APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1990, p. 1683–1688
0099-2240/90/061683-06$02.00/0 Vol. 56, No. 6
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# Xylose Uptake by the Ruminal Bacterium *Selenomonas ruminantium*

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Received 20 November 1989/Accepted 25 March 1990

***Selenomonas ruminantium* HD4 does not use the phosphoenolpyruvate phosphotransferase system to transport xylose (S. A. Martin and J. B. Russell, J. Gen. Microbiol. 134:819–827, 1988). Xylose uptake by whole cells of *S. ruminantium* HD4 was inducible. Uptake was unaffected by monensin or lasalocid, while oxygen, *o*-phenanthroline, and $HgCl_2$ were potent inhibitors. Menadione, antimycin A, and KCN had little effect on uptake, and acriflavine inhibited uptake by 23%. Sodium fluoride decreased xylose uptake by 10%, while *N,N'*-dicyclohexylcarbodiimide decreased uptake by 31%. Sodium arsenate was a strong inhibitor (83%), and these results suggest the involvement of a high-energy phosphate compound and possibly a binding protein in xylose uptake. The protonophores carbonyl cyanide *m*-chlorophenylhydrazone, 2,4-dinitrophenol, and SF6847 inhibited xylose uptake by 88, 82, and 43%, respectively. The cations $Na^+$ and $K^+$ did not stimulate xylose uptake. The kinetics of xylose uptake were nonlinear, and it appeared that more than one uptake mechanism may be involved or that two proteins (i.e., a binding protein and permease protein) with different affinities for xylose were present. Excess (10 mM) glucose, sucrose, or maltose decreased xylose uptake <40%. Uptake was unaffected at extracellular pH values between 6.0 and 8.0, while pH values of 5.0 and 4.0 decreased uptake 28 and 24%, respectively. The phenolic monomers *p*-coumaric acid and vanillin inhibited growth on xylose and xylose uptake more than ferulic acid did. The predominant end products resulting from the fermentation of xylose were lactate (7.5 mM), acetate (4.4 mM), and propionate (5.1 mM), and the $Y_{XYLOSE}$ was 24.1 g/mol.**

*Selenomonas ruminantium* is a common, gram-negative ruminal bacterium that can account for 22 to 51% of the total viable bacterial counts in the rumen (4). Many different carbohydrates are fermented by *S. ruminantium*, and it can grow under a variety of dietary conditions (16). When it is grown in batch culture on glucose, lactate is the predominant fermentation product (13). Rapid growth and lactate production by *S. ruminantium* contribute to the acidity of rumen fluid, and it is one of the more-acid-tolerant ruminal bacteria (34).

*S. ruminantium* has high affinities for glucose, maltose, sucrose, and xylose (33), but glucose, sucrose, and xylose are preferentially used over maltose (32). *S. ruminantium* possesses a phosphoenolpyruvate-dependent phosphotransferase system (PEP-PTS) for glucose and sucrose but not for maltose (28). Maltose is hydrolyzed by an inducible extracellular maltase, and the resulting glucose is transported by the constitutive glucose PTS (28). Since the maltase is competitively inhibited by glucose and sucrose (28), these substrates are used before maltose. Little is known concerning xylose metabolism by *S. ruminantium*. PEP-dependent phosphorylation of xylose was negligible in toluene-treated cells of *S. ruminantium* (28), which suggests that the PEP-PTS does not mediate xylose uptake. In addition, uptake was inhibited by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and by chlorhexidine diacetate (28). Since uptake is often a key characteristic which determines the success of bacteria in natural environments (29), the following series of experiments were conducted to examine in greater detail factors that affect xylose uptake in *S. ruminantium*.

(This work was done by D. K. Williams in partial satisfaction of the requirements for the M.S. degree from The University of Georgia, Athens.)

## MATERIALS AND METHODS

**Organism and growth conditions.** The HD4 strain of *S. ruminantium* was used (3). Basal medium was prepared by previously described methods (25, 28). In certain cases, cells were grown in a minimal medium that contained (per liter) 292 mg of $K_2HPO_4$, 240 mg of $KH_2PO_4$, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 1 mmol of valerate, 4,000 mg of $Na_2CO_3$, 500 mg of cysteine hydrochloride, 100 mg of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 5.0 ml of microminerals (40), 10 ml of vitamins (7), 4.0 mg of biotin, and 100 mg of yeast extract (pH 6.7). Sugars were prepared as separate anaerobic solutions (20% [wt/vol]) under $O_2$-free $CO_2$ (glucose and xylose) or $N_2$ (sucrose and maltose), autoclaved, and added (6 g/liter) to the basal medium. Incubations were performed anaerobically under $CO_2$ at 39°C in batch culture.

