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Production of an Inducible Sucrase Activity by *Serpulina hyodysenteriae*

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Strains of *Serpulina hyodysenteriae* and *Serpulina innocens* produced a cell-associated sucrase activity when grown in a medium containing sucrose. *S. hyodysenteriae* B204 sucrase activity cleaved sucrose and, to a lesser extent, raffinose and had a pH optimum of 5.7 to 6.2. This is the first report of an inducible enzyme produced by either *S. hyodysenteriae* or *S. innocens*.

Serpulina hyodysenteriae is an anaerobic spirochete found in the large intestines of pigs, and it is the causative agent of the disease swine dysentery (7, 18). The pathogenesis of the disease requires that *S. hyodysenteriae* cells survive and multiply at the mucosal surface of the intestine. To persist, the spirochete must be able to utilize the substrates available to it in this environment for growth and energy production. However, little is known about the substrates used by *S. hyodysenteriae* during colonization of the swine intestinal tract. Such information would contribute to our understanding of the pathogenesis of the disease and could lead to the development of methods to control swine dysentery.

Sucrose utilization by *S. hyodysenteriae* was chosen as a system which would provide information about the ability of *S. hyodysenteriae* to ferment sugars other than glucose and about the regulation of the metabolic enzymes involved. Several other saccharolytic spirochetes can utilize sucrose as a carbon and energy source. These include *Treponema saccharophilum* (13), *Treponema bryantii* (15), and several *Spirochaeta* species (1, 4). However, there is no information concerning whether these spirochetes regulate their protein expression in the presence of sucrose.

We hope to gain an understanding of the mechanisms that *S. hyodysenteriae* employs in controlling protein expression by studying an enzymatic system that *S. hyodysenteriae* regulates on the basis of an external factor (i.e., the presence or absence of sucrose in the culture medium). Preliminary studies indicated that *S. hyodysenteriae* produced an intracellular sucrase activity when grown in media containing sucrose (8). This was the first identification of a regulated enzyme in *S. hyodysenteriae*. The work presented here describes the production and regulation of this *S. hyodysenteriae* sucrase activity.

Serpulina strains, their serotypes, and the countries in which they were isolated are presented in Table 1. *S. hyodysenteriae* and *Serpulina innocens* strains were routinely grown in brain heart infusion broth to which serum was added at 39°C under a 99% N_2–1% O_2 atmosphere as described previously (16). For studies examining the effects of different sugars on the production of sucrase activity, heart infusion broth containing 5% fetal calf serum (HIS) was used as a basal medium, since it did not contain any added carbohydrates (17). Sugars were added to the medium at a final concentration of 0.4% (wt/vol). Spirochetes were grown to an optical density at 620 nm (OD_{620}) of 0.8 to 1.2 (approximately 1×10^8 to 3×10^8 cells per ml; 18-mm lightpath).

Cell lysates were prepared by centrifuging (12,100 × *g*, 10 min, 4°C) cells from HIS cultures (7 ml). Cells were resuspended in 7 ml of Tris-buffered saline (10 mM Tris-HCl [pH 7.4] plus 150 mM NaCl), recentrifuged, resuspended in 2.0 ml of 10 mM Tris-HCl (pH 7.4) buffer, and sonicated (Branson model 250 Sonifier) with three 30-s bursts on setting number 1. Cells were cooled on ice for 1 min between the sonications. Cell debris was removed by centrifugation (12,100 × *g*, 30 min, 4°C). Cell lysates were either used immediately or frozen (−20°C) for future use.

