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Detection of Type 1 Fimbrial Genes in Fish Pathogenic and Non-pathogenic *Edwardsiella tarda* Strains by PCR

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ABSTRACT—Presence of fimbrial genes among fish pathogenic and non-pathogenic strains of *Edwardsiella tarda* was investigated by polymerase chain reaction (PCR). Four primer sets for PCR were designed to detect the four genes (*etfA*, *etfB*, *etfC*, *etfD*) of the type 1 fimbrial gene cluster of *E. tarda*. All the four genes were successfully amplified with the four primer sets in all the pathogenic strains. On the other hand, any of *etfA*, *etfB* and *etfC* were not detected in 13 of 14 non-pathogenic strains. The results suggest that fimbriae play a role in the pathogenicity of *E. tarda* and that detection of *etfA*, *etfB* and *etfC* by PCR is useful to distinguish between pathogenic and non-pathogenic strains of *E. tarda*.

Key words: *Edwardsiella tarda*, type 1 fimbrial gene, PCR, pathogenicity, edwardsiellosis

Edwardsiella tarda, a Gram-negative bacterium of the family Enterobacteriaceae, is known as the causative agent of edwardsiellosis in freshwater and marine fish[1,2]. The virulence varies among different strains[3]. Most *E. tarda* strains isolated from Japanese eel *Anguilla japonica* and Japanese flounder *Paralichthys olivaceus* were reported to be classified into serotype A by O-agglutination test[4,5]. Many pathogenic properties of this bacterium have been proposed, such as dermatotoxin production[6], anti-phagocytic killing[7], serum resistance, and adherence and invasion to epithelial cells[3,8]. However, the virulence determinants of *E. tarda* are largely unknown. Adherence of pathogenic bacteria to the surface of their host is a critical step in bacterial infection. Fimbriae or hemagglutinin is known to be involved in adherence to host[9,10]. We found that a fimbrial gene cluster, a homologue of type 1 fimbrial gene cluster found in Enterobacteriaceae, mediated hemagglutination of *E. tarda*[11]. Furthermore, the hemagglutination activity is reported to be correlated with the adherence to an epithelial cell line, HEp-2[12]. In the present study, we investigated four fimbrial genes composing the cluster in *E. tarda* strains isolated from diseased fish and aquaculture environment as well as in other *Edwardsiella* species by using polymerase chain reaction (PCR).

Materials and Methods

Four primer sets (A, B, C and D) were designed for PCR detection of respective genes (*etfA*, *etfB*, *etfC* and *etfD*) composing the fimbrial cluster (Table 1). The 17 fish pathogenic and 14 non-pathogenic *E. tarda* strains[13–15], and five strains of other *Edwardsiella* species, *E. hoshinae* and *E. ictaluri,* were used in this study (Table 2). Pathogenicity of these *E. tarda* strains was reported in the previous papers[13–15] by challenge test with Japanese eel, Japenese flounder, red sea bream *Pagrus major* or yellowtail *Seriola quinqueradiata*.

Bacterial genomic DNA was isolated using the Puregene® DNA Purification kit (Gentra Systems) according to the manufacturer's protocol. PCR was performed with TaKaRa Ex Taq (TaKaRa Bio Inc.) according to the following amplification protocol; an initial denaturation step (94°C, 2 min), 30 cycles of denaturation (95°C, 20 s), annealing (55°C, 20 s) and extension (72°C, 1 min), and a final extension step (72°C, 7 min). PCR products were analyzed by 1% agarose gel electrophoresis.

Results and Discussion

Results of PCR amplification with four primer sets are shown in Table 2. PCR products of the predicted size were detected in all fish pathogenic *E. tarda* strains by using every four primer set. No PCR products were amplified in 13 of 14 non-pathogenic strains with primer sets A, B and C, although a 445-bp amplicon (*etfD*) was obtained with primer set D in almost half of non-pathogenic strains. Only one exception among non-pathogenic strains was strain SU28, from which all four genes were detected.

Fimbrial genes (*etfB*, *etfC*) targeted by primer sets B and C encode proteins having high homology with fimbrial chaperon and usher protein, respectively[11]. We supposed that the biosynthesis pathway of *E. tarda* fimbriae was chaperon-usher system, since the biosynthesis of fimbriae needs involvement of chaperon and usher proteins[16]. These genes may be damaged or missing in most non-pathogenic *E. tarda* strains. An *E. tarda* strain with mutation in *fimA* (*etfA*) showed decrease in virulence[17]. We assumed that the fimbriae participated in hemagglutination by contacting with *N*-acetylneuraminic acid (NANA) on erythrocytes[18]. Adherence of *E.*

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Table 1. Primers for detection of *Edwardsiella tarda* type 1 fimbrial gene cluster

Primer set	Sequence	Positions*	Target gene (Encoding protein)	Predicted product size (bp)
A	5′-CGGTAAAGTTGAGTTTACGGGTG-3’	2160–2182	*etfA*	415
	5′-TGTAACCGTGTTGGCGTAAG-3’	2555–2574	(Major fimbrial subunit)	
B	5′-CTATATGGTGCAGACCTG-3’	2811–2828	*etfB*	493
	5′-GCTGAAGGAGACTGTATTG-3’	3285–3303	(Fimbrial chaperon protein)	
C	5′-AACACCGGTATCAGCGGAAC-3’	5223–5242	*etfC*	357
	5′-GTTGAATCGGTATGGCGTC-3’	5561–5579	(Fimbrial usher protein)	
D	5′-GGTAACCTGATTTGGCGTTC-3’	6171–6190	*etfD*	445
	5′-GGATCACCTGGATCTTATCC-3’	6596–6615	(Fimbrial subunit)	

*Sequence positions according to Sakai *et al.*[11] (DDBJ accession number AB100170).

