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Molecular Characterization of *Serpulina* (*Treponema*) *hyodysenteriae* Isolates Representing Serotypes 8 and 9

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The study described here was carried out to further characterize reference strains of *Serpulina* (*Treponema*) *hyodysenteriae* representing serotypes 8 and 9. Results obtained from restriction fragment length polymorphism analysis, enteropathogenicity testing, and endotoxin profiles confirmed their identifications. Electron microscopy indicated that both strains were covered with a thin layer of capsule-like material. Immunoblot analysis indicated that an antigen in the 19-kDa region of proteinase K-digested whole cells reacted only with homologous antiserum. The serotype-specific antigens were sensitive to periodate oxidation but resistant to proteinase K digestion and migrated in the same region as purified lipopolysaccharides. Immunoblotting with proteinase K-digested whole cells appeared as useful as immunodiffusion with extracted lipopolysaccharide for the serological classification of *S. hyodysenteriae*. Immunogold labeling of whole cells and purified periplasmic flagella showed strong cross-reactions between *S. hyodysenteriae* and *Serpulina innocens*. Outer membrane preparations of strains representing serotypes 8 and 9 contained four major proteins which reacted with antisera against both species, and one major protein with a molecular mass of 46 kDa which reacted only with antisera against *S. hyodysenteriae*, irrespective of the serotype. Our findings suggest that periplasmic flagella and some outer membrane proteins are antigens common to both *S. hyodysenteriae* and *S. innocens*, whereas a 46-kDa outer membrane protein may be a species-specific antigen of *S. hyodysenteriae*. Finally, we propose immunoblotting as an alternative method to immunodiffusion for the serotyping of *S. hyodysenteriae*.

Serpulina (*Treponema*) *hyodysenteriae*, a gram-negative anaerobic spirochete, is the causative agent of swine dysentery (9, 34, 35). The virulence factors of this organism have not been fully defined, but lipopolysaccharide (LPS)-endotoxin and hemolysin may be important in pathogenesis (5, 6, 24, 27). The most distinctive characteristics of *S. hyodysenteriae* are its strong beta-hemolytic activity and its enteropathogenicity in swine (20). *Serpulina innocens* (19) is morphologically identical to *S. hyodysenteriae* (11, 19), but it is weakly beta-hemolytic and is nonenteropathogenic for swine (19), and it shares only about 40% DNA sequence homology with *S. hyodysenteriae* (35). We have previously described the use of hemolysis and the ring phenomenon test in conjunction with an indole spot test for differentiation of the two species (3). Recently, oligodeoxynucleotide probes complementary to a unique region of *S. hyodysenteriae* 16S rRNA have been developed and used for the identification of this spirochete (14).

S. hyodysenteriae LPS is responsible for serological specificity (25, 33). LPS may also play a role in protection against *S. hyodysenteriae* infection since protection has been shown to be serotype specific (17). Seven serotypes of *S. hyodysenteriae* have been described on the basis of agar gel double immunodiffusion precipitation with extracted LPS and hyperimmune rabbit antisera (2, 25). Serogrouping of *S. hyodysenteriae* has also been proposed (8). Cross-reactions between *S. hyodysenteriae* and *S. innocens* often occurred in several serological tests (11, 12, 15). These cross-reactions were probably due to protein antigens (16). Examination of outer membrane (33, 38), outer envelope (4, 32), or sonicated preparations (16) of porcine *Serpulina* spp. by immunoblotting revealed several cross-reactive protein antigens between the two species as well as proteins unique to *S. hyodysenteriae*. These species-specific antigens may be potentially important for the development of molecular markers for diagnostic purposes, the development of a subunit vaccine, or both.

We have recently reported two new serotypes of *S. hyodysenteriae*, serotypes 8 and 9 (23). These serotypes, which represented 70% of the isolates tested, were the major serotypes found in the province of Quebec (23). The purpose of the study described here was to further characterize the two serotypes by using restriction fragment length polymorphism (RFLP) analysis, endotoxin profiles, and enteropathogenicity testing in rabbits; such experiments enabled us to confirm the identification of *S. hyodysenteriae* and differentiate it from the nonpathogenic *S. innocens*. Surface components were also studied in order to identify serotype- and species-specific antigens as well as antigens common to both *S. hyodysenteriae* and *S. innocens*. These studies may provide useful information for the development of molecular markers for the rapid identification of field isolates of *S. hyodysenteriae*.

