Detection of Coagulase Activity in *Erysipelothrix rhusiopathiae*

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Coagulase activity was detected in 99% of 225 strains of *Erysipelothrix rhusiopathiae*. These strains included isolates from a variety of animal and environmental sources. Activity could be detected by the tube or slide technique, with the tube reactions being easier to interpret. Coagulation of rabbit and/or bovine plasma was observed, with most strains reacting in both. The activity appeared to be a common characteristic of the species and may be useful in differentiating *E. rhusiopathiae* from *Listeria* and *Corynebacterium* species, which fail to demonstrate the activity. There was no correlation noted between coagulase activity and the serotype, source, geographic origin, or virulence (as detected by mouse pathogenicity tests) of the isolates.

Coagulase activity is most often associated with pathogenic staphylococci or yersiniae and is not ordinarily reported as a property of *Erysipelothrix rhusiopathiae* (9, 11, 14, 17). The existence of coagulase activity in *E. rhusiopathiae* was noted by Chang and Wu (M. H. Chang and S. F. Wu, Letter, Can. Vet. J. 21:335, 1980) and by Wu (20). Earlier reports of coagulase activity in *E. rhusiopathiae* were published by Nikolov (12, 13). Because coagulase may be correlated with virulence of certain organisms (9, 11, 14), it seemed appropriate to investigate the matter further. The objectives of the present study included (i) documenting the existence of coagulase activity in *E. rhusiopathiae* and (ii) detecting any difference in coagulase activity based on serotype, geographic distribution, host species of origin, or virulence.

A total of 225 *E. rhusiopathiae* strains were supplied by R. A. Packer; by The National Animal Disease Center, Ames, Iowa; and by the Iowa State University Veterinary Diagnostic Laboratory, Ames. Many had been maintained on agar slants without transfer or as lyophilized cultures for as long as 40 years. Other organisms used included *Staphylococcus aureus* Baum as a positive coagulase control. This strain was chosen because of rapid coagulation of rabbit and bovine plasma in both tube and slide procedures. A strain of *Staphylococcus epidermidis* isolated from a horse was used as a negative control for all experiments. Various strains of *Listeria monocytogenes*, *Corynebacterium renale*, and *Corynebacterium pseudotuberculosis* were also included as negative controls.

Blood agar plates were prepared from blood agar base supplemented with 5% citrated bovine blood. Serum broth was prepared from brain heart infusion broth (37 g/liter) in distilled water. Sterile horse serum (50 ml) was added to the autoclaved medium. All basal medium components were obtained from BBL Microbiology Systems, Cockeysville, Md.

Lyophilized rabbit plasma was purchased from BBL. It contained approximately 0.85% sodium citrate and 0.85% sodium chloride; EDTA was not added. Bovine plasma was collected from clinically normal animals housed at the Iowa State University Veterinary Teaching Hospital. Sodium citrate (18 g) dissolved in 72 ml of sterile distilled water was placed in a collection flask assembly and sterilized. Blood was aseptically collected to an approximate volume of 800 ml. Plasma was harvested after the cells had settled overnight at 4°C. *E. rhusiopathiae* strains were incubated for 24 to 48 h on blood agar plates and transferred into serum broth. Broth cultures were incubated at 37°C overnight or until a turbidity matching a McFarland nephelometer standard of 5 (approximately 10⁸ to 10¹⁰ cells per ml) was obtained. Coagulase activity could not be detected in suspensions containing fewer than 10⁶ cells per ml. Serum broth cultures were used directly for the tube coagulate test. Cells harvested from a 48-h blood agar culture and suspended in 0.5 ml of sterile saline were used for the slide procedures. Pelleted cells used in certain procedures were obtained by centrifugation of serum broth cultures (1,500 to 2,000 × g; 15 to 20 min).

