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Fermentation of glucose and xylose in *Prevotella ruminicola* AR29

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ABSTRACT

Prevotella ruminicola is a fibrolytic rumen bacterium that degrades complex carbohydrates and ferments the resulting hexoses and pentoses. Cultures of strain AR29 grew more rapidly on glucose than on xylose, but produced almost the same metabolite profiles on both carbon sources. Glucose in glucose-plus-xylose medium was used preferentially. The production of cell dry matter, growth yields of dry matter and protein, and cell composition (cell carbohydrate and protein) were not significantly different in glucose- and xylose-grown cultures. High aldolase (EC 4.1.2.13) activity was found both in glucose- and xylose-grown cells of the strain AR29. A very low phosphoketolase (EC 4.1.2.9) activity was detected in cells cultured on xylose. In contrast, enzymes of the Entner-Doudoroff pathway (6-phosphogluconate dehydrase and 2-keto-3-deoxy-6-phosphogluconate aldolase) could not be evidenced.

KEY WORDS: *Prevotella ruminicola*, metabolism, carbohydrates

INTRODUCTION

Anaerobic bacteria belonging to the species *Prevotella ruminicola* represent one of the most numerous bacterial groups inhabiting the rumen (Stewart and Bryant, 1988). The predominance of this organism can be explained by its ability to use a variety of substrates and also by its efficient energy metabolism. Strains that have been isolated from the rumen ferment soluble sugars including soluble cellodextrins, polysaccharides, and decompose protein, peptides and amino acids. Several studies attempted to elucidate carbohydrate metabolism of *P. ruminicola*. Experiments by Joyner and Baldwin (1966) and Mountfort and Roberton (1978)

showed that glucose was predominantly fermented via the Embden-Meyerhof glycolytic pathway. Studies with ^{14}C-arabinose indicated that pentose was fermented by a pentose phosphate cycle plus glycolysis, with a minor contribution of a phosphoketolase-type pathway (Turner and Roberton, 1979). The pectin metabolism of *P. ruminicola* is not fully understood. Previous experiments showed that cultures with pectin and glucose differed considerably in the composition of fermentation end-products and in production of cell dry matter (Marounek and Kalachnyuk, 1995).

The purpose of this study was to extend our knowledge of carbohydrate fermentation in *P. ruminicola*. Strain AR29 was grown on glucose and xylose to determine fermentation stoichiometry, growth yields and activity of intracellular enzymes involved in metabolism of hexoses and pentoses. Glucose and xylose are the principal monomeric units of plant polysaccharides. In addition, xylose is supposed to be an intermediate of uronic acid catabolism (Leng, 1970).

MATERIAL AND METHODS

Bacteria and culture conditions

P. ruminicola AR29 was kindly supplied by Dr. K. Gregg from The University of New England, Australia. The bacterium was grown on a medium containing in 1L: $K_2HPO_4 \cdot 3H_2O$ – 5.9 g, KH_2PO_4 – 4.5 g, $NaHCO_3$ – 3.0 g, $(NH_4)_2SO_4$ – 2.9 g, NaCl – 0.9 g, $MgSO_4 \cdot 7H_2O$ – 0.09 g, $CaCl_2$ – 0.09 g, yeast extract – 1.0 g, pancreatic casein hydrolysate – 1.0 g, clarified rumen fluid – 100 ml. A trace metal solution and a vitamin solution were also added. The medium was reduced by a mixture of 0.025% cysteine·HCl and 0.025% $Na_2S \cdot 9H_2O$ and sterilized at 110°C for 1 h. Substrates were added at 4 g/L. Glucose and xylose were autoclaved separately. *Lactobacillus plantarum* 185 was used as a positive control for phosphoketolase activity, and was supplied from the Milcom Comp. (Prague, Czech Republic). It was grown in MRS nutrient broth (Harrigan and McCance, 1966). *Pseudomonas fluorescens* DBM 3056, an organism fermenting hexoses by the Entner-Doudoroff pathway, was grown as described by Van Dijken and Quayle (1977). The pseudomonad was grown on fructose (4 g/L).

Fermentation of glucose and xylose by *P. ruminicola* AR29 was followed in 0.8 L batch cultures at pH 6.5 and 39°C in an O_2-free CO_2 atmosphere. Two LF3 fermentors (CSAV Works, Prague, Czech Republic) were used. Bacterial growth was monitored turbidimetrically at 640 nm. The incubation was completed after reaching the stationary phase. The ability of strain AR29 to utilize other carbohydrates was assessed on the basis of visible turbidity or change in culture pH following incubation.

