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NOTES

Partial Purification of a Specific Antigen of *Treponema hyodysenteriae*

D. H. BAUM AND L. A. JOENS*

National Animal Disease Center, Agricultural Research, Science and Education Administration, U. S. Department of Agriculture, Ames, Iowa 50010

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A specific antigen of pathogenic *Treponema hyodysenteriae* was extracted from lyophilized cells of isolant B169 with hot phenol-water, isolated, and semipurified by starch block electrophoresis.

Serological tests (4; L. A. Joens, J. M. Kinyon, D. H. Baum, and D. L. Harris, Proc. 5th Int. Pig Vet. Congr., Zagreb, Yugoslavia, Abstr H29, 1978) indicate that *Treponema hyodysenteriae* (3, 10, 13) and *T. innocens* (9, 11) have similar surface antigens. Results from agglutinating antibody tests (5) (L. A. Joens and D. L. Harris, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C12, p. 38) and fluorescent antibody studies (4) have demonstrated that a possible antigenic difference exists between the two organisms. However, the two species are still most readily and reliably differentiated by the hemolysis each produces in blood agar (9-11).

The object of this study was to test our hypothesis that *T. hyodysenteriae* cells possess an antigen that is distinct from antigens in *T. innocens* for use in differentiating the two species.

Pathogenic *T. hyodysenteriae* isolants A-1, B78, B140, B169, B204, B234, B9605, and Dys 7 and *T. innocens* isolants 4/71, B256, B297, B6571, Dys 676, 1555a, 6580, and 6830 were obtained from the Veterinary Medical Research Institute, Iowa State University, Ames, through J. M. Kinyon; *T. hyodysenteriae* isolants T3 and T4 were obtained from Joe Weller, Dallas Center, Iowa; and T5, T6, and T7 were from our own collection. All isolants were frozen broth cultures.

Trypticase soy broth was prepared as previously described for tubes (8) and flasks (6). All broth received a 10% supplement of fetal calf serum (NADC; GIBCO Laboratories, Grand Island, N.Y.) at the time of inoculation. Broth cultures were incubated under an atmosphere of deoxygenated H_2/CO_2 (50:50) at 37°C. Flask cultures were incubated on a reciprocating shaker (ca. 90 rpm).

Whole-cell bacterins were prepared from 36- to 48-h, 200-ml cultures of each isolant of *T. hyodysenteriae* and used to hyperimmunize White New Zealand rabbits as previously described (2). Serum samples were passed through sterile membrane filters (0.22 μm average pore diameter) and stored at −20°C.

A frozen culture of *T. hyodysenteriae* isolant B169 was thawed at 37°C, transferred to tubed Trypticase soy broth (11% inoculum), and subcultured into 1,000 ml of Trypticase soy broth in 2,000-ml round-bottomed flasks (6). After 36 to 42 h, flask cultures were incubated, harvested, washed, and lyophilized.

Lyophilized cultures of isolant B169 were extracted with hot phenol-water (2, 14). The aqueous phases were aspirated and discarded, whereas the phenol phase was combined with cell debris and dialyzed against distilled water for 48 h at 4°C with at least six changes of water. The cell debris was then homogenized in the dialysate and removed by centrifugation at 16,000 × *g* for 30 min at 0°C. The resulting supernatant was decanted and concentrated to one-tenth of its original volume by negative pressure dialysis (385-mm Hg vacuum). This dialyzed fraction contained approximately 10 mg of protein per ml as determined by the Lowry method (12), using a bovine serum albumin standard. This sample was then dialyzed against 100 volumes of a borate buffer (pH 8.6, 0.15 M) (1).

Antigens and antisera were reacted in double immunodiffusion precipitin tests as previously described (2) and read after 24 h. When the concentrated phenol phase was reacted with antisera, two lines of precipitate formed between the phenol phase and each antiserum against *T. hyodysenteriae*. However, only one precipitate line formed between the phenol phase and each antiserum against *T. innocens* (Fig. 1).

The dialyzed phenol phase was applied to a potato starch block (28 by 8 by 1.3 cm^3), electrophoresed (250 V, 12 mA), and eluted as previously described (1). Elution fractions were concentrated to 1.0 ml by negative pressure dialysis (385 mm of Hg) against a Veronal buffer gradient (7) at 4°C.

Concentrated elution fractions were tested in double immunodiffusion with an antiserum against a *T. hyodysenteriae* isolate and a *T. innocens* isolate. A fraction specific for *T. hyodysenteriae* migrated 5 to 6 cm from the site of sample application (7 cm from the cathode) toward the anode. An extreme case is shown (Fig. 2) where this specific fraction migrated over nine centimeters in the starch block.

