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Hemagglutination Properties of *Streptococcus suis*

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A total of 49 strains (23 reference strains and 26 field isolates) of *Streptococcus suis* were tested for their ability to agglutinate erythrocytes from different animal species. Ten different hemagglutination patterns were established. Thirty-three strains (67%) did not agglutinate any of the erythrocytes tested; sixteen strains (33%) agglutinated erythrocytes from one or more animal species. Different strains belonging to the same capsular type presented different hemagglutination patterns. No correlation was found between the tissue origin and/or the virulence (evaluated in 4-week-old mice) of different field isolates and their hemagglutination activity. Hydrophobic surface properties were also evaluated. All *S. suis* strains studied appeared to possess a hydrophilic cell surface. Morphologically similar fimbriae were observed on hemagglutinating as well as on nonhemagglutinating strains of *S. suis*. This study provides evidence that certain strains of *S. suis* possess hemagglutinating properties which do not appear to involve hydrophobic interactions. The possible role of fimbriae in hemagglutination remains unclear.

Streptococcus suis is a major problem in pig production, in which it has been associated with meningitis, septicemia, pneumonia, arthritis, and other conditions (5, 13, 14). It is also known to cause meningitis in humans (4). Up to now, 23 capsular types have been described, and most of them are present in North America (4; R. Higgins and M. Gottschalk, Proc. Assoc. Swine Pract., 1990). The mechanisms of pathogenicity of *S. suis* are not really known; however, the capsular material and some surface proteins were suspected as virulence factors (2, 15). Recently, fimbriae were described on cells of capsular types 1 to 8, but their physiopathogenic functions are not known (6).

It is accepted that the interaction of bacteria with mammalian cells is important for pathogenicity (1). The ability of bacteria to attach to and agglutinate erythrocytes may be used in vitro for studying host-bacterium interaction and mechanisms of attachment (10). Recently, some *S. suis* strains were shown to agglutinate human group B erythrocytes (8), but a complete description of hemagglutination properties of the different *S. suis* capsular types have not, to our knowledge, been thoroughly examined.

The aim of this study was to investigate the hemagglutination patterns (HAPs) of different capsular types of *S. suis* by using erythrocytes from several different animal species and to correlate them with fimbriation, surface hydrophobicity, and virulence in mice.

S. suis reference strains (capsular types 1 to 22 and 1/2) were obtained from R. Higgins. A total of 26 field isolates originating from diseased (18 isolates) and clinically healthy (8 isolates) pigs were also studied. They belonged to the following capsular types: type 2 (14 isolates), type 3 (4 isolates), type 19 (4 isolates), and type 22 (4 isolates). The isolates were all biochemically identified as *S. suis* and serotyped by using the coagglutination and capsular reaction tests (4, 5). These capsular types are frequently isolated from diseased pigs (capsular types 2, 3, and 22) and from clinically healthy pigs (capsular types 3 and 19) in Canada (5; M. Gottschalk et al., submitted for publication). For this study, they were grown overnight on bovine blood agar plates and in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) at 37°C. Dense bacterial suspensions (approximately 10^{10} CFU/ml) were prepared in phosphate-buffered saline (0.01 M; pH 7.2).

The hemagglutination test was performed as previously described (7). Blood was collected in Alsever's solution from the following species: sheep, cattle, goat, pig, horse, rabbit, guinea pig, chicken, dog, rat, mouse, and human (types A, B, and O). Erythrocytes were washed twice in phosphate-buffered saline and suspended to obtain a final concentration of 3%. Bacterial suspension (1 drop) was added to 1 drop of erythrocyte suspension and 1 drop of saline in the circular depression of a glass slide, which was rocked at room temperature (10 min) and at 4°C (10 min). *Escherichia coli* 8a (O115K-F1+) was used as a positive control. A parallel test, in which a drop of 1.5% (wt/vol) D-mannose was substituted for the drop of saline in the mixture, was used to determine whether the activity was mannose susceptible. To verify reproducibility, the hemagglutination assay was repeated three times under the same conditions.

Of the 49 strains studied, 10 different HAPs were found (Table 1). Thirty-three strains (67%) did not agglutinate any of the erythrocytes tested; sixteen strains (33%) agglutinated erythrocytes of one or several animal species. One reference strain (capsular type 17) and one field isolate of capsular type 19 agglutinated all types of erythrocytes tested. Human erythrocytes of groups O and B were the most frequently agglutinated. Among reference strains, most hemagglutinating strains belonged to the recently described capsular types 9, 10, 11, 13, 15, 17, 18, 19, and 22. Of the previous capsular types, only capsular type 1 showed hemagglutination activity. Capsular type 4 reference strain, which agglutinated human group B erythrocytes in a previous report (8), was negative for all types of erythrocytes in this study. This discrepancy could be explained by a possible phase variation of the strain, the origin of erythrocytes, and/or differences in experimental conditions. Different strains belonging to the same capsular type presented different HAPs. None of the capsular type 3 strains agglutinated any of the types of erythrocytes tested. However, because of the low number of strains of each capsular type, no definitive conclusion could be reached about the possible correlation between capsular

