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Chemotactic Response to Mucin by *Serpulina hyodysenteriae* and Other Porcine Spirochetes: Potential Role in Intestinal Colonization

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Chemotaxis of porcine spirochetes towards a variety of mucins was measured quantitatively by a capillary method. A chemotaxis buffer consisting of 0.01 M potassium phosphate buffer (pH 7.0) and 0.2 mM L-cysteine hydrochloride was necessary for chemotaxis of spirochetes. The optimum incubation time and incubation temperature were 1 h and 40°C, respectively. The mucin concentration also affected the chemotaxis observed, and a concentration of 1% (wt/vol) was near the optimum. Virulent *Serpulina hyodysenteriae* strains were chemotactic towards 1% (wt/vol) hog gastric mucin and 1% (wt/vol) porcine colonic mucin but not towards 1% (wt/vol) bovine submaxillary mucin. Virulent *S. hyodysenteriae* strains were significantly more chemotactic than avirulent strains of *S. hyodysenteriae* (SA3 and VS1), *Serpulina intermedius*, and *Serpulina innocens*. Other spirochetes belonging to the proposed group of spirochetes *Anguillina coli* were also not chemotactic. Pathogenicity of *S. hyodysenteriae* strains that cause swine dysentery may, in part, be attributed to their attraction to porcine intestinal mucus.

Motility and chemotaxis have been shown to play an important role in bacterial colonization of several environments. A chemotactic response to formate by *Campylobacter concisus* was implicated in gingival colonization (34), whereas motility alone was important in intestinal colonization of suckling mice by *Campylobacter jejuni* (32). Both chemotaxis and motility towards mucosal slices were essential for virulence of *Vibrio cholerae* in mice and rabbits (10–13, 16).

Spirochetes have a unique system for motility, and the chemotaxis of some of these organisms has been studied (3, 4). *Spirochaeta aurantia* has been used as a model for spirochete chemotaxis (7, 8, 15, 20). Many of its chemoattractants and repellants are known. *Spirochaeta aurantia* is attracted to a number of different sugars and is aerotactic (15), whereas acids, alcohols, and sulfides were effective chemorepellants (20). Recently, Yuri et al. (38) have demonstrated chemotaxis of virulent leptospires, but not avirulent strains, towards hemoglobin.

Swine dysentery is a mucohemorrhagic diarrheal disease of pigs caused by *Serpulina hyodysenteriae* (37). Electron microscopy studies have shown that the spirochetes associate closely with the colonic mucosa, in particular within the crypts of Lieberkühn, and are present in and around necrotic epithelial cells and laminae propriae (22). However, there is no evidence that deep invasion of the tissues occurs.

Kennedy et al. (22) proposed three different mechanisms by which spirochetes could colonize the mucosa: (i) specific or nonspecific adhesion to the epithelium or mucus, (ii) coadhesion to adherent microorganisms, or (iii) chemotactic and/or motility-regulated mucus association. These investigators observed *S. hyodysenteriae* to be highly motile in intestinal mucus and also demonstrated chemotaxis of in vivo-grown *S. hyodysenteriae* B204 to 1% (wt/vol) hog gastric mucin (HGM). Attempts to study chemotaxis by using in vitro-grown *S. hyodysenteriae* were not successful, in part because of a lack of motility of the spirochetes in such cultures.

The purpose of this investigation was to characterize the chemotaxis of selected porcine spirochetes, including *S. hyodysenteriae* strains, in order to evaluate the role that this property may play in colonization of the porcine colonic mucosa.

Bacterial strains and culture conditions. The porcine spirochete strains used in this study are shown in Table 1. The bacteria were grouped according to their electrophoretic types on the basis of multilocus enzyme electrophoresis as described by Lee et al. (28, 29). Multilocus enzyme electrophoresis was used to determine the genetic diversity of the porcine spirochetes. Fifteen enzymes from each strain were examined, and the mobility variants were equated with alleles at the structural gene loci. Each group with the same alleles at all loci was referred to as being an electrophoretic type. Enteropathogenicity in pigs, when known, is also shown. Two *S. hyodysenteriae* spirochetes that have been shown to be nonpathogenic in pigs are VS1 (2) and SA3 (29). These strains also do not appear to be pathogenic in the mouse model of swine dysentery (36). All other *S. hyodysenteriae* strains have been considered pathogenic either because they have produced dysentery in pigs or because they have been isolated from pigs with dysentery.

