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Utilization of Glucose and Xylose in Ruminal Strains of *Butyrivibrio fibrisolvens*

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The dual-substrate utilization pattern in cultures of five ruminal strains of *Butyrivibrio fibrisolvens* growing on glucose and xylose was investigated. Strains ATCC 19171 and 86 utilized glucose and xylose simultaneously. Other strains exhibited diauxic growth. Strains X1 and CE 51 exhibited classical diauxic growth in which glucose was utilized during the first phase. Strain X2D62 displayed atypical diauxic growth in which slow utilization of xylose was followed by rapid utilization of glucose after the xylose depletion. The ATP-dependent phosphorylation of glucose was found in all strains tested. The phosphoenolpyruvate-dependent phosphorylation of glucose was detected only in *B. fibrisolvens* CE 51.

Glucose and xylose are two major monomeric units of carbohydrate constituents that occur in plants (4). Both sugars are released in the process of microbial digestion of plant matter in the rumen and are eventually utilized inside microbial cells. The purpose of the present study was to investigate the utilization of glucose and xylose in cultures of *Butyrivibrio fibrisolvens* to determine the dual-substrate utilization pattern in this bacterium. This report also shows how *B. fibrisolvens* strains phosphorylate glucose. *B. fibrisolvens* is ubiquitous in the gastrointestinal tracts of mammals (2). In the rumen, *B. fibrisolvens* is one of the most common bacteria in animals fed widely different diets (3, 10). Five strains of *B. fibrisolvens* were used in this study. Type strain 19171 was obtained from the American Type Culture Collection. Strain 86 was a gift from the Hannah Research Institute, Ayr, Scotland. Strain X1 was isolated from the rumen fluid of a sheep at this institute. Strains CE 51 and X2D62 were obtained from the National Chemical Research Laboratory, Pretoria, South Africa. Bacteria were grown at pH 6.5 in LF2 fermentors on a vitamin-mineral medium with 10% rumen fluid. Growth medium and culture conditions were described previously (11). Glucose and xylose were added at 2 g/liter each. At regular time intervals samples were removed for the measurement of optical density and the determination of glucose and xylose concentrations. Glucose was estimated by the glucose oxidase-peroxidase method with a commercial kit (Lachema, Brno, Czech Republic), and xylose was estimated by using the orcinol reagent (5).

The method used to measure ATP- and phosphoenolpyruvate (PEP)-dependent phosphorylation of glucose was that of Martin and Russell (12). The assay was conducted with cells which were harvested at the end of the logarithmic phase, resuspended in a 0.1 M phosphate buffer, and treated with a toluene-ethanol mixture. Sodium PEP and ATP were purchased from Sigma (St. Louis, Mo.), and D-[U-^{14}C]glucose (8,262 MBq/mmol) was purchased from the Institute for Research, Production and Application of Radioisotopes (ÚVVVR) (Prague, Czech Republic). All incubations were performed in triplicate. Protein from 1 M NaOH-hydrolyzed cells was determined according to the method of Lowry presented by Herbert et al. (5).

Two strains (ATCC 19171 and 86) utilized glucose and xylose simultaneously. Strains X1 and CE 51 preferred glucose to xylose. Strain X2D62 preferred xylose to glucose (Fig. 1). Only strain CE 51 showed PEP-dependent phosphorylation of