**Xylose uptake.** $^{14}C$-labeled-xylose uptake was examined in xylose-grown cells (40 ml) that were harvested anaerobically during exponential growth (optical density at 600 nm, approximately 1.0) by centrifugation (10,000 × *g*, 15 min, 4°C). Cells were washed once with $O_2$-free 100 mM sodium-potassium phosphate buffer (pH 7.2) plus 5 mM $MgCl_2$ and suspended in 10 ml of buffer. The reaction mixture (1.0 ml) contained 100 mM sodium-potassium phosphate buffer, 5 mM $MgCl_2$, and 100 μl of intact cells. The reaction was started by the addition of 1 mM xylose that contained 0.2 μCi of D-[U-$^{14}C$]xylose. After incubation at 39°C for 15 min,

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the reaction was stopped by placing the reaction tubes in an ice bath and adding 5 ml of ice-cold buffer. Approximately 5 min after the addition of ice-cold buffer, the cells were collected by filtration through a 0.45-μm-pore-size membrane filter (Millipore Corp., Bedford, Mass.) and rinsed with 5 ml of buffer. No leakage of xylose from the cells was observed, and the rate of xylose uptake was linear for at least 15 min under these conditions. The filters were air dried and counted in an LS-9800 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Xylose uptake was also examined in cells grown on glucose, maltose, or sucrose. All incubations were performed anaerobically and in duplicate, and variation was indicated by standard deviation. Initial rates of uptake were determined by using a 30-s incubation period.

**Inhibitors.** The volume of anaerobic buffer in the reaction mixture was reduced (by 10 μl or 50 μl) to accommodate the volume of inhibitor added. Cells were preincubated at room temperature in buffer plus inhibitor for 3 min prior to xylose addition (26, 28). Inhibitors were dissolved in either deionized water or 2% (vol/vol) ethanol. When ethanol-soluble inhibitors were used, control incubations contained the same final concentration of ethanol.

**Monovalent cations.** Xylose-grown cells were harvested, washed, and suspended in either $O_2$-free 100 mM potassium phosphate buffer (pH 7.2) plus 5 mM $MgCl_2$ or 100 mM sodium phosphate buffer (pH 7.2) plus 5 mM $MgCl_2$. Each 1.0-ml reaction mixture contained 100 mM $O_2$-free phosphate buffer, 5 mM $MgCl_2$, 0 to 150 mM NaCl or KCl, 100 μl of intact cells, and radiolabeled xylose. Cells were preincubated in buffer plus salt for 10 min prior to the addition of xylose.

**Phenolic monomers.** The effects of *trans-p*-coumaric acid, *trans*-ferulic acid, and vanillin on the growth of *S. ruminantium* HD4 in xylose (4 g/liter) medium were examined. Weighed amounts of each phenolic compound were incorporated into the basal medium at 0.01, 0.05, and 0.1% (wt/vol), and the medium was sterilized by autoclaving (6, 27). The pH of the medium was not altered by the addition of these compounds (27). Butyl rubber-stoppered roll tubes that contained 9.7 ml of basal medium, phenolic compound, and xylose were inoculated with 0.3 ml of an overnight xylose-grown culture. Control tubes without added phenolic monomers were also run. The $A_{600}$ was read against a blank of uninoculated medium with a Spectronic 20D spectrophotometer (Milton Roy Co., Rochester, N.Y.). All incubations were performed in duplicate, and variation between tubes was less than 10%. To examine the effects of the three phenolic monomers on xylose uptake, cells were preincubated in buffer plus phenolic compound for 3 min before xylose addition.

