Conjugative Transposition of Tn916 and Detection of Tn916-Like Transposon in *Erysipelothrix rhusiopathiae*

Manao OZAWA¹⁾, Kinya YAMAMOTO¹⁾, Akemi KOJIMA¹⁾, Masami TAKAGI¹⁾ and Toshio TAKAHASHI¹⁾

¹⁾National Veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries, 1-15-1 Tokura, Kokubunji, Tokyo 185-8511, Japan

(Received 1 October 2008/Accepted 12 July 2009)

ABSTRACT. In order to investigate the origin of tetracycline resistance in *Erysipelothrix rhusiopathiae*, conjugative transpositions of Tn916 were tested. The frequency of transfer between strains of *E. rhusiopathiae* was about 10-fold higher than that between *Enterococcus faecalis* and *E. rhusiopathiae*. In addition, detection of a Tn916-like transposon was performed by PCR assay and DNA sequencing in *E. rhusiopathiae* field isolates. Of 49 tetracycline-resistant isolates, 38 (77.6 %) carried a Tn916-like transposon, while 11 (22.4 %) carried *tet*(M) only. These results suggested that Tn916-like transposon may be widely present in the *E. rhusiopathiae* field isolates resistant to tetracycline.

KEY WORDS: antimicrobial resistance, *Erysipelothrix rhusiopathiae*, tetracycline, Tn916.

J. Vet. Med. Sci. 71(11): 1537–1540, 2009

Erysipelothrix rhusiopathiae is the causative agent of swine erysipelas, which causes great economic loss in pig husbandry throughout the world. *E. rhusiopathiae* is also an important pathogen in public health as it causes erysipeloid in humans [19]. The tetracycline group of antibiotics is frequently used for growth promotion or treatment of various infections in pigs. The emergence of strains resistant to oxytetracycline (OTC) was first reported in 1984 [16]. Recently, Yamamoto *et al.* [20] demonstrated that 53.3% of *E. rhusiopathiae* strains isolated from 1988 to 1998 were resistant to OTC. They detected the *tet*(M) gene in *E. rhusiopathiae* and indicated that the nucleotide sequence of the *tet*(M) gene had 99% identity with the *tet*(M) gene from Tn916 of *Enterococcus faecalis* [21]. Tn916, initially detected in *E. faecalis*, is a broad-host-range conjugative transposon that carries the *tet*(M) gene [5, 15]. Tn916 and related elements are found in a wide variety of Gram-positive and a few Gram-negative bacterial species [13]. It has been recognized that Tn916 is transferable from *Enterococcus* and *Listeria* to *Erysipelothrix* by *in vitro* conjugation [14, 17]. In this study, we investigated conjugative transposition from *E. rhusiopathiae* to *E. rhusiopathiae* and distribution of Tn916-like transposon in *E. rhusiopathiae* field isolates, to clarify how *tet*(M) gene is spread in *E. rhusiopathiae* strains in the field.

The main characteristics of the bacterial strains used in this study are listed in Table 1. The strain Fujisawa of *E. rhusiopathiae* was used as a recipient [9]. Tn916 was transposed from *E. faecalis* CG110 [6] to *E. faecalis* OG1X [7] and this strain was designated OG1X::Tn916 and used as the donor for Tn916. The spontaneous mutant strains resistant to dihydrostreptomycin (DSM) were obtained from a bacterial suspension in tryptose phosphate broth (TPB;

Difco Laboratories, MI, U.S.A.) that contained 0.1% Tween 80 (pH 7.6) (TPB-T80) and dihydrostreptomycin (100 µg/ml). These mutant strains were designated T17-DSMR and C4-DSMR. In detection of Tn916-like transposon, 68 *E. rhusiopathiae* field isolates were used. They were isolated from cases of swine erysipelas between 1994 and 2001 in Japan. Of 68 isolates, 49 (72.1%) were OTC-resistant [minimum inhibitory concentration (MIC) range, 16–64 µg/ml]. MICs were determined as described previously [8].

Filter matings were carried out as follows. Overnight cultures of donor and recipient cells in TPB-T80 broth were mixed at a ratio of 1:10. One millilitre of the mating mixture was inoculated on a membrane filter placed on TPB-T80 agar. After 20 hr incubation at 37°C, the cells were suspended in 2 ml TPB-T80 broth and plated on agar that contained appropriate antibiotics to select transconjugants. In filter matings between *E. faecalis* and *E. rhusiopathiae*, agar containing kanamycin (250 µg/ml) and OTC (10 µg/ml) was used. In filter matings between strains of *E. rhusiopathiae*, agar containing DSM (100 µg/ml) and OTC (10 µg/ml) was used. PCR for termini of circular intermediate of Tn916