Tests for coagulase activity were conducted by following standard procedures to test coagulase activity in *S. aureus*, except that plasma was not diluted for the tube tests (9, 11, BBL Manual of Products and Laboratory Procedures, 5th ed., BBL Microbiology Systems, Cockeysville, Md., 1968). The tube procedure was done with 0.5-ml volumes of plasma. Approximately 0.5 ml of broth culture or 5 drops of resuspended pellet was added to rabbit or bovine plasma. Tubes were incubated at 37°C and observed at various times for clot formation. The degree of coagulation was noted as complete lack of clot formation, partial or incomplete clot formation, or complete or firm clot formation. The slide procedure involved mixing cells from a blood agar plate in saline on a clean microscope slide. The mixture was stirred with a wooden applicator and rocked back and forth for approximately 1 min.

Each isolate was serotyped according to previously published procedures (18) by testing an autoclaved extract against reference sera in agarose gel double diffusion.

Mouse pathogenicity data were available for 121 of the 225 strains and were determined by previously published procedures (19).

Coagulase activity was detected in 223 of 225 strains (99.1%) of *E. rhusiopathiae* tested (Table 1). Reactions could be detected in both bovine and rabbit plasmas. The property seemed to be a stable characteristic, not affected by prolonged storage or repeated transfer of the isolates. On rare occasions, a strain was positive in plasma from one source and negative in plasma from the other.

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TABLE 1. Reactions of 225 E. rhusiopathiae strains in rabbit and bovine plasmas as detected by the tube and slide techniques

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<th>Rabbit plasma</th>
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a Positive control, S. aureus; negative controls: S. epidermidis, L. monocytogenes, C. renale, P. pseudotuberculosis, salmon. serum, serum broth, fetal calf serum, and pooled bovine serum.

b Coagulation was determined by 24 h; each test was run in duplicate.
c Coagulation was determined by 1 to 3 min; each test was run in duplicate.

procedure were determined by 24 h; most strains tested failed to show any reactivity until at least 8 to 12 h after inoculation and incubation. All coagulase-positive strains produced coagulation by 24 h. Extension of the incubation period beyond 24 h did not alter the results. There was no dissolution of clots once they were formed, indicating that E. rhusiopathiae does not produce a fibrinolyisin, as seen occasionally with S. aureus or Streptococcus pyogenes (9, 10). Slide reactions were more difficult to interpret but could be read in between 1 and 3 min. Most positive strains showed coagulation within 1 min; strains tested in bovine plasma produced reactions similar in appearance to agglutination reactions. It has been noted with the staphylococci that agglutinationlike reactions or clumping may be produced and that these may be comparable to reactions with the clumping factor described for S. aureus (2, 9, 11). The reactions noted in the present study may indicate the presence of a factor analogous to this clumping factor but require further investigation before conclusions are drawn.

There was no correlation noted between coagulase activity of the strains and serotype, mouse pathogenicity, geographic location, or the host species or sources of their initial isolations.

At present, E. rhusiopathiae is identified in clinical samples by its colonial and cellular morphology, Gram stain reaction, production of hydrogen sulfide in fresh triple sugar iron slants, and the production of a "test tube" reaction in nutrient gelatin (5, 7, 15, 17). At times, especially on preliminary Gram stain examinations or primary plating media, it may be confused with other gram-positive bacilli. Coagulase activity may be useful as part of the characterization of the organism, because it does not appear to be present in L. monocytogenes or Corynebacterium species.

Coagulation of plasma has been documented for many bacteria other than S. aureus, including Serratia marcescens, Escherichia coli, Bordetella pertussis, and group D streptococci (1, 3, 4, 6–8, 16). These organisms do not produce a true coagulase enzyme but are able to break down the anticoagulant citrate and thus initiate the natural clotting pathways. E. rhusiopathiae does not use citrate as a carbon source (7) and thus would be unable to cause coagulation by this method.

The relationship to pathogenicity is difficult to interpret. There was no correlation found between coagulase activity and pathogenicity for mice. Of the 121 strains tested, 111 (92%) were shown to be virulent for mice, causing the deaths of all four mice in each test group within 7 days. Three strains were weakly virulent for mice, causing death after 7 days or killing only some of the animals. Seven strains were avirulent for mice.

Coagulase may be considered a virulence factor for certain bacteria and might play a role in the pathogenicity associated with E. rhusiopathiae. Further studies are required to determine the actual role, if any, of the enzyme before the impact of coagulase production by virulent E. rhusiopathiae strains may be fully evaluated.

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LITERATURE CITED