The spirochetes were grown in Trypticase soy broth supplemented with 5% (vol/vol) rabbit serum and incubated at 40°C under an anaerobic gas mix (10% CO_2–90% N_2), as described by Kent et al. (23). Actively motile cultures, as determined by phase-contrast microscopy, in mid-logarithmic growth phase were used in the chemotaxis assays.

Mucins. HGM and bovine submaxillary mucin (BSM) were commercial preparations (Sigma, Poole, United Kingdom). Porcine colonic mucin (PCM) was prepared from healthy conventionally reared pigs according to the method of Marshall and Allen (30). Solutions of 1% (wt/vol) mucin were prepared in phosphate-buffered saline (PBS; per liter, 10 g of NaCl, 0.25 g of KCl, 0.1438 g of Na_2HPO_4, 0.25 g of KH_2PO_4 [pH 7.2]) or in chemotaxis buffer (0.01 M potassium phosphate

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TABLE 1. Differentiation of porcine spirochete strains

Strain	ET[a]	EP[b]
S. hyodysenteriae		
B78[c]	9	+
KF9	12	+
P18A	13	+
B204	15	+
B169	16	+
NSW2	22	NT1
SA3	29	–
ACK300/8[d]	NT	NT1
VS1	NT	–
S2	NT	+
S. intermedius[e]		
2818.5	30	NT2
PWS/A[c]	34	–
889	35	NT2
S. innocens		
B256[c]	38	–
4/71	43	–
Anguillina sp.[e]		
155-20	52	NT2
P43/6/78	60	NT2
3295	61	NT2
M1	62	–
LL3	NT	NT3

[a] ET, electrophoretic type by multilocus enzyme electrophoresis (28, 29). NT, not tested.
[b] EP, enteropathogenicity testing in pigs. NT1, not tested but isolated from pigs with swine dysentery; NT2, not tested but isolated from pigs with forms of diarrhea; NT3, not tested but isolated from pigs with no clinical signs (2, 6, 29).
[c] Type strain.
[d] This strain has also been called JWPM 300/8.
[e] Proposed by Lee et al. (29).

buffer [pH 7.0], 0.2 mM L-cysteine hydrochloride) used for *Spirochaeta aurantia* chemotaxis experiments (15).

Chemotaxis assay. The technique for the measurement of chemotaxis was based on a modification (22) of the original method of Adler (1). Capillary tubes (5 μl) were filled with 1% (wt/vol) mucin and placed in 0.5 ml of cell culture (approximately 10^8 cells per ml) in sterile glass test tubes (10 by 75 mm). In all experiments, chemotaxis buffer alone was included as a control. The tubes were capped and incubated at 40°C in an anaerobic cabinet (Don Whitley Scientific, Shipley, United Kingdom). After incubation, the contents of the capillaries were expelled into 0.5-ml Microfuge tubes (Eppendorf) containing 45 μl of chemotaxis buffer and mixed. The total number of spirochetes present was determined by using a Thoma counting chamber. Chemotaxis was expressed as the ratio (R_{che}) of the number of bacteria in the mucin capillary to the number in the control capillary (21). Calculation of the R_{che} value was used to normalize experimental or day-to-day variation in data (33). Individual strains were examined in two separate experiments, using fresh inocula, and for each experiment, duplicate tests were performed and the mean and standard error of the mean were calculated.

Conditions for chemotaxis. Conditions for the optimum chemotaxis of *S. hyodysenteriae* P18A to HGM were established. Adler (1) suggested that a chemotaxis buffer should contain K^+ or Na^+ salts at pH 7.0. Figure 1 shows the relative chemotaxis of P18A to HGM (0.1 to 1.0% [wt/vol]) in PBS and *Spirochaeta aurantia* chemotaxis buffer (15). Significant levels of chemotaxis, i.e., an R_{che} of >2, were achieved only in the

