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# Analysis of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* by Multilocus Enzyme Electrophoresis

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**The genetic diversity of 74 Australian field isolates of *Erysipelothrix rhusiopathiae* and 22 reference strains for serovars of *E. rhusiopathiae* or *Erysipelothrix tonsillarum* was examined by multilocus enzyme electrophoresis. Four serovar reference strains of *E. tonsillarum* (strains KS 20 A, Wittling, Lengyel-P, and Bano 107 for serovars 25, 3, 10, and 22, respectively) were genetically distinct from *E. rhusiopathiae*. However, the *E. tonsillarum* reference strain for serovar 14 (Iszap-4) and the reference strain for serovar 13 (Pecs-56), which has been said to represent a new genomic species, were found to cluster with typical isolates and reference strains of *E. rhusiopathiae*. Our reference strain for serovar 7 (Rotzunge) was also genetically typical of *E. rhusiopathiae*, thus indicating that these serotype reactivities cannot be relied upon as a means of identifying isolates as *E. tonsillarum*. Australian field isolates of *E. rhusiopathiae* were genetically diverse. Those recovered from sheep or birds were more diverse than those isolated from pigs, and isolates of serovar 1 were more diverse than those of serovar 2. The diversity found among isolates of the same serovar and the presence of isolates of different serovars in the same electrophoretic types (ETs) indicated that serotyping of *E. rhusiopathiae* was unreliable for use as an epidemiological tool. Some ETs contained isolates recovered from different animal species. ET 41 contained 32.2% of the field isolates and two reference strains, indicating that this clone of *E. rhusiopathiae* is both widespread and commonly associated with disease in various species of animals.**

*Erysipelothrix rhusiopathiae* is a gram-positive, slender bacillus that is responsible for a range of diseases in a variety of animal species (16). Its main economic impact is as a cause of septicemia and polyarthritis in pigs, lambs, calves, turkeys, and ducks; in Australia it is an emerging problem in farmed emus (4).

Heat-stable antigens can be extracted from the cell wall of the bacteria (6). These are used as the basis for serotyping isolates in agar gel double-immunodiffusion precipitation tests with hyperimmune rabbit serum. To date, serovars 1 through 26 have been described among isolates of *E. rhusiopathiae* as well as type N strains, which do not induce precipitating antibody (3, 8–10, 17–19). The current serovar 25, represented by strain KS 20 A, was originally named serovar 23 (10). Subsequently, Norrung and Molin (9) reclassified it as serovar 25 after they realized that serovars 23 and 24 had been described previously (18, 19). Strain KS 20 A is still identified as serovar "23" in some recent publications (e.g., see references 13 and 14). Serovars 1 and 2 can be subtyped into 1a and 1b and into 2a and 2b, respectively. Most isolates recovered from diseased animals are either serovar 1 or 2, although serovars 5, 15, and 21 are commonly recovered from sheep in Australia (1, 2) and serovar 21 has caused a number of cases of septicemia in farmed emus (4).

Recently, a group of avirulent isolates, all of serovar 7, were recovered from the tonsils of healthy pigs and were shown by DNA-DNA homology studies to be a distinct species named *Erysipelothrix tonsillarum* (13). Members of this species are morphologically and biochemically almost indistinguishable from *E. rhusiopathiae*, apart from their ability to ferment sucrose (14). Subsequently, strains of serovars 3, 7, 10, 14, 20, 22, and "23" were shown to exhibit more than 66% hybridization with the type strain of *E. tonsillarum*, but less than 27% homology with the type strain of *E. rhusiopathiae* (14). These strains were therefore considered to be *E. tonsillarum* and not *E. rhusiopathiae*. Strains of serovars 1, 2, 4 to 6, 8, 9, 11, 12, 15 to 17, 19, and 21 and type N, however, showed more than 73% hybridization with the type strain of *E. rhusiopathiae* and less than 24% hybridization with the type strain of *E. tonsillarum*, confirming that they were *E. rhusiopathiae* (14). Finally, the DNAs of strains of serovars 13 and 18 exhibited low levels of hybridization with the DNAs of type strains of both species, suggesting that they were members of a separate and new genomic species (14). *E. tonsillarum* is considered to be nonpathogenic (14), although recently, certain isolates of those serovars that are said to be characteristic of the species have been shown to be capable of inducing disease (3).