Analyses and calculations

When growth on glucose or xylose had ceased, cell dry weight was determined after centrifugation of the culture, washing and drying at 105°C overnight. Cell carbohydrate and protein were measured by the phenol-sulphuric acid and Lowry methods, respectively (Herbert et al., 1971). Remaining analyses were done on frozen samples. Formate was determined colorimetrically (Sleat and Mah, 1984), other metabolites by gas chromatography: acetate and propionate at 140°C on a column of the Chromosorb WAW with 15% SP1220 and 1% H_3PO_4; lactate, fumarate and succinate on a programmed (140-180°C) column of the Supelcoport with 3% SP 2340 (Supelco, USA), after methylation. Residual xylose was estimated by the orcinol reagent (Herbert et al., 1971) and glucose enzymatically, using a commercial kit (Lachema, Czech Republic). Carbon content was determined in freeze-dried cells by means of elemental analyzer Perkin Elmer 2400.

Phosphoketolase activity (EC 4.1.2.9) was determined as described previously (Marounek and Petr, 1995). The method is based on conversion of xyluloso-5-phosphate to acetyl phosphate, and acetate determination in acidified samples. Aldolase (EC 4.1.2.13) was selected as a representative of glycolysis. Its activity was measured using a commercial kit (Sigma; procedure No. 752). The enzymes, 6-phosphogluconate dehydrase (EC 4.2.1.16) and 2-keto-3-deoxy-6-phosphogluconate aldolase (EC 4.1.2.14), were chosen as representatives of the Entner-Doudoroff pathway (Touster, 1969). Enzyme assays were carried out according to Kovachevich and Wood (1955 a,b). The method is based on conversion of 6-phosphogluconate to pyruvate. Pyruvate was determined on a column of the Carbopack B-DA with 4% Carbowax 20M (Supelco, USA) at 175°C.

Growth yields and metabolic parameters were computed from the difference between the beginning and the end of the incubation. Carbon recovery was calculated from the metabolic products and carbon content of cells. Culture maximum specific growth rates were calculated by taking the slope of the linear portion of the log-transformed optical density vs time curve. The significance of differences was evaluated by the *t*-test.

RESULTS

P. ruminicola AR29 fermented glucose, galactose, mannose, fructose, arabinose, ribose, xylose, cellobiose, lactose, maltose, sucrose, trehalose, raffinose, glucuronic acid, glucosamine, N-acetylglucosamine, pectin and starch. Sorbose, dulcit, mannit, carboxymethylcellulose, inulin and xylan were fermented weakly. No growth was observed in cultures with arabit and galacturonic acid.

TABLE I

Parameters of growth and metabolism of *P. ruminicola* AR29 on glucose and xylose

	Glucose	Xylose
Growth rate, h^{-1}	0.40 ± 0.10	0.27 ± 0.05*
Residual substrate, $mg.l^{-1}$	77 ± 21	69 ± 66
Substrate used, $mg.l^{-1}$	3792 ± 24	3889 ± 71*
Metabolites, $mmol.l^{-1}$		
formate	2.7 ± 0.9	2.3 ± 2.2
acetate	10.4 ± 1.2	11.2 ± 1.6
propionate	1.7 ± 0.3	3.3 ± 1.5
lactate	1.8 ± 0.5	1.0 ± 0.9
succinate	1.1 ± 0.7	11.7 ± 1.9
fumarate	1.1 ± 0.2	0.4 ± 0.3*
Cell dry weight, $mg.l^{-1}$	1028 ± 148	117 ± 62
Cell composition		
carbohydrate, mmol g.u. per g DM	1.44 ± 0.52	1.43 ± 0.29
protein, % DM	58.9 ± 6.7	52.8 ± 3.9
Yields, mg per g substrate used		
DM	271 ± 40	287 ± 17
protein	160 ± 37	152 ± 23
Carbon recovery, %	92.3 ± 6.0	96.0 ± 5.4