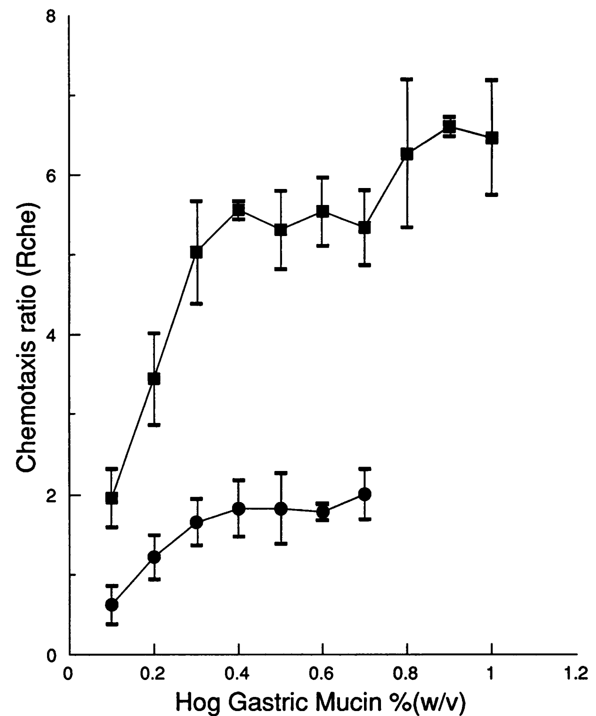

FIG. 1. Chemotaxis of *S. hyodysenteriae* P18A to HGM at 0.1 to 1.0% (wt/vol) in either PBS (●) or chemotaxis buffer (■). The spirochetes were incubated for 60 min at 40°C. Error bars indicate standard errors of the means.

Spirochaeta aurantia chemotaxis buffer. In this buffer, chemotaxis of *S. hyodysenteriae* P18A increased with increasing concentrations of HGM, reaching a maximum R_{che} of 6.6 at 0.8 to 1.0% (wt/vol) HGM. Kennedy et al. (22) used 0.85% saline as the diluent in their chemotaxis experiments with *S. hyodysenteriae*. Chemotaxis of B204 cells grown in vivo was observed; however, no chemotaxis was observed with cells grown in vitro. In this study, chemotaxis of cells grown in vitro was demonstrated with a phosphate buffer containing L-cysteine hydrochloride. The presence of L-cysteine hydrochloride maintained reduced conditions, which may have facilitated chemotaxis of this facultative anaerobic spirochete.

When the effect of incubation time on chemotaxis of P18A to 1.0% (wt/vol) HGM was examined, a significant R_{che} was observed after 20 min and reached a maximum in 60 min (Fig. 2). The optimum temperature for chemotaxis was 40°C, when an R_{che} of 7.8 was obtained. Significant chemotactic responses were not observed for temperatures below 28°C or above 50°C (Fig. 3). The optimum conditions for chemotaxis of porcine spirochetes were similar to those described for other motile bacteria (15, 34). The optimum temperature for *Spirochaeta aurantia* chemotaxis was 25°C, whereas the optimum temperature for *S. hyodysenteriae* was approximately 40°C, which is the optimum growth temperature for this organism (31).

When 1% (wt/vol) HGM was present in the bacterial culture as well as the capillaries, the R_{che} was 1.2. This result indicated that only low numbers of cells migrated into the capillaries and suggested that migration towards the mucin was a result of chemotaxis rather than chemokinesis (34).

Chemotaxis of porcine spirochetes to HGM, PCM, and BSM. All virulent *S. hyodysenteriae* strains gave similar chemotactic responses (R_{che} of 4.5 to 7.5) to HGM (Fig. 4). *Serpulina*

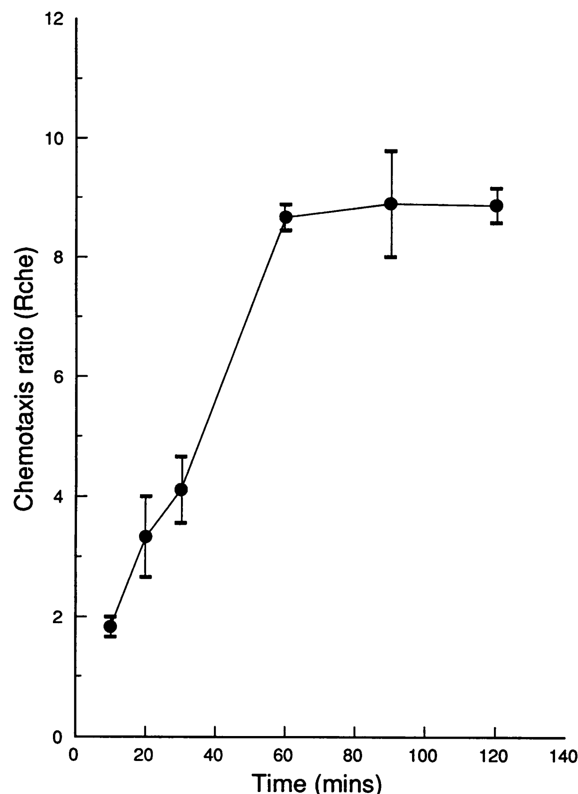

FIG. 2. Effect of incubation time on chemotaxis of *S. hyodysenteriae* P18A to 1% (wt/vol) HGM in chemotaxis buffer. Error bars indicate standard errors of the means.

