Differentiation of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Cell Proteins

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The protein patterns of whole cells of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* were studied by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The protein patterns of the 16 strains of *E. rhusiopathiae* and *E. tonsillarum* studied, including the type strains of these two species, resembled each other, except that there were 71-, 41-, 34-, and 26-kDa proteins in the *E. rhusiopathiae* pattern and 74-, 44-, 36-, and 25-kDa proteins in the *E. tonsillarum* pattern. This observation indicates that there is some phenotypic heterogeneity in the genus *Erysipelothrix*. In addition, the protein patterns of *E. rhusiopathiae* serotype reference strains representing serotypes 1 through 23 and type N were compared. The protein patterns of serotype 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 19, and 21 and type N strains were similar to the pattern of the type strain of *E. rhusiopathiae* (strain ATCC 19414). Conversely, the protein patterns of serotype 3, 7, 10, 14, and 20 strains were very similar to the pattern of the type strain of *E. tonsillarum* (strain ATCC 43339). An atypical pattern was observed in serotype 13, 17, 18, 22, and 23 strains. These results suggest that this method may be used as an aid in studying the taxonomy of these bacteria.

Erysipelothrix rhusiopathiae is the causative agent of swine erysipelas (15). This organism is also found in the tonsils of healthy swine, in slime on fish, and in the environment. *E. rhusiopathiae* strains are classified into serotypes 1 (with subtypes 1a and 1b) through 23 and type N; type N strains do not produce any precipitating antibody against homologous and heterologous heat-stable extracts in rabbits (6, 11). The genus *Erysipelothrix* was originally thought to consist of a single species despite the wide range of hosts and wide variety of serological characteristics of *E. rhusiopathiae* (2).

Recently, we reported that a cluster of avirulent serotype 7 strains found in porcine tonsils was genetically distinct from *E. rhusiopathiae*, and a new species was proposed for serotype 7 strains, *Erysipelothrix tonsillarum* (8). Moreover, serotypes 1 through 23 and type N of *E. rhusiopathiae* were studied, and two distinct DNA relatedness groups were identified on the basis of the results of DNA-DNA hybridization experiments performed with the type strains of these two species (9). However, phenotypically, *E. tonsillarum* appeared to be identical to *E. rhusiopathiae* except that it was able to produce acid from saccharose (8, 9).

Since cell proteins are genetically directed, their electrophoretic patterns tend to express genetic relationships among microorganisms (7). The purposes of this study were to compare the electrophoretic patterns of whole-cell proteins from strains of *E. rhusiopathiae* and *E. tonsillarum* and to identify phenotypic characteristics that distinguish these two species.

MATERIALS AND METHODS

Bacterial strains. Eight field strains identified phenotypically and genetically as *E. rhusiopathiae* (representing serotypes 2, 6, 11, 12, and 16) and six field strains identified

phenotypically and genetically as *E. tonsillarum* (representing serotype 7) (8, 10) were used. These strains were isolated from tonsils of apparently healthy slaughter swine in 1984. The type strains of *E. rhusiopathiae* (strain ATCC 19414) (serotype 2) and *E. tonsillarum* (strain ATCC 43339) (serotype 7) were obtained from the American Type Culture Collection, Rockville, Md. To compare the protein patterns of all of the serotypes, serotype reference strains identified phenotypically as *E. rhusiopathiae* representing serotypes 1 through 23 and type N (9, 11) were also used. These strains segregated into two main DNA-DNA hybridization groups, *E. rhusiopathiae* and *E. tonsillarum* (9). Serotyping of the strains was performed by the method described previously (6, 11).

