The genus *Serpulina* of intestinal spirochetes consists of at least three species. The first two species, originally classified as *Treponema*, have been reclassified based on DNA:DNA reassociation and 16S rRNA sequencing as a unique genus, *Serpulina* (3, 32, 34). The first species identified was *Serpulina hyodysenteriae*, the etiologic agent of swine dysentery (11), a diarrheal disease of growing and finishing pigs that has worldwide economic importance (27). The spirochete colonizes the cecum and the colon, resulting in severe mucos hemorrhagic typhlocolitis, which can lead to dehydration and death if the pigs are left untreated. The second species of intestinal spirochetes, which is biochemically similar to *S. hyodysenteriae* but produces a weak beta-hemolysis when cultured anaerobically on agar medium containing blood. Because it is present in the cecum of healthy swine, it was the name *Serpulina innocens* (15). The third species of intestinal spirochetes, which is also weakly hemolytic, was recently described as *Serpulina pilosicoli* (34). Strains of *S. pilosicoli* are associated with colonic spirochetal infections of humans, pigs, and dogs (3, 18, 34).

*S. hyodysenteriae* has been classified into serogroups based on the reactivity of lipooligosaccharide antigens with antisera raised against whole cells (1, 6–9, 22, 24). More recently, Pettersson and coworkers proposed a classification of porcine intestinal spirochetes based on phylogenetic analysis of the 16S rRNA sequence (28). At least four phenotypes within three phylogenetic clusters were identified. One cluster included phenotype I, representing the strongly beta-hemolytic variants that were biochemically similar to *S. hyodysenteriae*. A second cluster, represented by phenotype II, was the most variable, with three subgroups, designated IIa, IIb, and IIc, of *S. innocens* strains. The third cluster consisted of phenotype IV and included weakly beta-hemolytic spirochetes similar to strain P43/6/78, the type strain for *S. pilosicoli* (28, 34). Through D are represented by *S. hyodysenteriae* strains which have been identified by Baum and Joens (1) as type strains for serotypes 1 through 4. The serotyping system of Baum and Joens (1), which is also based on lipooligosaccharide antigens, was expanded to include serotypes 5 through 9 by cross-adsorption of antisera to differentiate cross-reactive serotypes (22, 24). More recently, Pettersson and coworkers proposed a classification of porcine intestinal spirochetes based on phylogenetic analysis of the 16S rRNA sequence (28). At least four phenotypes within three phylogenetic clusters were identified. One cluster included phenotype I, representing the strongly beta-hemolytic, pathogenic *S. hyodysenteriae*, whereas phenotype II comprised weakly beta-hemolytic variants that were biochemically similar to *S. hyodysenteriae*. A second cluster, represented by phenotype III, was the most variable, with three subgroups, designated IIIa, IIIb, and IIIc of *S. innocens* strains. The third cluster consisted of phenotype IV and included weakly beta-hemolytic spirochetes similar to strain P43/6/78, the type strain for *S. pilosicoli* (28, 34). A characteristic morphologic feature of spirochetes is the presence of flagella in the periplasmic space. The periplasmic flagella (PF) are responsible for motility and thus are important virulence attributes of pathogenic spirochetes. The motility of *S. hyodysenteriae* allows penetration of the colonic mucus layer and establishment of the spirochete in the crypts of Lieberkühn and the surface epithelium (13). The PF of *S. hyodysenteriae* are composed of five proteins: two outer-sheath proteins with molecular masses of 44 and 35 kDa and three core proteins with molecular masses of 37, 34, and 32 kDa (14, 16). The genes encoding the 37- and 34-kDa core proteins have been designated *flaB1* and *flaB2* (5, 19). Similarly, the gene encoding the 44-kDa sheath protein, designated *flaA1*, has been cloned and sequenced (17). Single *flaA1* and *flaB1* and dual *flaA1* *flaB1* mutants have altered motility compared to...
the wild type and had attenuated virulence in a murine model of swine dysentery (30, 31).