**Sampling and analyses.** To determine the fermentation acids and cell yields on xylose medium, cells were removed from the medium by centrifugation (10,000 × *g*, 4°C, 15 min). The cell pellet was washed, recentrifuged, and suspended in deionized $H_2O$. The dry weight of the washed cell suspension was determined after drying on aluminum pans at 105°C. The cell-free supernatants were stored at −20°C prior to analysis. Xylose was analyzed by the method of Schneider (41), and lactate was assayed by the method of Hohorst (14). Volatile fatty acids were measured by gas-liquid chromatography with a model 3400 gas chromatograph (Varian, Palo Alto, Calif.) (S. A. Martin and D. J. Nisbet, J. Anim. Sci., in press). Washed cells were hydrolyzed in 0.2 N NaOH (100°C, 15 min), and protein was determined by the method of Lowry et al. (24) or Bradford (2). Bovine serum albumin was the standard; it was treated similarly.

TABLE 1. Effect of various metabolic inhibitors on xylose uptake by whole cells of *S. ruminantium*

| Inhibitor[a] | % Inhibition |
|---|---|
| $O_2$ | 95 |
| 2,4-Dinitrophenol (1 mM) | 82 |
| CCCP[b] (8 μM) | 88 |
| SF6847[b] (1.3 μM) | 43 |
| DCCD[b] (8 μM) | 31 |
| Sodium arsenate (10 mM) | 83 |
| $HgCl_2$ (0.5 mM) | 96 |
| *o*-Phenanthroline[b] (1 mM) | 96 |

[a] Specific activity values of controls were 90 and 84 nmol/mg of protein per min for untreated and ethanol-treated cells, respectively. All inhibitors that were dissolved in ethanol were compared with ethanol-treated controls. The following inhibitors exhibited little effect (<24% inhibition of xylose uptake): acriflavine (1 mM), antimycin A (50 μM), KCN (10 mM), lasalocid (0.01 mM), menadione (20 μM), monensin (0.01 mM), and NaF (15 mM). All of these inhibitors except KCN and NaF were dissolved in ethanol.

[b] Dissolved in ethanol.

**Chemicals.** D-[U-$^{14}$C]xylose (76 μCi/μmol; 1 μCi = 37 kBq) was obtained from Amersham Corp., Arlington Heights, Ill. 3,5-Di-*tert*-butyl-4-hydroxybenzylidene-malononitrile (SF6847) was obtained from Wako Chemicals USA, Inc., Dallas, Tex. *N,N*-dicyclohexylcarbodiimide (DCCD), 2,4-dinitrophenol, and CCCP were from Sigma Chemical Co., St. Louis, Mo. All other chemicals were of the highest purity commercially available.

## RESULTS AND DISCUSSION

The PEP-PTS is favorable to anaerobic bacteria because it mediates the simultaneous transport and phosphorylation of a sugar without expending additional ATP in a kinase reaction. In general, pentoses are not transported by the PTS, but pentitol-specific PTSs have been reported in *Lactobacillus casei* (22, 23). The lack of significant PEP-dependent phosphorylation of xylose by toluene-treated cells of *S. ruminantium* indicated that a PTS was probably not used for the uptake of this sugar (28).

**Effect of growth sugars on xylose uptake.** When *S. ruminantium* was grown on glucose, maltose, or sucrose, xylose uptake was low (<1.7 nmol/mg of protein per min) compared with that of xylose-grown cells (94.3 nmol/mg of protein per min). Therefore, xylose uptake in this bacterium is inducible. Glucose PTS activity in *S. ruminantium* was constitutive, and the sucrose PTS was inducible (28). Another predominant ruminal bacterium, *Streptococcus bovis*, also possesses a constitutive glucose PTS, while PTS activity was inducible for cellobiose, maltose, and sucrose (26).

**Metabolic inhibitors.** Several strains of *S. ruminantium*, including HD4, are able to grow on glucose in the presence of the common feed additives monensin and lasalocid (5). The insensitivity of *S. ruminantium* to these ionophores is further supported by the lack of significant inhibition of xylose uptake by either monensin or lasalocid (Table 1). Both ionophores are capable of disrupting sodium or potassium gradients or both across bacterial cell membranes (36, 37), but xylose uptake in *S. ruminantium* is not dependent on either of these cations (Fig. 1). Glucose uptake by the ruminal cellulolytic bacterium *Bacteroides succinogenes* was strongly inhibited by both monensin and lasalocid (10), while sodium-dependent uptake of amino acids was inhibited by monensin in *S. bovis* (37).

