Classification of *Erysipelothrix* Strains on the Basis of Restriction Fragment Length Polymorphisms

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Restriction fragment length polymorphisms of the 16S rRNA genes of *Erysipelothrix* strains were studied by cleavage of the chromosomal DNA with restriction endonuclease *EcoRI*, followed by hybridization to a 420-bp internal fragment of the 16S rRNA gene. Thirty-two *Erysipelothrix* type and reference strains were classified, together with seven field strains. Reference strains of all serotypes and the type strains of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* were included. Nine ribopatterns were observed. Pattern A was represented by 16 strains and included strains of serotypes 1b, 2 to 8, 11 to 13, 15 to 17, 19, and 23. Pattern B was represented by two strains (serotypes 1a and 9). Pattern C was represented by five strains (serotypes 5, 6, and 21). Pattern D was represented by one strain of serotype 4. Pattern E was represented by 11 strains of serotypes 2, 7, 10, 20, 22, 24, and 25. Patterns F, G, H, and I were each represented by a single strain of serotypes 26, 2, 18, and 3, respectively. All the different ribopatterns had some bands in common. Patterns B, C, and D were most similar to pattern A, while patterns F, G, H, and I resembled pattern E. Partial sequencing of the 16S rRNA gene of nine selected strains resulted in three different sequences, i.e., the typical *E. rhusiopathiae* sequence, the *E. tonsillarum* sequence, and a third sequence found for two strains. Strains of the same serotype were found to have different ribopatterns as well as different partial 16S rDNA sequences.

Erysipelothrix rhusiopathiae is the causative agent of swine erysipelas and also is known to cause a variety of diseases in warm- and cold-blooded animals (2, 12). The genus *Erysipelothrix* comprised only *E. rhusiopathiae* (8) until 1987, when Takahashi et al. (18) proposed the inclusion of *Erysipelothrix tonsillarum*. Known strains of the new species were not pathogenic to pigs. *Erysipelothrix* strains have for long been divided into serotypes, the most recent of which is serotype 26 (10). Strains of the different serotypes have been grouped by means of DNA-DNA hybridization (19) and polyacrylamide gel electrophoresis (20). They have, according to DNA-DNA hybridization results, been placed into the *E. rhusiopathiae* group (serotypes 1a, 1b, 2, 4 to 9, 11, 12, 15 to 17, 19, and 21), the *E. tonsillarum* group (serotypes 3, 7, 10, 14, 20, 22, and 23) or outside the two described species (serotypes 13 and 18) (19, 20).

Determination of restriction fragment length polymorphisms (RFLPs) of genes coding for rRNA (rDNA), also called ribotyping, has been used for taxonomic studies of many different bacteria (9, 16). The method has proven to be a useful tool in differentiating between species as well as subspecies (3, 6, 13). Its use in epidemiology has also been proposed because of great intraspecies variations in the ribopatterns (22).

The aim of this study was to classify *Erysipelothrix* strains of all the described serotypes by the use of RFLPs of the 16S rRNA gene. Nine selected strains were subjected to partial sequencing of their 16S rDNAs.

MATERIALS AND METHODS

Bacterial strains. The test strains are listed in Table 1. Strains *E. rhusiopathiae* CCUG 221^T, *E. tonsillarum* CCUG 31352^T, and *E. tonsillarum* CCUG 31353 were obtained from the Culture Collection of the University of Gothenburg, Gothenburg, Sweden; the field strains were isolated from pork loin (strains K5, K30, and K44) and from fish (strains F1, F3, F7, and F20 [17]). The remaining strains were all obtained from the collection of the National Veterinary Laboratory, Copenhagen, Denmark.

Serology. All strains were serotyped by the capillary tube method, using autoclave-prepared antigen extracts and type 1 through 26 antisera as described by Wellmann et al. (21).

DNA preparation and restriction endonuclease digestion. The strains were grown in brain heart infusion broth supplemented with horse serum (5%). Chromosomal DNA was extracted by the method of Ståhl et al. (15). One microgram of the DNAs was digested with 10 U of *EcoRI* (Boehringer Mannheim, Scandinavia AB, Bromma, Sweden) for 4 h at 37°C in the buffer supplied by the manufacturer.

RFLPs. Gels were run for 18 h as described earlier (15, 16). Phage λ DNA, cleaved with *HindIII* and digoxigenin labeled (Boehringer Mannheim), was used as a fragment size marker. The gels were blotted onto a positively charged nylon membrane (Boehringer Mannheim) with LKB 20016 Vacugene (Pharmacia LKB Biotechnology AB, Bromma, Sweden) according to the manufacturer's instructions.

