The Journal of Veterinary Medical Science

NOTE

Bacteriology

Genetic analysis of an *Erysipelothrix rhusiopathiae* swine isolate determined to be serovar 2 by a gel double diffusion test but serovar 1a/2 by a serotyping PCR assay

Kazumasa SHIRAIWA[1)], Yohsuke OGAWA[1)], Sayaka NISHIKAWA[1)], Momoko NAKAYAMA[1)], Masahiro EGUCHI[1)] and Yoshihiro SHIMOJI[1,2)]*

[1)]National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan

[2)]Research Institute for Biomedical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan

J. Vet. Med. Sci.
82(9): 1376–1378, 2020
doi: 10.1292/jvms.20-0387

Received: 23 June 2020
Accepted: 17 July 2020
Advanced Epub:
27 July 2020

ABSTRACT. We previously developed a multiplex PCR assay for the differentiation of serovar 1a, 1b, 2 and 5 strains of *Erysipelothrix rhusiopathiae*. In this study, we analyzed the serovar-defining chromosomal region of a serovar 2 swine isolate, which was PCR-positive for both serovars 1a and 2 by the multiplex PCR assay. Genetic analysis of the chromosomal region revealed that, as in serovar 1a strains, the ERH_1440 gene, which is usually truncated or missing in serovar 2 strains, was intact in this strain. This paper first shows an *E. rhusiopathiae* serovar 2 strain possessing an intact ERH_1440 gene and suggests that care may be needed when determining the serovar of such rare strains by PCR assay.

KEY WORDS: ERH_1440, *Erysipelothrix rhusiopathiae*, serovar 1a, serovar 2

Erysipelothrix rhusiopathiae is a gram-positive zoonotic pathogen and the etiological agent of erysipelas in a variety of animals, including swine, turkeys, and chickens [6]. In humans, it also causes a skin disease known as erysipeloid, which can lead to septicemia and then endocarditis [7]. At present, based on a conventional gel double diffusion test, *Erysipelothrix* species have been classified into 28 serovars. It has been well acknowledged that specific serovars, namely, 1a, 1b, and 2, are associated with disease in pigs, poultry, and humans [1–3, 8, 13, 14]. Recently, we reported a multiplex PCR assay for the identification of *E. rhusiopathiae* serovars 1a, 1b, 2 and 5, the last of which is often isolated from wild animals [12]. The PCR assay was developed based on the sequence of the chromosomal region involved in the virulence of a serovar 1a strain of *E. rhusiopathiae* [5]. In this study, we analyzed the serovar-defining chromosomal region of an isolate from a 10-month-old growing-finishing pig that died from septicemia.

Serotyping of the isolate, designated Ishikari, was performed using heat-stable peptidoglycan antigens and type-specific rabbit antiserum, as previously described [4], and the isolate was determined to belong to serovar 2. Multiplex PCR was performed as previously described [12], and the results showed that the strain was PCR positive for both serovars 1a and 2 (Fig. 1). Using three primer pairs [5], seq 1F and seq 1R', seq 2F and seq 2R, and seq 3F and seq 3R, the 17.8-kb chromosomal region that defines serovar antigenicity of the strain was sequenced (accession number LC556333). Sequence analysis revealed that except for the ERH_1440 gene, the genetic structure and contents were identical with those of R32E11 (a serovar 2 reference strain), with >97.9% amino acid sequence identity between each gene (Fig. 2); R32E11 possesses a truncated ERH_1440 gene, which encodes CDP-glycerol:poly (glycerophosphate) glycerophosphotransferase, whereas the Ishikari strain has an intact ERH_1440 gene, similar to other serovar 1a strains. This is the first observation that a serovar 2 strain possesses an intact ERH_1440 gene. In the previously analyzed sixteen strains of serovar 2 (Tochigi-20, Nagano 11-2, and SE-9) [5], serovar 2/15 (Niigata 05-67, Toyama 10-5, and 262) [12], serovar 2/21 (ATCC 19414, Toyama 02-2, Kanazawa 02-3, 17C, 20C, 28C, 33C, and Wakayama) [11], and untypeable (Ishikawa 02-26 and Nagano 11-1) [5], both of which were thought to be derived from serovar 2 strains, the ERH_1440 was missing or truncated. Furthermore, testing 190 serovar 2 strains, we confirmed that multiplex PCR with the 1a-R primer designed to detect serovar 1a strains does not amplify a DNA fragment from the serovar 2 strains, suggesting that the Ishikari strain is a rare serovar 2 strain that possesses an intact ERH_1440 gene.

