Journal of Proteomics 131 (2016) 177–189

Contents lists available at ScienceDirect

Journal of Proteomics

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

Comparative proteomics of uropathogenic *Escherichia coli* during growth in human urine identify UCA-like (UCL) fimbriae as an adherence factor involved in biofilm formation and binding to uroepithelial cells

Daniël J. Wurpel [a], Makrina Totsika [b], Luke P. Allsopp [c], Richard I. Webb [d], Danilo G. Moriel [a], Mark A. Schembri [a,*]

[a] Australian Infectious Disease Research Centre, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia
[b] Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia
[c] MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, Imperial College London, London, United Kingdom
[d] Centre for Microscopy and Microanalysis, The University of Queensland, Brisbane, Australia

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form 23 October 2015
Accepted 2 November 2015
Available online 3 November 2015

Keywords:
F17-like
Chaperone-usher
Pili
Proteome
Outer membrane
Vesicles
UPEC
Adhesion

ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) are the primary cause of urinary tract infection (UTI) in humans. For the successful colonisation of the human urinary tract, UPEC employ a diverse collection of secreted or surface-exposed virulence factors including toxins, iron acquisition systems and adhesins. In this study, a comparative proteomic approach was utilised to define the UPEC pan and core surface proteome following growth in pooled human urine. Identified proteins were investigated for subcellular origin, prevalence and homology to characterised virulence factors. Fourteen core surface proteins were identified, as well as eleven iron uptake receptor proteins and four distinct fimbrial types, including type 1, P, F1C/S and a previously uncharacterised fimbrial type, designated *UCA-like* (UCL) fimbriae in this study. These pathogenicity island (PAI)-associated fimbriae are related to UCA fimbriae of *Proteus mirabilis*, associated with UPEC and exclusively found in members of the *E. coli* B2 and D phylogroup. We further demonstrated that UCL fimbriae promote significant biofilm formation on abiotic surfaces and mediate specific attachment to exfoliated human uroepithelial cells. Combined, this study has defined the surface proteomic profiles and core surface proteome of UPEC during growth in human urine and identified a new type of fimbriae that may contribute to UTI.

1. Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections affecting humans. These infections cause significant morbidity and mortality, with an estimated global incidence of approximately 150 million cases per year [1–3]. Symptomatic UTIs typically present as bladder infection (cystitis), but may also manifest as kidney infection (acute pyelonephritis) and lead to urosepsis. It is estimated that more than half of all women will experience at least one UTI episode in their lifetime and one in four will undergo a recurrent infection within six months [4]. Antibiotic therapy is the first choice of treatment and as a result UTIs represent the second predominant reason for antibiotic prescription worldwide. However, the rapid rise in the incidence of UTIs caused by multidrug resistant bacteria has highlighted an urgent need for the development of novel therapeutic strategies [5–7].

Uropathogenic *Escherichia coli* (UPEC) are the predominant etiological agents of UTI, accounting for more than 80% of community acquired and 50% of nosocomial infections [2,8]. Due to their role in pathogenesis, UPEC surface-exposed virulence factors represent attractive targets for the development of new diagnostic, drug and vaccine applications. As a result of the extensive genetic and phenotypic heterogeneity that exists between individual UPEC strains, however, the development of novel broadly protective therapeutic solutions to prevent UPEC-mediated UTI has been challenging [9]. Accordingly, a comparative analysis of the UPEC cell surface is paramount for the identification of ubiquitous surface antigens that can serve as common targets for treatment.