In view of these important findings, we thought it timely both to examine the genetic structure of a large collection of Australian isolates of *E. rhusiopathiae* and to compare these with reference strains of serovars of *E. rhusiopathiae* and *E. tonsillarum*. Multilocus enzyme electrophoresis (MEE) was selected as an appropriate technique for the purpose of this analysis, because it is readily applicable to the study of population structures of large collections of bacteria (11).

## MATERIALS AND METHODS

**Bacteria.** The sources of the 96 field isolates and strains of *E. rhusiopathiae* and *E. tonsillarum* used in the study are summarized in Table 1. These comprised 74 isolates from Australian animals or the environment and 22 reference strains of various serovars (3, 8–10, 17–19). Five of these

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TABLE 1. ETs, sources, and serotypes of *Erysipelothrix* spp.

| ET | Strain or isolate designation[a] | Origin[b] | Source | Tissue | Serovar[c] |
|---|---|---|---|---|---|
| 1 | CJSF-14-2* | People's Republic of China | Fish | NR[d] | 24 |
| 2 | KS 20 A* | Denmark | Slurry | | 25 |
| 3 | Wittling* | Germany | Fish | NR | 3 |
| 4 | Lengyel-P* | Poland | Squirrel | NR | 10 |
| 5 | Bano 107* | Argentina | Sheep dip | | 22 |
| 6 | 1186 | NSW | Pig | Liver | 1a |
| 6 | 1227 | NSW | Penguin | Liver | 5 |
| 7 | 1069 | NSW | Sheep | Joint | 1a |
| 7 | 1259; 786 | NSW | Pig | Joint | 1a |
| 7 | 653 | NSW | Pig | Blood | 1b |
| 7 | 1258 | NSW | Duck | Liver | 1b |
| 7 | 2271; 2381 | WA | Emu | Liver | 21 |
| 8 | E* | NR | NR | NR | 9 |
| 9 | 2285 | WA | Effluent | | 1b |
| 10 | 2286 | WA | Effluent | | 1b |
| 11 | 247 | NSW | Pig | Kidney | 1a |
| 11 | 2275 | WA | Emu | Liver | 1b |
| 12 | 468 | NSW | Frogmouth | Blood | 1b |
| 13 | 1226 | NSW | Frogmouth | Liver | 8 |
| 14 | Iszap 4* | Hungary | Pond mud | | 14 |
| 15 | 1054 | NSW | Sheep | Joint | 5 |
| 16 | P 100* | Hungary | Pig | NR | 11 |
| 17 | $B_4$* | Hungary | NR | NR | 2a |
| 18 | Pecs 3597* | Hungary | Pig | Tonsil | 15 |
| 19 | Tanzania III* | Hungary | Parrot | NR | 16 |
| 20 | P-92* | Japan | Fish | Tonsil | 8 |
| 21 | Heilbutt* | Germany | Fish | NR | 4 |
| 22 | 1224 | NSW | Swallow | Feces | 6 |
| 23 | CJPT-91-1* | People's Republic of China | NR | NR | 23 |
| 24 | 1250 | NSW | Sheep | Joint | 21 |
| 25 | 222 | NSW | Sheep | Joint | 1b |
| 26 | EW-2* | NR | NR | NR | 1b |
| 26 | 2272 | WA | Pig | Lymph node | 1a |
| 26 | 325 | NSW | Pig | Skin | 1a |
| 26 | 228 | NSW | Pig | Tonsil | 2 |
| 26 | 292 | NSW | Pig | Joint | 2b |
| 26 | 252 | NSW | Sheep | Joint | 2 |
| 26 | 313 | NSW | Sheep | Joint | UT |
| 26 | 933 | NSW | Frogmouth | Heart | 6 |
| 27 | 226 | NSW | Pig | Joint | 1b |
| 28 | 941 | NSW | Frogmouth | Blood | UT |
| 29 | 1116 | NSW | Blackbird | Liver | 21 |
| 30 | 943 | NSW | Snake | Peritoneum | 21 |
| 31 | 286B | NSW | Pig | Blood | 1a |
| 32 | Rotzunge* | Germany | Fish | NR | 7 |
| 32 | 545* | United States | Pig | Spleen | 17 |
| 33 | P 56* | Hungary | Pig | NR | 13 |
| 34 | 2017* | United States | Pig | Spleen | 19 |
| 34 | 2360 | NSW | Pig | Joint | 2 |
| 35 | 1257 | NSW | Duck | Liver | 1b |
| 36 | 1117 | NSW | Pig | Kidney | 1a |
| 37 | 1106 | NSW | Pig | Liver | 2b |
| 38 | $M_2$* | Hungary | Cow | NR | 12 |
| 39 | 1105 | NSW | Pig | Kidney | 2b |
| 40 | 334 | NSW | Pig | Lung | 1a |
| 41 | A360* | NR | NR | NR | 1a |
| 41 | Seelachs* | Germany | Fish | NR | 2b |
| 41 | 298; 329; 452 | NSW | Pig | Liver | 1a |

