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Preliminary assessment of the capability of the rumen bacterium, *Butyrivibrio fibrisolvens,* to utilize fructose polymers for growth

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ABSTRACT

The rumen bacterium *Butyrivibrio fibrisolvens* was able to grow *in vitro* when timothy grass fructan, inulin, inulooligosaccharides, sucrose, or fructose was the sole source of carbon in the culture medium, and utilized 89.9, 47.7, 80.9, 87.9 and 94.5% of the carbohydrate, respectively. The distinctly lower utilization of inulin than other carbohydrates was accompanied by a considerably lower bacteria count in the cultures. Examination of an extract from bacterial cells showed that its fructanolytic activity was closely related to the carbon source in the growth medium. The number of fructanolytic enzymes synthesized by the bacteria depended on the carbon source in the growth medium.

KEY WORDS: *Butyrivibrio fibrisolvens*, fructans, fructanolytic enzymes

INTRODUCTION

The bacterium *Butyrivibrio fibrisolvens* represents a significant part of the microbial population in the rumen and its role in the digestion of such plant carbohydrates as cellulose, xylan, pectin, and starch has been widely studied (Stewart et al., 1997). In contrast, very little is known about the ability of these bacteria to utilize fructans (Ziołecki et al., 1992), which are important storage carbohydrates in grasses and *Compositae* and consist of fructofuranosyl residues linked by either β-2,6 (levans) or β-2,1 (inulins) glycosidic bonds. The objective of this study was, therefore, to examine the ability of *B. fibrisolvens* to utilize various fructose polymers.

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MATERIAL AND METHODS

Butyrivibrio fibrisolvens strain 3 bacteria isolated from ovine rumen (Ziołecki et al., 1992) were cultured in an anaerobic medium (Hungate, 1969) supplemented with 0.5% (w/v) of timothy grass fructan, inulin, inulo-oligosaccharides, sucrose, or fructose as the sole source of energy. The bacteria were grown for 24 h. At the end of the incubation period, the optical density of the cultures was determined by measuring absorbance at 660 nm and the carbohydrate concentration in the medium was determined by the anthrone method.

The fructanolytic properties of bacteria were evaluated by quantification of reducing sugars released from the particular carbohydrates following incubation (20 h, 40°C) with a cell-free extract in 0.02 M sodium phosphate buffer (pH 6.0). The cell-free extract was prepared by sonication and centrifugation (23 000 *g* for 15 min at 4°C) of disrupted bacteria.

Reducing sugars were determined using the dinitrosalicylic acid reagent (Miller et al., 1960). Identification of fructanolytic enzymes was performed by native polyacrylamide gel electrophoresis (PAGE) in combination with zymogram (Gabriel and Wang, 1969).

RESULTS AND DISCUSSION

Bacteria were able to grow on all five carbohydrates used as sole sources of carbon in culture media. The density of the bacterial population grown on inulin and fructose was, however, significantly lower ($P<0.05$) when compared with the culture maintained on timothy grass fructan, inulooligosaccharides, or sucrose (Table 1).

Table 1. Bacterial population density (absorbance at 660 nm) and substrate utilization (% of initial content) after 24 h of incubation of *B. fibrisolvens* on media supplemented with different carbohydrates. Mean values (n=4)

Item	Carbon source in the culture medium					
	T.g. f[1]	inulin	I.o.s.[2]	sucrose	fructose	mean SD
Population density	1.07[a]	0.69[b]	1.20[a]	1.07[a]	0.81[b]	0.107
Substrate utilization	89.9[a]	47.7[c]	80.9[b]	87.9[ab]	94.5[a]	5.75

[1] timothy grass fructan; [2] I.o.s. inulooligosaccharides
values in rows marked with different letters differ significantly ($P<0.05$)

The lower number of bacteria growing on inulin was accompanied by significantly restricted utilization of this fructan ($P<0.05$). A similar relationship was found earlier in the rumen bacteria, *Treponema* sp. (Kasperowicz, 2005).

Utilization of fructose was not, however, lower despite the restricted growth of bacteria on the medium with this carbohydrate. Further studies are thus needed.

Identification of fructanolytic enzymes of *B. fibrisolvens* revealed that their synthesis was closely related to the carbon source in the growth medium (Table 2).

Table. 2 Fructanolytic enzyme activity of the bacterial cell extract in relation to the carbohydrate added to growth medium (nM fructose /mg protein/h)

Carbon source in growth medium	Degraded carbohydrates		
	T.g. fructan	inulin	sucrose
T.g. fructan	2529.7	235.7	1563.6
Inulin	151.2	142.3	2475.3
Sucrose	66.7	77.1	2400.4
Fructose	6.9	18.4	213.7

This suggests the inducible character of this protein(s), with the exception of a nonspecific enzyme capable of degrading both the β-2,1 and β-2,6 glycosidic linkages in the chain of fructose residues (Uchiyama, 1993). This suggestion is supported by the results of zymography (Figure 1).

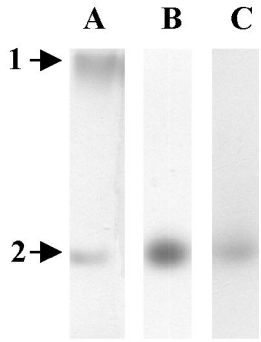

Figure 1. Zymogram of timothy grass fructan (A) and sucrose (B,C) degrading enzymes of *Butyrivibrio fibrisolvens* bacteria growing in a medium supplemented either with timothy grass fructan (A,B) or sucrose (C). 1- specific enzyme, 2 - nonspecific enzyme

It was found that bacteria growing on the medium with timothy grass fructan synthesized an enzyme specifically degrading this polysaccharide and a second one that was able to digest timothy grass fructan and sucrose. The former enzyme was not, however, present in the cell-free extract when bacteria were grown on sucrose. Thus, *Butyrivibrio fibrisolvens* strain 3 differs from some strains of ruminal treponemes that synthesize specific and nonspecific fructanolytic enzymes regardless of the carbon source in the growth medium (Kasperowicz, 2005).

CONCLUSIONS

Butyrivibrio fibrisolvens strain 3 belongs to species utilizing fructose polymers for growth. The obtained results suggest that it synthesizes an enzyme specifically digesting β-2,6 glycosidic linkages between fructose residues and also an enzyme splitting β-2,6 and β-2,1 linkages. On the other hand, the performed studies suggest that the carbon source present in the medium used for cultivation of bacteria can be the factor inducing the synthesis of some fructanolytic enzymes.

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