MATERIALS AND METHODS

Bacteria and growth conditions. *S. hyodysenteriae* reference strains representing serotypes 1 (B234), 2 (B204), 3 (B169), and 4 (A-1) were provided by L. A. Joens, Department of Veterinary Science, University of Arizona, Tucson. *S. hyodysenteriae* serotypes 5 (B8044), 6 (B6933), and 7 (Ack 300/8) and *S. innocens* B256 were obtained from M. J.

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Wannemuehler, Veterinary Medical Research Institute, Iowa State University, Ames. *S. hyodysenteriae* serotypes 8 (FM 88-90) and 9 (FMV 89-3323) were from our collection (23). Nineteen isolates of *S. hyodysenteriae* and 11 isolates of *S. innocens* (3, 23) were originally obtained from S. Messier, Agriculture Canada, Saint-Hyacinthe, Quebec, Canada. 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 at 37°C for 4 days in jars (Oxoid) by using a GasPak Plus generator atmosphere (BBL, Beckton Dickinson and Co., Cockeysville, Md.) (3).

Enteropathogenicity. The enteropathogenicities of isolates of *S. hyodysenteriae* and *S. innocens* were evaluated in rabbit ligated ileal loops as described by Knoop (21). Approximately 10^9 organisms were inoculated into each ligated loop. Results were obtained 18 h postchallenge.

RFLP analysis of spirochete rRNA genes. Partially purified *S. hyodysenteriae* B204 16S and 23S rRNAs were isolated as described previously (28) and were labeled with photobiotin (Photoprobe Biotin; Vector Laboratories, Burlingame, Calif.) by following the supplier's instructions. Genomic DNA was isolated from spirochetes as described previously (35), except *S. hyodysenteriae* FM 88-90 and FMV 89-3323 DNA were isolated by the guanidinium thiocyanate method of Pitcher et al. (30). Genomic DNA was digested with the restriction enzyme *Sau*3A, electrophoresed on a 1% agarose gel, and blotted onto a nylon membrane (Hybond N; Amersham Corp., Chicago, Ill.) by capillary transfer (31). The membrane was prehybridized and hybridized in HP4 buffer (14) at 50°C for 3 and 20 h, respectively. The hybridization buffer contained photobiotin-labeled *S. hyodysenteriae* B204 RNA (50 ng/ml). After hybridization, the membrane was washed with 2× SSC (1× SSC is 150 mM NaCl plus 15 mM citric acid [pH 7.0])–0.1% sodium dodecyl sulfate (SDS) buffer twice for 1 min each time at room temperature and once for 20 min at room temperature. Blots were developed by using a streptavidin-alkaline phosphatase-based method (BluGene Nonradioactive Nucleic Acid Detection System; GIBCO Bethesda Research Laboratories, Gaithersburg, Md.).

Antiserum production. Antisera against whole cells of each reference strain or isolate of *S. hyodysenteriae* and *S. innocens* were produced in rabbits as described by Li et al. (23). Antisera were not adsorbed with other serotypes before use in immunoblot analysis.

Outer membrane preparations. Extraction of the outer membrane from *S. hyodysenteriae* was performed by a method that used both Triton X-100 and Sarkosyl. Briefly, cells were first extracted with Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) by the method of Penn et al. (29). The crude outer membrane preparations were separated from cell debris by centrifugation at 25,000 × *g* for 2 h. The supernatants were then centrifuged at 100,000 × *g* for 2 h at 4°C, and the pellets were resuspended in distilled water and extracted with Sarkosyl (sodium *N*-lauryl sarcosinate; Sigma) as described previously (1, 33). Contamination of preparations by periplasmic flagella was determined by electron microscopy and negative staining (see below).

Periplasmic flagella preparations. Periplasmic flagella from *S. hyodysenteriae* were purified by the method of Kent et al. (18) by using 10 mM Tris-HCl (pH 7.6) instead of a phosphate-buffered saline.

Extraction of LPS and endotoxin. Extraction of LPS from *S. hyodysenteriae* and *S. innocens* was performed with hot phenol-water (2, 23). Extraction of endotoxin was performed with butanol-water (6).