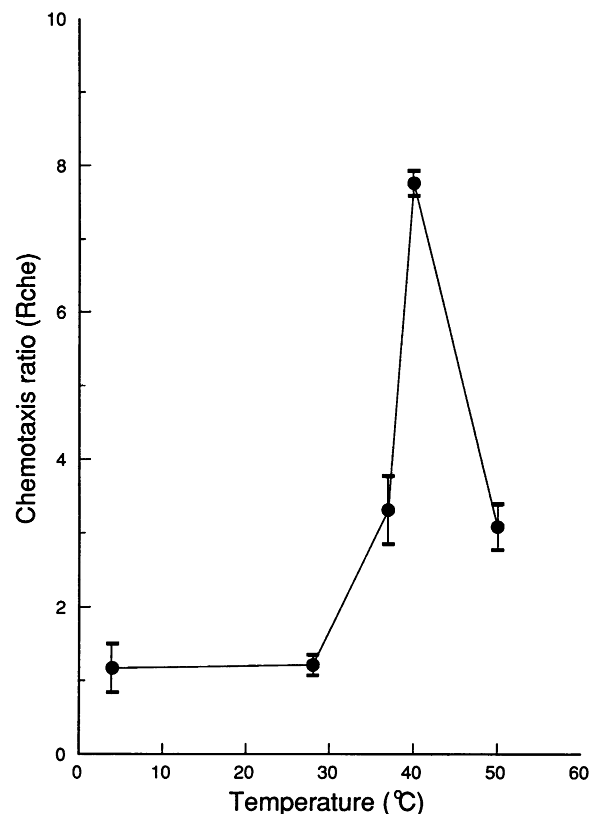

FIG. 3. Effect of incubation temperature on chemotaxis of *S. hyodysenteriae* P18A to 1% (wt/vol) HGM in chemotaxis buffer. Error bars indicate standard errors of the means.

intermedius strains (PWS/A, 2818.5, and 889) were weakly chemotactic (R_{che} of 2 to 3). *Serpulina innocens* strains (B256 and 4/71) and other porcine spirochetes were not chemotactic (R_{che} < 2). Virulent strains of *S. hyodysenteriae* showed significant chemotaxis to 1% (wt/vol) HGM. However, avirulent strains of *S. hyodysenteriae* and *S. intermedius* strains, all of which are considered to be nonpathogenic (29), had reduced chemoattraction. All other nonpathogenic porcine spirochetes were not attracted to this mucin. There were no significant differences ($P > 0.05$) between virulent *S. hyodysenteriae* strains, but, in general, these strains were significantly different ($P = 0.005$) from the other spirochetes. *S. intermedius* strains were not significantly different ($P > 0.05$) from either the *S. innocens* strains or other nonpathogenic spirochetes.

In studies with *V. cholerae*, Freter et al. (13) showed that chemotactic *V. cholerae* cells actively invaded the mucus gel covering the rabbit small intestine. On the other hand, motile but nonchemotactic mutants invaded the mucus at the same rate as inert particles. Since only virulent porcine spirochetes showed chemoattraction, it may be that this property enables these spirochetes to infect the colonic mucosa more easily than other spirochetes.

In order to examine this further, porcine colonic mucus was collected, and the glycoprotein was prepared. In addition, a readily available source of submaxillary mucin (BSM) was also used in chemotaxis assays to demonstrate that the response to intestinal mucin was specific and was not caused by the viscosity of the medium. The relative chemotaxis of eight different spirochetes to BSM and PCM is shown in Table 2. None of the strains was significantly attracted to BSM. However, chemotaxis of the spirochete strains towards PCM was similar to that observed with HGM, and only the virulent *S. hyodysenteriae* strains were significantly chemotactic. Although only two virulent *S. hyodysenteriae* strains were tested, the difference between these strains and the remaining spirochetes was statistically significant ($P = 0.005$). However, other virulent strains of *S. hyodysenteriae* may not be similarly chemotactic.

The similar results obtained when either HGM or PCM was

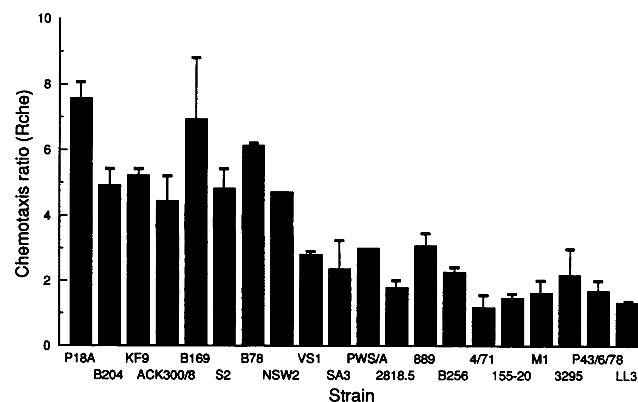

FIG. 4. Chemotaxis of various porcine spirochete strains to 1% (wt/vol) HGM in chemotaxis buffer. Error bars indicate standard errors of the means.

