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Antiserum to the 33,000-Dalton Periplasmic-Flagellum Protein of "*Treponema phagedenis*" Reacts with Other Treponemes and *Spirochaeta aurantia*

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"*Treponema phagedenis*" periplasmic flagella (PF) have two major protein bands at molecular weights of 33,000 and 39,800 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (R. J. Limberger and N. W. Charon, J. Bacteriol. 166:105–112, 1986). By use of Western blotting and a polyclonal antiserum directed toward the 33,000-molecular-weight PF protein, cell lysates of 12 species of spirochetes were surveyed for reactivity. Eight species of *Treponema* as well as *Spirochaeta aurantia* were positive. The results suggest that epitopes residing on the 33,000-molecular-weight PF protein of "*T. phagedenis*" are evolutionarily well conserved among the spirochetes.

Spirochetes constitute a diverse group of helically shaped bacteria which are classified into five genera, i.e., *Cristispira*, *Spirochaeta*, *Borrelia*, *Leptospira*, and *Treponema* (6, 17, 19). In addition, other poorly characterized spirochetes have been found in association with the hindgut of termites and with protozoans (6, 17, 19). These spirochetes all have periplasmic flagella (PF) between the outer membrane sheath and cell cylinder (6, 17, 19). The PFs are involved in the motility of *Leptospira* spp. (3, 5, 8), *Spirochaeta* spp. (13, 24), and *Treponema* spp. (20). Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, we found that the PFs of "*Treponema phagedenis*" have two major protein bands with molecular weights of 33,000 and 39,800 (20). The 33,000-molecular-weight band consists of at least two closely related protein species (S. J. Norris, N. Charon, and R. Limberger, manuscript in preparation). Based on evidence from enrichment of PFs from cell lysates, others have found that multiple proteins constitute the PFs of "*T. phagedenis*" (4, 15, 28, 29; D. R. Blanco, J. D. Radolf, A. E. Urquhart, M. A. Lovett, and J. N. Miller, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, E70, p. 108). These results could be explained by degradation of one or more of these proteins during isolation. However, we recently obtained genetic evidence that multiple major proteins do indeed constitute the PFs of "*T. phagedenis*." Monoclonal and polyclonal PF-specific antisera, Western blotting of wild-type and PF mutants, and immunoelectron microscopy were used in this analysis (20). Moreover, the multiple protein species were found to reside together on a given PF (20).

The antigenic similarity among the PFs of various species of *Treponema* is well established (16, 23, 29). In fact, the PFs of "*T. phagedenis*" have been used as a test antigen for the diagnosis of syphilis (23, 26, 27). The molecular basis for this antigenic similarity is unknown. We present the results of Western blot analysis of one of the previously described PF-specific antisera (20) against cell lysates of various spirochetes. The antiserum was rabbit polyclonal derived and was directed toward and specific for the denatured 33,000-molecular-weight PF protein of "*T. phagedenis*" (20).

(A preliminary report of this work has been presented [R. J. Limberger and N. W. Charon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, D99, p. 71]).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, molecular weight determination, and Western blotting were performed as previously described (20). The laboratory origins and growth of "*T. phagedenis*" Kazan 5, *Leptospira biflexa* serovar patoc strain Patoc I, and "*Leptospira illini*" 3055 have been previously described (7, 20). "*T. phagedenis*" Reiter and "*Treponema refringens*" Nichols were obtained from D. Cox, Texas College of Osteopathic Medicine, Fort Worth. Cells were grown in a peptone-yeast extract-glucose rabbit serum medium (20). "*Treponema vincentii*" N9, *Treponema pectinovorum* P5, and "*Treponema denticola*" W and II were obtained from R. Nauman, University of Maryland, Baltimore. These oral treponemes were grown in peptone-yeast extract-glucose rabbit serum medium supplemented with 0.33% thiamine pyrophosphate (19). Glucuronic acid (0.3%) was added to the growth medium of *T. pectinovorum* (31). *Escherichia coli* K-12 ATCC 12435 was grown in nutrient broth (Difco Laboratories). The following strains were provided as frozen cell pellets: *Spirochaeta aurantia* M1 (14) from E. P. Greenberg, Cornell University, Ithaca, N.Y.; *Treponema hyodysenteriae* B204 and *Treponema innocens* B256 (18) from L. Joens, University of Arizona, Tucson; *Borrelia hermsii* and *Borrelia burgdorferi* B31 (1) from R. C. Johnson, University of Minnesota, Minneapolis; and *Treponema pallidum* subsp. *pallidum* Nichols (15) grown in rabbit testes and isolated by the procedure of Hanff et al. (15) by B. Steiner, University of Texas, Houston. *T. pallidum* subsp. *pallidum* Nichols was also grown and isolated by the procedure described by Fitzgerald and Repesh (11) by T. Fitzgerald, University of Minnesota, Duluth (11).

