

Broadening the Scope of Fluorescent Sugar Probes to Track Bacterial Monosaccharide Uptake

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While modern medicine brought cures for previously untreatable bacterial diseases, these same ‘miracle drugs’ are contributing to a new crisis. Due to the overuse of broad-spectrum antibiotics, pathogenic bacteria have become resistant to modern antimicrobial drugs, presenting an imminent threat to public health. Promising targets for the next generation of antibiotics are glycans, complex polysaccharides that coat the surface of all living cells. Because bacterial glycans are involved in pathogenicity, therapeutics targeting glycan structure and function may act as highly specific, potent antimicrobial drugs.¹ To this end, the Dube Lab has pioneered the use of fluorescent monosaccharide probes, termed NBD-sugars, to study how pathogenic bacteria take up monosaccharides from their environment, which is the critical first step in glycan construction. Prior research by students in the Dube Lab has shown that these bacteria take up fluorescent analogs of rare monosaccharides in different ways, suggesting that NBD-sugar probes can be used to elucidate bacterial monosaccharide uptake mechanisms.^{2,3}

This summer, my research focused on expanding the number of bacterial species and monosaccharide probes to study sugar uptake mechanisms more thoroughly. To do this, live bacterial cells were incubated with novel NBD-sugars, then subjected to a subcellular fractionation procedure to isolate the bacterial cytoplasm and confirm probes were transported into the cell. The fluorescence of each fraction was measured using a fluorescent plate reader, indicating whether the bacteria took up the given NBD-sugar into each subcellular compartment.

Utilizing the previously untested probe NBD-Bacillosamine (NBD-Bac) and NBD-Glucose (NBD-Glc), which has been shown to be taken up by a variety of pathogenic bacteria,⁴ I observed increases in fluorescence in both the whole cell and cytoplasmic fractions for the pathogens *Helicobacter pylori* and *Bacteroides fragilis* (Figure 1). This indicated that these species took up NBD-Bac, demonstrating the ability for rare monosaccharides to be studied using their corresponding NBD-sugar analogs. Future work will focus on expanding the number of probes and bacterial species tested even further, as well as glucose-competition assays to determine whether NBD-Bac is taken up by glucose transporters in different bacterial species. These findings serve to ease the study of monosaccharide uptake systems, which could then be targeted by next-generation antibiotics.

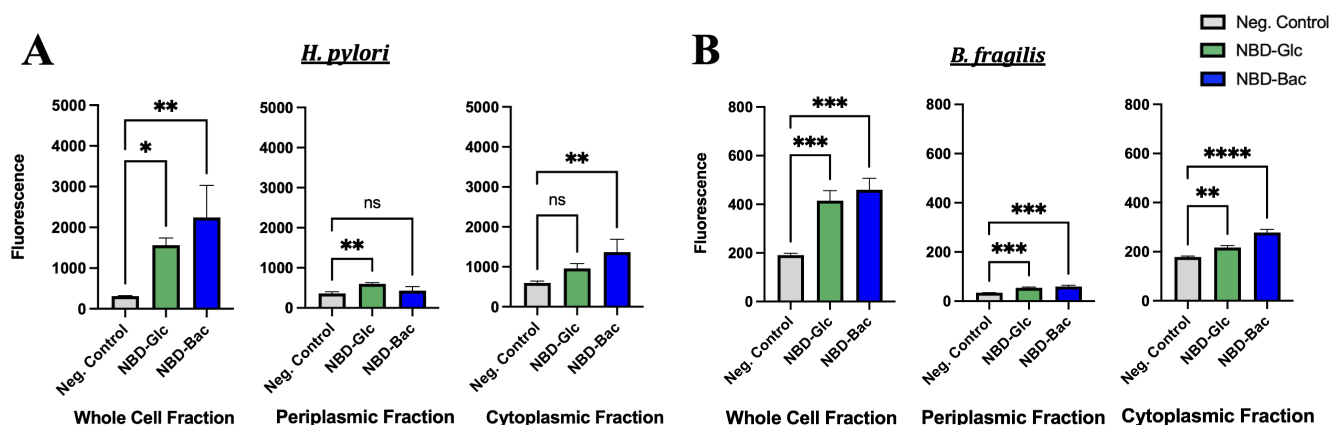


Figure 1. Fluorescence of subcellular fractions in *H. pylori* (A) and *B. fragilis* (B) incubated with NBD-Glc and NBD-Bac. Negative control fractions were isolated from bacteria not treated with NBD-sugars to give baseline fluorescence values.

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