means of four (glucose) or five (xylose) incubations ± SD

g.u., Glucose unit; DM, dry matter

* significantly different from the corresponding glucose value at $P<0.05$

Data on the metabolism of glucose and xylose, composition of cells and growth yields of *P. ruminicola* AR29 are summarized in Table 1. Growth on glucose was more rapid than growth on xylose ($P<0.05$). Succinate and acetate were the principal fermentation end-products both in glucose- and xylose-grown cultures. The nature of the substrate had no effect on molar production of formate, acetate, propionate, lactate and succinate. Fumarate production was significantly lower on xylose than on glucose (0.4 vs 1.1 mmol/L, on average). No significant differences were found in cell composition, production of cell dry matter or growth yields of dry matter and protein. Growth yields expressed on a molar basis were 48.8 and 43.1 g of cell dry matter per mole of glucose and xylose utilized, respectively. Cells of *P. ruminicola* AR29 contained 44.7% carbon. Carbon recovery in *P. ruminicola* AR29 cultures was incomplete, i.e. lower than 100%. Several potential products were assayed for, but not found (ethanol, acetoin, diacetyl, butyrate, valerate). Traces of methylglyoxal were sometimes detected. The utilization of xylose in glucose-plus-xylose medium was slow until the glucose concentration decreased to below 20% of its initial level (Figure 1).

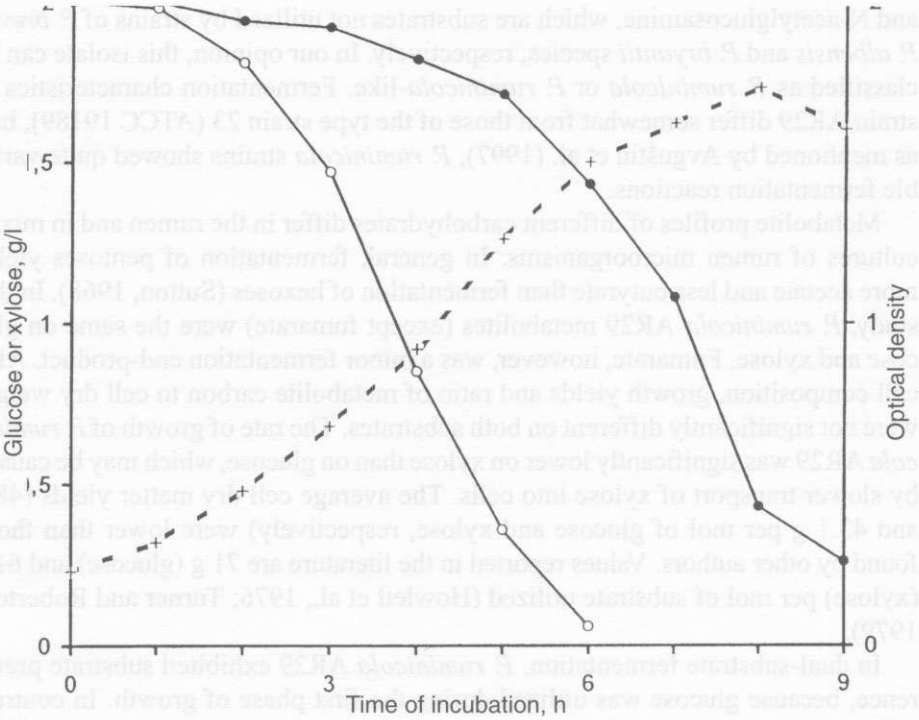

Figure 1. Time course of glucose (o) and xylose (•) utilisation in culture of *P. ruminicola* AR29. Optical density (+) of culture was monitored turbidimetrically at 640 nm

Aldolase activity was found both in glucose- and xylose-grown cells of strain AR29. Cells cultured on xylose also possessed very low phosphoketolase activity : 1.4 nmol of substrate broken down per min per mg of protein. The corresponding value in *L. plantarum* 185 was fifty times higher. Activity of enzymes of the Entner-Doudoroff pathway was found in the indicator organism (*P. fluorescens* DBM 3056), but not in *P. ruminicola* AR29.

DISCUSSION

It has been found that *P. ruminicola*, followed by *Butyrivibrio fibrisolvens*, generally predominates in the rumen over a wide range of diets (Van Gylswyk and Murphy, 1993). Numbers of both species increase on high-roughage rations (Oshio et al., 1987). According to Avguštin et al. (1997), ruminal isolates of the *Prevotella* genus belong to two redefined (*P. ruminicola, P. brevis*) and two newly defined species (*P. bryantii, P. albensis*). The strain AR29 ferments xylose, sucrose

and N-acetylglucosamine, which are substrates not utilized by strains of *P. brevis*, *P. albensis* and *P. bryantii* species, respectively. In our opinion, this isolate can be classified as *P. ruminicola* or *P. ruminicola*-like. Fermentation characteristics of strain AR29 differ somewhat from those of the type strain 23 (ATCC 19189), but, as mentioned by Avguštin et al. (1997), *P. ruminicola* strains showed quite variable fermentation reactions.

