation of hydrolysis products of greater degree of polymerization than maltoheptaose, and these were of minor importance on the basis of the results of TLC analysis. When cocultured with either *B. ruminicola* or *B. fibrisolvens*, *S. ruminantium* was able to utilize the oligosaccharide products of starch hydrolysis and grow to high numbers. However, the cell numbers of *S. ruminantium* in *S. bovis*-containing cocultures were low and the pattern of oligosaccharide accumulation differed little from that of *S. bovis* pure cultures. Obviously, the ability of *S. ruminantium* to compete for available maltooligosaccharides with *B. ruminicola* or *B. fibrisolvens* and *S. bovis* differed.

In batch cultures, maximum specific growth rate is likely to be the major determinant of competitive fitness (12). Since oligosaccharides accumulated in all three pure cultures of amylolytic bacteria, amylase activity was not limiting the growth of these cultures, and growth rates on maltooligosaccharides should reflect the maximum capacity for growth of these species under these conditions. Such a comparison showed that *S. bovis* grew most rapidly ($\mu = 1.62\ h^{-1}$) and was followed by *S. ruminantium* ($\mu = 1.03\ h^{-1}$), while *B. fibrisolvens* and *B. ruminicola* grew the most slowly (approximately equal, $\mu = 0.7\ h^{-1}$). The ability of *S. ruminantium* to grow more rapidly than *B. ruminicola* or *B. fibrisolvens* is consistent with its ability to grow in coculture with these species, while its inability to grow as rapidly as *S. bovis* explains why it was unsuccessful when cultivated with strain JB1.

Under conditions of restricted growth (e.g., continuous culture or in rumen) one might expect additional physiological growth characteristics such as maintenance energy expenditures or substrate affinities to be important determinants of competitive success. In starch-fed chemostat cultures of *S. bovis* and *Megasphaera elsdenii*, the numbers of *S. bovis* were always greatest regardless of the culture dilution rate or pH (14). These results generally followed what was predicted on the basis of the substrate affinities and maintenance requirements of the competing species, but the quantitative explanation of the relative numbers of each species required the consideration of several other factors (e.g., crossfeeding of lactate, ratio of substrate affinity to maximum specific growth rate, K_s/μ_{max}). Clearly, understanding what controls the composition of mixed bacterial cultures is quite complex. It is interesting to note that in the cultures mentioned above, it was assumed that maltose was the sole product of starch hydrolysis by *S. bovis*. On the basis of the results of this study, maltooligosaccharides were probably the substrates available for crossfeeding to *M. elsdenii* in these cultures. We have since confirmed that *M. elsdenii* is able to grow on corn syrup dextrins.

This study has dealt with the degradation of soluble starch, the generation of a pool of oligosaccharide intermediates, and the utilization of these intermediates as substrates for growth by a second species of bacterium. Ruminant diets, however, contain little soluble starch and instead are composed of raw starches which would be degraded more slowly. The slower digestion of more natural starch substrates might in turn affect the accumulation of intermediates and therefore the substrates available for crossfeeding. Preliminary results in this laboratory indicate that maltooligosaccharides are detectable in culture fluids of *B. fibrisolvens* cultivated on raw wheat, corn, or rice starches. In addition, evidence from animal feeding studies demonstrate that the degradation of starch in vivo can be quite rapid and that soluble carbohydrates accumulate during digestion (8, 9). Overall, it seems that crossfeeding of maltooligosaccharides between species of bacteria is a likely occurrence and that such crossfeeding is partially responsible for the complexity of bacterial types found in the rumen.

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