Veterinary Microbiology 141 (2010) 149–154

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

DNA adenine methylase is involved in the pathogenesis of *Edwardsiella tarda*

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ARTICLE INFO

Article history:
Received 23 March 2009
Received in revised form 23 August 2009
Accepted 4 September 2009

Keywords:
Antisense RNA
DNA adenine methylase
Edwardsiella tarda
RNA interference
Virulence

ABSTRACT

Edwardsiella tarda is a serious aquaculture pathogen that can infect many cultured fish species. The aim of this study was to investigate the potential importance of DNA adenine methylase (Dam) in *E. tarda* pathogenesis. The *E. tarda dam* gene (dam_{Et}) was cloned from a pathogenic strain, TXD1, isolated from diseased fish. Dam_{Et} shares high (70.2%) sequence identity with the Dam proteins of *Yersinia enterocolitica* and several other bacterial species. Recombinant Dam_{Et} is able to complement a *dam*-deficient *Escherichia coli* strain and methylate the genomic DNA. Attenuation of dam_{Et} expression by antisense RNA interference had no apparent effect on the growth of TXD1, but caused significant attenuation of overall bacterial virulence and altered several stress responses including spontaneous mutation, recovering from UV radiation and H_2O_2 exposure, binding to host mucus, and dissemination in host blood and liver. In addition, attenuation of dam_{Et} expression increased *luxS* expression and AI-2 activities in *E. tarda*. These results indicate that Dam_{Et} is a virulence determinant and plays a role in the pathogenesis of TXD1, and that temporal expression of dam_{Et} is essential for optimal bacterial infection.

1. Introduction

Edwardsiella tarda is a Gram-negative bacterium of the family *Enterobacteriaceae*. It is an opportunistic pathogen of humans, animal, and fish. *E. tarda* can infect a wide range of fish species and cause edwardsiellosis, a systematic disease that often leads to mortality. A number of virulence systems have been identified in *E. tarda*, notably the type III secretion system (TTSS) (Tan et al., 2005), the type VI secretion system (T6SS) (Zheng and Leung, 2007), and the EthA/EthB haemolysin system (Hirono et al., 1997). In addition, recent studies have found that the LuxS/AI-2 quorum sensing system is also involved in the pathogenesis of *E. tarda* and that a virulence-associated protein, EthR, regulates the expression of both the LuxS/AI-2 quorum sensing system and the EthA/EthB haemolysin system (Zhang et al., 2008a; Wang et al., 2009a).

DNA adenine methylase (Dam) is an enzyme that catalyzes the methylation of N-6 of the adenine residue in GATC sequences. Dam is known to function in multiple cellular processes, including DNA replication, gene expression, and mismatch repair (Casadesus and Low, 2006; Lobner-Olesen et al., 2005). Methyl-directed DNA mismatch repair system corrects base mismatches by cleaving, via the endonuclease MutH, the unmethylated daughter strand at a nearby GATC site. Overproduction of Dam is known to cause untimely methylation of the daughter strand and prevent the cleavage of MutH. As a result, strains overexpressing *dam* exhibit enhanced mutation frequency. On the other hand, *dam* mutation can also hamper the action of the mismatch repair system (Marinus, 1996). Hence, in the

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doi:10.1016/j.vetmic.2009.09.004

Table 1
Bacterial strains, plasmids, and primers used in this study.

