Microbial Pathogenesis 95 (2016) 216–223

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

The role of regulator Eha in *Edwardsiella tarda* pathogenesis and virulence gene transcription

Daqing Gao [a, *], Yuhong Li [a], Zeyan Xu [b], Ankang Sheng [a], Enjin Zheng [a], Zeye Shao [c], Nian Liu [a], Chengping Lu [d]

[a] Department of Pathogenic Microbiology and Immunology, Southeast University School of Medicine, China
[b] JiangSu University School of Medicine, China
[c] Department of Clinical Lab, Southeast University School of Medicine, China
[d] College of Veterinary Medicine, NanJing Agricultural University, China

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form
20 March 2016
Accepted 22 March 2016
Available online 31 March 2016

Keywords:
Edwardsiella tarda
eha gene
Virulence
Regulator

ABSTRACT

Edwardsiella tarda is a pathogen with a broad host range that infects both animals and humans. Eha is a new transcriptional regulator identified in ET13, which is involved in the bacterial hemolytic activity. This study explored the effect of the Eha in the pathogenesis of *E. tarda* and the transcriptional regulation of the bacterial virulence genes (*eseC*, *fliC*, *pagC* and *fim*A). Our results found that the virulence of the eha mutant was 2.5-fold less than the one of its wild ET13 by LD_{50} in a murine model of i.p. infection, and the bacterial loads of the mutant displayed a different profile from the one of the wild strain. Most significantly, the mice infected with the mutant have greatly reduced acute inflammation in the liver, spleen and kidney compared to the ones infected with the wild. We further demonstrated that *eseC*, *fliC* and *pagC* were regulated directly by the Eha with qRT-PCR and β-Galactosidase assay, but *fim*A wasn't done. The promoter regions of the genes modulated and the *cly* gene reported before had been found to contain a common conserved motif by using software. In addition, we found that the wild strain was more toxic to RAW264.7 macrophages, and induced less the host cell apoptotic responses than the *eha* mutant did. Altogether, these data suggested that the Eha was required for the bacterial infection and the transcriptive regulation of the important virulence genes of *E. tarda*.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Edwardsiella tarda (*E. tarda*), an important causative agent of edwardsiellosis in freshwater and marine fish, is widely distributed in aquatic environments and a variety of animals [1,2]. In humans, *E. tarda* is the only recognized pathogenic species of *Edwardsiella* primarily associated with sporadic cases of gastroenteritis [3]. Other clinical syndromes include soft tissue infection, septicaemia, hepatobiliary infection, meningitis, peritonitis, osteomyelitis, endocarditis, tubo-ovarian abscess and salpingitis [2]. These diseases usually occur in the patients with hepatobiliary disease, diabetes, malignancy, immunosuppression, and iron overload syndrome. *E. tarda* is a virulent pathogen and, when associated with bacteremia, can carry up to 50% mortality [4].

As an important pathogen, *E. tarda* evolves through a complex mechanism to cause intestinal and extraintestinal infections in both fish and human [5,2]. It also is the pathogenic bacterium which could replicate in macrophages to cause host systemic infection [6,7]. Intracellular pathogens can regulate programmed the macrophages apoptosis during intra-macrophage infection. The bacterium is reported to modulate the host's apoptotic responses to promote its intracellular survival [8]. Pathogenesis of *E. tarda* involves in some virulent factors, which have been named such as secretion systems, pili, hemagglutinins, chondroitinases, hemolysins, iron scavenging systems, the flagella and so on [6,9,10]. Very little information is currently available concerning what regulating factors control the virulence factors in *E. tarda*.

Recently, we identified a new regulating factor of *E. tarda*, designated Eha (*E. tarda* hemolysin activator) from a virulent strain ET13 [11]. ET13 strain was isolated from the feces of a patient [4]. Our results showed that the Eha should be the positive regulator, which involved in activating the *cly* gene in *Escherichia coli* (*E. coli*),

* Corresponding author. Ding JiaQiao Street 87 Southeast University School of Medicine, Nanjing 210009, China.
E-mail address: dgao2@seu.edu.cn (D. Gao).

http://dx.doi.org/10.1016/j.micpath.2016.03.010
0882-4010/© 2016 Elsevier Ltd. All rights reserved.

as evidenced by gel shift assay and primer extension assays [11]. The Eha is a member of the transcriptional regulators of MarR family [12]. Eha analogous proteins (such as SlyA) found in some pathogenic Enterobacteriaceae have been shown to regulate their virulence factors [13,14]. For example, the SlyA in *Salmonella typhimurium* was required for survival in macrophages and virulence in mice [15]. RovA in *Yersinia enterocolitica* played a key role in the pathogenesis of a *Y. enterocolitica* infection [16]. In addition, the ClyA is a cryptic hemolysin in *E. coli*, *Salmonella enterica* (*S. enterica*) and *Shigella flexneri* (*S. flexneri*) [17], but the presence of the *clyA* has not yet been confirmed in *E. tarda*. In *Salmonella typhi* (*S. typhi*), the *clyA* mutant can produce the more invasion rate, and the less bacterial cytotoxicity, comparing the wild [18]. We had constructed the *eha* mutants from ET13, and observed that the wild strains produced more hemolytic activities than the eha mutants did [11]. However, the effect of the Eha in *E. tarda* pathogenesis and its virulence gene regulated has not yet been explored. Here, our results showed that the Eha was very required for the infection of *E. tarda* with murine models and macrophages.