We surveyed several lysates of bacteria for reactivity to the PF-specific antiserum. The Western blot reactions of cell lysates of various spirochetes probed with a polyclonal antiserum to the 33,000-molecular-weight PF protein of "*T. phagedenis*" are illustrated in Fig. 1. The antiserum showed extensive cross-reactivity with the other spirochetes. All the treponemes had cross-reacting proteins with molecular

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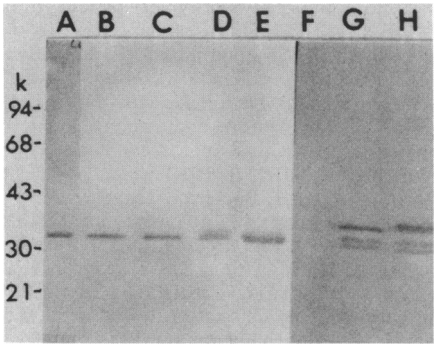

FIG. 1. Western blot of cell lysates of various spirochetes by using the polyclonal antiserum directed to the 33,000-molecular-weight PF protein of "*T. phagedenis*" Kazan 5. Lanes: A, *T. pectinovorum* P5; B,"*T. denticola*" 11; C, "*T. denticola*" W; D, "*T. vincentii*" N9; E, "*T. phagedenis*" Kazan 5; F, "*L. illini*" 3055; G, *T. hyodysenteriae* B204; H, *T. innocens* B256. Numbers at left indicate molcular weight in thousands.

weights between 32,000 and 37,000. Some had multiple protein bands. No reaction was detected with "*L. illini.*" *S. aurantia*, "*T. phagedenis*" Reiter, and "*T. refringens*" were also reactive (Table 1). *Spirochaeta halophila* was also found to be positive with this antiserum (B. Brahamsha and E. P. Greenberg, personal communication). The results (Fig. 2) indicate that a protein band in *T. pallidum* with a molecular weight of 33,000 reacted with this antiserum. No reactions were noted with *L. biflexa* serovar patoc, *B. hermsii*, *B. burgdorferi*, and *E. coli*. The results are summarized in Table 1. Our results with *Borrelia* spp. agree with those of Barbour et al. (2). They found that monoclonal antibodies to *Borrelia* PFs failed to react with those of other spirochetes.

The results indicate that epitopes similar to those residing on the 33,000-molecular-weight PF protein of "*T. phagedenis*" are found in other *Treponema* species and in *S. aurantia*. The nature of the immunizing antigen is important in considering the type of cross-reactivity observed. The antigen used for production of this polyclonal antiserum was the denatured 33,000-molecular-weight PF protein (20).

TABLE 1. Western blot reactions of cell lysates of various bacteria probed with a polyclonal antiserum directed to the 33,000-molecular-weight PF protein of "*T. phagedenis*" Kazan 5

Organism	Mol wt
"*T. phagedenis*" Kazan 5 and Reiter	33,000
T. pectinovorum P5	33,000
"*T. denticola*" 11 and W	33,000
"*T. vincentii*" N9	33,000, 34,000
T. hyodysenteriae B204	32,000, 35,000, 37,000
T. innocens B256	32,000, 35,000, 37,000
"*T. refringens*" Nichols	33,000
T. pallidum subsp. *pallidum* Nichols	33,000
"*L. illini*" 3055	
L. biflexa serovar patoc strain Patoc I	
B. hermsii	
B. burgdorferi B31	
S. aurantia M1	32,000, 36,500[a]
E. coli K-12	

[a] B. Brahamsha and E. P. Greenberg (personal communication) found molecular weights of 31,500, 33,000, 34,000, and 36,000 by using this antiserum against both cell lysates and purified PFs of *S. aurantia*.