strains have recently been considered to be *E. tonsillarum*, and another strain has been considered to be of a new genomic species (14). The Australian bacteria were isolated or identified from referred material at the Elizabeth Macarthur Agricultural Institute during the period from 1970 to 1991 (1, 2).

**Serotyping.** The isolates were serotyped in agar gel precipitation tests by using rabbit antisera raised against reference strains of serotypes 1 to 25 as described previously (1, 2).

**Bacterial growth and enzyme preparation.** Isolates were subcultured from Oxoid B.A. Base No. 2 agar containing 5%

TABLE 1—*Continued*

| ET | Strain or isolate designation[a] | Origin[b] | Source | Tissue | Serovar[c] |
|---|---|---|---|---|---|
| 41 | 326; 451; 652 | NSW | Pig | Heart | 1a |
| 41 | 312 | NSW | Pig | Skin | 1a |
| 41 | 586 | NSW | Pig | Joint | 1a |
| 41 | 251; 453 | NSW | Pig | Liver | 1b |
| 41 | 470; 477 | NSW | Pig | Joint | 1b |
| 41 | 248 | NSW | Pig | Lymph node | 1b |
| 41 | 314 | NSW | Pig | Kidney | 1b |
| 41 | 315 | NSW | Pig | Spleen | 1b |
| 41 | 2272 | WA | Emu | Liver | 1b |
| 41 | 227 | NSW | Pig | Joint | 2 |
| 41 | 462 | NSW | Pig | Liver | 2 |
| 41 | 2329 | NSW | Pig | Blood | 2 |
| 41 | 1064 | NSW | Pig | Blood | 2a |
| 41 | 459 | NSW | Pig | Liver | 2b |
| 41 | 1067 | NSW | Sheep | Lymph node | 5 |
| 41 | 2359 | TAS | Turkey | NR | 5 |
| 42 | 225B | NSW | Sheep | Joint | 1a |
| 43 | 942 | NSW | Frogmouth | Heart | n.n. |
| 44 | 293 | NSW | Sheep | Joint | 1b |
| 44 | 460 | NSW | Pig | Lymph node | 2b |
| 44 | 2270 | WA | Emu | Liver | 21 |
| 45 | 478 | NSW | Pig | Skin | 1b |
| 46 | 223 | NSW | Pig | Tonsil | 1b |
| 47 | 233 | VIC | Sheep | Joint | 2b |
| 48 | 934 | NSW | Sheep | Joint | 1b |
| 48 | 2361 | TAS | Emu | Blood | 2 |
| 48 | 231 | NSW | Sheep | Joint | 5 |
| 49 | 788 | NSW | Sheep | Joint | 1a |
| 50 | 847 | NSW | Sheep | Joint | n.n./5 |

