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Is Fiber Digestion in the Rumen Reduced by Catabolite Repression?

Kevin L. Anderson and Vincent H. Varel¹

Introduction

Bacteria which degrade cellulose play a key role in animal digestion of plant material. As with all bacteria, these cellulolytic bacteria are able to regulate their growth and enzymatic activity by a number of mechanisms. One of these regulatory mechanisms may be catabolite repression.

This repression refers to the ability of certain bacteria to stop the metabolism of one substrate in preference to a second substrate. Sometimes termed "glucose effect," this repression is observed, for example, as the bacterium *Escherichia coli* grows on lactose. When glucose is then added to the growth medium, *E. coli* will stop utilizing lactose and use glucose instead. Thus, glucose causes a repression of lactose utilization.

Since soluble carbohydrates may be present in the microecosystem of cellulolytic bacteria, especially near the site of plant degradation, there is a potential for catabolite repression. This would negatively affect bacterial cellulolytic activity, thereby reducing efficiency of ruminal plant digestion. However, it is not clear which, if any, cellulolytic bacteria are capable of catabolite repression. Previous studies have not been conclusive because of a variety of confounding factors resulting from the experimental design. These include difficulty of adequately measuring substrate depletion, metabolic effect of other regulatory systems, and inhibition of cellulolytic activity by a decreasing pH of the growth medium.

Glucose analogs are chemical compounds which have the ability to "trick" the bacterial cell into thinking it is a food substrate, when in fact the analog is an imitation compound which the cell cannot metabolize for growth. Therefore, a small concentration of an analog can simulate the regulatory effect of glucose without serving as a growth substrate or interfering with carbohydrate transport. Since energy metabolism and bacterial growth will result only from cellulose degradation, many confounding factors are eliminated.

Procedure

Several strains of ruminal and nonruminal anaerobic (growth without oxygen) cellulolytic bacteria were studied (see Table 1). All strains were grown in a basal medium containing 20% incubated rumen fluid. As the sole carbohydrate source, 0.5% glucose, fructose, xylose, maltose, or 1% balled-milled cellulose was added to the medium.

All bacterial strains were grown for at least 12 generations on a noncellulose carbohydrate substrate prior to inoculation of cellulose medium. When a strain could grow on a sugar other than glucose, this compound was used as an inducing substrate (see Table 1).

Each strain was then inoculated into six bottles of the basal medium (30 ml) containing 1% cellulose. As a treatment group, the analog methyl-glucose was then added to three of these bottles. All bottles were incubated at 102°F while being continually agitated.

Periodically 1.5 ml was removed from each bottle, filtered, and analyzed for fermentation products. Since growth resulted only from cellulose degradation, the rate of production of these fermentation products was used as an indicator of metabolic activity. Concentrations of this metabolite from the treatment group were compared to those of the respective control group. Only treatment groups showing almost complete repression of metabolic activity for at least a 24 h period were identified as catabolite repression.

Results

Eight species of anaerobic cellulolytic bacteria were studied for their ability for catabolite repression. Under the experimental conditions used, only the rumen strain *C. longisporum* OC4 was found to exhibit catabolite repression. This repression was most pronounced when OC4 was grown on the sugar fructose (see Figure 1), apparently because this required the cells to induce the maximum number of cellulolytic genes while in the presence of the glucose analog. Although *C. longisporum* OC4 is a ruminal isolate, it is not routinely found in the rumen. Its susceptibility to catabolite repression offers one explanation why it does not appear to be a common cellulolytic bacterium of the rumen.

This study suggests that catabolite repression is not a significant factor in cellulolytic degradation. In fact, several of the strains studied grew better on cellulose than on soluble carbohydrates. This suggests that cellulose was preferred over other substrates, even glucose. Furthermore, *Clostridium* sp. 54408 and *C. polysaccharolyticum* B do not use glucose as a substrate. Therefore, their lack of response to the glucose analog was predictable.

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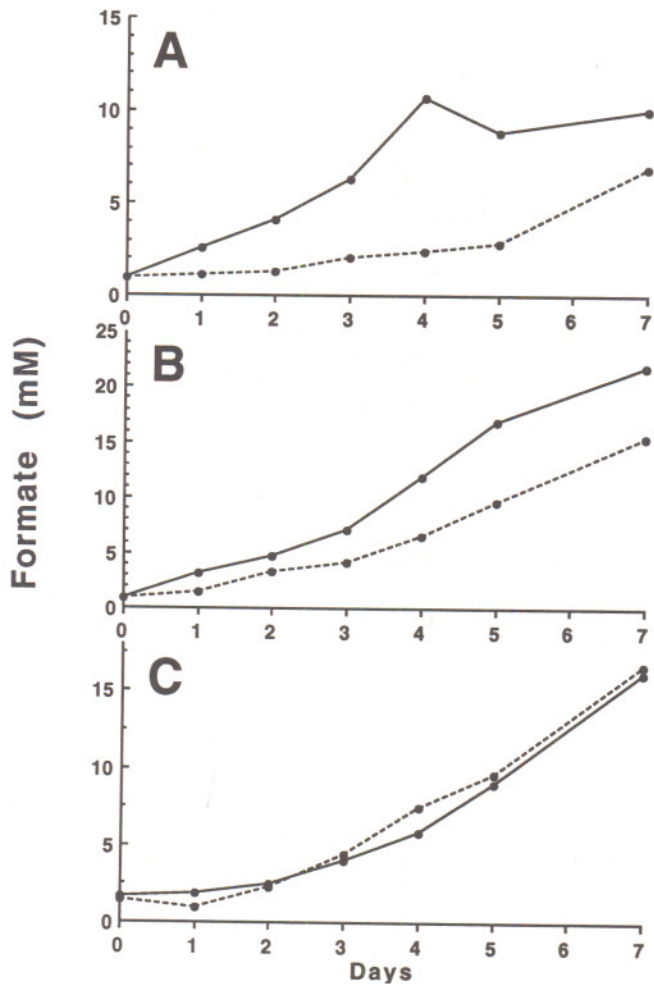


Figure 1 – Formate production by *Clostridium longisporum* OC4 grown on cellulose (—) and cellulose + 2 mM methyl glucose (---). Cells were grown at least 12 generations on a) fructose, b) glucose, c) cellobiose before inoculation into cellulose medium.

Table 1—Cellulolytic bacterial strains studied. Cells were fully induced (12 generations) on respective substrates prior to inoculation into cellulose medium. Growth on cellulose was determined by measuring the increase of the respective metabolite

Bacterium	Substrate	Metabolite	Repression ^a
<i>Costridium cellulolyticum</i> H10	fructose	acetate	-
<i>Clostridium cellobioparum</i> 3359	glucose	acetate	-
<i>Clostridium polysaccharolyticum</i> B	xylose	formate	-
<i>Clostridium longisporum</i> OC4	fructose	formate	+
<i>Clostridium</i> sp. 54408	maltose	butyrate	-
<i>Eubacterium cellulosolvens</i> 5494	fructose	butyrate	-
<i>Ruminococcus albus</i> 7	fructose	acetate	-
<i>Fibrobacter succinogenes</i> S85	glucose	succinate	-

^a Detection of catabolite repression: (-) not detected, (+) detected.