Confirming glycoprotein visualization in *Ralstonia pickettii* via glycan modifying reagents

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The increasing ineffectiveness of existing antibiotics due to the evolution of microbial resistance has become a primary concern in the healthcare industry. Even when antibiotics are effective at treating a bacterial infection, these treatments are non-selective and tend to cause detrimental effects to one's microbiome. The World Health Organization has listed resistant bacteria as "High Priority Pathogens" that need alternative treatments due to their severe resistance to current antibiotics[1]. As well as in the High Priority Pathogens, resistance to antibiotics is increasing among many species of bacteria. For example, *Ralstonia*, a genus that contains a variety of species that range from human pathogens to plant pathogens, has developed innate resistance towards multiple antibiotics, thus supporting the concerns about future antibiotic resistance[2]. Therefore, the investigation of new therapeutic methodologies that targets specific bacterial populations is paramount.

A potential target for new treatments for bacterial infections are glycoproteins, the sugar-modified proteins on the surface of bacterial and human cells. These sugars are unique to the cells that they modify. Bacterial glycoproteins are intriguing targets because they are produced only by select bacteria. Their structures are markedly different from their eukaryotic counterparts and even vary between bacterial species. Previous work in the Dube lab illustrates the ability to target these sugar-modified proteins by employing metabolic oligosaccharide engineering (MOE). In essence, MOE metabolically incorporates an unnatural sugar inside the cell, and the newly formed glycan structure can later be used for the visualization and targeting of the bacteria. By implementing this technique in my senior thesis project, we saw that *Ralstonia pickettii* can be labeled with the peracetylated, azide-containing sugar *N*-azidoacetylglucosamine (Ac_4GlcNAz), a variant of the dominant sugar on the bacterium's surface, *N*-acetylglucosamine (GlcNAc). This azidosugar can then undergo a selective reaction which links the azide with a biochemical probe that can be detected. This approach works in various bacteria by using a different sugar to label the bacteria's dominant sugar on the bacterium's surface[3]. Using this technique, my thesis project took the initial steps to identify a protein glycosylation system present in *R. pickettii.* The ability of *R. pickettii* to process different sugars analogs into their glycans could reflect underlying differences in glycosylation machinery.

Due to the lack of current, well-characterized glycoproteins in *Ralstonia pickettii*, this summer we set out to further profile *R. pickettii's* glycoproteins. To characterize the nature of *R. pickettii*'s glycoproteins that were labeled with the azide sugar, we subjected the azide-labeled glycoproteins to treatment with glycan-modifying reagents. These glycan modifying reagents would begin to distinguish whether we are truly observing the metabolic incorporation of sugars on a glycoprotein and, furthermore, distinguish how these glycans are linked to the proteins, such as N-linked glycans, a motif commonly found in human cells, or O-linked glycans, a motif specific to bacteria. The enzymatic treatment used would remove the azide label that is incorporated into the glycan, thus allowing us to compare the incorporation of the azide label before and after treatment. After treating *R. pickettii* with a cocktail of enzymes, our results provided strong evidence that the observed metabolic labeling are indeed glycoproteins. To assess whether the azide label is present in O-linked glycans, azide-labeled *R.* pickettii was subjected to β-elimination with NaOH, a chemical process that leads to the removal of glycans that are O-linked, a motif commonly found in bacteria glycoproteins. Unfortunately, this characterization was inconclusive due to constant protein degradation from harsh chemical treatment. In future experiments, we will want to determine how the azidosugar is incorporated and specifically identify the glycoproteins. This work sets the stage for providing further insights into bacterial glycobiology and the roles of glycans in pathogenesis. Further understanding of these ideas can lead to the design of possible new therapeutics to combat the rise of antibiotic resistant bacteria.

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References

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