The probe used was a 420-bp (positions 506 to 926) fragment of the 16S rRNA gene of *E. rhusiopathiae* CCUG 221^T obtained by PCR (Perkin-Elmer Cetus). It was labeled by the digoxigenin DNA labeling technique described by the manufacturer (Boehringer Mannheim). The amount of probe used for each hybridization reaction was between 50 and 200 ng.

Partial 16S rDNA sequencing. Sequencing was done by the method of Pettersson et al. (11), and a partial sequence from nucleotide 356 to 915, according to the numbering of the *Escherichia coli* 16S rRNA gene, was determined. This method for directed automated solid-phase sequencing of in vitro-amplified segments of 16S rDNA was based on amplification by PCR and previously described immobilization and sequencing techniques (4, 5). Sequences were compared with those available from GenBank in May 1994.

Mathematical analysis. The RFLP data sets were compared by the Jaccard coefficient (S_j [14]). The similarity distance was defined as $D_j = 100(1 - S_j)$.

RESULTS

RFLPs. Nine different ribopatterns were found (Table 1; Fig. 1). The majority of strains showed either ribopattern A, which included the type strain of *E. rhusiopathiae*, or ribopat-

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TABLE 1. Ribopatterns of *Erysipelothrix* strains with different serotypes^a

Pattern	Label ^b	Strain	Serotype ^c	<i>D_J</i> to pattern:	
				A	E
A	<i>E.r.</i>	422/I ^d	1b	0	75
A	<i>E.r.</i>	CCUG 221 ^{Td}	2	0	75
A	<i>E.r.</i>	NF4 ^d	2	0	75
A	<i>E.sp.</i>	K5	2	0	75
A	<i>E.sp.</i>	F20	5	0	75
A	<i>E.sp.</i>	K44	6	0	75
A	<i>E.sp.</i>	Rotzunge	7	0	75
A	<i>E.r.</i>	Goda ^d	8	0	75
A	<i>E.r.</i>	IV12/8 ^d	11	0	75
A	<i>E.sp.</i>	M2	12	0	75
A	<i>E.sp.</i>	Pecs 18	13	0	75
A	<i>E.r.</i>	Pecs 3597 ^d	15	0	75
A	<i>E.r.</i>	Tanzania ^d	16	0	75
A	<i>E.r.</i>	545 ^d	17	0	75
A	<i>E.r.</i>	2017 ^d	19	0	75
A	<i>E.sp.</i>	CJPT97	23	0	75
B	<i>E.r.</i>	ME-7 ^d	1a	44	73
B	<i>E.r.</i>	Kaparek ^d	9	44	73
C	<i>E.r.</i>	Pecs 67 ^d	5	33	75
C	<i>E.sp.</i>	F1	5	33	75
C	<i>E.sp.</i>	K30	6	33	75
C	<i>E.sp.</i>	Vadkasca	6	33	75
C	<i>E.r.</i>	Bano 36 ^d	21	33	75
D	<i>E.sp.</i>	Heilbutt	4	33	75
E	<i>E.sp.</i>	F7	2	75	0
E	<i>E.t.</i>	CCUG 31352 ^{Td}	7	75	0
E	<i>E.t.</i>	CCUG 31353 ^d	7	75	0
E	<i>E.t.</i>	T-131 ^d	7	75	0
E	<i>E.t.</i>	P-43 ^d	7	75	0
E	<i>E.t.</i>	Lengyel-P ^d	10	75	0
E	<i>E.t.</i>	Iszap-4 ^d	14	75	0
E	<i>E.t.</i>	2553 ^d	20	75	0
E	<i>E.t.</i>	Bano 107 ^d	22	75	0
E	<i>E.sp.</i>	CJSF 14-2	24	75	0
E	<i>E.sp.</i>	KS20A	25	75	0
F	<i>E.sp.</i>	L136	26	75	57
G	<i>E.sp.</i>	F3	2	62	57
H	<i>E.sp.</i>	715 ^d	18	75	57
I	<i>E.t.</i>	Wittling ^d	3	75	33

^a The restriction endonuclease used was *EcoRI*, and the fragments were hybridized to a probe against 420 bp of the 16S rRNA gene of *E. rhusiopathiae* CCUG 221^T. *D_J* = 100 (1 - *S_J*); *S_J* is the Jaccard coefficient (14).

