International Journal of Medical Microbiology 307 (2017) 44–56

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

Strain-specific impact of the high-pathogenicity island on virulence in extra-intestinal pathogenic *Escherichia coli*

Mounira Smati[a,b,c,1], Giuseppe Magistro[d,1], Sandrine Adiba[e], Andreas Wieser[f], Bertrand Picard[a,b,c], Sören Schubert[f,2], Erick Denamur[a,g,h,*,2]

[a] *INSERM, IAME, UMR1137, Paris, France*
[b] *Univ Paris Nord, IAME, UMR1137, Sorbonne Paris Cité, Bobigny, France*
[c] *APHP, Hôpitaux Universitaires Paris Seine Saint-Denis, Site Avicenne, Bobigny, France*
[d] *Department of Urology, Ludwig-Maximilians-University of Munich, Munich, Germany*
[e] *Institut de Biologie de l'Ecole Normale Supérieure, ENS, CNRS UMR8197, INSERM U1024, Paris, France*
[f] *Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, München, Germany*
[g] *Univ Paris Diderot, IAME, UMR1137, Sorbonne Paris Cité, Paris, France*
[h] *APHP, Hôpitaux Universitaires Paris Nord Val de Seine Saint-Denis, Paris, France*

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form
16 November 2016
Accepted 20 November 2016

Keywords:
Escherichia coli
High pathogenicity island
Experimental virulence
Genetic background

ABSTRACT

In order to clarify the role of the high-pathogenicity island (HPI) in the experimental virulence of *Escherichia coli*, we constructed different deletion mutants of the entire HPI and of three individual genes (*irp2*, *fyuA* and *ybtA*), encoding for three main functions within the HPI. Those mutants were constructed for three phylogroup B2 strains (536-STc127, CFT073-STc73, and NU14-STc95), representative of the main B2 subgroups causing extra-intestinal infections. Transcriptional profiles obtained for the selected HPI genes *irp2*, *fyuA* and *ybtA* revealed similar patterns for all strains, both under selective iron-deplete conditions and in intracellular bacterial communities *in vitro*, with a high expression of *irp2*. Deletion of *irp2* and *ybtA* abrogated yersiniabactin production, whereas the *fyuA* knockout was only slightly impaired for siderophore synthesis. The experimental virulence of the strains was then tested in amoeba *Dictyostelium discoideum* and mouse septicaemia models. No effect of any HPI mutant was observed for the two more virulent strains 536 and CFT073. In contrast, the virulence of the less virulent NU14 strain was dramatically diminished by the complete deletion of the HPI and *irp2* gene whereas a lesser reduction in virulence was observed for the *fyuA* and *ybtA* deletion mutants. The two experimental virulence models gave similar results. It appears that the role of the HPI in experimental virulence is depending on the genetic background of the strains despite similar inter-strain transcriptional patterns of HPI genes, as well as of the functional class of the studied gene. Altogether, these data indicate that the intrinsic extra-intestinal virulence in the *E. coli* species is multigenic, with epistatic interactions between the genes.

1. Introduction

Iron acquisition is critical for the survival of pathogenic bacteria during infection. The high-pathogenicity island (HPI) is a 36- to 43-kb pathogenicity island (PAI), which encodes one of the major bacterial iron uptake systems (Garcia et al., 2011; Garenaux et al., 2011). The structure of the HPI has been extensively studied and several genes of interest have been described: an integrase gene (*int*), the genes *irp1*, *irp2* (iron repressible protein) encoding the siderophore yersiniabactin, the gene *fyuA* encoding the yersiniabactin receptor involved in iron uptake and the regulator gene *ybtA* (Buchrieser et al., 1998; Carniel et al., 1996). The HPI is widely distributed among the *Enterobacteriaceae* (Bach et al., 2000), particularly in *Yersinia*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Salmonella*, *Serratia* and *Escherichia coli*. It is highly conserved between species with homologies from 98 to 100% between the genes of *Yersinia* and *E. coli* (Dobrindt et al., 2002; Lesic and Carniel, 2005; Schubert et al., 1998). In *E. coli*, the HPI is more common in strains responsible for septicaemia (83%) and urinary tract infections (70%) and in enteroaggregative strains (92%) (Schubert et al., 1998). But it is also found in 27% of strains producing Shiga toxin (STEC) (Karch

