BCHM 421/422 **Bacterial Adhesins** **2024-25 Davies Lab**

Projects #1 & #2 : Bacteria use a variety of extracellular adhesin structures to bind to surfaces they colonize. RTX adhesins found in many Gram-negative bacteria are one such type formed from a particularly long (2 – 9,000 residues) polypeptide chain. At the distal end of RTX adhesins are a set of ligand-binding domains that enable the bacteria to occupy their niches and cause infections. In the example of *Vibrio cholerae*, the causative agent of cholera, the bacterium uses a glycan-binding domain to attach to human cells and a peptide-binding domain to anchor to the biofilm formed during colonization. These interactions can be blocked by specific sugars and peptides that compete for the ligand binding sites and could be used as reagents to counter bacterial infections. In this project we will be expanding our analyses of RTX adhesins to help control other bacteria that are human pathogens and agricultural pests.

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Project Title: Preventing bacterial infections by blocking adhesins.

Keywords (3-5):

1. DNA Cloning
2. Recombinant protein purification
3. Ligand binding analysis
4. Structural biology
5. Bacteria – cell interactions

Project Goals: To characterize one or more RTX adhesins in terms of their predicted protein structure; to identify their ligand-binding domains and ligands; and to develop inhibitors that can block binding of the bacteria to their targets.

Experimental Approaches: The structures of RTX adhesins can be reliably predicted using AlphaFold2. Ligand-binding domains at the C-terminal end of the adhesin will be identified based on structural and bioinformatic analyses. These domains will then be produced as recombinant proteins in *E. coli* and purified by affinity and other chromatographies. Their ligands will be identified using glycan and peptide arrays to enable blocking reagents to be designed. The effectiveness of these reagents will be studied by microscopy using fluorescently tagged ligand-binding domains in the presence of the cell types the bacteria colonize.

References:

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