^b *E.r.*, *E. rhusiopathiae*; *E.sp.*, *Erysipelothrix* sp.; *E.t.*, *E. tonsillarum*.

^c All strains were serotyped by the National Veterinary Laboratory, Copenhagen, Denmark.

^d Strains investigated by DNA-DNA hybridization by Takahashi et al. (19).

tern E, which included the type strain of *E. tonsillarum* (Table 1; Fig. 1). These two ribopatterns had two fragments in common, resulting in a similarity distance between them of 75 (Table 1; Fig. 1). The remaining strains had ribopatterns with similarity distances ranging between 33 and 75 from ribopatterns A and E (Table 1; Fig. 1).

Partial 16S rDNA sequencing. The partial 16S rDNA sequence was determined for *E. rhusiopathiae* CCUG 221^T and *E. tonsillarum* CCUG 31352^T together with seven selected *Ery-*

FIG. 1. Schematic representation of the different *EcoRI* 16S rRNA gene restriction patterns obtained in this investigation for *Erysipelothrix* strains. Phage λ DNA, cleaved with *HindIII*, was used as a fragment size marker (leftmost column).

sipelothrix strains (Table 1; Fig. 2). The sequence found for *E. rhusiopathiae* CCUG 221^T was identical to that reported in GenBank. This sequence was also found for *Erysipelothrix* strains Rotzunge, CJPT97, and 715 (Fig. 2). *E. tonsillarum* CCUG 31352^T, had the same partial 16S rDNA sequence as strains F7 and L136, but this sequence differed from that of *E. rhusiopathiae* in three positions at three different locations along the gene (Fig. 2). *Erysipelothrix* strains F3 and Vadkasca showed a third sequence. It differed from the *E. rhusiopathiae* sequence in two positions at one location (Fig. 2).

DISCUSSION

The ribopatterns of 39 strains of *Erysipelothrix*, representing all 26 serotypes, were relatively homogenous. All patterns had two fragments in common, which is unusual among ribopatterns of different species.

Thirteen of the strains examined had been designated *E. rhusiopathiae* after DNA-DNA hybridizations (19). These strains showed, in the present study, ribopatterns A (nine strains), B (two strains), and C (two strains). Ribopatterns B and C are, together with pattern D, those with the shortest similarity distances to ribopattern A (Table 1). Furthermore, eight strains that previously were shown by Takahashi et al. (19) to have DNA-DNA homologies of more than 70% to *E. tonsillarum* CCUG 31352^T exhibited ribopattern E (seven strains) or ribopattern I (one strain). Pattern I has the shortest similarity distance to pattern E among the patterns compared (Table 1).

Strain 715, with 47% DNA-DNA homology to the type strain of *E. rhusiopathiae* and 16% DNA-DNA homology to the type strain of *E. tonsillarum* (19), exhibited a unique ribopattern (H) which was distinct from both pattern A and pattern E (Table 1; Fig. 1). However, this strain had the same partial 16S rDNA sequence as *E. rhusiopathiae* CCUG 221^T (Fig. 2). The fact that two separate species have the same 16S rRNA sequence is uncommon but has been described previously for *Lactobacillus plantarum* and *Lactobacillus pentosus* (1, 7).

The partial 16S rDNA sequences for strains of ribopattern A (*E. rhusiopathiae* CCUG 221^T, strain Rotzunge, and strain CJPT97) were identical (Fig. 2). The same was true for strains of pattern E (*E. tonsillarum* CCUG 31352^T and strain F7). The coinciding ribopatterns and partial 16S rDNA sequences indicate that strains with ribopattern A and strains with ribopattern E can be regarded as *E. rhusiopathiae* and *E. tonsillarum*, respectively. This observation is in agreement with the results of the DNA-DNA hybridizations done by Takahashi et al. (19) for those of the strains that were part of their investigation (pattern A: *E. rhusiopathiae* CCUG 221^T, 422/I, NF4, Goda,

