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Short Communication

Spa type of *Erysipelothrix* strains and its association with virulence of *Erysipelothrix* strains in mice and swine

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We investigated the prevalence of *spaA*, *spaB* and *spaC* in 90 virulent or avirulent *Erysipelothrix* strains. All *Erysipelothrix rhusiopathiae* strains (n = 58) harboured either *spaA* or *spaB*, whereas all other sp.-2 (n = 3) strains harboured *spaC*. None of the other species, including *Erysipelothrix tonsillarum* (n = 25) and other sp.-1 (n = 2) and sp.-3 (n = 2), harboured *spa*-type genes. In mice, *spa*-type was not associated with virulence. In swine, erythema-eliciting strains were more prevalent in *spaA*-, *spaB*-, or *spaC*-positive groups than in *spa*-negative groups. Notably, strains eliciting systemic manifestations were found only in the *spaA*-positive group.

Key words: *Erysipelothrix*, spa type, swine, virulence.

INTRODUCTION

Erysipelothrix rhusiopathiae is a primary pathogen of swine as well as a cause of sporadic disease outbreaks in humans and other animals. The organism is distributed worldwide and has been isolated from the organs of many wild and domestic mammals, birds, reptiles, amphibians and fish (Wood and Henderson, 2006). Erysipelas commonly occurs in swine, and is characterized by urticarial diamond-shaped lesions that can rapidly progress to an acute septicaemic infection or death.

At present, the genus *Erysipelothrix* contains two main species: *E. rhusiopathiae* (including serovars 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, and 21 and type N) and *Erysipelothrix tonsillarum* (serovars 3, 7, 10, 14, 20, 22, and 23) (Takahashi et al., 1992). In addition, we previously reported other three minor species: other sp.-1 (serovar 13), other sp.-2 (serovars 9, 10, and 18), and other sp.-3 (serovar 7) (Takahashi et al., 2008). In that study, *Erysipelothrix* strains belonging to all the serovars and species were evaluated for virulence in mice and swine according to the serovar types and species.

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Surface proteins of Gram-positive bacteria play pivotal roles in virulence (Navarre and Schneewind, 1999). The surface protective antigen A (spa A) of *E. rhusiopathiae* is considered the major immunogenic antigen (Imada et al., 2003). Recently, 2 additional types of spa-related genes were detected: *spaB* and *spaC* (To and Nagai, 2007). The Spa proteins, as well as surface proteins of other bacteria, are considered to play a specific role in virulence (Shimoji, 2000); however, the association of Spa proteins with virulence for animals remains to be evaluated. In previous studies, the prevalence of Spa proteins has been investigated mainly in serovar reference strains of *Erysipelothrix* species (To and Nagai, 2007; Shen et al., 2010), and thus, the distribution of Spa proteins among numerous field strains is unclear.

MATERIALS AND METHODS

In this study, we investigated the prevalence of *spa*-type among our collection and evaluated the association of the *spa*-type genes with virulence for mice and swine.

In total, 90 strains from our collection of *Erysipelothrix* spp. (Takahashi et al., 2008) were used: 58 *E. rhusiopathiae*, 25 *E. tonsillarum*, 2 other sp.-1, 3 other sp.-2, and 2 other sp.-3; serovars 1a, 1b, 2 through 23, and type N were included (Table 1).

Table 1. Serovars, pathogenicity for mice and swine, species, Spa-type of *Erysipelothrix* strains in this study.