Genetic analyses of the PF genes and proteins of Serpulina species have shown that the core proteins are highly conserved among spirochetes, whereas the sheath proteins may be more diverse (5, 18, 23). Using hyperimmune rabbit serum produced against the 44-kDa antigen of *S. hyodysenteriae*, Li and coworkers (23) investigated *S. hyodysenteriae*, *S. innocens*, and some other uncharacterized weakly beta-hemolytic porcine intestinal spirochetes and suggested that the FlaA sheath protein was unique to *S. hyodysenteriae*. This result may provide a molecular basis for the development of serologic methods for detection of swine exposed to *S. hyodysenteriae*. However, after the work of Li and coworkers, Koopman and coworkers reported (28) that the *S. hyodysenteriae* species as a potential antigen for detection by serology of swine exposed to *S. hyodysenteriae*. This possibility was further evaluated for serological reactivity against the wild type and had attenuated virulence in a murine model of swine dysentery (30, 31).

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0.1% SDS, pH 8.5 (Tris-Gly-SDS Sepraduf; Integrated Separation Systems), at 30 mA of constant current per gel until the dye marker was within 2 to 3 mm of the bottom of the gel (21). After separation, proteins were electrotransferred to nitrocellulose (0.2-μm pore size; Pharmacia, Uppsala, Sweden) in 25 mM glycine–192 mM Tris-HCl–20% methanol with graphite electrodes (NovaBot; Pharmacia) at a constant current of 0.8 mA/cm² for 1 h (33). Duplicate gels were stained with Coomassie blue R250. The gels were dried between sheets of cellophane (gel drying system; Novex, San Diego, Calif.). Western blotting (immunoblotting). The immunoblots were completed as previously described (23). Briefly, the membranes were blocked with 2% nonfat dry milk in TBS (MTBS) for 2 h at 4°C. The MTBS was removed, and a 44-kDa-specific rabbit PAb (kindly provided by M. Jacques, Faculté de Médecine Vétérinaire, Université de Montréal, Quebec, Canada) (23) or MAb 7G2 in MTBS was added. The PAb was incubated for 16 to 18 h at 4°C with rocking. The dried gels and membranes were analyzed by laser densitometry (BioImage scanner; Waters, Ann Arbor, Mich.).

RESULTS

Immunoblotting of S. hyodysenteriae. Immunoblotting of the purified PF from strain B204 with the PAb revealed a single band of approximately 44 kDa (data not shown). Immunoblotting of the TXI fractions of reference strains of S. hyodysenteriae serogroups with the PAb revealed a band of approximately 44 kDa for all the strains (Fig. 1A). The 44-kDa immunoreactive band was present in the TXS fractions of S. hyodysenteriae B78, B204, B169, and A1 but not in the Australian strains, WA-6, Q16, VIC2, and NSW-1 (data not shown). A weakly immunoreactive band of unknown significance also was present at approximately 75 kDa in the TXI fractions of strains B78, B204, and B169 (Fig. 1A). Immunoblotting of purified PF from strain B204 and of TXS extracts from reference strains of S. hyodysenteriae serogroups with MAb 7G2 revealed three bands corresponding to the core flagellar proteins FlaB1 (37 kDa) and FlaB2 (34 kDa) and the 32-kDa FlaB protein (Fig. 1B).

Immunoblotting of S. innocens. Immunoblotting of TXI fractions of S. innocens strains with the PAb revealed an immunoreactive band of approximately 44 kDa in all the strains (Fig. 2A). While strains B256 and C301 appeared to have a slightly slower-migrating band, strain C378 had a faster-migrating band, and strains 4/71 and C336 had a mixture of both. Additionally, a weakly immunoreactive band of unknown significance was present at approximately 75 kDa in the TXI fractions of strains B256, 4/71, C336, and C301. Immunoblotting of the TXS extracts from the reference S. innocens strains with the MAb 7G2 revealed three bands corresponding to the core flagellar proteins FlaB1 (37 kDa) and FlaB2 (34 kDa) and the 32-kDa FlaB protein (Fig. 2B) seen in the reference S. hyodysenteriae strains and in the purified PF from S. hyodysenteriae B204.