<i>Erysipelothrix rhusiopathiae</i> ^a	AGTAGGGAATTTTCGGCAATGGGGAAACC	<i>Erysipelothrix rhusiopathiae</i> ^a	CGTGATGCCATAGAACTGGTAGACTAGAG
F3 & Vadkasca ^b	AGTAGGGAATTTTCGGCAATGGGGAAACC	F3 & Vadkasca ^b	CGTGATGCCATAGAACTGGTAGACTAGAG
<i>Erysipelothrix tonsillarum</i> ^c	AGTAGGGAATTTTCGGCAATGGGGAAACC	<i>Erysipelothrix tonsillarum</i> ^c	CGTGATGCCATAGAACTGGTAGACTAGAG
<i>Erysipelothrix rhusiopathiae</i> ^a	CTGACCGAGCAACGCCGCTGAGTGAAGAC	<i>Erysipelothrix rhusiopathiae</i> ^a	TGCAGGAGAGGTTAGTGAATTCATGTGT
F3 & Vadkasca ^b	CTGACCGAGCAACGCCGCTGAGTGAAGAC	F3 & Vadkasca ^b	TGCAGGAGAGGTTAGTGAATTCATGTGT
<i>Erysipelothrix tonsillarum</i> ^c	CTGACCGAGCAACGCCGCTGAGTGAAGAC	<i>Erysipelothrix tonsillarum</i> ^c	TGCAGGAGAGGTTAGTGAATTCATGTGT
<i>Erysipelothrix rhusiopathiae</i> ^a	GGCCTTCGGGTTGTAAAGCTCTGTTGAAG	<i>Erysipelothrix rhusiopathiae</i> ^a	AGCGGTAAATGCGTAGATATATGGAGGAA
F3 & Vadkasca ^b	GGCCTTCGGGTTGTAAAGCTCTGTTGAAG	F3 & Vadkasca ^b	AGCGGTAAATGCGTAGATATATGGAGGAA
<i>Erysipelothrix tonsillarum</i> ^c	GGCCTTAGGGTTGTAAAGCTCTGTTGAAG	<i>Erysipelothrix tonsillarum</i> ^c	AGCGGTAAATGCGTAGATATATGGAGGAA
<i>Erysipelothrix rhusiopathiae</i> ^a	GGAAGAACGATAGGAAGAGGGGAATGCTTCT	<i>Erysipelothrix rhusiopathiae</i> ^a	CACCAGTGGCGAAGGCGGCTAACTGGCCTG
F3 & Vadkasca ^b	GGAAGAACGATAGGAAGAGGGGAATGCTTCT	F3 & Vadkasca ^b	CACCAGTGGCGAAGGCGGCTAACTGGCCTG
<i>Erysipelothrix tonsillarum</i> ^c	GGAAGAACAATAAGAGAGGGGAATGCTTCT	<i>Erysipelothrix tonsillarum</i> ^c	CACCAGTGGCGAAGGCGGCTAACTGGCCTG
<i>Erysipelothrix rhusiopathiae</i> ^a	TATATGACGGTACCTTACCAGAAAGCCACG	<i>Erysipelothrix rhusiopathiae</i> ^a	TAACTGACGCTGAGGCTCGAAAGCGTGGGG
F3 & Vadkasca ^b	TATATGACGGTACCTTACCAGAAAGCCACG	F3 & Vadkasca ^b	TAACTGACGCTGAGGCTCGAAAGCGTGGGG
<i>Erysipelothrix tonsillarum</i> ^c	TATATGACGGTACCTTACCAGAAAGCCACG	<i>Erysipelothrix tonsillarum</i> ^c	TAACTGACGCTGAGGCTCGAAAGCGTGGGG
<i>Erysipelothrix rhusiopathiae</i> ^a	GCTAACTACGTGCCAGCAGCCGCGTAATA	<i>Erysipelothrix rhusiopathiae</i> ^a	AGCAAAATAGGATTAGATACCCCTAGTAGTCC
F3 & Vadkasca ^b	GCTAACTACGTGCCAGCAGCCGCGTAATA	F3 & Vadkasca ^b	AGCAAAATAGGATTAGATACCCCTAGTAGTCC
<i>Erysipelothrix tonsillarum</i> ^c	GCTAACTACGTGCCAGCAGCCGCGTAATA	<i>Erysipelothrix tonsillarum</i> ^c	AGCAAAATAGGATTAGATACCCCTAGTAGTCC
<i>Erysipelothrix rhusiopathiae</i> ^a	CGTAGGTGGCAAGCGTTATCCGGAATTATT	<i>Erysipelothrix rhusiopathiae</i> ^a	ACGCCGTAAACGATGGATACTAAGTGTGG
F3 & Vadkasca ^b	CGTAGGTGGCAAGCGTTATCCGGAATTATT	F3 & Vadkasca ^b	ACGCCGTAAACGATGGATACTAAGTGTGG
<i>Erysipelothrix tonsillarum</i> ^c	CGTAGGTGGCAAGCGTTATCCGGAATTATT	<i>Erysipelothrix tonsillarum</i> ^c	ACGCCGTAAACGATGGATACTAAGTGTGG
<i>Erysipelothrix rhusiopathiae</i> ^a	GGGCGTAAAGGAGCGCAGGCGGTTTATCA	<i>Erysipelothrix rhusiopathiae</i> ^a	AGAAATTCAGTGTCTAGTTAACGCAATAA
F3 & Vadkasca ^b	GGGCGTAAAGGAGCGCAGGCGGTTTATCA	F3 & Vadkasca ^b	AGAAATTCAGTGTCTAGTTAACGCAATAA
<i>Erysipelothrix tonsillarum</i> ^c	GGGCGTAAAGGAGCGCAGGCGGTTTATCA	<i>Erysipelothrix tonsillarum</i> ^c	AGAAATTCAGTGTCTAGTTAACGCAATAA
<i>Erysipelothrix rhusiopathiae</i> ^a	AGTTTATGGTTAAAGTTCGGGGCTTAACCC	<i>Erysipelothrix rhusiopathiae</i> ^a	GTATCCCGCTGGGGAGTATGCGCGCAAGC
F3 & Vadkasca ^b	AGTTTATGGTTAAAGTTCGGGGCTTAACCC	F3 & Vadkasca ^b	GTATCCCGCTGGGGAGTATGCGCGCAAGC
<i>Erysipelothrix tonsillarum</i> ^c	AGTTTATGGTTAAAGTTCGGGGCTTAACCC	<i>Erysipelothrix tonsillarum</i> ^c	GTATCCCGCTGGGGAGTATGCGCGCAAGC
<i>Erysipelothrix rhusiopathiae</i> ^a	GTAAAACTCAAA	<i>Erysipelothrix rhusiopathiae</i> ^a	GTAAAACTCAAA
F3 & Vadkasca ^b	GTAAAACTCAAA	F3 & Vadkasca ^b	GTAAAACTCAAA
<i>Erysipelothrix tonsillarum</i> ^c	GTAAAACTCAAA	<i>Erysipelothrix tonsillarum</i> ^c	GTAAAACTCAAA