[a] Reference strains are marked with an asterisk. These were originally made available to the Elizabeth Macarthur Agriculture Institute by G. Kucsera, Institute for the Control of Veterinary Serobacteriological Products, Budapest, Hungary (via G. Simmons of the Department of Primary Industries, Yeerongpilly, Queensland, Australia), V. Norrung, State Veterinary Serum Laboratory, Copenhagen, Denmark, and R. L. Wood, U.S. Department of Agriculture, Ames, Iowa.
[b] NSW, New South Wales; WA, Western Australia; VIC, Victoria; TAS, Tasmania.
[c] UT, untypeable in agar gel doubled diffusion tests with sera against serovars 1-25; n.n., isolate reacting with an antiserum raised against strain Seehecht, formerly serovar 3 (2).
[d] NR, not recorded.

citrated ovine blood into 500-ml aliquots of Erysipelothrix Growth Factor broth (2). These were cultured for 16 h at 35°C on a rocking platform under an atmosphere of 10% carbon dioxide in air. Samples from the broth were plated out to ensure the absence of contamination.

The bacteria were then harvested by centrifugation at 20,000 × *g*. The pellet was resuspended in TE buffer (10 mM Tris, 1 mM EDTA [pH 6.8]) and centrifuged again. This washing procedure was repeated twice. The pellet was then resuspended in 4 ml of TE buffer, and the cells were disrupted by a total of 12 cycles of 5-s sonications by using a Branson B30 sonicator with ice cooling. The sonicate was then centrifuged at 20,000 × *g* for 10 min at 4°C, and the supernatant was collected and stored at −70°C until it was used for electrophoresis.

**Electrophoresis.** The bacterial lysates were subjected to electrophoresis in 11.4% horizontal starch gels. Optimal electrophoretic conditions for each enzyme were determined by testing various buffer systems and variables as recommended by Selander et al. (11). Forty enzymes were screened for activity, but only the following 12 gave consistent banding patterns for all isolates: phosphoglucomutase, phosphoglucose isomerase, L-leucyl-glycylglycine, L-leucyl-L-tyrosine, nucleoside phosphorylase, fructose-1,6-diphosphatase, 6-phosphogluconate dehydrogenase, glucose 6-phosphate dehydrogenase, arginine phosphokinase, adenylate kinase, adenosine deaminase, and superoxide dismutase.

Phosphoglucomutase, phosphoglucose isomerase, nucleoside phosphorylase, L-leucyl-glycylglycine, and L-leucyl-L-tyrosine were analyzed on gels made with Tris-citrate (pH 8.0) buffer. 6-Phosphogluconate dehydrogenase, arginine phosphokinase, and adenylate kinase were electrophoresed on Tris-maleate (pH 7.4) gels, while glucose 6-phosphate dehydrogenase, superoxide dismutase, and adenosine diaminase were analyzed on Tris-citrate (pH 6.0), Tris-borate (pH 8.0), and lithium hydroxide (pH 8.1) gels, respectively.

For each isolate, the allelic form for each enzyme was determined at least twice, and the sonicates from all isolates showing the same allele for an enzyme were run together on the same gel to confirm that they were the same. MEE analysis was conducted on the isolates without prior knowledge of their origins or serotyping results. Distinctive mobility variants for each enzyme were numbered in order of decreasing anodal migration and were interpreted as products of different alleles at the corresponding structural gene locus. Isolates were characterized by the combination of alleles at the 12 enzyme loci, grouped according to these allele profiles, and designated a distinct electrophoretic type (ET).

**Analysis.** Genetic diversity at each locus ($h$) was calculated as follows: $h = (1 - \Sigma x_i^2)(n/n - 1)$, where $x_i$ is the frequency of the $i$th allele among ETs, and $n$ is the number of ETs or isolates (7). Total genetic diversity ($H$) was calculated as the mean of $h$ over all loci. The mean genetic

diversity per locus was also calculated separately for the porcine, ovine, and avian isolates and for the isolates of serovars 1 and 2.

The genetic distance between pairs of ETs was expressed by the proportion of loci fixed for different alleles. A phenogram of relationships between ETs was constructed for a matrix of distance coefficients by the unweighted pair group method with average clustering strategy (12).

