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Evaluation of the An-Ident System and an Indole Spot Test for the Rapid Differentiation of Porcine Treponemes

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The An-Ident strip system (Analytab Products, Inc., St-Laurent, Quebec, Canada) was evaluated for its ability to differentiate *Treponema hyodysenteriae* from *Treponema innocens*. Of the 20 tests included on this strip, 15 yielded identical results for the two species. Among the other five assays, none could be considered as a positive discriminator for the two species. However, when an indole spot test with 1% *p*-dimethylaminocinnamaldehyde was used in parallel, all reference strains and 97% of the isolates of *T. hyodysenteriae* were positive, whereas all isolates of *T. innocens* were negative. Our results indicate that An-Ident is of little value for the differentiation of the two species. Our results also suggest that a rapid and simple differentiation between *T. hyodysenteriae* and *T. innocens* can be achieved by using the hemolysis and ring phenomenon tests in conjunction with an indole spot test.

Treponema hyodysenteriae is the etiological agent of swine dysentery, a mucohemorrhagic enteritis confined to the large intestine of pigs, and is present in herds throughout most swine-raising areas of the world (5–7, 26). Seven serotypes have been described so far (1, 16). This microorganism is not easily differentiated from *Treponema innocens*, which is generally considered to be nonpathogenic (12). The type of hemolysis produced on blood agar is useful for the presumptive identification of *T. hyodysenteriae*, since this spirochete produces areas of complete hemolysis, whereas *T. innocens* produces only partial hemolysis (12, 23). Another characteristic of *T. hyodysenteriae* is the ring phenomenon (4, 19), in which a more pronounced hemolysis zone around a removed agar plug is observed, whereas *T. innocens* exhibits no such change in hemolysis (Fig. 1). The test consists of removing an agar plug with a pipette tip or cutting an incision into a previously inoculated blood agar plate with a scalpel blade and incubating the plate anaerobically at 37°C for 48 h. The ring phenomenon is not fully understood, but this test is different from the CAMP reaction (15), in which a lytic phenomenon is seen at the junction of two different organisms. Biochemical tests such as fructose fermentation and indole production may also help in differentiating between pathogenic and nonpathogenic treponemes (12, 21, 23). Rapid slide agglutination (3) and growth inhibition by disks soaked in antiserum (14) were used to differentiate both species, but these techniques require antisera and medium not routinely available in diagnostic laboratories. An enteropathogenicity test in pigs is more reliable but is cumbersome (12, 13). Joens et al. (11) suggested the use of a mouse model as an inexpensive test for enteropathogenicity.

Currently, there is considerable interest in the use of rapid identification tests for anaerobes (20). Many of these tests detect preformed enzymes and thus yield results rapidly. The miniaturized An-Ident system (Analytab Products, Inc., Saint-Laurent, Quebec, Canada) has been developed and used for the identification of anaerobes of human origin (2, 8, 17, 22, 25). In this study, we investigated the possibility of using the An-Ident system to differentiate *T. hyodysenteriae* from *T. innocens*.

T. hyodysenteriae reference strains representing serotypes 1 (B234, ATCC 31287), 2 (B204, ATCC 31212), 3 (B169), and 4 (A-1) were provided by L. A. Joens from the Department of Veterinary Science, University of Arizona, Tucson. Serotypes 5 (B8044), 6 (B6933), and 7 (ACK 300/8) of *T. hyodysenteriae* and *T. innocens* B256 (ATCC 29796) and 4/71 (12) were obtained from M. J. Wannemuehler, Veterinary Medical Research Institute, Iowa State University, Ames. These reference strains were recently used by Jensen et al. (10) for the identification of *T. hyodysenteriae* by using oligodeoxynucleotide probes. All *T. hyodysenteriae* reference strains were strongly beta-hemolytic and positive for the ring phenomenon test, whereas the two *T. innocens* strains were weakly beta-hemolytic and negative for the ring phenomenon test. Bacteria were grown on solid medium by using blood agar base no. 2 (Oxoid Ltd., Hampshire, England) containing 5% bovine blood. Plates were incubated anaerobically for 4 days in anaerobic jars (Oxoid) by using a GasPak Plus generator atmosphere (BBL, Beckton Dickson & Co., Cockeysville, Md.). Cells were harvested with a swab and suspended in 3 ml of sterile distilled water. The suspension was adjusted to a McFarland no. 5 turbidity standard. Inoculation, incubation, and reading of the An-Ident strip test were conducted by following the instructions of the manufacturer. Treponemes were tested for indole production by using the Kovac reagent (Analytab Products).

The nine reference strains of *Treponema* showed identical reactions for 15 of the tests. β-Galactosidase, β-glucosidase, and indoxyl acetate were positive for all bacteria, whereas *N*-acetylglucosaminidase, α-glucosidase, α-fucosidase, arginine utilization, and pyroglutamic acid arylamidase were always absent. Aminopeptidase tests, leucine, proline, tyrosine, alanine, histidine, phenylalanine, and glycine, were negative for all bacteria tested. A catalase test was not performed because bacteria were harvested with a swab on plates containing erythrocytes, which could give a false-positive reaction. Various percentages of positivity were observed with indole production, α-arabinosidase, alkaline phosphatase, α-galactosidase, and arginine aminopeptidase (Table 1).

