Investigation on uropathogenic *Escherichia coli* membrane proteins and their effects on phenotype and behaviour.

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Introduction

Uropathogenic *Escherichia coli* (UPEC) is a causative agent for over 80% of urinary tract infections (UTIs), including cystitis, pyelelonephritis and bacteraemia. It is a major burden on the healthcare system with over 150 million cases of UTI reported worldwide each year, and an estimated 4 billion pounds spent on care and treatment.[1] It is therefore important to investigate the mechanism of UPEC colonisation in order to be able to devise effective treatments against this pathogen.

NF-kB pathway

NF-kB protein is responsible for the activation of immune response, characterised by the secretion of cytokines and antimicrobial peptides towards pathogenic invasion (UPEC). (Figure 1)

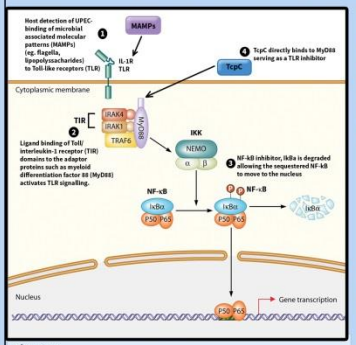


Figure 1
(1-3) describes NF-kB signalling pathway and how the binding of MAMP to TLR signalls MyD88 ligand binding to TIR causing the degradation of IkBa and the NF-kB activation. (4) describes how TcpC subverts the NF-kB pathway. Image adapted from Picard C et.al (2011).[2]

Some UPEC strains also possess **molecules to subvert the innate immune response, facilitating bacterial proliferation and invasion of the host urinary tract.** This is the focus of this research project.

TcpC

TcpC is a protein secreted by UPEC strain CFT073 which interferes with the NF-kB mechanism by directly binding to MyD88 serving as a TLR inhibitor (TIR homologous protein).[2] (Figure 1)

EnvZ and OmpR

In response to osmotic changes, EnvZ regulates the phosphorylation state of the transcription factor OmpR. This in turn controls the expression levels of outer membrane porin proteins OmpF and OmpC. (Figure 2)

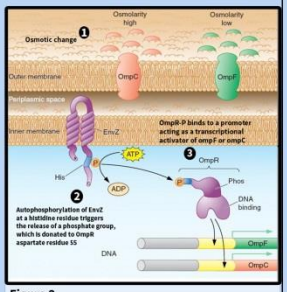


Figure 2
(1-3) describes the EnvZ-OmpR two-component regulatory system essential for responding to osmotic stress. Image adapted from Feng, X.H. et. al (2003).[3]

Aims

-To investigate the TcpC, EnvZ and OmpR dependent inhibition of the innate immune response (NF-kB pathway)
-To investigate the effects of TcpC, EnvZ and OmpR on UPEC phenotype

Methods

Construction of mutant strains in UPEC clinical isolate

4 mutant strains were constructed: (*tcpC, tcpC-envZ, envZ* and *ompR*). A protocol by Datsenko and Wanner (2000) exploits a recombinase transformed into UPEC followed by a transformation of a PCR product in order to inactivate the desired gene. Transformed colonies were selected using LB with kanamycin the colonies with the inserted cassette including kanamycin resistance. (Figure 3)

Visualisation of 3 mutant strain morphologies using microscopy

The 3 mutants (*tcpC, tcpC-envZ* and *envZ*) with controls were grown in a culture. A sample from each culture was then taken and fixed on a microscope slide to be viewed.

Isolation of TcpC protein

The gene encoding TcpC was induced with IPTG to produce recombinant TcpC protein. The culture solution was separated into non-induced versus induced, which was fractioned into secreted, whole lysate, soluble and insoluble to isolate the TcpC protein. SDS page gel electrophoresis was used to determine where the protein is located.

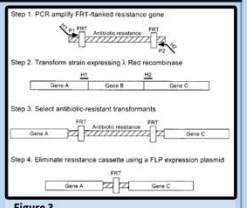


Figure 3
A method of gene inactivation proposed by Datsenko KA (2000). Using a red recombinase plasmid which includes 3 genes: γ (Gam), exo and β (Bet), Gam subverts RecBCD exonucleus of the host allowing Bet and exo to access the chromosome and allow the recombination of a PCR product.[4]

Results

Construction of mutant strains in UPEC clinical isolate

Among the 4 mutant strains, 3 (Δ*tcpC* mutant strain, Δ***tcpC***Δ*envZ* mutant strain and Δ*envZ* mutant strain) were successfuly constructed. These were verified by growing them on check plates (kanamycin LB plates)

Visualisation of 3 mutant strain morphologies using microscopy

Expected results were different to the observed results. It was expected that mutant *envZ* strain would result in a deformed UPEC phenotype as no mechanism would be available to the bacteria to regulate membrane pores as a result of osmolarity change. No phenotypic effect was expected from the knockout *tcpC* mutant strain. Visualisation of the 3 mutant strains using microscopy showed no obvious morphological difference to the control strains . This suggests that both *envZ* and *tcpC* do not affect the phenotype of UPEC. (Figure 4)

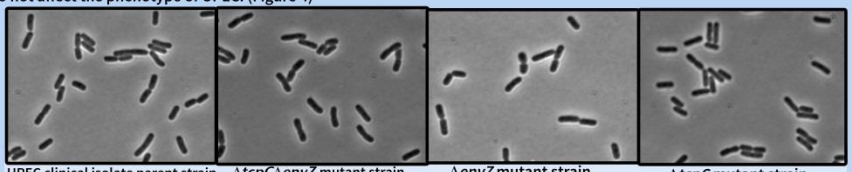
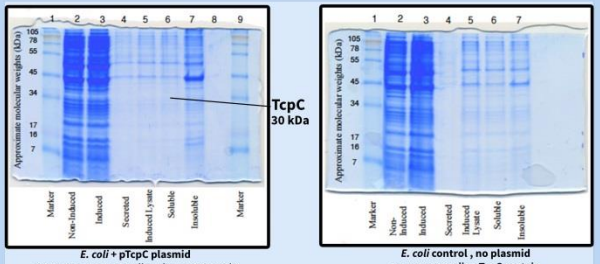


Figure 4
Images from the visualisation using microscopy of the control + mutant strains: UPEC clinical isolate strain, Δ*tcpC*Δ*envZ* mutant strain, Δ*tcpC* mutant strain and Δ*envZ* mutant strain.

Isolation of TcpC protein

From the SDS gel obtained, little or none of the TcpC protein was found. Several repeats were made in order to verify the result. (Figure 5)



***E. coli* + pTcpC plasmid**
possesses gene encoding the TcpC protein

***E. coli* control , no plasmid**
no gene encoding TcpC protein

Figure 5
SDS-page gels of the induced control UPEC strain and the model UPEC strain CFT073

Conclusion and further research

UPEC possesses molecules which may subvert the host innate immune response which can make the diagnosis and treatment of UTI difficult.
The *envZ* mutant did not show a strong morphological difference to the wild type suggesting that UPEC utilisation of EnvZ regulation does not follow a classic view (Figure 2). TcpC did not have any effect on bacterial phenotype and when attempting to isolate the TcpC protein, little or none was found. Further research may be done to upregulate the UPEC TcpC production, isolate this protein and to use these proteins to challenge epithelial cells for NF-kB response or inhibition. Comparison among clinical isolate alleles of TcpC for changes in NF-kB inhibition may also a possible avenue of research.

References

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[2] Picard et. al (2011) Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IkappaBalpha deficiency. Clin Microbiol Rev;24:490–7.
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[4] Datsenko and Wanner (2000). One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. 97:6640–6645.