## RESULTS

**Enzyme activities and genetic diversity.** All 12 enzyme loci were polymorphic, with a range of between 2 and 5 alleles and a mean of 3.75 alleles per locus. Isolates lacking enzyme activity were recorded as having null alleles at that locus. This occurred in 64 of a possible 4,800 instances (1.33%). Null alleles were recorded only if there was no activity in two separate lysate preparations in which these lysates had activities at other loci. The only loci without null alleles were L-leucyl-glycylglycine, L-leucyl-L-tyrosine, nucleoside phosphorylase, and fructose-1,6-diphosphate. No isolate had null alleles at more than two loci.

Fifty ETs were identified, with a mean genetic diversity per locus of 0.314, or 0.264 when the number of isolates in each ET was used in the calculations. The phenogram that was created from the data is shown in Fig. 1. Two distinct genetic clusters were apparent; they were separated at a genetic distance of 0.629. Cluster A was made up of ETs 1 through 5, with one isolate in each ET. These were reference strains for serovars 24, 25, 3, 10, and 22, respectively, the last four of which have been described as being *E. tonsillarum* (14). Cluster B included ETs 6 through 50 and had a mean genetic diversity per locus of 0.316, or 0.232 when the number of isolates in each ET was included in the calculation of diversity. The main loci responsible for the division of the isolates into two clusters were nucleoside phosphorylase, arginine phosphokinase, and adenylate kinase. The presence of allele 2 for the enzymes arginine phosphokinase and adenylate kinase and allele 3 for nucleoside phosphorylase was found only in the five strains in cluster A (*E. tonsillarum*).

**Diversity according to animal species of origin.** The sources of the bacteria used in the study are recorded in Table 1. The Australian isolates from animals included 40 from pigs, 14 from sheep, 17 from a variety of wild or farmed birds, and 1 from a snake. The isolates from pigs, sheep, and birds were located in 15, 12, and 14 ETs, respectively, and were distributed throughout cluster B on the phenogram. Twenty-two of the 40 porcine isolates (62.5%), however, were located in ETs 39 to 41, and 5 of the 14 ovine isolates (35.7%) were located in ETs 47 to 50. Results of analysis of the genetic diversity of the three groups of species are presented in Table 2. The porcine isolates were less diverse than those from the other two groups of animals, particularly when diversity was based on the number of isolates having a particular allelic profile. This difference was mainly attributable to the large number of porcine isolates in ET 42 (20 of 40 porcine isolates). The overall genetic diversity for ETs of *E. rhusiopathiae* (ETs 6 through 50) was greater than that for isolates from each of the three main groups of animal species (0.316 compared with a mean of 0.236). However, when the number of isolates in each ET were included in the calculations, only the porcine isolates remained less diverse than the whole species (0.132 compared with 0.232). ETs 6, 7, 11, 28, 30, 42, 44, and 48 contained mixtures of isolates recovered from different animal species.