SDS-PAGE. SDS-polyacrylamide gel electrophoresis (PAGE) was conducted by using the discontinuous buffer system of Laemmli (22), with a 4.5% polyacrylamide stacking gel and a 12.5% polyacrylamide running gel. Endotoxin preparations, however, were run on a 15% polyacrylamide gel. Samples were boiled for 10 min in solubilization buffer (62.5 mM Tris-HCl [pH 6.8], 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.025% bromophenol blue), and 20 to 50 μg of protein was applied to each lane. Low-molecular-mass markers and prestained low-molecular-mass markers were obtained from Bio-Rad Laboratories (Richmond, Calif). Gels were run on a Mini-PROTEAN II vertical slab electrophoresis cell (Bio-Rad) and were either stained with Coomassie brilliant blue R-250 or transferred to nitrocellulose for immunoblotting (see below).

Whole cells of *S. hyodysenteriae* and *S. innocens* were suspended in sample solubilization buffer, heated in a water bath for 15 min at 100°C, and centrifuged to remove cell debris. The supernatants were treated with an equal volume of 1 mg of proteinase K (Sigma) per ml for 60 min at 60°C to obtain proteinase K-digested whole cells (PKWCs). Polyacrylamide gels containing samples of PKWCs, LPS, or endotoxin extracts were stained with silver nitrate (37).

Immunoblotting. Immunoblots (Western blots) were carried out as described by Towbin et al. (36). The samples (10 to 25 μg of protein per lane) separated by electrophoresis were transferred (Mini-PROTEAN II transfer system; Bio-Rad) to nitrocellulose membrane blotting filter paper (pore size, 0.2 μm; Bio-Rad) for 1 h at 100 V. The membrane was blocked with 2% skim milk in Tris-buffered saline (10 mM Tris [pH 7.4], 150 mM NaCl) for 1 h and incubated with diluted antisera in the blocking solution overnight at 4°C. The membrane was then washed in Tris-buffered saline, and bound antibodies were detected with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Bio-Rad) by using 4-chloro-1-naphthol as the chromogenic substrate.

In some experiments, nitrocellulose membranes were treated overnight at 4°C with 100 mM *m*-periodate (in 100 mM sodium acetate buffer [pH 4.5]) after electrotransfer to detect periodate-sensitive material.

Electron microscopy. (i) Negative staining. Dense bacterial suspensions or outer membrane or periplasmic flagella preparations were examined by negative staining. A drop of each preparation was placed on a 200-mesh Formvar-coated grid. A drop of 1% phosphotungstate (pH 7.0) was then applied to the grid. Grids were examined with an electron microscope (Philips 201) at an accelerating voltage of 60 kV.

(ii) Immunogold labeling. A drop of sample was placed on Formvar-coated grids. After blocking for 5 min with 1% egg albumin, the grids were incubated for 30 min with a suitable dilution of rabbit antisera and rinsed with distilled water five times. The grids were then incubated for 30 min with protein A-gold particles (10 nm; Sigma), rinsed, and negatively stained with 1% phosphotungstate for 10 s. Grids were observed as described above.

(iii) Thin sectioning. Bacterial cells were prepared for transmission electron microscopy following immunostabilization or polycationic ferritin labeling as described by Jacques et al. (13).