Sucrase assay reaction mixtures contained the following (in a 0.2-ml volume): citrate-PO_4 buffer (pH 6.0), 20 μmol; sucrose, 20 μmol; and diluted cell sonicate, 100 μl (5 to 15 μg of protein). Assays were incubated at 37°C for 60 min, and then the amounts of reducing sugars released were measured by the Nelson-Somogyi reducing sugar assay (12). The assay of sucrase activity was linear over the 60-min incubation period. Glucose was used as the reducing sugar standard. All assays were carried out in duplicate, with a portion of each cell lysate sample inactivated in a boiling water bath for 4 min and used as a negative control. When known amounts of reducing sugars (glucose or fructose) were added to heated and unheated cell lysates to measure the decrease in concentration of sucrase assay end products, no decrease in reducing sugar concentration was detected. One unit of sucrase activity is that amount of enzyme required to produce 1 μmol of reducing sugar equivalent per minute. The specific activity of sucrase is expressed as units of sucrase activity per milligram of protein. The same assay was used to determine the ability of *S. hyodysenteriae* B204 cell lysate to hydrolyze other disaccharides and trisaccharides by using raffinose, trehalose, or melezitose in place of sucrose. In all assays the sugar was present at a concentration of 20 μmol in a 0.2-ml reaction mixture. Protein concentrations were determined by using a modified Lowry protein assay (14).

For determinations of the pH optimum of *S. hyodysenteriae* sucrase activity, the citrate-PO_4 buffer was adjusted to pH values between 4.2 and 7.2 (6).

Sucrase activity was also assayed qualitatively by nondenaturing polyacrylamide gel electrophoresis. Cell lysates were mixed 1:1 with nondenaturing sample buffer (125 mM Tris-HCl [pH 6.8], 20% glycerol, 0.0025% bromophenol blue) and loaded onto a prepoured polyacrylamide gradient (4 to 15%) gel (Mini-PROTEAN II Ready Gels; Bio-Rad Laboratories, Richmond, Calif.). Gels were run at 100 V until the bromo-

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TABLE 1. *Serpulina* species used in sucrase activity studies

Strain	Serotype	Origin	Reference
S. hyodysenteriae			
B78[a]	1	United States	3
B204	2	United States	3
B169	3	Canada	3
A-1	4	United Kingdom	3
B8044	5	United States	11
B6933	6	United States	11
Ack 300/8	7	The Netherlands	11
S. innocens			
B256[a]	NS[b]	United States	10
4/71	NS	United Kingdom	10

[a] Type strain.
[b] NS, no serotype.

phenol blue dye reached the end of the gel (approximately 100 min) and then stained for sucrase activity as described by Gabriel and Wang (5).

Sucrase activity production over time was measured by growing *S. hyodysenteriae* in a medium (200 ml) consisting of HIS plus 0.2% glucose to an OD_{620} of 0.9. Then either sucrose (see Fig. 3A) or glucose (see Fig. 3B) was added to a final concentration of 0.4%. At 0, 30, 60, 90, 120, 240, and 480 min, the OD_{620} was measured and two 7.0-ml samples were removed. Sucrase activity was obtained by a chloroform extraction procedure (2) which releases all sucrase activity from *Serpulina* sp. cells.

***S. hyodysenteriae* and *S. innocens* sucrase activity.** *S. hyodysenteriae* strains representing seven serotypes from four countries, and two *S. innocens* strains, all produced sucrase activity when grown in HIS medium containing sucrose, but only low levels were produced in cells grown in HIS medium containing glucose (Fig. 1). The levels of sucrase activity produced varied among *Serpulina* strains. Increases in sucrase activity produced by sucrose-grown cells compared with that produced by glucose-grown cells ranged from 4.6-fold in *S. innocens* 4/71 to a high of 45.9-fold in *S. innocens* B256. *S. hyodysenteriae* B204 was used to further study the production of this inducible sucrase activity.