The role of the ERH_1440 gene in virulence remains unclear. We previously observed that the ERH_0432 gene putatively

*Correspondence to: Shimoji, Y.: shimoji@affrc.go.jp

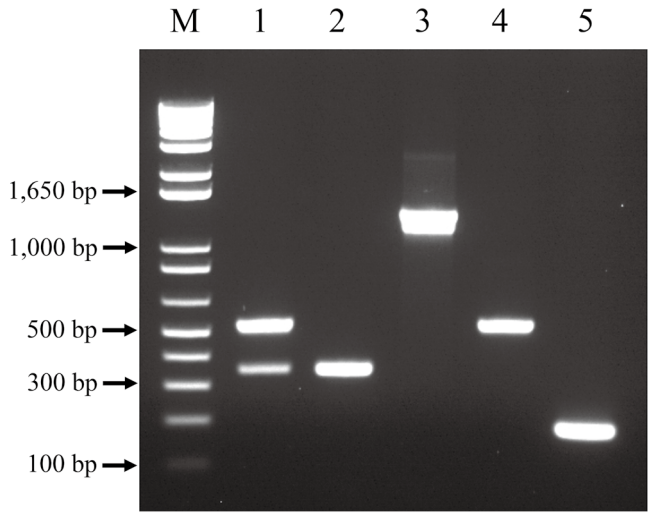

Fig. 1. Multiplex PCR assay to simultaneously detect and differentiate serovar 1a, 1b, 2, and 5 strains of *Erysipelothrix rhusiopathiae.* Lanes: 1, Ishikari strain (serovar 2); 2, Fujisawa strain (serovar 1a); 3, 422/1E strain (serovar 1b); 4, R32E11 strain (serovar 2); and 5, Pécs 67 strain (serovar 5). A molecular size marker (1-kb Plus DNA Ladder; Invitrogen) is shown to the left (M).