Table 1. Bacterial strains used in this study

Strains	Relevant properties	Reference
<i>E. faecalis</i>		
CG110	OTC ^r	6
OG1X	SM ^r	7
OG1X::Tn916	OTC ^r , SM ^r	This study
<i>E. rhusiopathiae</i>		
Fujisawa	Wild type (serotype 1a)	9
Fujisawa::Tn916	OTC ^r	This study
T17	Wild type (serotype 2)	This study
C4	Wild type (serotype 2)	This study
T17-DSMR	DSM ^r (spontaneous mutant)	This study
C4-DSMR	DSM ^r (spontaneous mutant)	This study

OTC^r, oxytetracycline resistant; SM^r, streptomycin resistant; DSM^r, dihydrostreptomycin resistant.

* CORRESPONDENCE TO: OZAWA, M., National Veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries, 1-15-1, Tokura, Kokubunji, Tokyo 185-8511, Japan.
e-mail: ozawa@nval.go.jp

Fig. 1. Electrophoretic analysis of PCR products (320 bp) obtained by amplification of primers designed on Tn916 termini. Lane M, DNA size marker (100 bp ladder), lane 1, Fujisawa, lane 2, OG1X::Tn916, lane 3, Fujisawa::Tn916, lane 4, T17-DSMR, lane 5, C4-DSMR, lane 6, T17-DSMR::Tn916, lane 7, C4-DSMR::Tn916.

were performed to confirm conjugative transposition of Tn916. DNA extraction of *E. faecalis* and *E. rhusiopathiae* strains was performed as previously described [10]. PCR primers were designed on Tn916 termini [11]. Their positions in the Tn916 nucleotide sequence [4] (GenBank Accession No. U09422) were as follows: Tn916-F1, 5'-AACAGGAGCGTCTTGTGCT-3' (17836–17855); Tn916-R1, 5'-CGTCGTATCAAAGCTCATTCA-3' (117–97). The PCR mixture consisted of 100 ng template DNA, 10 pmol each primer, 1 × PCR buffer (Mg²⁺ free), 2.5 mM MgCl₂, 0.2 mM dNTP mixture, and 1.25 U Ex Taq DNA polymerase (TaKaRa Ex Taq; Takara Bio Inc., Shiga, Japan). DNA amplification was carried out as follows: 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 56°C, 1 min at 72°C and a final extension of 10 min at 72°C. The PCR products were electrophoresed through a 1% agarose gel and visualized by ethidium bromide staining. The circular forms of Tn916 could be detected in the transconjugants, whereas they were not present in the parent strains Fujisawa, T17-DSMR and C4-DSMR (Fig. 1). The frequencies of transfer are shown in Table 2. In the filter mating using *E. faecalis* OG1X::Tn916 as donor with *E. rhusiopathiae* Fujisawa as recipient, the frequency of transfer was 10⁻⁶. On the other hand, in the filter matings using Fujisawa::Tn916 as donor with T17-DSMR and C4-DSMR as recipients, the frequencies of transfer was 10⁻⁵. The frequency of transfer between strains of *E. rhusiopathiae* was about 10-fold higher than that between *E. faecalis* and *E. rhusiopathiae*.

Detection of *tet*(M) gene and Tn916-like transposon in the *E. rhusiopathiae* field isolates was performed using PCR methods as previously described [1, 18]. PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Valencia, CA, U.S.A.) for use in sequencing reactions. Dye terminator cycle sequencing was performed with the BigDye Terminator Cycle sequencing kit and ABI PRISM 310 genetic analyzer (Applied Biosystems). The restriction enzyme HincII (Takara Bio Inc.) was used to

Table 2. Conjugative transfer frequency of Tn916

Donor	Receipient	Transposon transfer frequency (transconjugants/donor)
OG1X::Tn916	Fujisawa	7.8×10^{-6}
Fujisawa::Tn916	T17-DSMR	2.3×10^{-5}
Fujisawa::Tn916	C4-DSMR	7.6×10^{-5}

evaluate the specificity of the PCR assay for the detection of Tn916-like transposon. Of the 49 OTC-resistant isolates, 38 (77.6%) gave the expected PCR products of 2835 bp using primers linking *tet*(M) to Xis-Tn genes of Tn916. Of the seven isolates with low MIC values (16 µg/ml), six (85.7%) did not have a Tn916. All of the OTC-resistant *E. rhusiopathiae* isolates had *tet*(M). The parts of the PCR product linking *tet*(M) to Xis-Tn genes of Tn916 from four OTC-resistant field isolates were sequenced and had 99.8% and 100% homology with Tn916 of *E. faecalis* respectively (U09422, positions 13907–14337 and 15882–16401). They also had 99.8% homology with Tn916 of *Streptococcus suis* (EF432727, positions 16637–17067 and 18611–19130). To evaluate the specificity of the PCR assay for detection of the Tn916-like transposon, the amplified DNA fragments of OTC-resistant *E. rhusiopathiae* strains and *E. faecalis* OG1X::Tn916 were digested with Hinc II. All of the amplified DNA fragments digested with Hinc II generated two fragments (1623 and 1212) (Fig. 2).

