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## Conjugative Plasmid Transfer in Biofilms of Pathogenic Escherichia coli

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## **RCEU21-BYS-TS-03**

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**Project Title** - Conjugative plasmid transfer in biofilms of pathogenic *Escherichia coli*

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**Proposal Identifier** - RCEU21-BYS-TS-03

**Project Description** - The rapid and global spread of drug resistance genes amongst bacteria is largely attributed to horizontal gene transfer through conjugative transfer of resistance plasmids. Increasing prevalence of multidrug resistant *Enterobacteriaceae* such as uropathogenic *Escherichia coli* (MDR UPEC) makes even uncomplicated urinary tract infections significantly harder if not impossible to treat. During the urinary tract infections, the UPEC bacteria invade bladder epithelial cells and form intracellular bacterial communities (IBCs) resembling *E. coli* biofilms. Formation of the IBCs is critical for infection progression and for initiation of recurrent infections. It is poorly understood though how transfer of resistance plasmids can occur in *E. coli* biofilms. In this project we will create a system to test for conjugation in the UPEC biofilms to answer this question.

In particular, several UPEC strains will be created to carry conjugative plasmids encoding for a fluorescent protein, expression of which is activated by conjugation. We next will grow biofilms from the obtained strains and observe the transfer of the plasmid by measurement of fluorescence, using confocal fluorescent microscope. Using this system, we will quantify plasmid transfer in biofilms grown from one or more *E. coli* strains and under different conditions. The transfer in biofilms will be compared with transfer in liquid cultures. As a result, we will establish influence of the biofilm environment on conjugative transfer of resistance plasmids by uropathogen *E. coli*.

**Student Duties, Tangible Contributions, and Specific Outcomes** - This project will require full

time presence in the lab for 32-40 hours per week, for 10-12 weeks. During this time the student will learn basic microbiological procedures and several functional assays. In particular, this project will entail preparing solutions and plates; working with uropathogenic *Escherichia coli*; grow biofilms; conduct conjugation assays; and image bacteria using confocal microscope. In addition to practical work, the researcher will read the peer-reviewed scientific articles that is required for understanding and developing of the project at hand. The student will also learn how to document the experimental work and keep laboratory notebook with the detailed description of the performed experiments. The student will be encouraged to prepare a poster for consecutive presentation at a scientific conference. At the end, the student researcher will be required to write a report with suggestions for future development of the project and analyses of the obtained results that can be used in a future publication. This report will be presented at a lab meeting to share the results and experiences with the whole group.

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**Student Selection Criteria** - The student (from freshman to senior) should have completed some basic microbiology courses at UAH or as an AP course. While a good academic standing is not a requirement, the applicant has to have a sincere interest in the topic of microbiology in general or in bacterial genetics or pathogenesis in particular.

**Project Mentorship** - Dr. Sysoeva will provide one-on-one meetings to introduce to the project, supervise literature review, discussions and analyses of the experimental results. In addition, weekly group meetings will be held to share the results and solve any ongoing issues. She will ensure close daily supervision and training in the laboratory setting for the hands-on work at the bench, including safety training and microbiological work. Dr. Sysoeva will guide the student researcher in writing up the report of the obtained results and preparing for the final presentation. In addition, four MS students and an experienced undergraduate researcher working on related projects will be conducting their research in the laboratory and assist with microbiological work, such as media preparation, autoclaving, inoculations. Two of the MS students have experience teaching microbiology. It is particularly important that one of the MS students has recently completed the summer RCEU project and thus is familiar with the program goals and requirements.

**Safety and Contingency Plan** - Student awarded will complete required general lab safety and biosafety trainings at the UAH OEHS website. This would include any COVID-19 related training as well. As part of our research group the RCEU student will follow the developed reduced density standard procedures that were approved by the COS earlier in 2020 to allow the re-start of the wet lab work in the summer of 2020.

If due to public health concerns the experimental wet lab work on the UAH campus would not be allowed, the next RCEU project will take place. We will proceed with bioinformatic analysis of the related homologs of conjugative proteins from diverse *E. coli* plasmids, sequences of which are available in the lab and in the online databases. In particular, the focus will be on the homologs of transcriptional factors, such as TraJ. We will construct multiple sequence alignment (MSA) of the proteins and identify proposed functional and structural residues, conserved and hypervariable regions. Using the established MSAs, we will test for correlations of the conserved residues to

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predict interacting proteins and their domains *in silico*. The outcome of this bioinformatic project will guide later experimental inquiries and result in teaching the RCEU student basics of protein sequence work, conservation and structure analyses.