TABLE 2. Chemotaxis of various porcine spirochetes towards purified colonic mucin (1% [wt/vol]) and BSM (1% [wt/vol])

Strain	No. of flagella	R_{che} ± SEM	
		Colonic	Submaxillary
S. hyodysenteriae			
P18A	10[a]	5.00 ± 0.60[b]	2.03 ± 0.14
B78	7–10[c]	5.93 ± 0.68[b]	1.55 ± 0.26
SA3	7–9[d]	1.90 ± 0.40	2.03 ± 0.90
VS1	12[e]	1.94 ± 0.35	1.60 ± 0.55
S. intermedius			
PWS/A	9[e]	1.37 ± 0.08	1.01 ± 0.21
2818.5	9–14[d]	1.35 ± 0.02	1.59 ± 0.08
S. innocens			
B256	7–10[c]	1.32 ± 0.09	1.44 ± 0.04
4/71	10–14[c]	1.24 ± 0.04	1.25 ± 0.25

[a] Reference 18.
[b] Statistically significantly different from the ratio of other strains at P of 0.005 (n = 4).
[c] Reference 25.
[d] Reference 27.
[e] The number of endoflagella determined by negative staining according to the method of Kent et al. (24).

used and the absence of chemotaxis to BSM may be attributed to the different compositions of these glycoproteins. Porcine gastric mucin and PCM have similar compositions, approximately 83% carbohydrate, 3% ester sulfate, and 13% protein (percent [dry weight] glycoprotein) (30, 35), but their molecular weights are different (2×10^6 for gastric mucin and 15×10^6 for PCM). All submaxillary mucins, regardless of species, have typical compositions: 60 to 65% carbohydrate, 30 to 40% protein, and no ester sulfate (percent [dry weight] glycoprotein) (14, 17). The submaxillary mucins, although similar to one another, are clearly distinct from the gastric and colonic mucins, a major feature being the greater glycosylation of HGM and PCM. However, differences in the composition and/or structure of the HGM, PCM, and BSM may account for the observed differences in chemotaxis, but this is not understood.

It was possible that differences in motility of the spirochetes, as a result of different numbers of endoflagella, could have resulted in significant differences in chemotaxis to HGM and PCM. All strains possessed similar numbers of endoflagella (Table 2); therefore, motility probably had no effect on the differences in chemotaxis observed.

The association of bacteria with specific tissues in vivo is a complex process. Freter (9) has proposed the involvement of a number of steps: (i) chemotactic attraction of motile bacteria to the surface of the mucus gel, (ii) penetration of and trapping within the mucus gel, (iii) adhesion to receptors in the gel or to mucosa-associated layers of the indigenous microflora, (iv) adhesion to epithelial cell surfaces, and (v) multiplication of the bacteria. Successful negotiation of each step may require the expression of a distinct set of virulence determinants. However, no single pathogen needs to employ all these mechanisms, because association with the mucus could be the final step in colonization.

The effectiveness of colonization of sites within the intestine is probably due to the interplay between motility, chemotaxis, and adhesion. Cohen et al. (5) postulated that the importance of chemotactic or motility-regulated mucus association was dependent on the particular microorganism, the presence of normal flora, and the site of colonization. Motility and chemotaxis were not important for colonization by *Escherichia coli* or *Salmonella typhimurium*. However, Hugdahl and Doyle (19) found that mucus was chemotactic for *C. jejuni*, and Lee et al. (26) proposed that a major determinant of pathogenicity of *C. jejuni* was its ability to colonize intestinal mucus. This organism was similar to *S. hyodysenteriae* in that it rapidly tracked along intestinal mucus and ultimately colonized the intestinal crypts, but evidence of adhesion to the epithelial surface was not observed.

It could be hypothesized that mucus colonization alone may be the sole mechanism by which *S. hyodysenteriae* associates with the intestinal epithelium, and so, adhesion may be unnecessary. Adhesion to the colonic mucosal epithelium is not a major feature of infection by *S. hyodysenteriae*, but adhesion and penetration of epithelial cells have been observed (22). Chemotaxis alone could enable *S. hyodysenteriae* to colonize the mucosal surface in numbers high enough to induce a pathogenic effect on the host.

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