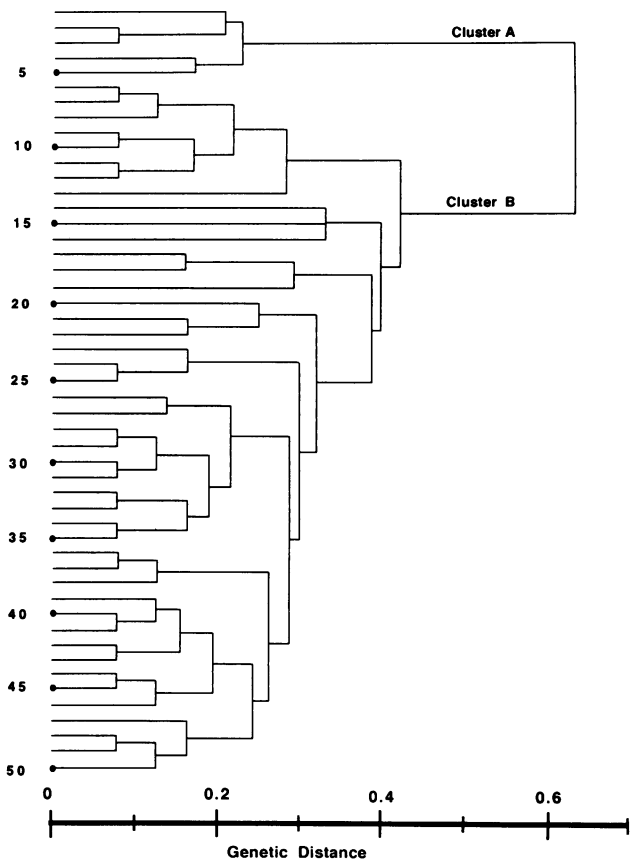


FIG. 1. Phenogram of genetic distance (expressed as percent fixed allelic differences) among 50 ETs of *Erysipelothrix* spp. clustered by the unweighted pair group method with averages strategy. Cluster A contains five strains of *E. tonsillarum*, and cluster B contains the other 92 field isolates and reference strains of *E. rhusiopathiae*.

**Diversity according to serovar.** Most of the Australian isolates (74.3%) either were of serovar 1 or 2 or were subtypes of these. The collection of isolates of serovar 1 was more genetically diverse than those of serovar 2 (Table 2), but both groups were less diverse than the whole collection of *E. rhusiopathiae* isolates. Subtypes a and b of serovars 1 and 2 were found in some of the same ETs (particularly ET 41), and isolates belonging to other different serovars were also found together in ETs 6, 7, 26, 32, 34, 41, 44, and 48.

**Genetic relationships between reference strains.** Reference

TABLE 2. Mean genetic diversity of Australian isolates of *E. rhusiopathiae* according to host species of origin and serovar

| Category | No. of ETs | No. of isolates | Genetic diversity | |
|---|---|---|---|---|
| | | | ETs | Isolates |
| Pigs | 15 | 40 | 0.219 | 0.132 |
| Sheep | 12 | 14 | 0.242 | 0.224 |
| Birds[a] | 14 | 17 | 0.248 | 0.233 |
| Serovar 1[b] | 20 | 40 | 0.268 | 0.189 |
| Serovar 2[c] | 8 | 15 | 0.143 | 0.109 |

[a] Includes wild birds, poultry, and farmed emus.
[b] Includes subtypes 1a and 1b.
[c] Includes subtypes 2a and 2b.

strains for *E. tonsillarum* serovars 25 (KS 20 A), 3 (Wittling), 10 (Lengyel-P), and 22 (Bano 107) were located in cluster A, in ETs 2 to 5, respectively, while the serovar 14 strain (Iszap-4) was located in ET 14 in cluster B. Our isolate of Pecs-56 (serovar 13), which is said to be a new genomic species of *Erysipelothrix* (14), was located in ET 33. Strain CJSF-14-2 (serovar 24) was located in ET 1, i.e., in cluster A with the four strains of *E. tonsillarum*. The other 15 reference strains were distributed through cluster B of the phenogram, with 6 strains in ETs 16 to 21, respectively. Reference strains for serovars 7 (Rotzunge) and 17 (545) were located together in ET 32, and reference strains for serovars 1a (A360) and 2b (Seelachs) were found together in ET 41.

## DISCUSSION

The study of a collection of bacteria resembling *E. rhusiopathiae* described here demonstrated that these include a distinct genetic group of five strains (cluster A) corresponding to the newly described species *E. tonsillarum* (13, 14). Four of these five reference strains have previously been shown to be *E. tonsillarum* (14). In that study, strain KS 20 A was identified as being serovar 23, although it is now more correctly considered to be serovar 25 (9). The current study demonstrates that reference strain CJSF-14-2, for serovar 24 (18, 19), clusters with these other four reference strains and is therefore also *E. tonsillarum*. Our study differs from that of Takahasi et al. (14) in finding that strains Iszap-4 and Pecs-56 are clustered with the main group of *E. rhusiopathiae* isolates (in ETs 14 and 33, respectively, in cluster B). Takahashi et al. (14) suggested that these two strains were *E. tonsillarum* and a representative of a new genomic species, respectively, but we consider them to be typical *E. rhusiopathiae*. These findings require confirmation with strains acquired from other sources.