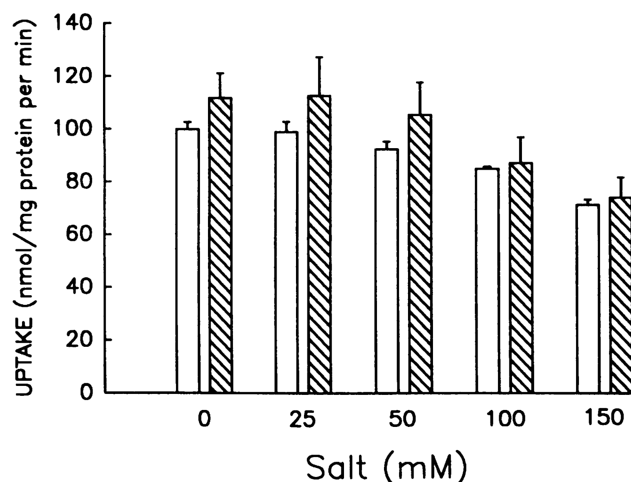


FIG. 1. Effect of KCl (▨) and NaCl (□) on xylose uptake by whole cells of *S. ruminantium*.

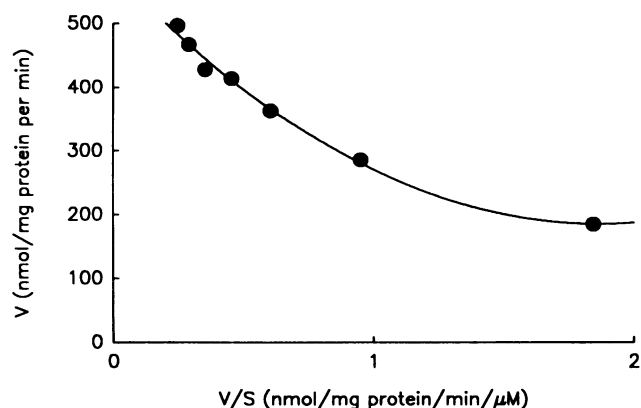


FIG. 2. Eadie-Hofstee plot of xylose uptake by whole cells of *S. ruminantium*. V, Velocity; S, substrate concentration.

Incubation of cells in buffer under an $O_2$ atmosphere almost completely inhibited (95%) xylose uptake (Table 1). Sensitivity of xylose uptake to $O_2$ is not surprising, considering the strictly anaerobic nature of this bacterium (21). Other potent (96%) inhibitors of xylose uptake by *S. ruminantium* were *o*-phenanthroline and $HgCl_2$. The strong inhibition by both compounds suggests that an electron carrier protein may be involved in the uptake of xylose. Even though KCN also reacts with iron-containing proteins (10), xylose uptake was inhibited by only 5%.

The electron transport inhibitors menadione and antimycin A had little effect on xylose uptake by *S. ruminantium*, and acriflavine reduced uptake by only 23% (Table 1). The lack of inhibition by menadione agrees with the finding that menaquinones and ubiquinones are absent in *S. ruminantium* (42). When *S. ruminantium* was grown in medium that contained an electron transport inhibitor, chlorpromazine or 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide, little change in growth was observed (8). The inhibition of xylose uptake by acriflavine suggests that flavin compounds may be involved in the transfer of electrons in *S. ruminantium*, possibly associated with the fumarate reductase (30). The electron transport chain of *S. ruminantium* PC18 contains a flavoprotein (8).

Studies with oral streptococci have indicated that the enzyme which converts 2-phosphoglycerate to PEP is fluoride sensitive (19). Since fluoride could inhibit the production of endogenous ATP, the effect of this compound on xylose uptake by *S. ruminantium* was evaluated. Only a 10% decrease in xylose uptake was observed (Table 1), and these results supported previous research that suggested that *S. ruminantium* was insensitive to fluoride (25).

To our knowledge, no information is available showing the presence of ATPases in *S. ruminantium*. The ATPase inhibitor DCCD inhibited xylose uptake by 31% (Table 1), and these results provide indirect evidence that an ATPase may play a role in the energetics of xylose uptake in this organism. However, further research is needed to support these indirect observations. Growth rate and cell yields of *S. ruminantium* PC18 were not altered when DCCD was incorporated into the growth medium (8).