Strain	Serovar[a]	Pathogenicity for mice (log LD50)[b]	Pathogenicity for swine[c]		Species[d]	Spa type
			Erythema	Systemic		
ME-7*	1a	1.0	+	+	*E. rhusiopathiae*	A
Sayo-SP	1a	1.1	+	+	*E. rhusiopathiae*	A
YamagataFD	1a	1.5	+	+	*E. rhusiopathiae*	A
Kanagawa96-1	1a	1.9	+	+	*E. rhusiopathiae*	A
422/1E1*	1b	1.0	+	+	*E. rhusiopathiae*	A
NKB-105	1b	1.9	+	+	*E. rhusiopathiae*	A
82-517	1b	1.9	+	+	*E. rhusiopathiae*	A
NF4E1	2	1.3	+	+	*E. rhusiopathiae*	A
R32E11*	2	1.5	+	+	*E. rhusiopathiae*	A
ATCC19414 [t]	2	1.4	+	+	*E. rhusiopathiae*	A
Kohriyama5-41	2	2.1	-	-	*E. rhusiopathiae*	A
K-209	2	1.7	+	-	*E. rhusiopathiae*	A
Witlling*	3	0.5	-	-	*E. tonsillarum*	-
Tama94-160	3	0.6	-	-	*E. rhusiopathiae*	A
NKF-245	3	1.6	-	-	*E. rhusiopathiae*	B
NKF-403	3	1.1	-	-	*E. rhusiopathiae*	B
NKF-246	3	1.6	-	-	*E. rhusiopathiae*	B
NKF-247	3	1.8	-	-	*E. rhusiopathiae*	B
NKF-402	3	1.6	-	-	*E. rhusiopathiae*	B
Doggerscharbe	4	>7	-	-	*E. rhusiopathiae*	B
NKF-425	4	1.6	+	-	*E. rhusiopathiae*	B
NKF-336	4	1.5	-	-	*E. rhusiopathiae*	B
Pécs 67*	5	1.4	+	-	*E. rhusiopathiae*	A
Dolphin S-1	5	2.2	-	-	*E. rhusiopathiae*	B
S-101	5	2.2	-	-	*E. rhusiopathiae*	A
Agata	5	2.0	-	-	*E. rhusiopathiae*	A
Tuzok*	6	0.8	-	-	*E. rhusiopathiae*	B
Dolphin E-1	6	0.4	-	-	*E. rhusiopathiae*	B
S-146	6	2.3	-	-	*E. rhusiopathiae*	A
NKB-2	6	1.9	-	-	*E. rhusiopathiae*	B
P-43*	7	1.8	-	-	*E. tonsillarum*	-
ATCC43339t	7	5.3	-	-	*E. tonsillarum*	-
ATCC43338	7	5.7	-	-	*E. tonsillarum*	-
L1-3	7	0.9	-	-	*E. tonsillarum*	-
L1-4	7	0.8	-	-	*E. tonsillarum*	-
Nairiku-21	7	0.8	-	-	*E. tonsillarum*	-
NG-37	7	1.0	-	-	*E. tonsillarum*	-
T-131	7	1.2	-	-	*E. tonsillarum*	-
Kanagawa-7	7	1.9	-	-	*E. tonsillarum*	-
NKB-13	7	1.2	-	-	*E. tonsillarum*	-
NKB-41	7	1.4	-	-	*E. tonsillarum*	-
NKB-183	7	1.6	-	-	Other sp. 3	-
NKB-199	7	3.5	-	-	Other sp. 3	-
266/719	7	1.3	-	-	*E. tonsillarum*	-

Table 1. Continued.