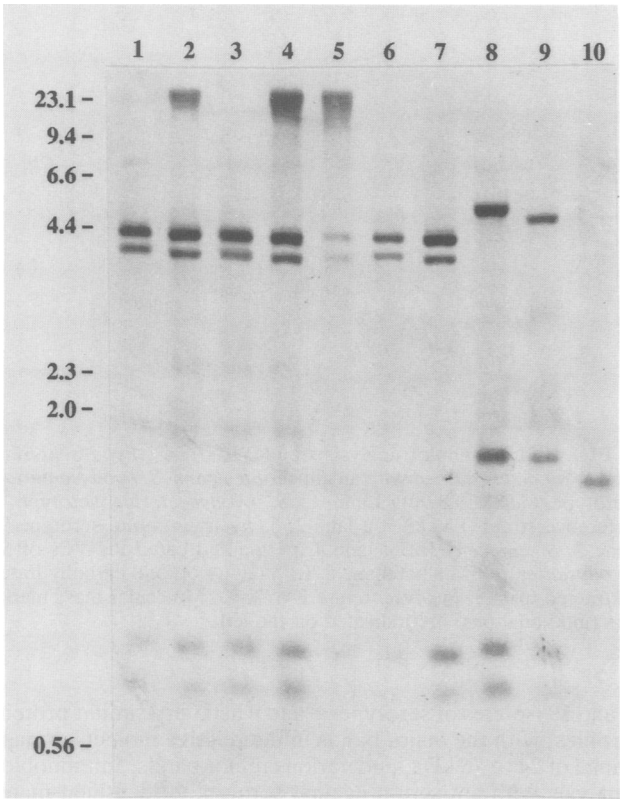

FIG. 1. rRNA gene restriction patterns of porcine intestinal spirochetes. Genomic DNA was cut with the restriction enzyme *Sau*3A, electrophoresed, transferred to a nylon membrane, and probed with photobiotin-labeled *S. hyodysenteriae* B204 rRNA. Lanes 1 to 7, *S. hyodysenteriae* B78, B204, B169, A-1, Ack 300/8, FM 88-90, and FMV 89-3323, respectively; lane 8, *S. innocens* B256; lane 9, *S. innocens* 4/71; lane 10, *T. succinifaciens* 6091. Numbers along the left of the figure represent size markers (in kilobases).

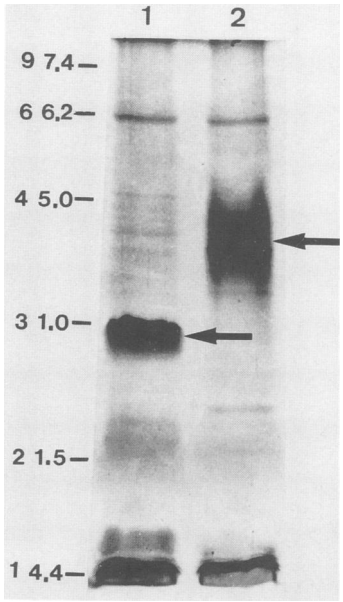

FIG. 2. SDS-PAGE profile of endotoxin preparations (20 μg of total carbohydrate per lane) separated with a 15% acrylamide gel and stained with silver nitrate. Lane 1, *S. hyodysenteriae* serotype 8 (FM 88-90); lane 2, *S. innocens* B256. Arrows indicate the differences in mobilities between the two species.

RESULTS

RFLP analysis of spirochete rRNA genes. *S. hyodysenteriae* strains from a variety of countries including Canada (B169), England (A-1), The Netherlands (Ack 300/8), and the United States (B78 and B204) all had the same rRNA gene restriction pattern when genomic DNA was digested with the restriction enzyme *Sau*3A (Fig. 1, lanes 1 to 5). The two bands between 3.6 and 4.3 kb were characteristic of all *S. hyodysenteriae* strains tested. *S. innocens* B256 and 4/71 and *Treponema succinifaciens* 6091 had patterns that were markedly different from those of *S. hyodysenteriae* strains (Fig. 1, lanes 8 to 10). *S. hyodysenteriae* FM 88-90 and FMV 89-3323 both had an rRNA gene restriction pattern which identified them as strains of *S. hyodysenteriae* (Fig. 1, lanes 6 and 7).

Enteropathogenicity. Nineteen isolates of *S. hyodysenteriae* serotypes 8 and 9, as well as the two reference strains (FM 88-90 and FMV 89-3323), were tested for their enteropathogenicities in rabbit ligated ileal loops. All isolates and both reference strains of serotypes 8 and 9 of *S. hyodysenteriae* were enteropathogenic as determined by the accumulation of intestinal fluid (data not shown). On the other hand, the 11 *S. innocens* isolates tested were nonenteropathogenic.

Endotoxin profiles. Endotoxin profiles of reference strains and isolates of serotypes 8 and 9 were similar to previously reported *S. hyodysenteriae* endotoxin profiles (5) but were quite different from the *S. innocens* B256 endotoxin preparation, which was less mobile in the gel (Fig. 2).