Metabolite profiles of different carbohydrates differ in the rumen and in mixed cultures of rumen microorganisms. In general, fermentation of pentoses yields more acetate and less butyrate than fermentation of hexoses (Sutton, 1968). In this study, *P. ruminicola* AR29 metabolites (except fumarate) were the same on glucose and xylose. Fumarate, however, was a minor fermentation end-product. Also cell composition, growth yields and ratio of metabolite carbon to cell dry weight were not significantly different on both substrates. The rate of growth of *P. ruminicola* AR29 was significantly lower on xylose than on glucose, which may be caused by slower transport of xylose into cells. The average cell dry matter yields (48.8 and 43.1 g per mol of glucose and xylose, respectively) were lower than those found by other authors. Values reported in the literature are 71 g (glucose) and 61 g (xylose) per mol of substrate utilized (Howlett et al., 1976; Turner and Roberton, 1979).

In dual-substrate fermentation, *P. ruminicola* AR29 exhibited substrate preference, because glucose was utilized during the first phase of growth. In contrast with this, *P. ruminicola* B_{14} (currently classified as *Prevotella bryantii*) utilized glucose and xylose simultaneously (Strobel, 1993).

There is no doubt that glycolysis is the major pathway of fermentation of hexoses and pentoses in the rumen. The radioisotope studies of Wallnöffer et al. (1966) indicated that in rumen fluid, 75% of ^{14}C-xylose was metabolized via hexose synthesis and 25% via the phosphoketolase route. Attempts to identity rumen organisms with phosphoketolase activity met with limited success. The possibility of minor phosphoketolase activity in *P. ruminicola* B_{14} was suggested by Turner and Roberton (1979), but rejected later by Matte et al. (1992). Phosphoketolase activity was found in *B. fibrisolvens* CE51 cultured on xylose, but not in five other *B. fibrisolvens* strains tested (Marounek and Dušková, 1995). High phosphoketolase activity was detected in four strains of *Fibrobacter succinogenes* with ribose-5-phosphate as the substrate (Matheron et al., 1997). In contrast, the authors report the absence of the Entner-Doudoroff pathway in these strains, as observed also in strain *P. ruminicola* B_{14} (Mountfort and Roberton, 1978), and in *P. ruminicola* AR29 in the present study. In our opinion, the different composition of xylose metabolites in mixed cultures of rumen microorganisms is attributable rather to the selection of microbial strains than to the involvement of metabolic routes other than glycolysis.

CONCLUSIONS

It can be concluded from our results that *P. ruminicola* AR29 fermented glucose and xylose via the Embden-Meyerhof glycolytic pathway. To a negligible extent, the phosphoketolase route participated in the metabolism of xylose. Glucose in glucose-plus-xylose medium was used preferentially. Both carbohydrates were converted to the same metabolites with the same growth yield of cells. No evidence for the presence of the Entner-Doudoroff pathway in *P. ruminicola* AR29 was found.

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STRESZCZENIE

Fermentacja glukozy i ksylozy przez *Prevotella ruminicola* AR29

Prevotella ruminicola, fibrolityczna bakteria żwaczowa, rozkłada kompleks węglowodanowy i fermentuje powstające podczas tego procesu heksozy i pentozy. Kultury szczepu AR29 rosły szybciej na podłożu z glukozy niż ksylozy, lecz wytwarzały niemal takie same metabolity na obydwóch źródłach węgla. Glukoza z pożywki glukoza + ksyloza była wykorzystywana preferencyjnie. Produkcja suchej masy komórek, wydajność przyrostów s.m. i białka oraz skład komórek (węglowodany i białko) nie różniły się istotnie między kulturami hodowanymi na podłożu z glukozyą lub ksylozą. W obydwóch przypadkach stwierdzono wysoką aktywność aldolazy (EC 4.1.2.13), natomiast aktywność fosfoketolazy (EC 4.1.2.9) była bardzo niska w komórkach hodowanych na ksylozie. Nie stwierdzono aktywności enzymów przemian Entner-Doudoroff (dehydrazy 6-fosfoglukonianowej i aldolazy 2-keto-3-deoxy-6-fosfoglukonianowej).