FIG. 2. Partial nucleotide sequence of 16S rDNA from *E. rhusiopathiae*, strains F3 and Vadkasca, and *E. tonsillarum*. The F3 and Vadkasca sequence is compared with both the *E. rhusiopathiae* and *E. tonsillarum* sequences; differences (X) and similarities (:) are marked. The sequences correspond to the 16S rDNA sequence of *E. coli* from nucleotide 356 to 915. a, sequence shared by strains *E. rhusiopathiae* CCUG 221^T, Rotzunge, CJPT97, and 715; b, sequence shared by strains F3 and Vadkasca (GenBank accession number U21666); c, sequence shared by strains *E. tonsillarum* CCUG 31352^T, F7, and L136 (GenBank accession number U21665).

IV12/8, Pecs 3597, Tanzania, 545, and 2017; pattern E: *E. tonsillarum* CCUG 31352^T, *E. tonsillarum* CCUG 31353, T131, P-43, Lengyel-P, 2553, and Bano 107 [Table 1; Fig. 1]).

Strain L136 showed the same partial sequence as the type strain of *E. tonsillarum* but had a ribopattern that was relatively different from both pattern E and pattern A (Table 1; Fig. 1). There are no hybridizing data for this strain. It should also be pointed out that the sequences determined in the present investigation, even though representing regions of great variability, are only partial sequences of the 16S rRNA gene.

It has been suggested by Takahashi et al. (19) that the serotypes are linked to the species, e.g., strains of serotype 7 are of *E. tonsillarum* and serotype 2 strains are of *E. rhusiopathiae*. We found, however, that strains of serotype 7 could be of either *E. tonsillarum* (strains CCUG 31352^T, CCUG 31353,

T-131, and P-43) or *E. rhusiopathiae* (strain Rotzunge) (Table 1; Fig. 2). Furthermore, strains of serotype 2 were shown to be of either *E. rhusiopathiae* (strains CCUG 221^T, NF4, and K5), *E. tonsillarum* (strain F7), or the third unnamed *Erysipelothrix* taxon (strain F3) (Table 1; Fig. 2). The fact that the same serotype was found in different species makes serotyping a somewhat questionable method for taxonomic and epidemiological studies of *Erysipelothrix* strains. A third partial 16S rDNA sequence differing from both that of *E. rhusiopathiae* and *E. tonsillarum* was found, perhaps indicating the existence of a third *Erysipelothrix* species.

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