Strain or plasmid or primer	Relevant characteristics[a]	Source or reference
Bacterial strains		
Edwardsiella tarda		
TXD1	Rif^R; fish pathogen	Sun et al. (2009)
Escherichia coli		
DH5α	F^-(*Φ*80d *lacZ* Δ*M15*) Δ(*lacZYA-argF*) U169 *recA1 endA hsdR17* (rk^-, mk^-) *supE44 thil*	Takara, Dalian, China
ER2925	*dam13::Tn9 xylA-5 mtl-1 thi-1 mcrB1*	New England Biolabs
Plasmids		
pBS-T	Ap^R: cloning vector	Tiangen, Beijing, China
pBSDP	Ap^R: carrying partial dam_{Et}	This study
pBT	Ap^R: cloning vector	Zhang et al. (2008a)
pBTDAM	Ap^R: carrying dam_{Et}	This study
pBTDI	Ap^R: carrying antisense dam_{Et}	This study
pJDAM	Ap^R: carrying dam_{Et}	This study
pJDRI	Ap^R: carrying antisense dam_{Et}	This study
pJRA	Ap^R: broad host range plasmid	Zhang et al. (2008a)
Primers	Sequences (5′ → 3′)[b]	
DAMF2	CGCGCTTTTTTGAAATGGGC	
DAMR1	GTGCATAAGGCGGATCGCA	
DAMF10	GATATCGCCCGTTGAGCCAG (EcoRV)	
DAMR9	GATATCTTAACGCTTGCCGTACA (EcoRV)	
ECF3	CTGGATAAGGTTGATGGGGAATC	
ECR3	TGCCGCTCTCCATCGTGCA	
EGF1	GACGCCCTGAGCCCAAAG	
EGR2	CCTGGCAGCCACACCGTA	

[a] Ap^R: ampicillin resistant; Rif^R: rifampicin resistant.
[b] Underlined nucleotides are restriction sites of the enzymes indicated in the brackets at the ends.

natural state of the cell, the number of Dam molecules is maintained at a controlled level (Boye et al., 1992).

In addition to participate in basic biological processes, Dam is also known to be a virulence factor in many pathogenic prokaryotes, and either its overexpression or mutation reduces the infectivity of the bacteria (Erova et al., 2006; Falker et al., 2007; Mehling et al., 2007; Oza et al., 2005; Pouillot et al., 2007).

In this study, we describe the cloning and analysis of the *dam* gene from a pathogenic *E. tarda* strain isolated from diseased fish. We found that interference with *dam* expression had drastic effects on bacterial capacities that are associated with infection.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterial strains used in this study are listed in Table 1. All strains were grown in Luria-Bertani broth (LB) medium (Sambrook et al., 1989) at 37 °C (for *Escherichia coli*) or 28 °C (for *E. tarda*). When appropriate, ampicillin and rifampicin were added at 50 and 30 μg/ml, respectively.

2.2. Examination of the methylation status of TXD1

The genomic DNA of TXD1 was prepared as described previously (Zhang and Sun, 2007) and digested with the restriction endonucleases DpnI, DpnII, and Sau3A1, respectively. All these enzymes have the sequence GATC as target site, but DpnI recognizes and cuts the target only when it is methylated, whereas DpnII cuts only when the target is unmethylated and Sau3AI cuts both methylated and unmethylated targets.

2.3. Cloning of dam_{Et}

An internal segment of dam_{Et} was cloned from TXD1 by degenerate PCR using primers DAMF2/DAMR1 (Table 1), which were designed based on the conserved sequences of known *dam* genes. The PCR products were ligated into pBS-T, resulting in pBSDP, which was subjected to DNA sequencing. Primers specific to dam_{Et} were designed based on the sequencing result of pBSDP and used to obtain the complete sequence of dam_{Et} by genome walking as described previously (Zhang and Sun, 2007).

2.4. Plasmid construction

The plasmids and primers used in this study are listed in Table 1. All PCR amplifications were performed with Pfu DNA polymerase (Tiangen, Beijing, PR China) to generate PCR products with blunt ends. To construct pJDAM, dam_{Et} was amplified by PCR with primers DAMF10/DAMR9, and the PCR products were inserted into pBT at the SmaI site; the resulting plasmid pBTDAM was digested with ScaI, and the fragment carrying dam_{Et} was inserted into pJRA at the EcoRV site, resulting in pJDAM. To construct pJDRI, the DNA corresponding to the region between positions 820 and −126 relative to the translational start of dam_{Et} was amplified by PCR with primers DAMR9/DAMF10; the PCR products were inserted into pBT at the SmaI site so that antisense dam_{Et} is constitutively expressed under the trc promoter (Zhang et al., 2008a); the resulting plasmid pBTDI was digested with ScaI, and the fragment carrying antisense dam_{Et} was inserted into pJRA at the EcoRV site, yielding pJDRI. pJDRI and pJRA were conjugated into TXD1 as described previously (Zhang et al., 2008a).