The *tet*(M) gene is common in *Enterococcus* and *Streptococcus* [22] and has also been found in *Listeria* [2, 12]. Yamamoto *et al.* [21] identified the *tet*(M) gene in the field isolates of *E. rhusiopathiae*. The wide distribution of this gene may occur because it is carried by broad-host range conjugative transposons that belong to the Tn916 family [13]. Our results suggest that the presence of *tet*(M) in *E. rhusiopathiae* is the outcome of acquisition of a Tn916 from *Enterococcus*. This proposal is based on the fact that Tn916 is transferable from *Enterococcus* and *Listeria* to *E. rhusiopathiae* by *in vitro* conjugation [14, 17]. In addition, Tn916

Fig. 2. The specificity of the PCR assay for the detection of the Tn916-like transposon. Lane M1, DNA size marker (1 kb ladder); lane 1, DNA fragment from *E. faecalis* OG1X::Tn916 (2,835 bp); lane 2, DNA fragment from *E. rhusiopathiae* T1 (oxytetracycline-resistant field isolate) (2,835 bp); lane 3, DNA fragment from *E. rhusiopathiae* C16 (oxytetracycline-resistant field isolate) (2,835 bp); lane 4, Hinc II-digested DNA fragment from *E. faecalis* OG1X::Tn916 (1,623 bp and 1,212 bp); lane 5, Hinc II-digested DNA fragment from *E. rhusiopathiae* T1 (1,623 bp and 1,212 bp); lane 6, Hinc II-digested DNA fragment from *E. rhusiopathiae* C16 (1,623 bp and 1,212 bp); lane M2, DNA size marker (200 bp ladder).

was also transferred between strains of *E. rhusiopathiae* in this study. The nucleotide sequence of the *tet(M)* gene from *E. rhusiopathiae* had 99% identity with that from Tn916 of *E. faecalis* [21]. Moreover, most OTC-resistant *E. rhusiopathiae* had a Tn916-like transposon. *Streptococcus* may also be a donor of Tn916 because of high similarity of sequence of Tn916 between *E. rhusiopathiae* and *S. suis* in this study. On the other hand, 22.4% of tetracycline-resistant isolates did not have Tn916 but they had *tet(M)* gene. Conjugative plasmids or transposons other than Tn916 may involved in the acquisition of *tet(M)* gene in these isolates.

Doucet-Populaire *et al.* [3] have demonstrated the transfer of Tn1545 from *E. faecalis* to *Listeria monocytogenes* in the digestive tract of gnotobiotic mice. *Enterococcus* and *Streptococcus* live naturally in the intestinal tract of pigs. The digestive tract is considered to be the most probable meeting point for these two bacterial species and *E. rhusiopathiae*. Therefore, the interspecific transfer of Tn916 might occur in the digestive tract.

In conclusion, we demonstrated conjugative transposition of Tn916 between *E. rhusiopathiae* strains and detected Tn916-like transposon in OTC-resistant *E. rhusiopathiae* field isolates. Further investigation of transfer of Tn916 *in vivo* is needed to clarify the reason of wide distribution of *tet(M)* gene in *E. rhusiopathiae* strains.