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TABLE 1. HAPs of 49 strains of *S. suis* grown overnight on bovine blood agar plates

HAP	No. of strains	Capsular type	Hemagglutination activity of erythrocytes from[a]:													
			B	Hr	Gp	H(B)	H(O)	H(A)	C	D	M	R	P	S	Go	Rb
I	33	1/2, 2–8, 12, 14, 16, 19–21, 22	−	−	−	−	−	−	−	−	−	−	−	−	−	−
II	1	1	+	−	−	−	−	−	−	−	−	−	−	−	−	−
III	1	2	−	+	−	−	−	−	−	−	−	−	−	−	−	−
IV	1	18	−	−	+	−	−	−	−	−	−	−	−	−	−	−
V	6	2, 9, 10, 22	−	−	−	+	+	−	−	−	−	−	−	−	−	−
VI	1	13	−	−	−	+	+	+	−	−	−	−	−	−	−	−
VII	1	15	−	−	−	+	+	+	+	−	−	−	−	−	−	−
VIII	1	22	−	−	−	+	+	+	−	+	+	+	−	−	−	−
IX	2	19	+	+	+	+	+	+	+	+	+	+	+	+	+	−
X	2	17, 19	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] B, Bovine; Hr, horse; Gp, guinea pig; H, human groups A, B, and O; C, chicken; D, dog; M, mouse; R, rat; P, pig; S, sheep; Go, goat; Rb, rabbit. −, No hemagglutination activity observed; +, hemagglutination activity observed.

type and hemagglutination activity. Furthermore, no relation was found between the tissue origin of different field isolates and their hemagglutination properties. Only one of the eight isolates recovered from the nervous system in cases of meningitis showed hemagglutination activity. None of the field isolates recovered from lungs (cases of pneumonia) and only 50% of the field isolates recovered from several tissues (cases of septicemia) were positive (Table 2). This is in agreement with previous information (8).

Hemagglutination was observed with microorganisms grown either on bovine blood agar plates or in Todd-Hewitt broth and was not inhibited by the addition of D-mannose. In most cases, the hemagglutination activity was temperature independent. These results are at variance with a previous work (8) in which hemagglutination was stronger at 0°C than at 22°C.

The cell surface hydrophobicity was measured by using the salt aggregation test (9). Serial dilutions were made in sodium phosphate (0.002 M; pH 6.8) to obtain $(NH_4)_2SO_4$ concentrations ranging from 4 to 0.2 M. Dense bacterial suspensions were mixed with an equal volume of salt solution in the depression of a glass slide, which was rocked for 2 min at room temperature, and visualized against a black background. A *Staphylococcus aureus* Cowan I strain was used as a positive control. All strains presented a hydrophilic surface and did not aggregate even at a high ammonium salt concentration (4 M). This is in accordance with the presence of a polysaccharidic capsule which has been shown to decrease surface hydrophobicity (12). Adhesion and hydrophobicity in group A streptococci is mediated, at least in part, by lipoteichoic acid of the bacterial surface (12). In spite of the presence of a lipoteichoic acid in all group D streptococci, it has been shown that *S. suis* lipoteichoic acid is located deep in the cell wall and is probably not exposed at the bacterial surface (3).

TABLE 2. Tissue origin of 26 hemagglutinating and nonhemagglutinating *S. suis* field isolates

Tissue origin	No. of isolates with[a]:	
	Positive hemagglutination activity	Negative hemagglutination activity
Brain	1	7
Multiple tissues[b]	2	4
Lung	0	4
Nose or tonsils[c]	3	5

[a] Negative activity corresponds to HAP I; positive activity corresponds to HAPs II to X.
[b] Probable septicemia.
[c] From clinically healthy pigs.

Tests for virulence were performed in 4-week-old mice by using the intraperitoneal route. Bacteria were grown in Todd-Hewitt broth, harvested in log-phase growth (6 to 7 h), and suspended in phosphate-buffered saline to a concentration of approximatively 10^9 CFU/ml. Only the 26 field isolates were evaluated. Virulence was estimated by determining the 50% lethal dose by the Reed-Muench method (11) with groups of five mice. Death or presence of clinical signs was monitored over the next 14 days. No correlation could be drawn between hemagglutination activity and virulence for mice. The 50% lethal dose of hemagglutinating isolates was 3.56 ($\pm$2.8) $\times 10^8$, and that of nonhemagglutinating isolates was 2.1 ($\pm$1.90) $\times 10^8$.

Dense bacterial suspensions prepared for hemagglutination were examined after negative staining. In this case, only hemagglutinating and nonhemagglutinating strains belonging to the same capsular type were tested. A drop of each preparation was placed on a 200-mesh Formvar-coated electron microscope grid and blotted partially dry. A drop of 2% (wt/vol) phosphotungstate (pH 7.0) was then applied to the grid. Grids were examined with a Philips 201 electron microscope (6). The presence of a peritrichous array of surface fimbriae extending outward from the cell wall was observed among hemagglutinating as well as nonhemagglutinating strains (Fig. 1). This is in accordance with previous results in which the presence of fimbriae in all capsular types 1 to 8 and 1/2 reference strains was demonstrated (6). None of these strains, with the exception of capsular type 1 reference strain, showed hemagglutination activity in this study. Despite the fact that fimbriae present in different strains were morphologically similar, they may be functionally different. Their composition and function have yet to be determined.