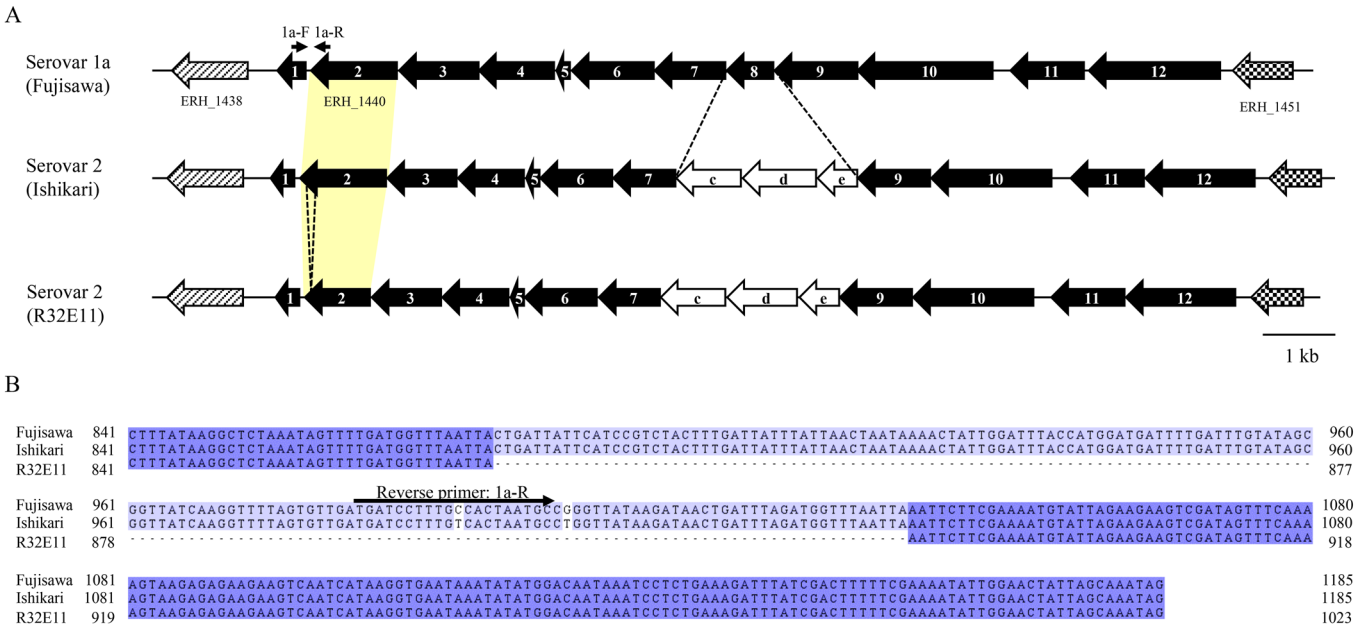

Fig. 2. (A) Schematic representation of the chromosomal region defining the antigenicity of the *Erysipelothrix rhusiopathiae* strains Fujisawa (serovar 1a), Ishikari (serovar 2), and R32E11 (serovar 2). Identical genes are indicated by the same numbers or letters. Small arrows indicate the locations of the primer pairs used for the multiplex PCR to detect *E. rhusiopathiae* serovar 1a strains [12]. (B) Sequence alignment of the ERH_1440 gene sequence from the Fujisawa, Ishikari, and R32E11 strains. Dark blue background, nucleotides identical among three strains; light blue background, nucleotides missing in R32E11 strain; dashes, gaps in the aligned sequences. The coding sequence from 841 to 1,185 nucleotides of the ERH_1440 gene of Fujisawa and Ishikari and the corresponding sequence of R32E11 are shown.

encoding the same protein, CDP-glycerol:poly (glycerophosphate) glycerophosphotransferase, was involved in capsule expression and virulence of the organism [9]. It has been shown that most of the isolates from acute septicemic cases belong to serovar 1a and that most of the isolates from subacute and chronic cases belong to serovar 2 [6]. Considering that the Ishikari strain was isolated from an acute case, it is intriguing to consider that serovar 2 strains with an intact ERH_1440 gene may be more virulent than other serovar 2 strains with a deletion in the gene. Previously, we confirmed that a serovar 1a mutant with deletion of the whole ERH_1440 gene is as virulent as its parental strain in mice and that virulence was not altered [5]. However, it is not surprising that *E. rhusiopathiae* exhibits different pathogenicity between pigs and other hosts [10, 15]; thus, it may be interesting to compare the

virulence in pigs between the Ishikari strain and its mutant with a 162-bp deletion in ERH_1440.

Finally, we found that although the ERH_1440 gene is usually truncated or missing in serovar 2 strains, some serovar 2 strains may possess an intact ERH_1440 gene. This finding is important in the differentiation of serovar 1a and 2 strains in the previously reported multiplex PCR assay [12], and care may be needed when determining the serovar of such rare strains.

CONFLICT OF INTEREST. The authors declare no potential conflicts of interest.

ACKNOWLEDGMENT. This work was supported by a grant from the National Agriculture and Food Research Organization (to Y. S.).