266/6171b	7	1.0	-	-	*E. tonsillarum*	-
266/6969	7	0.7	-	-	*E. tonsillarum*	-
266/7946	7	1.1	-	-	*E. tonsillarum*	-
266/7991	7	1.3	-	-	*E. tonsillarum*	-
Goda*	8	0.6	+	-	*E. rhusiopathiae*	A
NKF-263	8	1.7	-	-	*E. rhusiopathiae*	B
82-911	8	1.6	+	-	*E. rhusiopathiae*	B
Kaparek*	9	>6.9	-	-	*E. rhusiopathiae*	A
IT-401	9	1.8	+	-	Other sp. 2	C
IT-349	9	1.5	+	-	*E. rhusiopathiae*	B
IT-497	9	2.0	-	-	*E. rhusiopathiae*	B
14B	9	1.8	+	-	*E. rhusiopathiae*	B
Lengyel-P*	10	0.2	-	-	*E. tonsillarum*	-
2179	10	2.0	+	-	Other sp. 2	C
IV 12/8*	11	0.9	+	-	*E. rhusiopathiae*	B
K-503	11	>7.8	-	-	*E. rhusiopathiae*	A
NKB-60	11	2.0	+	-	*E. rhusiopathiae*	A
Pécs 9*	12	0.7	+	+	*E. rhusiopathiae*	A
NKF-1	12	>7.4	-	-	*E. rhusiopathiae*	B
Iida-4	12	1.9	+	-	*E. rhusiopathiae*	A
Pécs 18	13	>8.6	-	-	Other sp. 1	-
Shiribeshi-19	13	>7.1	-	-	Other sp. 1	-
Iszap-4*	14	0.8	-	-	*E. tonsillarum*	-
Pécs 3597*	15	1.2	-	-	*E. rhusiopathiae*	A
NKF-206	15	>6.9	-	-	*E. tonsillarum*	-
2909-H	15	1.9	-	-	*E. rhusiopathiae*	A
2909-S	15	2.0	+	-	*E. rhusiopathiae*	A
Tanzania*	16	1.4	-	-	*E. rhusiopathiae*	A
NKD-96	16	1.8	-	-	*E. tonsillarum*	-
NKB-97	16	1.4	-	-	*E. tonsillarum*	-
T-184	16	0.5	+	-	*E. rhusiopathiae*	B
545*	17	>6.5	-	-	*E. rhusiopathiae*	A
S-106	17	>7.7	-	-	*E. rhusiopathiae*	A
Shizuoka-55	17	>8.7	-	-	*E. rhusiopathiae*	A
715*	18	1.1	+	-	Other sp. 2	C
2017*	19	0.9	+	-	*E. rhusiopathiae*	B
NKB-78	19	1.7	-	-	*E. rhusiopathiae*	B
NKD-82	19	1.8	+	-	*E. rhusiopathiae*	A
2553*	20	1.5	+	-	*E. tonsillarum*	-
Baño 36*	21	0.8	+	-	*E. rhusiopathiae*	B
K-303	21	2.0	+	-	*E. rhusiopathiae*	A
82-533	21	2.0	-	-	*E. rhusiopathiae*	A
Baño 107*	22	>5.9	-	-	*E. tonsillarum*	-
KS20A*	23	1.4	-	-	*E. tonsillarum*	-
MEW 22*	N	1.0	-	-	*E. rhusiopathiae*	A
Shizuoka-12	N	2.0	+	-	*E. rhusiopathiae*	A

*Serovar reference strains. [a–d]Data from the previous study (Takahashi et al., 2008). [b]Mice were inoculated by subcutaneous injection of serially diluted broth culture of each strain. The LD_{50} values are expressed as the number of viable bacteria per mouse. [c]Swine were inoculated by intradermal injection of 0.1 ml of a broth culture (approximately 10^7 CFU) of each strain. Systemic manifestations indicate depression and anorexia.

Table 2. Prevalence of strains virulent for swine by *spa*-type.

Spa-type	Pathogenicity for swine (%)		
	None	Erythema[a]	Systemic[b]
A (n=35)	15 (42.9)	20 (57.1)*	12 (34.3)
B (n=23)	15 (65.2)	8 (34.8)*	0 (0)*
C (n=3)	0 (0)	3 (100)*	0 (0)
None (n=29)	28 (96.6)	1 (3.4)	0 (0)*

[a]Strains eliciting erythema for swine were significantly more prevalent in the SpaA-, SpaB-, and SpaC-positive group than in the Spa-negative group (*$P < 0.05$). [b]Strains eliciting systemic manifestations for swine were significantly more prevalent in the Spa-A than in Spa-B-positive and Spa-negative groups (*$P < 0.05$).

All strains were analyzed by multiplex-polymerase chain reaction (PCR) for the presence of the *spaA*, *spaB*, and *spaC* genes, as previously described (Shen et al., 2010). All PCR testing was performed in conjunction with the relevant positive and negative controls, and any ambiguous PCR results were clarified with repeat assays.

Virulence for mice and swine among the *spa*-types was compared using Ryan's multiple comparison test (Ryan, 1960). A *P* value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

According to previous studies (To and Nagai, 2007; Ingebritson et al., 2010; Shen et al., 2010), *E. rhusiopathiae* probably harbours some *spa*-type genes (notably, *spaA* and *spaB*). Similarly, we found that all 58 *E. rhusiopathiae* strains harboured either *spaA* (35 strains, 38.9%) or *spaB* (23 strains, 25.6%) (Table 1). Regarding the prevalence of the *spa*-type genes by serovar, only the *spaA* gene was detected in strains belonging to serovar 1a, 1b, 2, 15, and 17 and type N, whereas both *spaA* and *spaB* genes were prevalent in strains belonging to serovars 3, 5, 6, 8, 9, 11,12, 19 and 21. Recently, Coutinho et al. (2011a, b) reported the genetic diversity in major serovar groups of *E. rhusiopathiae*. This fact may possibly elucidate the variation of spa-types in *E. rhusiopathiae* strains belonging to specific serovars. Anyway, the present findings suggest that the prevalence of Spa types can differ according to *E. rhusiopathiae* serovar.