REFERENCES

1. Agersø, Y., Jensen, L.B., Givskov, M. and Roberts, M.C. 2002. The identification of a tetracycline resistance gene *tet(M)*, on a Tn916-like transposon, in the *Bacillus cereus* group. *FEMS Microbiol. Lett.* **214**: 251–256.
2. Charpentier, E., Gerbaud, G., Jacquet, C., Rocourt, J. and Courvalin, P. 1995. Incidence of antibiotic resistance in *Listeria* species. *J. Infect. Dis.* **172**: 277–281.
3. Doucet-Populaire, F., Trieu-Cuot, P., Dosbaa, I., Andreumont, A. and Courvalin, P. 1991. Inducible transfer of conjugative transposon Tn1545 from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts of gnotobiotic mice. *Antimicrob. Agents Chemother.* **35**: 185–187.
4. Flannagan, S.E., Zitzow, L.A., Su, Y.A. and Clewell, D.B. 1994. Nucleotide sequence of the 18-kb conjugative transposon Tn916 from *Enterococcus faecalis*. *Plasmid* **32**: 350–354.
5. Franke, A.E. and Clewell, D.B. 1981. Evidence for a chromosome-borne resistance transposon (Tn916) in *Streptococcus faecalis* that is capable of “conjugal” transfer in the absence of a conjugative plasmid. *J. Bacteriol.* **145**: 494–502.
6. Gawron-Burke, C. and Clewell, D.B. 1982. A transposon in *Streptococcus faecalis* with fertility properties. *Nature* **300**: 281–284.
7. Ike, Y., Craig, R.A., White, B.A., Yagi, Y. and Clewell, D.B. 1983. Modification of *Streptococcus faecalis* sex pheromones after acquisition of plasmid DNA. *Proc. Natl. Acad. Sci. U.S.A.* **80**: 5369–5373.
8. Ishiyama, S., Ueda, S., Kuwabara, N., Kosakai, G., Koya, M., Konno, M. and Fujii, R. 1968. On the standardization of method for determination of minimum inhibitory concentrations. *Chemotherapy (Tokyo)* **16**: 98–99.
9. Kucsera, G. 1972. Comparative study on special serotypes of *Erysipelothrix rhusiopathiae* strains isolated in Hungary and abroad. *Acta Vet. Acad. Sci. Hung.* **22**: 251–261.
10. Makino, S., Okada, Y., Maruyama, T., Ishikawa, K., Takahashi, T., Nakamura, M., Ezaki, T. and Morita, H. 1994. Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J. Clin. Microbiol.* **32**: 1526–1531.
11. Manganelli, R., Romano, L., Ricci, S., Zazzi, M. and Pozzi, G. 1995. Dosage of Tn916 circular intermediates in *Enterococcus faecalis*. *Plasmid* **34**: 48–57.
12. Poyart-Salmeron, C., Trieu-Cuot, P., Carlier, C., MacGowan, A.

- A., McLauchlin, J. and Courvalin, P. 1992. Genetic basis of tetracycline resistance in clinical isolates of *Listeria monocytogenes*. *Antimicrob. Agents Chemother.* **36**: 463–466.
13. Rice, L.B. 1998. Tn916 family conjugative transposons and dissemination of antimicrobial resistance determinants. *Antimicrob. Agents Chemother.* **42**: 1871–1877.
 14. Shimoji, Y., Yokomizo, Y., Sekizaki, T., Mori, Y. and Kubo, M. 1994. Presence of a capsule in *Erysipelothrix rhusiopathiae* and its relationship to virulence for mice. *Infect. Immun.* **62**: 2806–2810.
 15. Su, Y.A., He, P. and Clewell, D.B. 1992. Characterization of the *tet(M)* determinant of Tn916: evidence for regulation by transcription attenuation. *Antimicrob. Agents Chemother.* **36**: 769–778.
 16. Takahashi, T., Sawada, T., Ohmae, K., Terakado, N., Muramatsu, M., Seto, K., Maruyama, T. and Kanzaki, M. 1984. Antibiotic resistance of *Erysipelothrix rhusiopathiae* isolated from pigs with chronic swine erysipelas. *Antimicrob. Agents Chemother.* **25**: 385–386.
 17. Vicente, M.F., Baquero, F. and Perez-Diaz, J.C. 1988. Conjugative acquisition and expression of antibiotic resistance determinants in *Listeria* spp. *J. Antimicrob. Chemother.* **21**: 309–318.
 18. Warsa, U.C., Nonoyama, M., Ida, T., Okamoto, R., Okubo, T., Shimauchi, C., Kuga, A. and Inoue, M. 1996. Detection of *tet(K)* and *tet(M)* in *Staphylococcus aureus* of Asian countries by the polymerase chain reaction. *J. Antibiotics (Tokyo)* **49**: 1127–1132.
 19. Wood, R.L. 1992. Erysipelas. pp. 475–486. In: Diseases of Swine, 7th ed. (Lemen, A. D., Straw, B. E., Mengeling, W. L., D'Allaire, S. and Taylor, E. D. J. eds.), Iowa State University Press, Ames.
 20. Yamamoto, K., Kijima, M., Yoshimura, H. and Takahashi, T. 2001. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae* isolated from pigs with swine erysipelas in Japan, 1988–1998. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **48**: 115–126.
 21. Yamamoto, K., Sasaki, Y., Ogikubo, Y., Noguchi, N., Sasatsu, M. and Takahashi, T. 2001. Identification of the tetracycline resistance gene, *tet(M)*, in *Erysipelothrix rhusiopathiae*. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **48**: 293–301.
 22. Zilhao, R., Papadopoulou, B. and Courvalin, P. 1988. Occurrence of the *Campylobacter* resistance gene *tetO* in *Enterococcus* and *Streptococcus* spp. *Antimicrob. Agents Chemother.* **32**: 1793–1796.