Fimbriae are often described as hydrophobins, but that capacity depends mostly on the spatial disposition of hydrophobic amino acid residues (12). Moreover, in the case of *S. suis* strains, the hydrophobic nature of fimbriae should compete with the hydrophilic activity of the capsular material. Additional studies should be performed to characterize the hemagglutinins and to determine whether only some fimbriae, although all morphologically very similar, bear the hemagglutination activity. The nature of both hemagglutinins and fimbriae as well as their exact function in *S. suis* infection remains unclear.

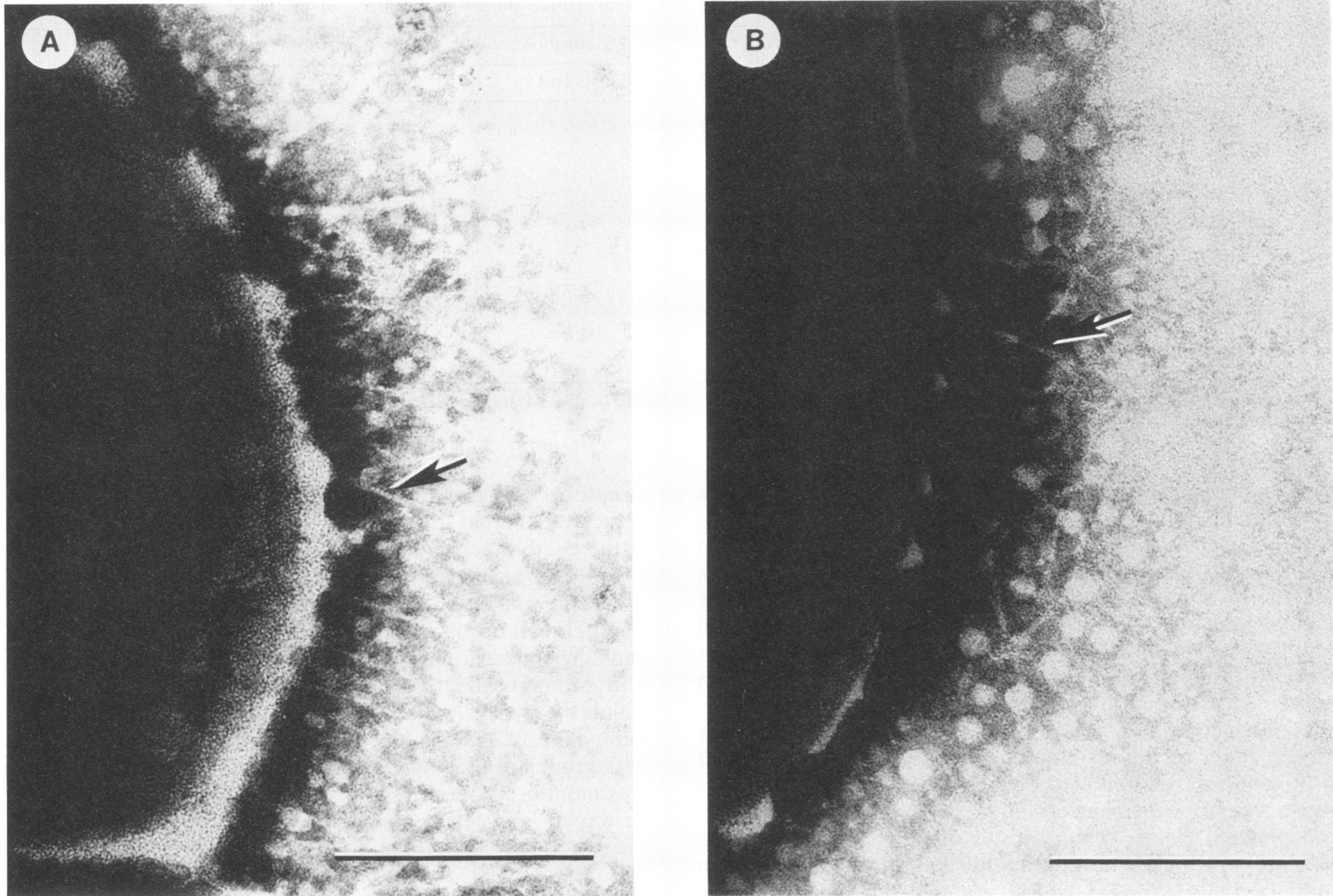

FIG. 1. Transmission electron micrographs of negatively stained preparation of *S. suis* grown on bovine blood agar plates. (A) Hemagglutination-positive isolate of capsular type 19 (HAP X); (B) hemagglutination-negative isolate of capsular type 19 (HAP I). Note the presence of fimbriae (◀) extending from the cell surface. Bars, 200 nm.

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