Sucrase activity produced by *S. hyodysenteriae* B204 had a

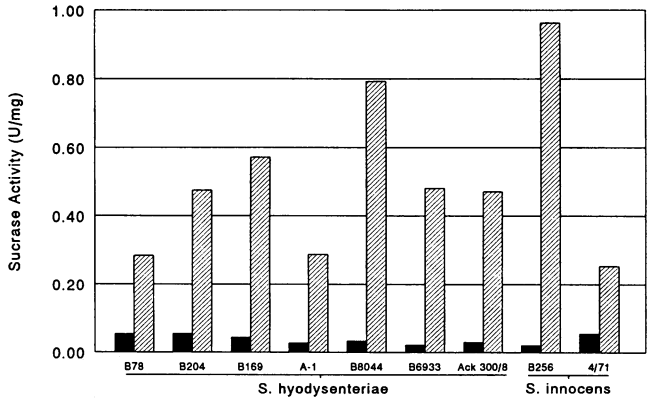

FIG. 1. Sucrase activity produced by *S. hyodysenteriae* and *S. innocens* strains grown with either glucose or sucrose as the carbon source. Spirochete strains were grown in HIS containing 0.4% (final concentration) sucrose or glucose. ■, glucose; ▨, sucrose.

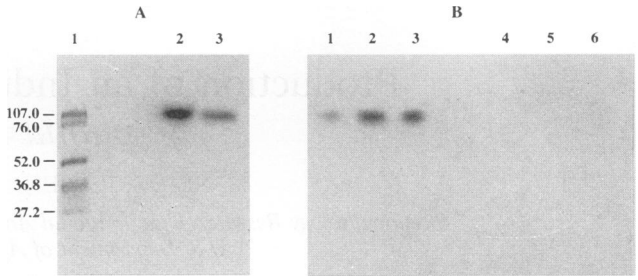

FIG. 2. Polyacrylamide gradient (4 to 15%) nondenaturing gel stained for sucrase activity. (A) Lane 1, low-molecular-weight prestained size markers; lane 2, 5.0 U of sucrase activity (229.4 μg of protein); lane 3, 2.0 U of sucrase activity (91.8 μg of protein). (B) Comparison of sucrase activity bands from *S. hyodysenteriae* cells grown in HIS plus sucrose (lanes 1 through 3) and *S. hyodysenteriae* cells grown in HIS plus glucose (lanes 4 through 6). Lanes 1 and 4, 57.25 μg of protein; lanes 2 and 5, 114.5 μg of protein; lanes 3 and 6, 229 μg of protein.

pH optimum of 5.7 to 6.2. No extracellular sucrase activity was detected in culture supernatant fluids following 10-fold concentration by ultrafiltration using a 10,000-molecular-weight filter (Centricon-10; Amicon, Inc., Beverly, Mass.), indicating that all sucrase activity was cell associated.

The abilities of sugars other than sucrose to induce sucrase activity production were tested by growing *S. hyodysenteriae* B204 in HIS medium containing sucrose, fructose, galactose, glucosamine, *N*-acetylglucosamine, glucose, glucuronate, mannose, or trehalose (0.4% final concentration). Sucrose was the only sugar to induce production of sucrase activity. Cells grown in HIS medium containing fructose contained a level of sucrase activity that was only 6.8% of the amount of sucrase activity produced by sucrose-grown cells, while growth on any other sugar resulted in sucrase activity levels that were less than 2.5% of the induced sucrase activity levels.

Cell lysates from *S. hyodysenteriae* B204 cells grown in HIS medium containing sucrose (0.4%) produced high levels of reducing sugars in enzyme assays containing sucrose as the substrate. Assays containing raffinose as the substrate resulted in a level of reducing sugars that was 28% of the amount of reducing sugars produced in sucrose-containing assays, while trehalose- and melezitose-containing assays produced little or no reducing sugars. Raffinose is a trisaccharide consisting of a sucrose molecule bound by an α-1,6-glycosidic bond to galactose. It is possible that the sucrase activity present in the cell lysate is able to cleave the α-1,4 bond in raffinose but is partially inhibited by the presence of the galactose molecule. Alternatively, *S. hyodysenteriae* may produce additional enzymes which can degrade raffinose. Lysates from cells grown in glucose-containing medium produced no reducing sugars in assays containing any of the four substrates.