Thus far, *spaC* was detected only in the reference strain of serovar 18 (that is, other sp.-2 strain 715) (To and Nagai, 2007; Shen et al., 2010). The present study found that *spaC* was detected in three other sp.-2 strains belonging to serovars 9 and 10, as well as serovar 18. To our knowledge, this is the first report of *spaC*-positive strains other than reference strain 715. SpaC is possibly the surface protein specific to other sp.-2 strains, and this may be true irrespective of the serovar type. To clarify this point, additional other sp.-2 strains should be investigated despite the low prevalence of this strain.

Other researchers (To and Nagai, 2007; Shen et al., 2010) failed to identify *spa*-type genes in *E. tonsillarum*. However, only reference strains of specific serovars were evaluated (that is, 3, 7, 10, 14, 20, 22, 24, 25, and 26), and thus, the prevalence of Spa proteins in field strains of this species is unknown. We did not find any *spa*-type genes in any of the field strains of *E. tonsillarum* as well as serovar reference strains. Moreover, 1 field strain of other sp.-1 specie (that is, strain Shiribeshi-19) harboured no *spa*-type genes. In addition, we firstly evaluated the prevalence of Spa proteins in other sp.-3 strains, belonging to serovar 7, and failed to detect *spa*-type genes in these strains. Thus, none of these species of *Erysipelothrix* are likely to harbour Spa proteins.

Possible relationships between *spa*-type and virulence for animals have been not yet elucidated. In this study, we compared virulence in mice and swine among groups positive for *spaA*, *spaB*, and *spaC* and groups negative for the *spa*-type genes. The present results are summarized in Table 1. In mice, no significant differences in the prevalence of strains exhibiting $LD_{50} < 2.0$, which is considered highly virulent (Takahashi et al., 2008), were observed among groups positive for *spaA* (21 strains, 60.0%), *spaB* (19 strains, 82.6%), *spaC* (2 strains, 66.7%), and negative for these genes (22 strains, 75.9%; $P > 0.05$). This finding suggests that Spa proteins are unlikely to be associated with virulence in mice.

On the contrary, we found a clear difference in the virulence for swine among the *spa*-types. Strains eliciting erythema were significantly more prevalent in groups positive for *spaA* (20 strains, 57.1%), *spaB* (eight strains, 34.8%), and *spaC* (three strains, 100%) than in groups negative for the *spa*-type genes (one strain, 3.6%) ($P < 0.05$). On the other hand, no significant differences were observed in the prevalence of strains eliciting erythema among strains positive for *spaA*, *spaB* and *spaC* (Table 2). Thus, Spa proteins might be related to virulence in the case of swine, irrespective of their types. Notably, strains eliciting systemic manifestations (that is, depression and anorexia) were prevalent in strains positive for *spaA* (12 strains, 34.3%) but not in other *spa*-type and *spa*-negative strains. This finding suggests that *spaA* is linked with a highly virulent type of swine erysipelas. Shimoji (2000)

reported that Spa A is very similar in its structure and amino acid sequences of its C-terminal region to choline-binding proteins of *Streptococcus pneumoniae*. These proteins play various roles in the virulence of this species, including facilitation of colonization (Bergmann and Hammerschmidt, 2006). Further studies are necessary to determine the exact role of Spa proteins in the virulence for swine.

Virulence of *Erysipelothrix* spp. is likely to depend on species and serovars (Takahashi et al., 2008); however, the reason has been not fully clarified. The present data raise the possibility that the prevalence of Spa proteins is closely linked with species, serovars, and virulence for swine. Notably, SpaA may be involved with high virulence in specific species and serovars of *Erysipelothrix* (for example, *E. rhusiopathiae* serovars 1a, 1b and 2). Prevalence of Spa proteins should be taken into consideration when evaluating the virulence of *Erysipelothrix* spp.

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