Genes encoding virulence factors are typically located on horizontally acquired mobile genomic elements called pathogenicity islands (PAIs) [10,11]. Although no single virulence gene is definitive for UPEC pathogenesis, a complementary array of factors including iron acquisition systems, toxins and adhesins facilitate bacterial colonisation and persistence within the human urinary tract. Adherence to uroepithelial cells is a critical initial step in uropathogenesis as it enables UPEC to resist the hydrodynamic forces of urine flow and promotes colonisation. In mice, UPEC adherence also leads to the invasion of superficial bladder

* Corresponding author at: School of Chemistry and Molecular Biosciences, Building 76, The University of Queensland, Brisbane QLD 4072, Australia.
E-mail address: m.schembri@uq.edu.au (M.A. Schembri).

http://dx.doi.org/10.1016/j.jprot.2015.11.001

epithelial cells and the subsequent formation of intracellular bacterial communities (IBCs) [12,13]. While UPEC possess a diverse array of adherence factors, fimbriae of the chaperone-usher (CU) secretion pathway such as type 1, P, F1C/S and AFA fimbriae represent the primary mediators for colonisation of the urinary tract [13–16]. Other factors that contribute to UPEC bladder colonisation include autotransporter adhesins and curli (for a review refer to [17]).

The CU pathway is a highly conserved secretion pathway in Gram-negative bacteria that facilitates the production of fimbriae. The biogenesis of CU fimbriae involves a dedicated periplasmic chaperone and an integral outer membrane (OM) usher protein, which functions as an assembly platform for the formation of the fimbrial heteropolymer [18,19]. The bulk of the fimbrial organelle consists of approximately 500 to 3000 major subunit monomers and typically contains a receptor-binding adhesin subunit at the distal tip [20]. The adhesin is composed of two distinct protein domains [18]; the C-terminal domain connects the adhesin to the main fimbrial shaft, sometimes aided by one or more minor subunits, while the N-terminal lectin domain mediates attachment to specific ligands, effectively determining the adhesive phenotype of the fimbriae [21]. The structural genes encoding CU fimbriae are almost invariably organised as polycistronic operons. Genomic analysis of the *E. coli* pan-genome has identified 38 distinct CU fimbrial types based on genomic locus position and usher phylogeny, and revealed that a single strain may contain up to 17 fimbrial operons [22].

Type 1 and P fimbriae play a key role in UPEC pathogenesis by mediating attachment to α-D-mannosylated proteins on the bladder epithelium and α-Gal(1–4)β-Gal receptor epitopes in the upper urinary tract, respectively [14,18,23]. Other CU fimbriae associated with UPEC include the AFA/Dr adhesins, involved in adherence to collagen IV in the interstitial compartments of the kidneys, and F1C/S fimbriae, which confer binding to GalNAcβ1-4Galβ glycolipids and sialyl galactoside glycoproteins, both present on epithelial cells lining the bladder and kidneys [15,24,25]. However, some UPEC strains are able to mediate attachment to uroepithelial cells in the absence of these well-characterised adhesins, indicating the existence of additional adherence factors involved in uropathogenesis [26].

In this study, we applied a method involving nanoscale liquid chromatography tandem mass spectrometry (nanoLC–MS/MS) of EDTA heat-induced outer membrane vesicles (OMVs) to characterise the surface proteome of five reference UPEC strains during *in vitro* growth in human urine. The analysis led to the identification of 173 unique proteins, which were characterised for subcellular origin, prevalence and homology to functionally characterised virulence factors. Of the predicted surface proteins identified, 14 were detected in all strains. Furthermore, we observed co-expression of up to nine distinct iron uptake systems in individual UPEC strains and four fimbrial types, including type 1, P, F1C/S and a previously uncharacterised fimbrial type, designated *UCA-like* (UCL) fimbriae in this work. We demonstrate that genes encoding UCL fimbriae are associated with UPEC strains and are phylogenetically related to UCA (Uroepithelial Cell Adhesin) fimbriae from *Proteus mirabilis* and F17/G fimbriae from *E. coli*. We also show that recombinant expression of these PAI-associated fimbriae mediates significant biofilm formation on abiotic surfaces and confer specific attachment to human exfoliated uroepithelial cells, suggesting a role in the colonisation of the human urinary tract.