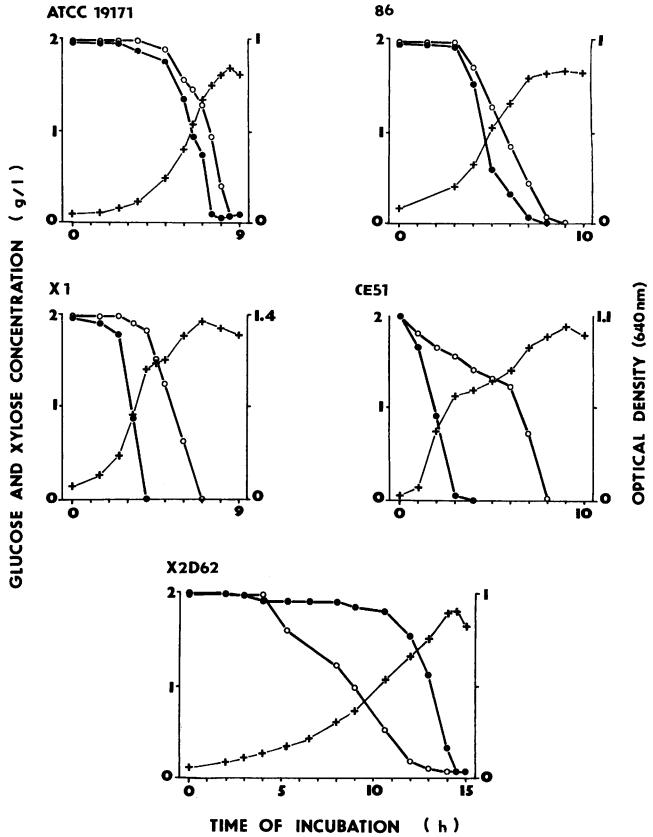

FIG. 1. Time course of glucose (●) and xylose (○) utilization in cultures of five strains of *B. fibrisolvens*. Cell density (+) was monitored turbidimetrically at 640 nm.

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TABLE 1. Specific activities of glucose phosphorylation with PEP or ATP as a phosphoryl donor in *B. fibrisolvens* strains

Strain	Specific activity[a] with donor		
	None	PEP	ATP
X1	0.25 ± 0.08	0.25 ± 0.06	0.64 ± 0.07
X2D62	1.57 ± 0.15	0.89 ± 0.02	106.81 ± 10.0
CE 51	0.59 ± 0.02	1.10 ± 0.07	1.21 ± 0.03
86	13.63 ± 0.90	1.55 ± 0.24	190.92 ± 9.99
ATCC 19171	2.55 ± 0.14	1.18 ± 0.11	16.25 ± 0.03

[a] Nanomoles of glucose phosphorylated per milligram of protein per minute. Values are means of three incubations ± standard deviations.

glucose, which is indicative of the PEP-glucose phosphotransferase system (PEP-PTS). The ATP-dependent phosphorylation of glucose was found in all strains (Table 1). In a previous study, Martin and Russell (12) found in *B. fibrisolvens* 49 very low PEP-PTS activity for glucose and relatively much higher ATP-dependent phosphorylation.

The mixed-substrate utilization pattern is of particular importance in the rumen, as microbes in their natural environment have more than one substrate at their disposal. When glucose was present in a mixture of substrates, the utilization of xylose was generally inhibited (1, 6–8). In many bacteria the sugar substrates of the PTS (e.g., glucose) inhibit the uptake of non-PTS substrates (e.g., xylose). The PEP-PTS, which is restricted to procaryotic organisms, is energetically beneficial because the sugar is transported and phosphorylated simultaneously (13, 15). This regulation allows the bacterium to select preferred carbon sources when more than one is present in the medium. In the rumen some microorganisms utilize glucose and xylose simultaneously. Russell and Baldwin (14) reported simultaneous utilization of glucose and xylose by the rumen bacteria *Selenomonas ruminantium* HD4 and *B. fibrisolvens* A38. Strain R1 of an anaerobic rumen fungus, a *Neocallimastix* sp., has also been reported to utilize glucose and xylose simultaneously (9). In this study, two of five strains of *B. fibrisolvens* utilized both substrates simultaneously, and three strains exhibited diauxic growth. Strain X1 exhibited classical diauxic characteristics, because glucose was utilized during the first phase of growth. Strain CE 51, which was the only strain with a PEP-PTS activity, utilized xylose slowly in the presence of glucose and rapidly when glucose disappeared from the medium. Strain X2D62 displayed atypical diauxic growth in which slow utilization of xylose was followed by rapid utilization of glucose after the xylose depletion. Our data suggest that *B. fibrisolvens* strains possess catabolic regulatory systems involved in the metabolism of carbohydrates with different modes of action. No general correlation between the dual-substrate utilization pattern and glucose phosphorylation was found.

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