Immunoblotting of S. pilosicoli. Although a constant protein content was loaded in each lane, the relative immunoreactivity of the 44- and 75-kDa bands varied among the TXI fractions of S. pilosicoli strains reacted with the PAb (Fig. 3A). While most strains appeared to have a faster-migrating 44-kDa band, strains P43/6/78, UNL-8, and B1555a had a slower-migrating 44-kDa band. Immunoblotting of TXS extracts from the reference strains and from field isolates of S. pilosicoli with MAb 7G2 revealed three bands corresponding to the core flagellar proteins FlaB1 (37 kDa) and FlaB2 (34 kDa) and the 32-kDa FlaB protein (Fig. 3B) seen in the reference S. hyodysenteriae strain B78 and in S. innocens B256.

N-terminal amino acid sequencing. To confirm the identity of the immunoreactive bands, the N-terminal amino acid sequences of the 37- and 44-kDa bands from the TXI fractions of S. hyodysenteriae B78, reacted with MAb 7G2 and the PAb, respectively, and the 44-kDa band from the TXI fraction of S. pilosicoli P43/6/78, reacted with the PAb, were sequenced through the first 10 amino acid residues. The resulting amino acid sequences were compared with available sequences for the FlaA1 and the FlaB1 proteins of S. hyodysenteriae C5 (Table 2) (16). The 44-kDa protein of S. hyodysenteriae B78 had 9 of 10 amino acid residues identical to those of the FlaA1 protein of S. hyodysenteriae C5 (90% identity) (16). The 44-kDa protein of S. pilosicoli P43/6/78 had 8 of 10 residues identical to those of the FlaA1 protein of S. hyodysenteriae C5 (80% identity). This confirmed that the PAb was specific for the PF sheath protein FlaA1 and that the FlaA1 protein was conserved among porcine Serpulina species. The 37-kDa protein from S. hyodysenteriae B78 had 10 of 10 amino acid residues identical to those of the PF core protein FlaB1 of S. hyodysenteriae C5 (16). This confirmed that MAb 7G2 reacted with the core FlaB proteins.
DISCUSSION

The banding patterns of the immunoreactive bands from the TXS fractions of porcine Serpulina species which were immunoblotted with the MAb 7G2 corresponded to those of the PF core proteins, the FlaB proteins, of S. hyodysenteriae. Comparisons of the N-terminal amino acids of the PF core proteins FlaB1 and FlaB2 of S. hyodysenteriae with those of the PF core proteins of Treponema pallidum, Treponema phagedenis, Spirochaeta aurantia, Borrelia burgdorferi, and Leptospira borgpetersenii have shown a high degree of sequence homology (16). The immunoblot reactivity of MAb 7G2 provided further evidence from strains obtained from pigs in North America, Europe, and Australia for the universal conservation of the PF core proteins among porcine intestinal spirochetes belonging to Serpulina species.