SDS-PAGE. To obtain cells for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), colonies from a 48-h agar plate culture of each strain were inoculated into tryptose phosphate broth (Difco) (pH 7.6). After incubation for 48 h at 37°C, the broth culture was centrifuged at 12,000 × *g* for 20 min. The bacterial cells were washed three times with physiological saline and were suspended in 62.5 mM Tris hydrochloride buffer (pH 6.8) containing 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate, 10% glycerol, and 0.002% bromphenol blue. SDS-PAGE was performed in 10% slab gels by the method of Laemmli (5). Approximate molecular masses were determined by comparing migration patterns with the migration patterns of proteins having known molecular masses (Fluka AG, Buchs, Switzerland). The gels were stained with Coomassie brilliant blue R-250.

Antisera. Antisera were prepared by hyperimmunizing rabbits with whole cells of the type strains of *E. rhusiopathiae* and *E. tonsillarum* (11).

To prepare strain-specific antisera, anti-ATCC 19414^T (T = type strain) serum and anti-ATCC 43339^T serum were each absorbed with the heterologous type strain by using the procedure to remove cross-reactive antibodies described below. Formalinized cells of strains ATCC 19414^T and ATCC 43339^T were washed three times with physiological

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FIG. 1. SDS-PAGE patterns of porcine strains of *E. rhusiopathiae*. Lane 1, strain ATCC 19414^T (serotype 2); lane 2, strain T-183 (serotype 2); lane 3, strain T-191 (serotype 2); lane 4, strain T-194 (serotype 2); lane 5, strain T-221 (serotype 2); lane 6, strain T-324 (serotype 6); lane 7, strain T-205 (serotype 11); lane 8, strain T-312 (serotype 12); lane 9, strain T-184 (serotype 16). KD, kilodaltons.

FIG. 2. SDS-PAGE patterns of porcine strains of *E. tonsillarum*. Lane 1, strain ATCC 43339^T; lane 2, strain T-228; lane 3, strain T-278; lane 4, strain T-285; lane 5, strain T-334; lane 6, strain T-339; lane 7, strain T-342. All of these strains belong to serotype 7. KD, kilodaltons.

saline and were mixed with antisera at a concentration of 100 mg/ml. After incubation at 37°C for 2 h and then at 4°C for 18 h, the antisera were separated by centrifugation. This procedure was repeated at least three times until the O antibodies against heterologous antigen disappeared. The resulting sera were stored at -20°C until they were used.

Western blotting (immunoblotting). Bacterial proteins were transferred to nitrocellulose membranes by electrophoresis at 100 V for 1 h with a transblot apparatus (Bio-Rad Laboratories, Richmond, Calif.), using a modification of the Western blot method of Towbin et al. (12). After the strips were treated with specific anti-ATCC 19414^T serum or anti-ATCC 43339^T serum, they were washed with Tris-buffered saline (20 mM Tris, 500 mM sodium chloride; pH 7.2), incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G goat serum (Cappel), and washed again. The strips were developed by using a color development reagent containing 4-chloro-1-naphthol (Bio-Rad).

RESULTS

Electrophoretic patterns of *E. rhusiopathiae* strains. The protein patterns obtained for whole cells of *E. rhusiopathiae* strains isolated from tonsils and type strain ATCC 19414 are shown in Fig. 1. Our interpretation of the specific patterns was based on molecular weights (R_f values) rather than on the intensities of the bands. The protein patterns of the *E. rhusiopathiae* strains contained approximately 20 bands, 10 of which were consistently more prominent in Coomassie brilliant blue-stained gels. The major proteins had molecular masses of 68, 56, 54, 41, 38, 32, 16, 15, 13, and 12 kDa. Within *E. rhusiopathiae*, there was a good deal of similarity among the major proteins of nine strains, including the type strain.

Electrophoretic patterns of *E. tonsillarum* strains. Figure 2 shows the protein patterns of six *E. tonsillarum* strains and type strain ATCC 43339; these strains were isolated from

tonsils. The protein patterns of *E. tonsillarum* strains contained approximately 24 bands, 11 of which were consistently more prominent in Coomassie brilliant blue-stained gels. The major proteins had molecular masses of 74, 68, 56, 54, 44, 38, 32, 16, 15, 13, and 12 kDa. The patterns were similar for all strains of *E. tonsillarum*, including the type strain.