REFERENCES

1. Bender, J. S., Irwin, C. K., Shen, H. G., Schwartz, K. J. and Opriessnig, T. 2011. *Erysipelothrix* spp. genotypes, serotypes, and surface protective antigen types associated with abattoir condemnations. *J. Vet. Diagn. Invest.* **23**: 139–142. [Medline] [CrossRef]
2. Chirico, J., Eriksson, H., Fossum, O. and Jansson, D. 2003. The poultry red mite, *Dermanyssus gallinae*, a potential vector of *Erysipelothrix rhusiopathiae* causing erysipelas in hens. *Med. Vet. Entomol.* **17**: 232–234. [Medline] [CrossRef]
3. Harada, K., Amano, K., Akimoto, S., Yamamoto, K., Yamamoto, Y., Yanagihara, K., Kohno, S., Kishida, N. and Takahashi, T. 2011. Serological and pathogenic characterization of *Erysipelothrix rhusiopathiae* isolates from two human cases of endocarditis in Japan. *New Microbiol.* **34**: 409–412. [Medline]
4. Imada, Y., Takase, A., Kikuma, R., Iwamaru, Y., Akachi, S. and Hayakawa, Y. 2004. Serotyping of 800 strains of *Erysipelothrix* isolated from pigs affected with erysipelas and discrimination of attenuated live vaccine strain by genotyping. *J. Clin. Microbiol.* **42**: 2121–2126. [Medline] [CrossRef]
5. Ogawa, Y., Shiraiwa, K., Nishikawa, S., Eguchi, M. and Shimoji, Y. 2018. Identification of the chromosomal region essential for serovar-specific antigen and virulence of serovar 1 and 2 strains of *Erysipelothrix rhusiopathiae*. *Infect. Immun.* **86**: e00324-18. [Medline] [CrossRef]
6. Opriessnig, T. and Wood, R. L. 2012. Erysipelas. pp. 750–759. *In*: Diseases of Swine, 10th ed. (Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J. and Stevenson, G. W. eds.), Wiley-Blackwell, Ames.
7. Reboli, A. C. and Farrar, W. E. 1989. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clin. Microbiol. Rev.* **2**: 354–359. [Medline] [CrossRef]
8. Schmitt, F., Schade, B., Böhm, B., Shimoji, Y. and Pfahler, C. 2014. Erysipelas in a free-range layer flock with conjunctival oedema as an unusual clinical sign. *Berl. Munch. Tierarztl. Wochenschr.* **127**: 183–187. [Medline]
9. Shimoji, Y., Ogawa, Y., Tsukio, M., Shiraiwa, K., Nishikawa, S. and Eguchi, M. 2019. Genome-wide identification of virulence genes in *Erysipelothrix rhusiopathiae*: use of a mutant deficient in a *tagF* homolog as a safe oral vaccine against swine erysipelas. *Infect. Immun.* **87**: e00673-19. [Medline] [CrossRef]
10. Shimoji, Y., Osaki, M., Ogawa, Y., Shiraiwa, K., Nishikawa, S., Eguchi, M., Yamamoto, T. and Tsutsui, T. 2019. Wild boars: A potential source of *Erysipelothrix rhusiopathiae* infection in Japan. *Microbiol. Immunol.* **63**: 465–468. [Medline] [CrossRef]
11. Shimoji, Y., Shiraiwa, K., Tominaga, H., Nishikawa, S., Eguchi, M., Hikono, H. and Ogawa, Y. 2020. Development of a multiplex PCR-based assay for rapid serotyping of *Erysipelothrix* species. *J. Clin. Microbiol.* **58**: e00315-20. [Medline] [CrossRef]
12. Shiraiwa, K., Ogawa, Y., Nishikawa, S., Eguchi, M. and Shimoji, Y. 2018. Identification of serovar 1a, 1b, 2, and 5 strains of *Erysipelothrix rhusiopathiae* by a conventional gel-based PCR. *Vet. Microbiol.* **225**: 101–104. [Medline] [CrossRef]
13. Shiraiwa, K., Ogawa, Y., Nishikawa, S., Kusumoto, M., Eguchi, M. and Shimoji, Y. 2017. Single nucleotide polymorphism genotyping of *Erysipelothrix rhusiopathiae* isolates from pigs affected with chronic erysipelas in Japan. *J. Vet. Med. Sci.* **79**: 699–701. [Medline] [CrossRef]
14. Takahashi, T., Nagamine, N., Kijima, M., Suzuki, S., Takagi, M., Tamura, Y., Nakamura, M., Muramatsu, M. and Sawada, T. 1996. Serovars of *Erysipelothrix* strains isolated from pigs affected with erysipelas in Japan. *J. Vet. Med. Sci.* **58**: 587–589. [Medline] [CrossRef]
15. Takahashi, T., Sawada, T., Seto, K., Muramatsu, M., Maruyama, T. and Kanzaki, M. 1985. Pathogenicity of *Erysipelothrix rhusiopathiae* strains of serovars 1a, 3, 5, 6, 8, 11, 21, and type N isolated from slaughter pigs affected with chronic erysipelas. *Nippon Juigaku Zasshi* **47**: 1–8. [Medline] [CrossRef]