Recent research demonstrated that the well-known uncoupling agent CCCP inhibited xylose uptake by 83% in intact cells of *S. ruminantium* HD4 (28). Similar results were observed in this study (Table 1), but CCCP has been shown to block sulfhydryl groups in membrane proteins of *Escherichia coli* and *Staphylococcus aureus*, so caution should be used in interpreting the effects of CCCP (18). Therefore, the effects of the uncouplers 2,4-dinitrophenol and SF6847 on xylose uptake were determined. Even though different concentrations were used, both compounds significantly inhibited xylose uptake (Table 1), suggesting that proton gradients may be involved in xylose transport.

Even though energy appears to be derived from the fumarate reductase in *S. ruminantium* (30), little is known concerning the contribution of this energy to nutrient uptake. The electron transport inhibitors used in this study inhibited xylose uptake by <24% (Table 1). Since this inhibition was not great, the role of the electron transport system in xylose uptake is at best ambiguous. On the basis of the inability of several electron transport inhibitors and uncouplers to inhibit the growth of *S. ruminantium* PC18, Dawson and co-workers (8) concluded that this microorganism generates ATP only by substrate-level phosphorylation. Furthermore, these researchers proposed that *S. ruminantium* PC18 utilizes electron transport solely for reducing equivalent disposal (8). The effects of various metabolic inhibitors on xylose uptake by *S. ruminantium* HD4 (Table 1) do not contradict this conclusion.

Sodium arsenate was a strong (83%) inhibitor of xylose uptake by *S. ruminantium* (Table 1), suggesting that a high-energy phosphate compound as well as binding proteins may be involved (1, 20, 44). In addition, inhibition by the uncouplers may implicate involvement of a proton motive force in xylose uptake; however, intracellular energy sources may be expended to regenerate the membrane potential, and the availability of ATP equivalents for uptake could be depleted (44). Maltose uptake in *E. coli* has been studied extensively, and uptake of this disaccharide is sensitive to uncouplers as well as arsenate (39). These results support uptake by a shock-sensitive system, but participation of a proton electrochemical gradient in the uptake process cannot be ruled out (39).

**Kinetics of xylose uptake.** When the initial rates of xylose uptake by *S. ruminantium* were determined, a nonlinear Eadie-Hofstee plot was obtained (Fig. 2). These nonlinear kinetics suggested that more than one mechanism of xylose uptake may be used by *S. ruminantium* or that one mechanism of uptake exists in which two proteins, such as a periplasmic binding protein and a permease protein, with different affinities for xylose are present. However, the latter

TABLE 2. Effect of unlabeled sugars on $^{14}C$-xylose uptake by xylose-grown cells of *S. ruminantium*[a]

| Sugar added (10 mM) | Sp act[b] | % Inhibition |
|---|---|---|
| None | 94.3 ± 7.8 | |
| Xylose | 12.0 ± 4.0 | 87 |
| Glucose | 57.9 ± 1.0 | 39 |
| Maltose | 79.6 ± 2.0 | 16 |
| Sucrose | 70.4 ± 3.4 | 25 |

[a] Each reaction mixture contained 100 mM sodium-potassium phosphate buffer (pH 7.2), 5 mM $MgCl_2$, 0 or 10 mM unlabeled competing sugars, 1 mM xylose that contained 0.2 μCi of $^{14}C$-xylose, and 100 μl of xylose-grown cells. The cells were preincubated with buffer and unlabeled sugars for 3 min prior to xylose addition.

[b] Nanomoles per milligram of protein per minute.

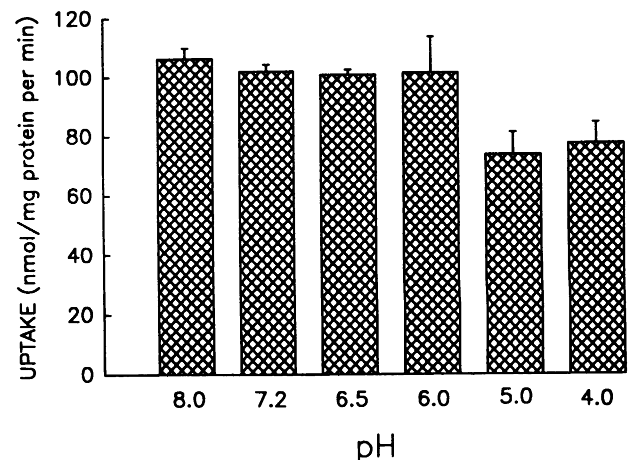


FIG. 3. Effect of extracellular pH on xylose uptake by whole cells of *S. ruminantium*. Anaerobic ($N_2$ atmosphere) sodium-potassium phosphate buffers were used at all pH values tested.