2. Methods

2.1. Strains, media, and culture conditions

Wild type *E. tarda* strain ET13 was kindly provided by Dr. Janda.(California Department of Health Services Microbial Diseases Laboratory). The plasmid pMP220 [19] and *E. coli* strain DH5a were kindly from Prof. Mao (Department of biochemistry Southeast University). The *eha* gene was deleted from ET13 by the suicide plasmid pHM5 and homologous recombinant method. The eha mutant strains (ET13 with the *eha* gene deleted) and the complementary strains ehaComp (the eha mutant containing complementary plasmid pACYC184-*eha*) were constructed previously [11]. Bacteria were cultivated in Luria-Bertani (LB) medium (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl). Appropriate antibiotic selection was carried out, using chloramphenicol (35 μg/ml), penicillin (100U/ml), gentamicin (100 μg/ml) and streptomycin (100U/ml).

2.2. Plasmid construction

The primers used in this study are listed in Table 1. To construct pMPF, the 422bp PCR product, which was amplified from the promoter region of the *fli*C, was inserted into the pMP220 at BglII and XbaI sites. To construct pMPE, the 203bp region of the *ese*C promoter digested with BglII and XbaI was inserted into the pMP220.

Table 1
Primer sequences and product sizes.

Gene	Primer	Sequence(5′to3′)	Size(bp)
eseC	eseC-F	GCTGTACAGTAACTGTATC	160
	eseC-R	TCTGAAAGTCCGGTTCGTC	
fliC	fliC-F	AGACTGGCATGTCCGTGTCT	120
	fliC-R	GGCAACTTCAGCCAGACGAC	
fimA	fimA-F	GGCGAAAGGCGTGGGTAT	154
	fimA-R	CGTGTTGGCGTAAGAGCG	
pagC	pagC-F	CGCTGGACTGGTATTGGG	242
	pagC-R	CGTGATCCTGCGAGGTGA	
16s rRNA	16s-F	TAGGTCGCTTAGGACATCTC	150
	16s-R	AGGGCCGGCTTGGCGACCGT	
fliC-promoter	fliC′	CAGATGGCACCAAGCGTTGTTTCTCG	203
	fliC′	CTCTAGAAGCGACAGGCTGTTGGTATT	
eseC-promoter	eseC′	CAGATCTTGTCAGCGTCTGGAGGAGCA	422
	eseC′	CTCTAGAATCGCCTGGTACTGCGGCTTGG	
pagC-promoter	pagC′-F	CAGATCTAGCCAGCGTTATTGACCAGC	348
	pagC′-R	CTCTAGACTCAGCGCAGATAAGAAACA	

F: forward primer, R:reverse primer.

To construct pMPP, the 348bp *pag*C promoter was inserted into the pMP220 at the BglII and XbaI site. The fragments inserted from the pMPF, pMPE and pMPP were sent to Sunshine Biotechnology Company for sequencing (Sunshine Biotechnology, Nanjing China).

2.3. Bacterial electrotransformation

The pMPF, pMPE and pMPP were introduced into the wild and *eha* mutant strains by electrotransformation, performed separately as described previously [20]. Transformants were screened on oxytetracycline plates.

2.4. Cell culture

RAW264.7 macrophages were provided from Research Institute of Beijing Chuanglian North Carolina Biotechnology (Shanghai, China) and were cultured in Dulbecco's minimal essential medium (DMEM) containing 10% fetal calf serum (FCS) at 37 °C in an atmosphere containing 5% CO_2. All culture reagents were obtained from Gibco (Invitrogen, Shanghai, China).

2.5. Animals

BALB/c females, 6 weeks of age and weighing between 18 g and 22 g, were obtained from laboratory animal center, Yangzhou University. Mice were maintained in specific-pathogen-free-conditions and allowed unlimited food and water. All animal experiments were approved by the Chinese Ethics Committee for animal Experiments.

2.6. LD_{50} and the bacterial loads from the tissues infected

A murine model of *E. tarda* infection was used to examine the virulence of the wild strain and its *eha* mutant [4]. Two groups of BALB/c mice were intraperitoneally infected in 200 μl doses with successive 5-fold dilutions of the bacterial suspension (5×10^6, 2.5×10^7, 5×10^7, 2.5×10^8, 5×10^8, 2.5×10^9 and 5×10^9 cfu/ml) of the wild or the *eha* mutant (Table 2). The mice were monitored twice daily for 4-week. The 50% lethal doses (LD_{50}) values were determined with RM6240 systems software of treated biologic signal.

The bacterial loads of their organs were determined when mice injected intraperitoneally in 200 μl doses with 5×10^6 cfu/ml amounts of the wild or the eha mutant strains. At various times post infection (1, 3, 5, 7, 9 or 11 days), every group of mice were sacrificed and organs (kidney, spleen, and liver) were removed. The organs were prepared as tissue homogenates in 1 ml sterile PBS/organ. A series of dilutions of the homogenates were plated onto LB plates and incubated at 37 °C overnight. The bacterial colony forming units (CFUs) per organ were counted on LB plates.

2.7. Histopathology

Mice of two groups were infected intraperitoneally in 200 μl doses with 5×10^6 cfu/ml amounts of the wild or the eha mutant strains, respectively. The third group of mice was injected at the same route with sterile PBS as a control. On 1, 3, 5, 7, 9 or 11 days postinfection, the mice were sacrificed and the spleen, liver, and kidney were removed and weighted. The tissues were fixed 24 h in 10% neutral buffered formaldehyde, and replaced with the fresh buffers, and stored at 4 °C. The tissues were embedded in paraffin wax. Sections (5 μm thick) were cut on a rotary microtome, stretched in a water bath, mounted on glass slides, and stained with hematoxylin and eosin (H&E). An experienced pathologist blinded to the nature of the slides examined and performed qualitative

Download English Version:

https://daneshyari.com/en/article/3416259

Download Persian Version:

https://daneshyari.com/article/3416259

Daneshyari.com