Sucrase activity-stained polyacrylamide gels. Nondenaturing gradient (4 to 15%) gels stained for *S. hyodysenteriae* B204 sucrase activity exhibited one band of activity (Fig. 2A). This sucrase activity band migrated to a position between that of phosphorylase B (107 kDa) and that of bovine serum albumin (76 kDa) in this gradient gel system. The presence of a single band of sucrase activity indicated either that one sucrase enzyme was produced or, if more than one was produced, that the sizes and charges of the enzymes produced were similar, resulting in one activity band on the gel.

The specificity of the gel assay was demonstrated by electrophoresing samples of cell lysates from sucrose-grown cells

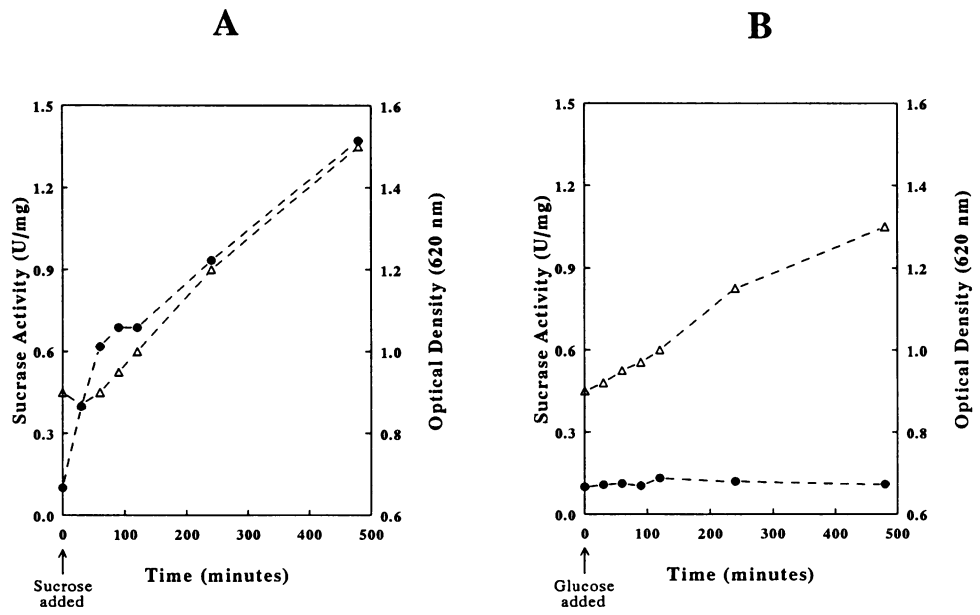

FIG. 3. Production of sucrase activity by *S. hyodysenteriae* over time. *S. hyodysenteriae* B204 was grown in a medium consisting of HIS plus 0.2% glucose to an OD_{620} of 0.9. Then either sucrose (A) or glucose (B) was added. OD_{620} (△) and sucrase activity (●) were measured at 0, 30, 60, 90, 120, 240, and 480 min after the addition of the sugars.

and glucose-grown cells on a gradient (4 to 15%) gel and staining it for sucrase activity. Lysates from sucrose-grown cells resulted in a strong band of activity (Fig. 2B, lanes 1 through 3), while glucose-grown cell lysates had no band of sucrase activity (Fig. 2B, lanes 4 through 6).

Induction of *S. hyodysenteriae* sucrase activity over time. Increased sucrase activity was detected in cultures of *S. hyodysenteriae* B204 within 30 min of sucrose addition (Fig. 3A). No change in levels of sucrase activity was found in cultures to which glucose (0.4% final concentration) was added (Fig. 3B). In both cases cell growth continued, as determined by OD_{620}, indicating that the cells were viable and metabolically active.

Sucrase activity and a recently described hydrogen peroxide-inducible catalase activity (9) are the only two *S. hyodysenteriae* enzymatic activities which have been shown to be inducible. It is not known what role sucrose utilization plays in the ecology and pathogenicity of *S. hyodysenteriae* in the pig intestine. The ability to grow on sucrose may be important to the survival of the spirochete either in the intestine or after it has been excreted in the feces. Or *S. hyodysenteriae* sucrase activity may serve to cleave other, unknown compounds along the mucosal surface of the intestine.

