An Observation of the Exoproducts in Media of Uropathogenic *E. coli*: Finding a Prospective Biological Mechanism for Uroepithelial Intracellular Colonization

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Abstract

In this study, a procedure was developed to search for proteins secreted by UPEC that could be involved with urinary tract infections. UPEC strain CFT073 was inoculated in urine and incubated at body temperature for a total of 48 hours to imitate natural conditions in the human body. The bacterial cells were removed through centrifugation, and possible secreted proteins were isolated, quantified and separated using various protein analysis kits and SDS-PAGE. The results of the CFT073 strain were compared to a laboratory strain, MG1655 (K-12) and sterile urine. The data shows that there were no recognizable secreted proteins present in the UPEC urine cultures. This could indicate that secreted proteins larger than 5 kiloDaltons (kDa) are not involved in UTIs or that uroepithelial cell tissue is required for UPEC to secrete proteins.

Introduction

Women are more than 50% likely to contract a urinary tract infection (UTI) at least once in their lifetime.[1] As antibiotic resistance increases, the current methods of UTI treatment using ciprofloxacin and other antibiotics are becoming outdated, leaving patients at risk for recurrent urinary tract infections. When a UTI is asymptomatic or not treated, the infection can move from the bladder to the kidneys via the ureters causing pyelonephritis. Chronic urinary tract infections are a risk factor for bladder cancer. A recent study, done by Kyle Richards from the University of Wisconsin-

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Figure 1. The image to the right depicts the electrophoresis of a precast gel loaded with samples. *Lane 1* - Urine Trial 2, *Lane 3* - CFT073 Trial 2, *Lane 5* - protein standard, *Lane 7* - CFT073 Trial 1, *Lane 9* - K-12 Trial 1, *Lane 11* - protein standard, *Lane 13* - K-12 Trial 2, *Lane 15* - Urine Trial 1

An Edvotek vertical gel apparatus was useb33(st)C0ET(5)7( )2a SDS-PAGE. A W33(s7( )2a)-3(k)6(i)-10(n)6(g)-5( )-122(L)9(o)-5(ab33(sin)7(g)-5( )-110(B33(su)-5((5)(5)er)-17( )-122(w)11(as)-9( )-110(

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gels were placed ins a stoaage solution hich consisteb336( )-171(o)-17(f)-3( )]TJETBT1 0 0 1 54 301.61 Tm[(0)-5(.)-2(0)-5(5)-5(% a)-2(

Figure 3. Average Total Extracellular Proteins in 48 hour cultures of CFT073 and K-12. The Standard Plot was determined with the Total Protein Assay Kit. The ñXò on the plot designates the average total protein found in sterile urine. This figure demonstrates that CFT073 does not always secrete proteins in urine. This does not include proteins under 5 kDa.

The SDS-PAGE experiments all yielded gels that looked similar to the gel shown in **Figure 4**.

Figure 4. Finished SDS-PAGE gel. The well contents are labeled just above the figure. T1 and T2 represent Trial 1 and Trial 2. The molecular weights in kDa of the Prestained SDS-PAGE Standards are to the right of the figure lined up to the corresponding band.

Discussion

Since both strains were inoculated in a similar fashion in the cell counting experiment, the

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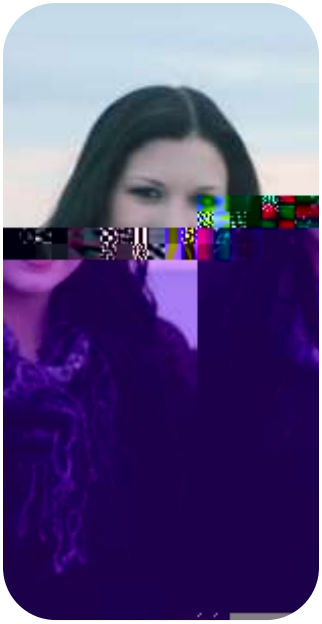

Brittany N. Carver is a senior double majoring in Forensic Science and Biology ï Pre Medical at UNH. She worked as a lab assistant for Anatomy and Physiology and currently for Microbiology.

Her interests are in epigenetics and environmental conservation, and she is interested in continuing on to medical school to eventually become a Forensic Pathologist or Medical Examiner.