Takahashi et al. (14) also implied that all isolates of serovars 3, 7, 10, 14, 20, 22, and "23" (≡25) were *E. tonsillarum*. However, we found the reference strain for serovar 14 (Iszap-4) in ET 14 and the reference strain for serovar 7 (Rotzunge) in ET 32, both in cluster B with typical *E. rhusiopathiae* isolates. Assuming that the strains in our collection were correctly identified and typed, the results presented above indicate that serotyping cannot be relied upon to identify isolates as *E. tonsillarum*.

All of the Australian isolates belonged to the species *E. rhusiopathiae* (cluster B). The diversity of the species (0.316, or 0.232 when the number of isolates was considered) was very similar to that previously found in our laboratory for *Actinobacillus pleuropneumoniae* (5). The diversity of those isolates recovered from sheep was very similar to that of the isolates recovered from birds, but both groups of isolates were more diverse than those recovered from pigs. The low level of diversity of the porcine isolates was in part a reflection of the fact that 50% of these isolates belonged to one ET. In addition, the porcine isolates were all only of serovar 1 or 2, unlike those from the other animal species, and therefore might be expected to be less diverse than those isolates. This explanation was probably correct, since when only serovars 1 and 2 were considered for the different animal groups, diversities for the remaining seven ovine and six avian isolates were reduced to 0.178 and 0.193, respectively. These figures were more similar to that calculated for the porcine isolates (0.132). Nevertheless, overall, the porcine isolates in the collection were less diverse than those recovered from the other species of Australian animals.

For the whole collection, isolates of serovar 2 were less diverse than those of serovar 1. Although these two serovars are the most common cause of erysipelas in most animal species, their natural history may be different. For example, according to Wood (16), serovar 1 (especially subtype 1a) is usually described as the predominant type isolated from animals with septicemic disease, while serovar 2 is the most common type recovered from animals with subacute and chronic cases of erysipelas. Both serovars, however, are considered capable of causing all forms of the disease, and this was confirmed in the present study. Serovar 2 isolates were also shown to represent 31.4% of *E. rhusiopathiae* isolates in pig and cattle slurries in a Danish study (10), while isolates of serovar 1 comprised only 2.3% of the total. Similarly, 31.7% of 63 isolates recovered from the tonsils of 600 apparently healthy Japanese slaughter pigs were of serovar 2, while no isolates of serovar 1 were recovered (15). Nevertheless, generalizations about the properties of serovars should be interpreted with caution. If there are consistent differences in the biology of the serovars, these differences are likely to be directly associated with or genetically linked to the serovar antigens. The differences in biological behaviors cannot reflect the overall genetic origins of the serovars, since these were diverse.

The substantial diversity found among isolates of the same serovars and subtypes makes serotyping an unreliable technique for tracing sources of isolates of *E. rhusiopathiae* in epidemiological studies. However, the presence of isolates of different serovars and subtypes in the same ET does suggest that serotyping could be useful in conjunction with MEE, since it further differentiates isolates of the same ET. Since only 12 enzyme loci were suitable for use in MEE, other means of subspecific differentiation may demonstrate further genetic differences between certain isolates grouped in the same ETs.

Some of the ETs contained isolates that were recovered from different animal species, and in the case of ETs 7, 26, and 41, some of these isolates from different species were also of the same serovar. This provided indirect evidence that *E. rhusiopathiae* may be transmitted between animal species in nature. ET 41 contained 25 of the 91 isolates of *E. rhusiopathiae* (27.5%), including 20 porcine isolates and one ovine isolate from New South Wales, one from a Western Australian emu, one from a Tasmanian turkey, and the overseas reference strains for serovars 1a and 2b. This clonal group of isolates is therefore common and widely disseminated. Both it and other ETs contained isolates from a variety of different animal tissues and from animals with different clinical manifestations of erysipelas, thus suggesting that given isolates are unlikely to have specific tissue tropism. Only three of the Australian isolates (in ETs 22, 26, and 46) were from healthy animals. It would have been useful to determine the virulences of these isolates by experimental inoculation of animals, but this was not done. The reference strains for serovars 3, 7, 12, 14, and 17, however, have been shown to lack virulence (3). These strains were located throughout the phenogram, as were the virulent clinical isolates. Virulence in *E. rhusiopathiae* therefore does not reside in only a few specific clones of the bacteria.

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