Comparison of the protein patterns of *E. rhusiopathiae* and *E. tonsillarum*. The protein patterns of *E. rhusiopathiae* and *E. tonsillarum* were compared (Fig. 3). The main differences in the protein patterns occurred in the 71-, 41-, 34-, and 26-kDa proteins of *E. rhusiopathiae* and the 74-, 44-, 36-, and 25-kDa proteins of *E. tonsillarum*. To determine whether proteins detected by SDS-PAGE are specific for each species, specific proteins were examined by Western blot analysis. Figure 4 shows the reactions of specific antisera against the type strains of the two species with the preparations described above. Anti-ATCC 19414^T serum reacted strongly with the 56- and 32-kDa protein bands from *E. rhusiopathiae* ATCC 19414^T and T-194 preparations. No reaction between this serum and the 56-kDa protein of *E. tonsillarum* was observed. In contrast, anti-ATCC 43339^T serum reacted broadly with the 32-kDa region from *E. tonsillarum* ATCC 43339^T and T-278 preparations. No reaction between this serum and the 32-kDa protein of *E. rhusiopathiae* was observed.

Comparison of the protein patterns of *E. rhusiopathiae* strains representing all serotypes. The protein patterns of *E. rhusiopathiae* serotype reference strains representing all 23 serotypes were compared with the protein patterns of the *E. rhusiopathiae* and *E. tonsillarum* type strains. The comparison of the protein patterns was carried out by examining the similarity in the patterns in the 73-, 43-, 35-, and 25-kDa regions. The taxonomic relationship between serotypes and protein patterns in the genus *Erysipelothrix* is summarized in Table 1. Strains representing serotypes 1 through 23 and type N segregated into two main groups on the basis of SDS-PAGE

FIG. 3. Comparison of SDS-PAGE patterns of *E. rhusiopathiae* (lanes 2 and 5) and *E. tonsillarum* (lanes 1 and 4). Lane 1, strain ATCC 43339^T; lane 2, strain ATCC 19414^T; lane 3, strain ATCC 43339^T plus strain ATCC 19414^T; lane 4, strain T-285; lane 5, strain T-194; lane 6, strain T-285 plus strain T-194. The arrowheads indicate the positions of different proteins of the two species. KD, kilodaltons.

type. Strains belonging to serotypes 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 19, and 21 and type N produced patterns similar to the pattern of the type strain of *E. rhusiopathiae*. In contrast, strains belonging to serotypes 3, 7, 10, 14, and 20 produced patterns similar to the pattern of the type strain of *E. tonsillarum*. Strains belonging to serotypes 13, 17, 18, 22, and 23 produced independent protein patterns which seemed to be unrelated to the pattern of either type strain.

DISCUSSION

The results of this study of the protein patterns of whole cells of *E. rhusiopathiae* and *E. tonsillarum* shows that

TABLE 1. Classification in the genus *Erysipelothrix*

Species	Serotypes and type as determined by:	
	Genetic techniques ^a	SDS-PAGE
<i>E. rhusiopathiae</i>	1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, N	1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, N
<i>E. tonsillarum</i>	3, 7, 10, 14, 20, 22, 23	3, 7, 10, 14, 20
Other <i>Erysipelothrix</i> species	13, 18	13, 17, 18, 22, 23

^a Strains belonging to serotypes 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, and 21 and type N exhibited 73 to 103% DNA relatedness to the type strain of *E. rhusiopathiae* and 18 to 24% DNA relatedness to the type strain of *E. tonsillarum*. Strains belonging to serotypes 3, 7, 10, 14, 20, 22, and 23 exhibited 66 to 100% DNA relatedness to the type strain of *E. tonsillarum* and 14 to 27% DNA relatedness to the type strain of *E. rhusiopathiae*. Strains belonging to serotypes 13 and 18 exhibited low levels of hybridization (16 to 47%) to both of the type strains (9).