The reactivity of the 44-kDa-protein-specific rabbit PAb with S. hyodysenteriae, S. innocens B256, and three uncharacterized weakly-beta-hemolytic porcine intestinal spirochetes suggested that the PF sheath protein FlaA1 was unique to S. hyodysenteriae (23). This was consistent with a report indicating that the FlaA1 protein has the highest degree of variation among PF proteins, suggesting less functional constraint for the sheath protein (16). In this study, we examined reference strains for the S. hyodysenteriae serogroups proposed by Hampson, with the exception of strain VIC1 of serogroup F, which was not available. The reactivity of the PAb with the TXS fractions of S. hyodysenteriae B78, B204, B169, and A1 was consistent with previous observations made by Li and coworkers (23) using the same antiserum reacted with Triton X-114 detergent extracts. However, when the Australian S. hyodysenteriae strains WA-6, Q16, VIC2, and NSW-1 were immunoblotted with the PAb, a band of approximately 44 kDa, presumably the FlaA1 protein, was present in the TXI fraction but not in the TXS fraction, as was seen in the reference S. hyodysenteriae serotypes 1 through 4. Initial experiments suggested that the Triton X-100 detergent provided a more consistent solubilization of the PF proteins, but further evaluation with the TXI fractions indicated variations in the solubilities of the FlaA1 proteins among porcine Serpulina species. Efforts to solubilize these proteins with Triton X-114 resulted in even more variable separation of the FlaA1 between the detergent and the aqueous phase (data not shown).

In solubility of the FlaA1 protein in Triton X-100 and Triton X-114 and the minor variations in the mobility of the 44-kDa immunoreactive band among Serpulina strains remain unexplained at this time. Nevertheless, we found that the reactivity of the PAb with the TXI fractions of S. innocens strains was similar to that seen with S. hyodysenteriae strains. This discrepancy with the data of Li and coworkers (23) may be attributable to variations in the solubility of the PF sheath protein FlaA1 among the porcine Serpulina species with Triton X-114 and Triton X-100 with or without SDS treatment.

The TXI fractions of some strains of S. hyodysenteriae and S. innocens that were reacted with the PAb had weakly immunoreactive bands of approximately 75 kDa, while the TXI fractions of five of the nine S. pilosicoli strains had 75-kDa immunoreactive bands that were stronger than the 44-kDa bands. The 75-kDa immunoreactive band was absent from purified PF but present in the TXI fraction of S. hyodysenteriae B204, we concluded that the origin of the 75-kDa antigen in detergent extracts was uncertain.

Currently, swine dysentery is diagnosed on the basis of results from bacteriologic culture of feces or mucosal scrapings or based on demonstration of S. hyodysenteriae-specific products after amplification of DNA sequences by PCR (4, 10). The PF of B. burgdorferi have been used as antigens for serologic diagnosis of Lyme disease in human beings; patients develop specific reactivity to the PF proteins early in the course of the infection (12, 37). The evaluation of additional strains of Serpulina species with a 44-kDa-protein-specific PAb provided evidence that this reagent reacted with the PF sheath protein FlaA1 and that, although it was more variable than the other PF proteins, FlaA1 was not unique to S. hyodysenteriae and was present in all porcine Serpulina species. The immunoblot reactivity of MAb 7G2 indicated that it was specific for the PF core FlaB proteins and further confirmed that these proteins were conserved among porcine Serpulina species. MAb 7G2 may be useful for the detection of spirochetes and in studies aimed at determining the role of FlaB in the pathogenesis of enteric diseases caused by porcine intestinal spirochetes.

TABLE 2. Comparison of the amino-terminal sequences of porcine Serpulina species

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. hyodysenteriae C5, FlaA1</td>
<td>LSNSTLIDFA</td>
<td>16</td>
</tr>
<tr>
<td>S. hyodysenteriae B78, 44-kDa band</td>
<td>LNXSTLIDFA</td>
<td>This report</td>
</tr>
<tr>
<td>S. pilosicoli P43/6/78, 44-kDa band</td>
<td>XXNSTLIDFA</td>
<td>This report</td>
</tr>
<tr>
<td>S. hyodysenteriae C5, FlaB1</td>
<td>MVINNNISAI</td>
<td>16</td>
</tr>
<tr>
<td>S. hyodysenteriae B78, 37-kDa band</td>
<td>MVINNNISAI</td>
<td>This report</td>
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the rabbit PAb specific for the 44-kDa PF sheath protein of S. hyodysenteriae.

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REFERENCES