hypothesis may depend on the substrate concentration available to each protein and whether the binding protein step is rate limiting. Several unsuccessful attempts were made to make spheroplasts as a method of determining whether a periplasmic xylose-binding protein was involved in uptake (10). Recently, Dean et al. (9) reported that methods used to routinely lyse *E. coli* cells were not successful for *S. ruminantium* HD4. Therefore, involvement of a periplasmic xylose-binding protein remains unclear, but arsenate was a strong inhibitor of xylose uptake (Table 1) and has been shown to inhibit shock-sensitive or binding protein active transport systems in other bacteria (1, 15, 44).

**Competition studies.** Unlabeled glucose, sucrose, and maltose inhibited uptake of radiolabeled xylose by 39, 25, and 16%, respectively (Table 2). Unlabeled xylose inhibited uptake of $^{14}C$-xylose by 87%, an amount similar to the theoretical dilution (91%). Since glucose PTS activity in *S. ruminantium* HD4 is constitutive (28), the inhibition of xylose uptake by glucose might be explained by regulation of the xylose carrier by the glucose PTS (39). Phosphotransferase system-mediated regulation may also be involved in the inhibition of xylose uptake by maltose and sucrose. While sucrose PTS activity was highest for sucrose-grown cells, xylose-grown cells also exhibited sucrose PTS activity, and this activity was 24% of that observed with sucrose-grown cells (28). This level of activity corresponds to the 25% level of inhibition of xylose uptake by sucrose. Low levels of maltase activity were detected in xylose-grown cells (28), so the constitutive glucose PTS may also be involved in the small inhibition of xylose uptake by excess maltose.

**Cations and xylose uptake.** Xylose uptake was unaffected by the removal of potassium or sodium from the transport assay (Fig. 1). Concentrations of $Na^+$ or $K^+$ of 50 mM or less had little effect on xylose uptake, while higher concentrations tended to decrease uptake. Similar results were observed for cells incubated in 50 mM triethanolamine buffer (pH 7.5) plus 5 mM $MgCl_2$ instead of potassium or sodium phosphate buffers (data not shown).

**Extracellular pH.** Even though the rumen is well buffered by bicarbonate, phosphate, protein, and volatile fatty acids, rumen fluid pH values can vary from approximately 7.0 to less than 5.0, depending on the dietary conditions (16). *S. ruminantium* is more acid tolerant than many other rumen bacteria (34, 35), but little information is available regarding the effects of extracellular pH on nutrient uptake. Little change in xylose uptake by *S. ruminantium* was detected when the extracellular pH was between 8.0 and 6.0, and uptake was reduced by only 28 and 24% at pH values of 5.0 and 4.0, respectively (Fig. 3). These results are consistent with the ability of this bacterium to grow under acidic conditions (34, 35).

**Phenolic monomers.** Ferulic acid, *p*-coumaric acid, and vanillin are low-molecular-weight phenolic compounds frequently isolated from forages (17). In addition, lower-quality plant fractions contain *p*-coumaric acid more often than they contain other phenolic compounds (11). High concentrations of free, mixed phenolic acids have been shown to be released by hydrolases in sorghum (45), and *p*-coumaric acid, ferulic acid, and vanillin were released upon treatment of ryegrass with NaOH and represented up to 0.54% of the dry matter (12).