Morphology. The cells of *S. hyodysenteriae* reference strains FM 88-90 and FMV 89-3323 averaged 0.3 to 0.4 μm in diameter and 5 to 9 μm in length, with eight to nine flagella inserted at each end of a cell (data not shown). Cells of both strains were exposed to antisera or labeled with polycationic ferritin and were prepared for transmission electron microscopy. Cells exposed to homologous antiserum were covered with an irregular layer of capsule-like material (Fig. 3A). This layer was absent on cells treated with preimmune serum or heterologous antiserum. Cells labeled with polycationic ferritin were covered with a layer of ferritin granules (Fig. 3B), whose thickness was about 20 nm for both reference strains.

Serotype-specific antigens. Whole cells of reference strains (representing serotypes 1 to 9) of *S. hyodysenteriae* and a reference strain of *S. innocens* (B256) were treated with proteinase K and tested by immunoblot analysis with antisera against these reference strains in order to locate serotype-specific antigens. When PKWCs of reference strains of *S. hyodysenteriae* and *S. innocens* were probed with an antiserum against serotype 8, only the serotype 8 PKWCs showed a band in the 19-kDa region (Fig. 4, lane 1). Treatment with periodate completely abolished this reaction (Fig. 4, lane 4). Similar results were obtained when purified LPS of serotype 8 was used (Fig. 4, lane 5). Proteinase K digests of all isolates of serotype 8 displayed similar profiles after SDS-PAGE and silver staining, showing one major band in the 19-kDa region. When these digests were probed with an antiserum against serotype 8, this major band reacted uniformly among all isolates (Fig. 5). The serotype-specific antigens were also detected by immunoblotting, in some cases, in the 30-kDa region (Fig. 4, lane 5; Fig. 5, lanes 1, 2, 6, and 7). Identical results, i.e., periodate-sensitive

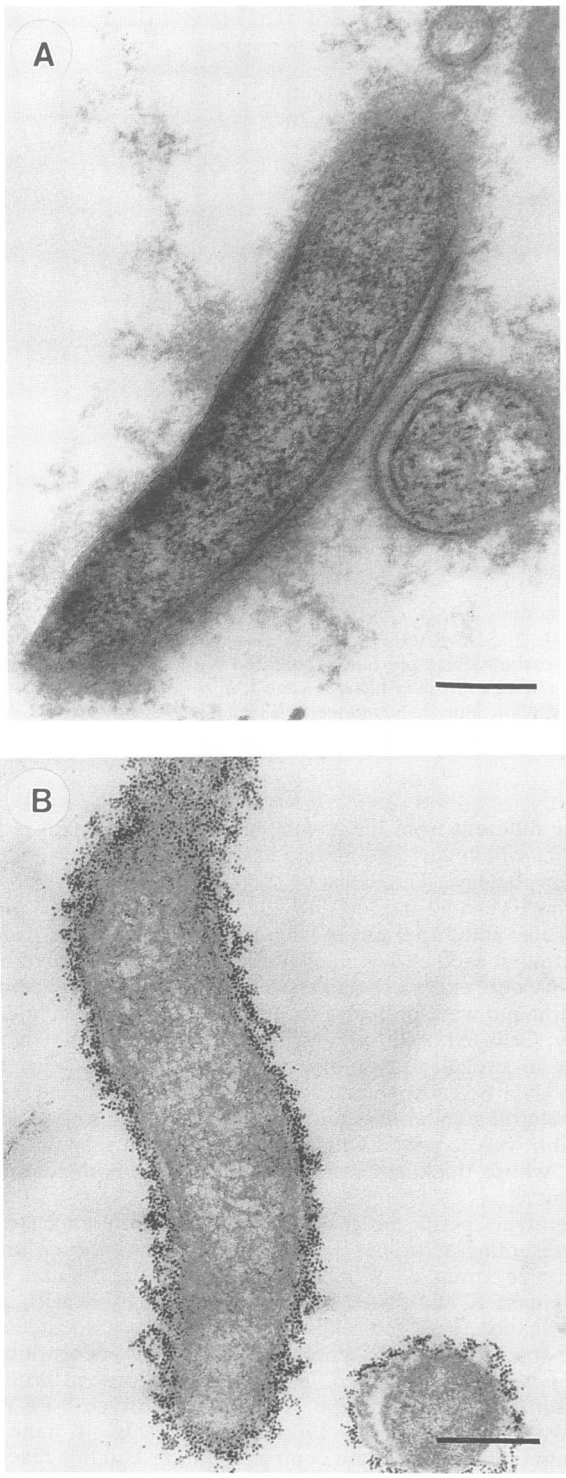

FIG. 3. Transmission electron micrographs of thin sections of *S. hyodysenteriae* serotype 9 (FMV 89-3323). Cells were stabilized with whole-cell homologous antiserum and stained with ruthenium red (A) or labeled with polycationic ferritin (B). Bars, 0.2 μm.

