Examining uptake of fluorescent sugar probes in bacteria

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Abstract

Pathogenic bacteria such as *H. pylori* are becoming more antibiotic resistant[1] as bacteria use an array of 700 rare monosaccharide building blocks[2]. These rare monosaccharides are essential for fitness and survival and build glycans, which are linked to bacterial pathogenicity. Indeed, the Dube lab has developed and is utilizing fluorescent sugar analogs termed NBD-sugars to probe, measure, and characterize selective monosaccharide uptake[3]. Preliminary data displays that bacterial cells become fluorescent after NBD-sugar incubation. However, cellular fluorescence is not direct evidence that the sugar analogs are taken up. To acquire more direct evidence of probe uptake, we performed subcellular fractionation. Fluorescence analysis of fractions revealed the presence of the fluorescent sugar analogs in cytoplasmic and periplasmic fractions of treated bacteria, suggesting successful probe uptake. An NAD-MDH assay revealed high levels of malate dehydrogenase (MDH) and NADH in whole cell and cytoplasmic fractions, and thus confirmed successful subcellular fractionation[4]. Taken together, these results reveal a successful use of subcellular fractionation as significant sugar uptake was located in cytoplasmic fractions of bacteria. Future work will characterize mechanism of sugar transport across bacteria species using these validated probes.

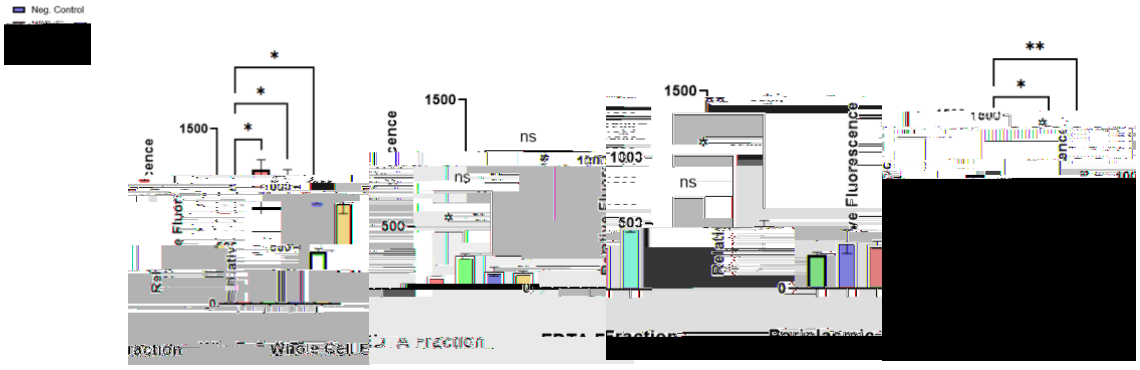
Methods:

- Subcellular fractionation of *B.fragilis* and *H. pylori*
- NAD-MDH Assay[6]

Bacterial cells from black, fluorescent 96-well plates were treated with corresponding NAD-MDH Assay kit agents to bring about electron coupling reagents, which showed bright orange in wells. Negative controls wells were used so that prior NBD fluorescence would not interfere with NAD-MDH fluorescence. Cells were transferred into clear, fluorescent 96-well plates (ThermoFisher) and fluorescence was measured using fluorescent plate reader (450 Elisa).

Results:

NBD-sugar analogs were observed mostly in cytoplasm of *H. pylori*



sugar analogs were observed mostly in cytoplasm of *B. fragilis*

NAD-MDH assay confirms success of subcellular fractionation

We tested the hypothesis that NBD-sugars are transported by a glucose transporter

Pilot glucose competition assay reveals no competition between glucose and non-glucose analogs in *H. pylori*, which leads to strong indication of a specific glucose transport protein

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References (if applicable)

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