* Corresponding author at: IAME, UMR1137, INSERM, Université Paris Diderot, Site Xavier Bichat, 16, rue Henri Huchard, 75018 Paris, France.
E-mail address: erick.denamur@inserm.fr (E. Denamur).
[1] These authors contributed equally to this work.
[2] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijmm.2016.11.004
1438-4221/

Table 1
List of the mutant and complemented strains of *E. coli* 536, CFT073 and NU14, as well as the control strains, with their main characteristics and origins.

Strain ID	Main characteristics	Origin
536	O6 : K15 : H31; pyelonephritis isolate	Berger et al. (1982)
536 ΔHPI	Deletion of the entire *hpi*; St^R	Diard et al. (2010)
536 *irp2*::Kn	Insertional *irp2* mutant; Kn^R	This study
536 *irp2*::Kn rec	Recomplemented mutant, pWKS30-P*irp2*; Kn^R Ap^R	This study
536 *fyuA*::Cm	Isogenic *fyuA* mutant; Cm^R	This study
536 *ybtA*::Kn	Isogenic *ybtA* mutant; Kn^R	This study
536 *ybtA*::Kn rec	Recomplemented mutant, pWKS30-*ybtA*; Kn^R Ap^R	This study
CFT073	O6:K2:H1; pyelonephritis isolate	Mobley et al. (1990)
CFT073 HPI::Cm	Deletion of the entire *hpi*; Cm^R	This study
CFT073 *ybtA*::Kn	Isogenic *ybtA* mutant; Kn^R	This study
NU14	O18 : K1 : H7 ; cystitis isolate	Hultgren et al. (1986)
NU14 HPI::Kn	Deletion of the entire *hpi*; Kn^R	This study
NU14 *irp2*::Kn	Insertional *irp2* mutant; Kn^R	This study
NU14 *irp2*::Cm	Isogenic *irp2* mutant; Cm^R	This study
NU14 Δ*irp2* rec	Recomplemented mutant, pWKS30-irp2; Ap^R	This study
NU14 *irp2*::Cm rec	Recomplemented mutant, pWKS30-P*irp2*; Cm^R Ap^R	This study
NU14 *fyuA*::Cm	Isogenic *fyuA* mutant; Cm^R	This study
NU14 *fyuA*::Cm rec	Recomplemented mutant, pACYC184-*fyuA*; Cm^R Tet^R	This study
NU14 *ybtA*::Kn 1	Isogenic *ybtA* mutant; Kn^R	This study
NU14 *ybtA*::Kn 2	Isogenic *ybtA* mutant; Kn^R	This study
NU14 *ybtA*::Kn rec 1	Recomplemented mutant, pWKS30-*ybtA*; Kn^R Ap^R	This study
NU14 *ybtA*::Kn rec 2	Recomplemented mutant, pCP1; Kn^R Cm^R	This study
DH5α	Laboratory K-12 *E. coli*	Hanahan (1983)
MG1655	Laboratory K-12 *E. coli*	Blattner et al. (1997)
REL 606	Laboratory *E. coli* B	Jeong et al. (2009)
WR1542	*S. enterica* serotype Typhimurium, pACYC5.3L	(Rabsch, Wernigerode)

*St: streptomycin, Kn: kanamycin, Cm: chloramphenicol, Ap: ampicillin, Tet: tetracyclin.

et al., 1999) and in 30% of isolates from faeces of healthy individuals (Bielaszewska et al., 2007).