reaction restricted to isolates of the same serotype, were obtained with serotype 9 (data not shown).

Common antigens. SDS-PAGE of whole cells indicated that all reference strains of *S. hyodysenteriae* serotypes 1 to 9 and 19 isolates of serotypes 8 and 9 had very similar protein profiles, with the major bands in the relative molecular mass range of 24 to 76 kDa, and various minor bands. Immunoblot analysis with antiserum against serotype 8 identified many bands common to all serotypes of *S. hyodysenteriae* (Fig. 6, lanes 1 to 5 and 7 to 10), some of which were also present in the *S. innocens* preparation (Fig. 6, lane 6). In addition, low-molecular-mass bands in the 19-kDa region, corresponding to the serotype-specific antigens, were observed (Fig. 6, lane 9).

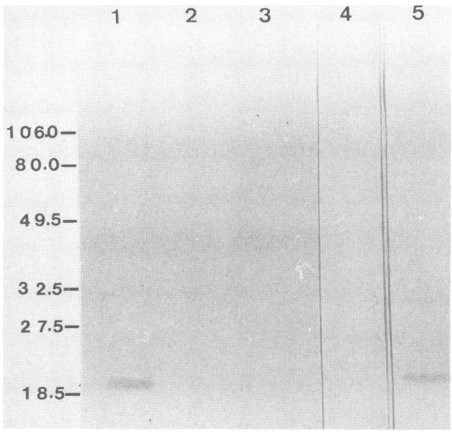

FIG. 4. Immunoblot analysis of PKWCs of *S. hyodysenteriae* and *S. innocens* probed with an antiserum against *S. hyodysenteriae* serotype 8 (FM 88-90). Lane 1, *S. hyodysenteriae* serotype 8 reference strain (FM 88-90); lane 2, *S. hyodysenteriae* serotype 9; lane 3, *S. innocens* B256; lane 4, periodate-treated PKWCs of *S. hyodysenteriae* FM 88-90; lane 5, LPS (20 μg of total carbohydrate) extracted from *S. hyodysenteriae* FM 88-90. Molecular mass markers (in kilodaltons) are indicated on the left.

Immunogold labeling of whole cells also revealed cross-reactions between the different serotypes of *S. hyodysenteriae* and between *S. hyodysenteriae* and *S. innocens*. These cross-reactions appeared, at least in part, to be due to periplasmic flagella, and immunogold labeling performed with purified periplasmic flagella clearly demonstrated

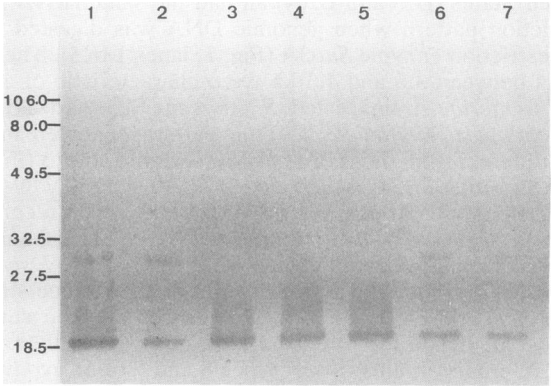

FIG. 5. Comparison of various isolates of *S. hyodysenteriae* serotype 8 by immunoblotting of PKWCs probed with antiserum against the serotype 8 reference strain (FM 88-90). Lanes 1 to 7, isolates FM 88-90, FM 88-116, FM 88-91, FM 88-86P7, FM 88-89, FM 88-77P6, and FM 88-95P6, respectively. Molecular mass markers (in kilodaltons) are indicated on the left.