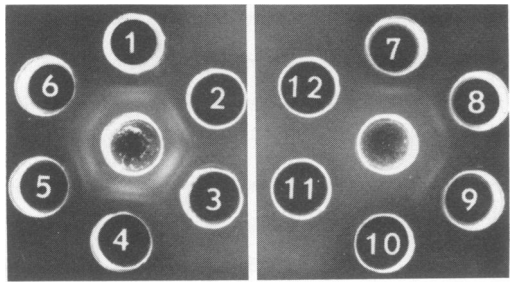

FIG. 1. *Demonstration of antigens extracted in the phenol phase from T. hyodysenteriae isolant B169. Antisera in peripheral wells were prepared against T. hyodysenteriae isolates: B234 (1), B78 (2), A-1 (3), B204 (4), T6 (5), B169 (6), and T. innocens isolates B256 (7), B297 (8), and 1555a (9). Wells 10, 11, 12 were filled with normal rabbit sera. Center wells contain the phenol phase from extraction of B169.*

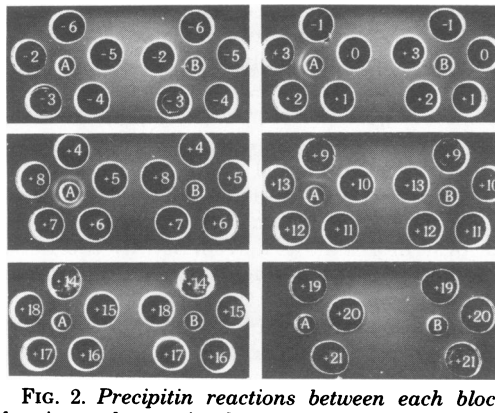

FIG. 2. *Precipitin reactions between each block fraction and an antipathogen serum and an antinonpathogen serum. Antisera are in the center wells: A, Anti-T. hyodysenteriae; B, anti-T. innocens. Concentrated fractions are in the peripheral wells, labeled as the distance (centimeters) from origin toward the cathode (negative numbers) or toward anode (positive numbers).*

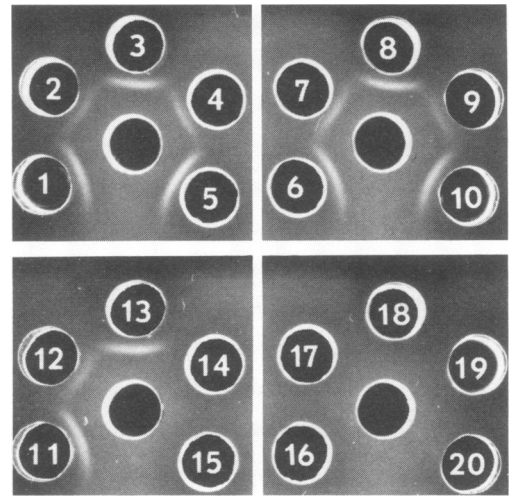

FIG. 3. *Demonstration by double immunodiffusion of the specificity of the antigen isolated from T. hyodysenteriae isolant B169. Center wells: combined fractions from electrophoresis. Antisera in peripheral wells were prepared against T. hyodysenteriae isolates B78 (1); B234 (2); dys 7 (3); T6 (4); T7 (5); B140 (6); B204 (7); T3 (8); T4 (9); T5 (10); 9605 (11); B169 (12); A-1 (13); T. innocens isolates 4/71 (14); B256 (15); B297 (16); B6571 (17); Dys 676 (18); 1555a (19); and 6580 (20).*

Fractions from six similar electrophoretic separations were pooled and tested against each antisera against *T. hyodysenteriae* and *T. innocens* in double immunodiffusion precipitin tests. The pooled fractions formed precipitin lines with antisera against *T. hyodysenteriae* isolates but not the *T. innocens* isolates (Fig. 3).

Phenol-water extraction of *T. hyodysenteriae* demonstrated the presence of an antigen common to *T. hyodysenteriae* and *T. innocens*. An antigen specific for *T. hyodysenteriae* was also extracted (Fig. 1) and partially purified by starch block electrophoresis (Fig. 2 and 3). We feel that this fraction will be useful in the surveillance of individual pigs for detecting asymptomatic carriers of *T. hyodysenteriae* and in the positive serodiagnosis of swine dysentery in individual animals.

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