The *E. coli* species is mainly clonal (Desjardins et al., 1995), with 7 main phylogenetic groups (A, B1, B2, C, D, E, and F) composed of numerous clones grouped in sequence type complexes (STc) or sub-groups (Clermont et al., 2013, 2015). The B2 group is the most diverse with at least 9 phylogenetic subgroups observed (Le Gall et al., 2007) and it is also the most frequently recovered group among the extra-intestinal pathogenic *E. coli* (ExPEC) strains responsible of human infections (Russo and Johnson, 2000). Interestingly, the HPI, which has spread within the *E. coli* species by homologous recombination, is not randomly distributed within the species but overrepresented within the B2 phylogroup (Schubert et al., 2009).

In *E. coli,* the studies on the role of the HPI in experimental virulence gave contrasting results according to strains and models. It appeared critical in some works. Thus, it has an important role during the bacteremic phase in a sepsis model in mouse (Schubert et al., 2002) and for preventing from grazing by the social haploid amoeba *Dictyostelium discoideum* (Adiba et al., 2010) for B2 phylogroup strains IAI51 and IAI52. These isolates belong to the subgroup IV (STc141) infrequently found in extra-intestinal pathologies (Clermont et al., 2014). Similarly, the HPI mutant of the CFT073 strain (B2 subgroup II, STc73) was outcompeted in the kidneys following transurethral competition with the wild type (Lloyd et al., 2009). In contrast, it has no impact in other works. No role has been reported in a neonatal meningitis model in newborn rat for B2 phylogroup strain C5 belonging to the subgroup IX (STc95) (Negre et al., 2004), in a mouse sepsis model (Tourret et al., 2010) and in a nematode (*Caenorhabditis elegans*) model (Diard et al., 2007) for the B2 phylogroup strain 536 (subgroup III, STc127). Lastly, discrepant results were obtained for the same strain 536 in two urinary tract infection (UTI) models: the competition between mutants and wild type in the classical murine model of ascending UTI (Garcia et al., 2011) and the monoinfection in infant mouse UTI followed by sepsis (Brzuszkiewicz et al., 2006).

This variable role of the HPI in experimental virulence could be explained by (i) the type of mutants analysed (deletions of various genes *versus* deletion of all the HPI), (ii) the utilisation in the inoculum of the models of monoinfection *versus* competition of mutant and wild type strains, (iii) the huge genetic diversity of the species in term of gene content, which could modify the effect of the mutations (Touchon et al., 2009) and (iv) the diversity of hosts and routes of inoculation in these experimental models of virulence, although a good correlation has been reported between the mouse, nematode and amoeba models (Adiba et al., 2010).

In order to disentangle these discrepancies in the role of the HPI in experimental virulence, we constructed different mutants of three individual genes (*irp2*, *fyuA* and *ybtA*) representative of the 3 principal functions of the HPI genes as well as of the entire HPI. The respective mutations were introduced in three B2 phylogroup strains representative of the major actual ExPEC subgroups (STc73, STc95 and STc127) of *E. coli*. Gene expression of the HPI under different conditions, as well as yersiniabactin production were analysed in the three prototypic UPEC strains and their respective HPI mutants. The virulence was then tested in two models of experimental virulence corresponding to two levels of integration: the resistance to phagocytosis by the amoeba *D. discoideum* model (Adiba et al., 2010) and a mammal model, the mouse septicaemia (Picard et al., 1999).

2. Materials and methods

2.1. Bacterial strains, media and in vitro growth conditions

Bacterial strains and plasmids used in the present work are listed in Table 1. Three uropathogenic *E. coli* (UPEC) strains of the phylogenetic group B2 were studied belonging to the subgroups II (STc73), III (STc127) and IX (STc95) (Clermont et al., 2015). The complete genome sequences of all three isolates are available. Strain 536 is an uropathogenic *E. coli* strain (O6:K15:H31), which belongs to the subgroup III. This strain was isolated from the urinary