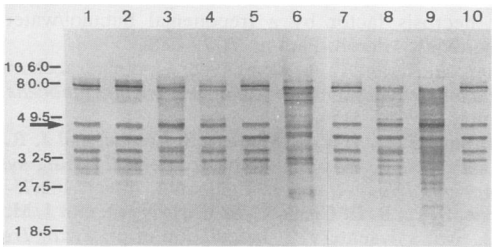

FIG. 6. Immunoblot analysis of whole cells of reference strains of *S. hyodysenteriae* and *S. innocens* probed with an antiserum against *S. hyodysenteriae* serotype 8 (FM 88-90). Lanes 1 to 5, *S. hyodysenteriae* serotype 1 to 5, respectively; lane 6, *S. innocens* B256; lanes 7 to 10, *S. hyodysenteriae* serotypes 6 to 9, respectively. Molecular mass markers (in kilodaltons) are indicated on the left. The arrow indicates the 46-kDa antigen.

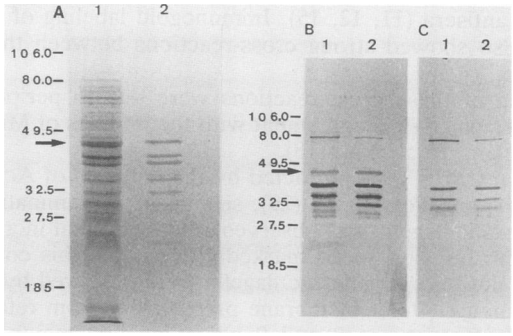

FIG. 7. SDS-PAGE profile and immunoblot analysis of OMPs of *S. hyodysenteriae* serotype 8 (FM 88-90). OMPs were extracted by the method that combined the use of Triton X-100 and Sarkosyl. (A) SDS-PAGE profile of *S. hyodysenteriae* FM 88-90. Lane 1, whole cells; lane 2, OMPs. (B) Immunoblot analysis of *S. hyodysenteriae* FM 88-90 probed with an antiserum against *S. hyodysenteriae* serotype 9 (FMV 89-3323). Lane 1, whole cells; lane 2, OMPs. (C) Immunoblot analysis of *S. hyodysenteriae* FM 88-90 probed with an antiserum against *S. innocens* B256. Lane 1, whole cells; lane 2, OMPs. Molecular mass markers (in kilodaltons) are indicated on the left. The arrows indicate the 46-kDa antigen.

strong cross-reactions between the two species (data not shown).

Species-specific antigen. One major band with a relative molecular mass of 46 kDa, which was present in all reference strains of *S. hyodysenteriae* probed with antiserum against each serotype of *S. hyodysenteriae* but was absent from the reference strain of *S. innocens*, was identified as a possible species-specific antigen (Fig. 6, arrow).

During this study, electron microscopic examination of outer membrane preparations extracted by the methods of Achtman et al. (1) or Penn et al. (29) indicated that both were contaminated with periplasmic flagella. Therefore, a centrifugation step of 25,000 × *g* for 2 h was added to separate crude outer membrane preparations from cell debris and periplasmic flagella. These outer membrane preparations were found to be free of periplasmic flagella, as determined by negative staining. The crude Triton X-100-extracted outer membrane preparations were then treated with Sarkosyl to selectively solubilize inner membrane proteins (1, 33).

SDS-PAGE revealed that outer membrane protein (OMP) preparations from reference strains of *S. hyodysenteriae* serotypes 8 and 9 contained five major bands in the relative molecular mass range of 32 to 46 kDa, and minor bands as well. Figure 7A shows the results of SDS-PAGE separation of whole-cell proteins (lane 1) and OMPs (lane 2) of strain FM 88-90. It appeared that Coomassie blue staining detected one band in the 46-kDa region of the OMP preparation (lane 2), but it detected a doublet in this region with the whole-cell proteins (lane 1). The immunoblots detected five major bands (relative molecular masses, 32 to 76 kDa) in FM 88-90 and FMV 89-3323 outer membrane preparations which reacted with antisera against all other serotypes of *S. hyodysenteriae* (Fig. 7B). Four of these bands also reacted with *S. innocens* B256 antiserum (Fig. 7C). The 46-kDa band was detected only with antisera against *S. hyodysenteriae*, irrespective of the serotype.