Several studies have shown that phenolic acids are inhibitory to ruminal microorganisms, including *S. ruminantium* (6, 27), and *p*-coumaric acid, ferulic acid, and vanillin were recently shown to inhibit rumen bacterial enzymes involved with fiber degradation (27). The ability of *S. ruminantium* HD4 to utilize xylose and cellodextrins (31), as well as the presence of an extracellular carboxymethylcellulase (31), implies that this bacterium may be closely associated with the particulate fractions within the rumen. Consequently, this bacterium may encounter high local concentrations of phenolic acids as cell wall degradation proceeds. Chesson et al. (6) reported that *S. ruminantium* WPL 151/1 was able to grow in a simple sugars medium that contained 10 mM (0.16%) or 20 mM (0.32%) *p*-coumaric acid or ferulic acid after 1 and 5 days, respectively. *p*-Coumaric acid and vanillin retarded the growth of *S. ruminantium* HD4 on xylose in a concentration-dependent fashion for up to 4 h (data not shown). However, after 6 h, the $A_{600}$ values for the 0.01 and 0.05% concentrations of both compounds were similar to those of the control incubations, while the 0.1% level continued to inhibit growth even after 120 h. Ferulic acid also retarded growth in a concentration-dependent fashion for up to 4 h, but after 6 h, the $A_{600}$ values for all three concentrations of ferulic acid were similar to control values. Uptake was inhibited by all three phenolic compounds, and the 0.1% concentration was the most inhibitory (Table 3). Both *p*-coumaric acid and vanillin inhibited uptake to a greater extent than ferulic acid did. These results suggest that the presence of phenolic monomers in rumen fluid could affect xylose utilization by altering its uptake in *S. ruminantium*.

**Fermentation products and cell yield.** When *S. ruminan-*

TABLE 3. Effect of phenolic monomers on xylose uptake by whole cells of *S. ruminantium*

| Phenolic monomer (% [wt/vol]) | Sp act[a] | % Inhibition |
|---|---|---|
| None | 125.9 ± 33.5 | |
| *p*-Coumaric acid | | |
| 0.01 | 109.0 ± 28.9 | 13 |
| 0.05 | 85.1 ± 18.5 | 32 |
| 0.10 | 68.0 ± 12.2 | 46 |
| Ferulic acid | | |
| 0.01 | 115.9 ± 33.5 | 8 |
| 0.05 | 108.2 ± 30.2 | 14 |
| 0.10 | 93.6 ± 25.5 | 25 |
| Vanillin | | |
| 0.01 | 108.4 ± 34.4 | 14 |
| 0.05 | 89.4 ± 28.4 | 29 |
| 0.10 | 63.2 ± 17.1 | 50 |

[a] Nanomoles per milligram of protein per minute.

*tium* was grown in minimal medium with xylose, lactate (7.5 mM), acetate (4.4 mM), and propionate (5.1 mM) were the predominant products. Small amounts of isobutyrate (0.4 mM), butyrate (0.3 mM), and isovalerate (0.08 mM) were also detected. These end products are consistent with the known fermentation products resulting from the metabolism of other sugars (43). Lactate is also a predominant fermentation product when *S. ruminantium* is grown in batch culture at high growth rates on glucose (13). Therefore, the rapid metabolism of xylose (growth rate of 0.53/h [28]) appears to result in a nearly homolactic fermentation. Previous studies by several laboratories reported $Y_{GLUCOSE}$ values between 29 and 100 g/mol for *S. ruminantium* (summarized in reference 38). Based on a bacterial dry weight of 0.40 mg/ml, the calculated $Y_{XYLOSE}$ was 24.1 g of cells per mol of xylose. This value agrees reasonably well with the previously reported values for glucose-grown cells, and one would expect a lower $Y_{XYLOSE}$ for a homolactic fermentation (38).

**Summary.** These experiments demonstrate that xylose uptake in *S. ruminantium* HD4 is inducible and may require a carrier protein and energy. Uptake is not dependent on $Na^+$ or $K^+$, and an extracellular pH of 4.0 reduced uptake by only 24%. *p*-Coumaric acid and vanillin inhibited growth and uptake more than ferulic acid did. Additional research with active membrane vesicles from *S. ruminantium* is needed to establish the role of periplasmic proteins and the electrochemical gradient in xylose uptake. Furthermore, studies are needed to identify the cytoplasmic enzymes involved in xylose metabolism by this predominant ruminal bacterium.

## ACKNOWLEDGMENTS

Funding for this research was provided by the Office of the Vice President for Research, New Faculty Grants Program, The University of Georgia, and by the Agricultural Experiment Station of The University of Georgia (Hatch project no. GE000512).

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