2. Methods

2.1. Bacterial strains, plasmids and culture conditions

Strains and plasmids used in this study are listed in Table 1. Five reference UPEC strains whose genome sequence is available on the NCBI database were used: 536 [9], CFT073 [27], F11 [28], UMN026 [29] and UTI89 [30]. *E. coli* strains from a community acquired urosepsis collection [31] and from the *E. coli* Reference Collection (ECOR) [32] have been described previously. Strains were routinely cultured at 37 °C on solid or in liquid Lysogeny Broth (LB) medium [33] or liquid M9 glucose minimal medium (42 mM Na_2HPO_4, 22 mM KH_2PO_4, 9 mM NaCl, 18 mM NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$ and 0.2% (*w*/*v*) glucose). Where appropriate, media were supplemented with ampicillin (Amp, 100 μg ml^{-1}), kanamycin (Kan, 100 μg ml^{-1}) or chloramphenicol (Cam, 30 μg ml^{-1}). To induce expression of UCL fimbriae from plasmid pUCL, culture media were supplemented with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG).

Table 1
Strains and plasmids used in this study.

E. coli strain or plasmid	Relevant characteristics	Reference
Strain		
536	Wild-type UPEC reference strain	Brzuszkiewicz et al. [9]
CFT073	Wild-type UPEC reference strain	Welch et al. [10]
F11	Wild-type UPEC reference strain	Rasko et al. [28]
UMN026	Wild-type UPEC reference strain	Touchon et al. [29]
UTI89	Wild-type UPEC reference strain	Chen et al. [30]
MS428	*E. coli* K-12 MG1655 *fim*	Kjaergaard et al. [47]
MS428(pSU2718)	pSU2718 in MS428, Cam^r	This study
MS428(pUCL)	pUCL in MS428, Cam^r	This study
MS428(pSU2718,pCO13)	pSU2718 and pCO13 in MS428, GFP^+ Kan^r Cam^r	This study
MS428(pUCL,pCO13)	pUCL and pCO13 in MS428, GFP^+ Kan^r Cam^r	This study
OS56	K-12 MG1655 *flu*, *attB*::*bla-gfp*, GFP^+ Amp^r	Sherlock et al. [75]
OS56(pSU2718)	pSU2718 in OS56, GFP^+ Amp^r Cam^r	This study
OS56(pUCL)	pUCL in OS56, GFP^+ Amp^r Cam^r	This study
Plasmid		
pSU2718	Cloning vector, Cam^r	Martinez et al. [76]
pUCL	ECP_3785–3782 (UCL_{536}) in pSU2718, Cam^r	This study
pCO13	*gfp* (GFP_{pKEN2}) containing plasmid, Kan^r	Ong et al. [77]

2.2. DNA manipulation and genetic techniques

Plasmid DNA was isolated using the QIAprep Spin Miniprep kit (Qiagen). Chromosomal DNA was purified using the GenomicPrep cell and tissue DNA isolation kit (GE Healthcare). General PCR reactions were performed using *Taq* DNA polymerase according to the manufacturer's instructions (Roche). For molecular cloning purposes, DNA was amplified using Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific). Oligonucleotide primers used in this study were purchased from Sigma-Aldrich and are listed in Table S1. The UCL fimbriae expression plasmid pUCL was constructed by Phusion High Fidelity PCR amplification of the entire 5 kb UCL gene cluster (ECP_3785–3782) from UPEC strain 536, using primers 3383 and 3384 containing 5′ XbaI and SphI restriction sites, respectively. The PCR product was then digested with XbaI and SphI and directionally cloned into the corresponding sites of cloning vector pSU2718, where expression of the *ucl* fimbrial operon on pUCL is controlled by the IPTG-inducible *lac* promoter. PCR products and plasmids were sequenced using the BigDye Terminator v3.1 cycle DNA sequencing kit according to the manufacturer's instructions (Life Technologies) at the Australian Equine Genome Research Centre. Plasmid transformations were mediated by electroporation.

2.3. Preparation of EDTA heat-induced outer membrane vesicles for mass spectrometric analysis

Bacterial strains were grown at 37 °C with shaking (250 rpm) to an optical density at 600 nm of approximately 0.8 in 50 ml of pooled, filter sterilised mid-stream urine (collected from 4 healthy female