DISCUSSION

In the study described here we further examined the reference strains and isolates of two new serotypes of *S. hyodysenteriae* that have recently been proposed (23). The two reference strains possessed properties typical of *S. hyodysenteriae*. They were strongly beta-hemolytic and enteropathogenic. They also had an endotoxin profile similar to that of *S. hyodysenteriae* but different from that of *S. innocens*, results similar to those reported by Greer and Wannemuehler (5). Finally, the use of RFLP analysis with *S. hyodysenteriae* rRNA as a probe confirmed that the two reference strains representing the new serotypes 8 and 9 were strains of *S. hyodysenteriae*.

Both reference strains were covered by a thin layer of capsule-like material. The fragility of this layer during preparation for electron microscopy might explain why it has not been described before with other strains of *S. hyodysenteriae*. Conventional fixation for thin-section electron microscopy does not preserve bacterial capsular material, a highly hydrated structure which collapses during dehydration, and stabilization procedures must be used (13). This layer is not unique to these two reference strains, and we also observed it on other reference strains and isolates of *S. hyodysenteriae*. To our knowledge, only Hovind-Hougen et al. (10) have mentioned the presence of a surface layer on the cells of some porcine spirochetes stained with ruthenium red.

Immunoblot analysis of proteinase K digests showed that serotype-specific antigens were present in the 19-kDa region. The same profile was observed when purified LPS was probed with serotype-specific antiserum. The sensitivity of the serotype-specific antigens to periodate and their resistance to proteinase K digestion, as well as their migration pattern on SDS-PAGE, strongly suggest that LPSs are the serotype-specific antigens, as reported previously for the other serotypes (7, 33, 38). Our results also suggest that immunoblotting of proteinase K digests with serotype-specific antiserum could be used for serotyping of *S. hyodysenteriae* isolates instead of immunodiffusion, which is time-consuming because of the need to extract LPS from the isolates. Therefore, we propose immunoblotting as an alternative technique for serotyping *S. hyodysenteriae* field isolates.

Hyperimmune rabbit antiserum raised against whole cells of *S. hyodysenteriae* contained antibodies against the serotype-specific antigens (LPS) as well as antibodies against several major proteins present in both *S. hyodysenteriae* and *S. innocens*. Antigens common to both species have been reported previously (4, 16, 32, 33), and this has resulted in problems with diagnostic methods that are based on poly-

clonal antisera (11, 12, 15). Immunogold labeling of whole cells also showed strong cross-reactions between the two species.

Some of these cross-reactions were due to periplasmic flagella, which is in agreement with the findings of Miller et al. (26) and Kent et al. (18).

When OMPs were extracted by the methods of Achtman et al. (1) and Penn et al. (29), significant contamination by periplasmic flagella was observed. We found that increasing the centrifugation speed markedly decreased this contamination until no periplasmic flagella were observed by negative staining. Outer membrane preparations from reference strains of serotypes 8 and 9 contained five major bands (relative molecular masses, 32 to 46 kDa), some of which reacted with antisera raised against both species.

Of particular interest was a major protein with a relative molecular mass of 46 kDa in OMP preparations; it was also present in whole cells of all serotypes of *S. hyodysenteriae* but not in whole cells of *S. innocens* and reacted only with antisera against serotypes of *S. hyodysenteriae*, suggesting that this protein could represent a species-specific antigen. Species-specific antigens with different molecular masses have been reported previously (4, 16, 32, 33). In our hands, however, only the 46-kDa protein reacted with antiserum against each of the nine serotypes of *S. hyodysenteriae*, but not with antisera against *S. innocens*. Thus, the 46-kDa protein may serve as a molecular marker for rapid identification of field isolates of *S. hyodysenteriae*.

The present study confirms the identification of the reference strains of *S. hyodysenteriae* representing serotypes 8 and 9. These strains were covered with a layer of capsule-like material. Our results indicate that although many antigens are shared by *S. hyodysenteriae* and *S. innocens*, a 46-kDa OMP appears to be an antigen specific to the species *S. hyodysenteriae*. Finally, our data identify LPS as the serotype-specific antigen of both serotypes 8 and 9, and we propose that immunoblotting of proteinase K digests be used for serotyping of *S. hyodysenteriae*.

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