Study of this system will lead to a better understanding of how *S. hyodysenteriae* controls its protein expression in response to environmental conditions. This could also provide valuable information concerning the pathogenic mechanisms of *S. hyodysenteriae*. The environmental conditions encountered by *S. hyodysenteriae* cells in the large intestines of pigs are quite different from the conditions encountered during growth in broth media. It is likely that proteins that are not detectable in in vitro-grown cells are expressed in vivo. Studies of the regulation of protein expression by *S. hyodysenteriae* and of the effects of environmental factors on protein expression will enable us to further understand the disease and will provide insights into how the spirochete survives in the pig intestine.

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REFERENCES

1. **Aksenova, H. Y., F. A. Rainey, P. H. Janssen, G. A. Zavarzin, and H. W. Morgan.** 1992. *Spirochaeta thermophila* sp. nov., an obligately anaerobic, polysaccharolytic, extremely thermophilic bacterium. Int. J. Syst. Bacteriol. **42**:175–177.
2. **Ames, G. F.-L., C. Prody, and S. Kustu.** 1984. Simple, rapid, and quantitative release of periplasmic proteins by chloroform. J. Bacteriol. **160**:1181–1183.
3. **Baum, D. H., and L. A. Joens.** 1979. Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect. Immun. **25**:792–796.
4. **Canale-Parola, E.** 1984. Genus I. *Spirochaeta*, p. 39–46. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
5. **Gabriel, O., and S.-F. Wang.** 1969. Determination of enzymatic activity in polyacrylamide gels. I. Enzymes catalyzing the conversion of nonreducing substrates to reducing products. Anal. Biochem. **27**:545–554.
6. **Gomori, G.** 1955. Preparation of buffers for use in enzyme studies. Methods Enzymol. **1**:138–146.
7. **Harris, D. L., R. D. Glock, C. R. Christensen, and J. M. Kinyon.** 1972. Swine dysentery. I. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. Vet. Med. Small Anim. Clin. **67**:61–64.
8. **Jensen, N. S., and T. B. Stanton.** 1992. Production and regulation of sucrase activity by *Serpulina* (*Treponema*) *hyodysenteriae*, abstr. K-133, p. 278. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992. American Society for Microbiology, Washington, D.C.
9. **Jensen, N. S., and T. B. Stanton.** 1993. Production of a hydrogen peroxide-inducible catalase activity by *Serpulina hyodysenteriae*, abstr. D-175, p. 126. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993. American Society for Microbiology, Washington, D.C.
10. **Kinyon, J. M., and D. L. Harris.** 1979. *Treponema innocens*, a new species of intestinal bacteria, and emended description of the type strain of *Treponema hyodysenteriae* Harris et al. Int. J. Syst. Bacteriol. **29**:102–109.
11. **Mapother, M. E., and L. A. Joens.** 1985. New serotypes of *Treponema hyodysenteriae*. J. Clin. Microbiol. **22**:161–164.

12. **Nelson, N.** 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153:**375–380.
13. **Paster, B. J., and E. Canale-Parola.** 1985. *Treponema saccharophilum* sp. nov., a large pectinolytic spirochete from the bovine rumen. Appl. Environ. Microbiol. **50:**212–219.
14. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. **83:**346–356.
15. **Stanton, T. B., and E. Canale-Parola.** 1980. *Treponema bryantii* sp. nov., a rumen spirochete that interacts with cellulolytic bacteria. Arch. Microbiol. **127:**145–156.
16. **Stanton, T. B., and C. P. Cornell.** 1987. Erythrocytes as a source of essential lipids for *Treponema hyodysenteriae*. Infect. Immun. **55:**304–308.
17. **Stanton, T. B., and D. F. Lebo.** 1988. *Treponema hyodysenteriae* growth under various culture conditions. Vet. Microbiol. **18:**177–190.
18. **Taylor, D. J., and T. J. L. Alexander.** 1971. The production of dysentery in swine by feeding cultures containing a spirochaete. Br. Vet. J. **127:**lviii–lxi.