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Carbohydrate Repression of Catalase Synthesis in *Bacteroides fragilis*

E. M. GREGORY,* B. J. VELTRI, DEBRA L. WAGNER, AND TRACY D. WILKINS

Department of Biochemistry and Nutrition and The Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

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Catalase formation by Bacteroides fragilis was immediately stopped upon addition of glucose to a culture growing in peptone medium. Each of eight other carbohydrates fermented by the organism also repressed catalase formation. Without added carbohydrate, the strains produced relatively large amounts of catalase (25 to 50 U/mg of protein).

Some anaerobic bacteria such as many of the propionibacteria produce appreciable amounts of catalase which, when present, can be useful for their identification. Catalase also is produced by *Bacteroides fragilis*, but only when the organism is supplied with sufficient amounts of hemin. Catalase formation by *B. fragilis* has been considered a variable characteristic (2), partly because the hemin requirement was not recognized until the last few years. During the course of some experiments designed to study the induction of catalase synthesis by hemin, we found that the carbohydrate composition of the medium was as important as the hemin concentration. This also has been briefly noted by Stargel et al. (3) in their recent report of tests using a miniaturized system for identification of anaerobic bacteria; a stronger bubbling reaction with H_2O_2 occurred in wells containing the control culture in basal peptone medium than in those containing a carbohydrate-based medium. We have investigated this phenomenon in more detail in regard to the actual changes in specific activity of the enzyme.

The type strain of *B. fragilis* (ATCC 25285, NCTC 9343) was used for all of the experiments reported in this note. These results have also been confirmed with five other strains of *B. fragilis* and five strains each of *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron*. These 20 strains were from the Virginia Polytechnic Institute (VPI) Anaerobe Laboratory culture collection. Cultures were transferred and maintained in prereduced media in CO_2-filled tubes; the composition of the media and details for anaerobic procedures are described in the VPI *Anaerobe Laboratory Manual* (2). All media contained 5 μg of hemin per ml. Induction experiments were performed in a fermenter (Microferm, New Brunswick, N.J.) with automatic pH control (pH 6.5; NaOH) with a constant slow flush of N_2. Cells were harvested by centrifugation at 16,300 $\times$ *g* for 10 min, washed in 50 mM sodium phosphate, pH 7.0, and disrupted by sonic treatment. The cell debris was removed by centrifugation at 34,800 $\times$ *g* for 15 min, and the supernatant was assayed for catalase activity by the method of Beers and Sizer (1). The protein content of the crude lysate was estimated from the optical density at 280 nm, assuming an extinction of 1 ml/mg per cm, and the specific activity of the enzyme was calculated.

B. fragilis was grown in peptone yeast extract and chopped meat broth (2), and the specific activity of catalase in the cells was compared with that of cells grown in peptone yeast extract-glucose and chopped meat-glucose broth. In repeated experiments, the cells grown with glucose (0.5%, wt/vol) varied from 0 to 6 U of catalase per mg of protein, but the cells grown without glucose had 25 to 50 U per mg of protein.

We then tested the effect of adding glucose to a culture growing in peptone yeast extract broth. This experiment was done in a fermenter with the pH controlled (pH 6.5) to insure that the effect of glucose was not due to the lower pH obtained in glucose cultures. Upon addition of glucose (0.5%, wt/vol), production of catalase by the cells appeared to stop almost immediately (Fig. 1). The residual catalase already in the cells was not destroyed but was diluted as the cells multiplied without producing more of the enzyme.

To determine whether this was a specific effect of glucose, we grew *B. fragilis* in tubes of peptone yeast extract broth supplemented with either lactose, fructose, raffinose, starch, mannose, sucrose, galactose, or maltose. Each of these carbohydrates was utilized by the organism, and all of them repressed the formation of catalase.

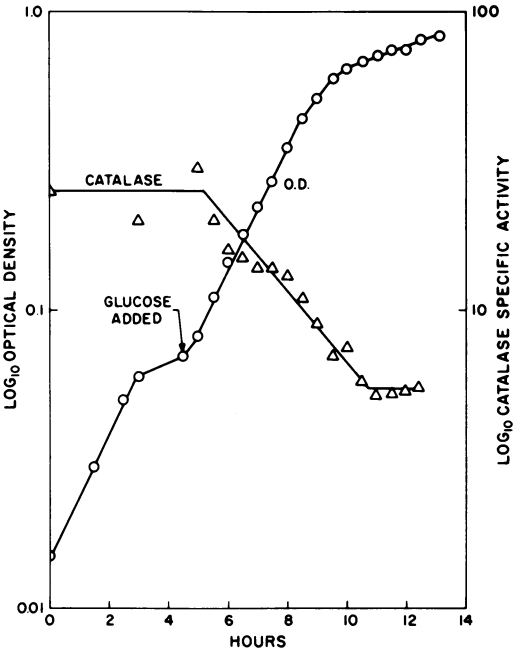

FIG. 1. *Effect on specific activity of catalase, caused by addition of glucose to a culture growing in peptone broth.*

This repression of catalase formation by fermentable carbohydrates must be taken into account when testing *Bacteroides* for catalase activity. We have found that chopped meat broth cultures are reproducibly catalase positive when equal volumes of culture and 3% H_2O_2 are mixed in either a small test tube or the well of a microtiter plate.

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