under standard conditions, the SDS-PAGE system can provide highly reproducible results that differentiate these two species. The protein patterns of *E. rhusiopathiae* and *E. tonsillarum* are species specific and correlate with serological type. This is surprising since serological specificity is due to soluble peptidoglycans of the cell walls (3), which are not involved in protein determinants in SDS-PAGE. *E. rhusiopathiae* strains produce a pattern that is distinct from the *E. tonsillarum* pattern in the 73-, 43-, 35-, and 25-kDa regions. We attempted to identify the specific proteins of these two species by Western blot analysis. Anti-ATCC 19414^T serum specifically reacted with 56-kDa proteins from the type strain of *E. rhusiopathiae*, while anti-ATCC 43339^T serum reacted broadly with 32-kDa proteins from the type strain of *E. tonsillarum*. These results indicate that *E. tonsillarum* can be distinguished from *E. rhusiopathiae* by both SDS-PAGE and Western blot analysis, as well as by the ability of *E. tonsillarum* to produce acid from saccharose.

Recently, we reported that two distinct DNA relatedness groups were found in the genus *Erysipelothrix* (9). Interestingly, the results of our comprehensive SDS-PAGE studies demonstrate that *E. rhusiopathiae* strains representing sero-

FIG. 4. Western blot analysis of anti-ATCC 19414^T serum (B) and anti-ATCC 43339^T serum (A) against the following antigens: strain ATCC 43339^T (lanes 1); strain ATCC 19414^T (lanes 2); strain ATCC 43339^T plus strain ATCC 19414^T (lanes 3); strain T-278 (lanes 4); strain T-194 (lanes 5); strain T-278 plus strain T-194 (lanes 6).

types 1 through 23 and type N derived from various sources also fall into two distinct groups. However, atypical patterns were obtained for strains belonging to serotypes 13, 17, 18, 22, and 23. Additional studies on the protein patterns of these serotypes will be necessary to elucidate the phenotypic differentiates among species in the genus *Erysipelothrix*.

White and Mirikitani reported that the protein patterns of *E. rhusiopathiae* strains were quite different, so that the SDS-PAGE method was judged to be unreliable for taxonomic purposes (13). Lachmann and Deicher reported that of the 20 separate bands obtained for *E. rhusiopathiae* T-28, two main proteins were found to migrate at molecular masses of 64 and 48 kDa (4). Recently, an immunoblot analysis showed that antisera against the recombinant protein that protected mice against challenge with *E. rhusiopathiae* recognized proteins having molecular masses of 66 and 43 kDa in whole-cell extracts and in Triton X-100-solubilized surface proteins of *E. rhusiopathiae* (1). Taking the information described above into consideration, our results show that *E. rhusiopathiae* contains two major protein antigens having molecular masses of 32 and 56 kDa, which were readily detectable by anti-ATCC 19414^T serum. In contrast, *E. tonsillarum*, which is not virulent, contains only a 32-kDa protein that can be recognized with anti-ATCC 43339^T serum. Therefore, the 56-kDa protein may play an important role in the pathogenesis of *E. rhusiopathiae*. To elucidate these points, additional studies of the 56-kDa protein of *E. rhusiopathiae* will be needed.

Our previous attempts to determine the serotypes of *E. rhusiopathiae* strains obtained from swine with various clinical symptoms of erysipelas (11) and from apparently healthy swine (10) showed that most strains belong to serotypes 1 and 2, as described by Wood (14). Strains belonging to these serotypes were experimentally highly virulent for swine and capable of inducing generalized urticarial lesions with depression and anorexia. On the other hand, most strains other than serotype 1 and 2 strains were nonvirulent or weakly virulent for swine. Therefore, the SDS-PAGE method in our study provided additional evidence that isolates belonging to serotypes 1 and 2 are indeed different from some strains belonging to nonvirulent serotypes.

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