These types of antibodies are notably different from those induced to intact PFs. For example, the polyclonal PF antiserum failed to react with intact PFs, based on immunoelectron microscopy (20). Similar results have been found with antisera directed towards *Bacillus subtilis* flagellin; these antibodies react weakly with intact flagellum filaments, but they react strongly to flagellin (10, 30). In addition, they show extensive cross-reactivity with the flagellin derived from several strains of *B. subtilis* (30). Such cross-reactivity is not as prevalent with antisera induced to intact flagella (10, 30).

Our results do not demonstrate that the proteins in the other spirochetes that reacted with the antiserum are PF proteins. However, most of the reactive proteins have molecular weights similar to those found in "*T. phagedenis*" PFs (Table 1). In addition, results with purified PFs from *S. aurantia* and from *T. pallidum* indicate that the reactive proteins are indeed PF proteins (B. Brahamsha, E. P. Greenberg, and S. J. Norris, personal communications).

Two explanations could account for the extensive immunological cross-reactivity we noted. First, the results could be a consequence of the 33,000-molecular-weight PF protein of "*T. phagedenis*" and certain proteins from *T. pallidum*, *S. aurantia*, and other treponemes having some common amino acid sequences. The extensive immunological cross-reactivity noted among flagellin from different strains of *B. subtilis* is likely the result of antibodies recognizing epitopes related to the primary structure of the proteins (30). Alternatively, the results could be explained by a common epitope resulting from posttranslational modification such as glycosylation (9). Along these lines, *T. pallidum* and "*T. phagedenis*" synthesize glycosylated proteins, with both species synthesizing at least one glycoprotein with a molecular weight of approximately 33,000 (22). In addition, because glycoproteins have also been found to constitute *Halobacterium halobium* flagella (32), it is at least possible that such modification occurs on spirochete PFs.

The extensive immunological cross-reactions with the "*T. phagedenis*" PF antiserum are intriguing. Many of the spirochetes found to be reactive have very little DNA homology with one another (21); they also have markedly different guanine-plus-cytosine content in their DNAs (19). For example, "*T. phagedenis*" and *T. pallidum* show ap-

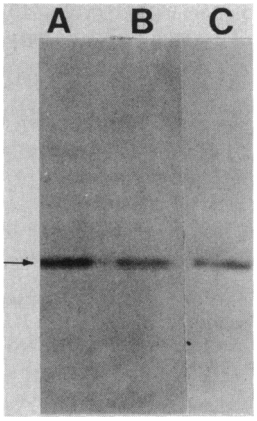

FIG. 2. Western blot of cell lysates of *T. pallidum* subsp. *pallidum* Nichols and "*T. phagedenis*" Kazan 5 by using the polyclonal antiserum directed to the 33,000-molecular-weight PF protein. Lanes: A, *T. pallidum* from B. Steiner; B, *T. pallidum* from T. Fitzgerald; C, "*T. phagedenis.*" The arrow indicates a protein molecular weight of 33,000.

proximately 5% DNA homology (21); the G+C content of "*T. phagedenis*" DNA is 38 to 39%, and that of *S. aurantia* is 61 to 65% (19). Oligonucleotide cataloging of 16S RNA suggests that the spirochetes are an ancient evolutionary branch of the eubacteria (12) and do not result from convergent evolution (25). The unpublished results of B. Brahamsha, E. P. Greenberg, and S. J. Norris indicate that purified PFs from *S. aurantia* and *T. pallidum* react with the antiserum directed to the 33,000-molecular-weight PF protein of "*T. phagedenis*." Given these results, the most likely explanation for this cross-reactivity is that the common epitopes are related to functions necessary for motility in at least these three species and are thus well conserved in evolution.

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