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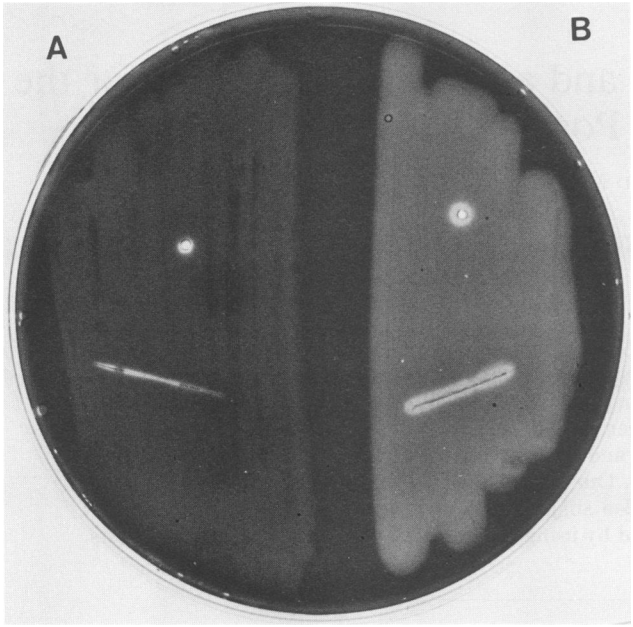

FIG. 1. (A) Strain 4/71 exhibiting a weak beta-hemolytic pattern and absence of the ring phenomenon. (B) Strain B204 with a strong beta-hemolytic pattern and presence of the ring phenomenon.

Arginine aminopeptidase, α-arabinosidase, and α-galactosidase could help to differentiate between *T. hyodysenteriae* and *T. innocens*, but only α-arabinosidase could discriminate between the two species. The majority of *T. hyodysenteriae* strains lacked α-galactosidase (71%) and arginine aminopeptidase (71%) activities; conversely, both *T. innocens* strains possessed these activities. Our results do not fully agree with those of Hunter and Wood (9) and Nibbelink and Wannemuehler (18), who used API ZYM and observed that α-galactosidase might be used to classify porcine treponemes.

The An-Ident indole reaction was positive for 86% of *T. hyodysenteriae* strains and one of the two *T. innocens* strains. Because the reaction was sometimes difficult to read, false interpretation of that test was possible with the An-Ident system. Sutter and Carter (24) found that an indole spot test with 1% *p*-dimethylaminocinnamaldehyde in 10% concentrated hydrochloric acid was a simple, reliable, and rapid method for detection of indole production by anaerobic bacteria. The spot test was performed by smearing the growth from a culture on a filter paper saturated with the indole reagent (REMEL; Regional Media Laboratories Inc., Lenexa, Kans.). Positive reactions were indicated by a blue color development, generally within 1 to 3 min. Negative reactions remained pink. All strains of *T. hyodysenteriae* were positive, whereas *T. innocens* strains were negative.

TABLE 1. Positive reactions of nine *Treponema* reference strains tested with the An-Ident system and an indole spot test

Enzyme or reaction	% Positive reactions	
	T. hyodysenteriae (n = 7[a])	*T. innocens* (n = 2)
Indole (spot test)	100	0
Indole (An-Ident)	86	50
Alkaline phosphatase	86	100
α-Arabinosidase	0	100
α-Galactosidase	29	100
Arginine aminopeptidase	29	100

[a] Number of reference strains tested.

Finally, a total of 40 field isolates of treponemes, obtained from rectal swabs, feces, or mucosal scrapings of colons, were collected from pigs from various herds in the Saint-Hyacinthe, Quebec, Canada, area. In these herds, swine dysentery was clinically apparent or was considered as a differential diagnosis. Isolates were obtained from S. Messier, Agriculture Canada, Saint-Hyacinthe, Quebec, Canada. The field isolates were tested with the An-Ident system and by the indole spot test. Thirty of them were strongly beta-hemolytic and positive for the ring phenomenon test, whereas the other 10 were weakly beta-hemolytic and negative for the ring phenomenon test.

α-Arabinosidase, which seemed to be a good discriminator between the reference strains of the two species, failed to differentiate the field isolates. All 30 isolates of *T. hyodysenteriae* were negative for α-arabinosidase, but only 10% of the *T. innocens* isolates were positive. The An-Ident strip system for anaerobes thus appears to be of little value for the differentiation of *T. hyodysenteriae* and *T. innocens*. However, as previously observed with the reference strains, all but one (97%) of the *T. hyodysenteriae* isolates were positive and all *T. innocens* isolates were negative by the indole spot test. Because it takes only 3 min to perform and is a simple test, we therefore suggest that an indole spot test with 1% *p*-dimethylaminocinnamaldehyde in 10% concentrated hydrochloric acid be used as a way to differentiate between these two species. Our data indicate that the combined results of hemolysis, the ring phenomenon, and the indole spot tests seem to allow the rapid and simple differentiation of porcine treponemes.

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