Table 2. *Edwardsiella tarda* strains and other *Edwardsiella* species used in this study, and the results of PCR with primer sets for detection of the fimbrial genes

Bacterium	Origin	Result of PCR			
		etfA	*etfB*	*etfC*	*etfD*
Fish pathogenic *E. tarda* strains					
E381	Tilapia, Niigata pref.	+[a]	+	+	+
Edk–1	Japanese eel, Shizuoka pref.	+	+	+	+
FPC495	Tilapia, Nagasaki pref.	+	+	+	+
FPC498	Japanese flounder, Nagasaki pref.	+	+	+	+
FPC503	Red sea bream	+	+	+	+
FPC615	Japanese flounder, Nagasaki pref.	+	+	+	+
NB8030	Red sea bream, Nagasaki pref.	+	+	+	+
NE8003	Japanese flounder, Nagasaki pref.	+	+	+	+
NUF49	Japanese eel, Nagasaki pref.	+	+	+	+
NUF251	Japanese flounder, Nagasaki pref.	+	+	+	+
NUF806	Japanese flounder, Nagasaki pref.	+	+	+	+
SU35	Japanese eel, Shizuoka pref.	+	+	+	+
SU53	Eel intestinal content, Shizuoka pref.	+	+	+	+
SU57	Shizuoka pref.	+	+	+	+
SU206	Eel pond water, Shizuoka pref.	+	+	+	+
SU226	Eel pond water, Shizuoka pref.	+	+	+	+
TC159	Red sea bream	+	+	+	+
Non–pathogenic *E. tarda* strains					
SU20	Eel pond water, Shizuoka pref.	–	–	–	+
SU28	Shizuoka pref.	+	+	+	+
SU100	Eel pond water, Shizuoka pref.	–	–	–	+
SU117	Eel pond water, Shizuoka pref.	–	–	–	–
SU119	Eel pond sediment	–	–	–	+
SU138	Eel intestinal content, Shizuoka pref.	–	–	–	–
SU157	Shizuoka pref.	–	–	–	–
SU240	Shizuoka pref.	–	–	–	+
SU244	Shizuoka pref.	–	–	–	–
SU270	Shizuoka pref.	–	–	–	–
SU349	Shizuoka pref.	–	–	–	–
SU401	Shizuoka pref.	–	–	–	+
SU425	Shizuoka pref.	–	–	–	+
SU428	Shizuoka pref.	–	–	–	+
Other *Edwardsiella* species					
E. hoshinae JCM1679	Puffin, JCM[b]	–	–	–	–
E. ictaluri JCM1680	Channel catfish, JCM,	–	–	–	+
E. ictaluri FPC1036	Striped catfish, Indonesia	–	+	+	+
E. ictaluri FPC1037	Striped catfish, Indonesia	–	+	+	+
E. ictaluri FPC1038	Striped catfish, Indonesia	–	+	+	+

[a] +, PCR positive; –, PCR negative
[b] Donor: JCM, Japan Collection of Microorganisms.

tarda to HEp-2 cell was markedly inhibited by NANA[12]. It is known that NANA is also present in the body mucus and on the surface of olfactory mucosa[19,20]. Pathogenic *E. tarda* strain was observed to adhere to the body mucus of blue gourami *Trichogaster trichopterus*[21]. Accordingly, pathogenic *E. tarda* strains may adhere to the host surface with fimbriae. However, in addition to the fimbriae, many potential virulence factors have been suggested in *E. tarda*[3, 6-8]. This may explain why all fimbrial genes were detected from a non-pathogenic strain SU28. Most *E. tarda* strains classified into serotype A showed high pathogenicity against eel[4]. In this study, however, pathogenic *E. tarda* strain SU35, which showed positive reaction in all PCR tests for fimbriae, was classified into serotype D by O-agglutination test (data not shown). Possession of type 1 fimbriae in *E. tarda* would not be related to O-serotype classification. In addition, siderophore production, which was essential for the pathogenicity of *E. tarda*, was not related to the serotype[22]. These results suggest that multiple pathogenic factors including fimbriae, siderophore and LPS are essential for the pathogenicity of *E. tarda*.

No PCR products with primer set A were observed in other *Edwardsiella* species. So, the PCR method using primer set A would be useful for rapid detection of the fish pathogenic *E. tarda* strains. It was reported that fimbriae were observed on the cell surface of *E. ictaluri*[23]. In this study, PCR products of the predicted size were observed in *E. ictaluri* isolated from diseased striped catfish *Pangasius hypophthalmus* using primer sets B, C and D. It is assumed that the fimbrial gene cluster of *E. ictaluri* is composed of the same genes as those of *E. tarda*, except for *etfA*.

This study suggests that fimbriae are an important factor in pathogenicity of *E. tarda*. Further studies are needed to determine the role of fimbriae in infection process of *E. tarda.*

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