Utilization of Lipid Anchor Analogue Probes to Study Bacterial Glycan Assembly

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Abstract

This study investigates the foundation for a streamlined polysaccharide purification protocol for vaccine production. The ability to use polysaccharides in vaccines to target pathogenic bacteria will aid in preserving the beneficial bacteria that live naturally within the human body. The current polysaccharide purification process utilizes time-consuming processes, resulting in expensive vaccines that are not fully accessible. To address this, fluorescently tagged probes to the lipid anchor that acts as the assembly platform for surface polysaccharides were introduced to bacteria for uptake into the membrane. Here, recombinant enzymes interacted with the probe to build the polysaccharide. This project utilized recombinant strains of C43 and genetically modified AM563 Escherichia coli and employed reverse phase high performance liquid chromatography to examine the in vivo uptake and modifications to the probe. The results showed that C43 E. coli treated with membrane permeabilizing agent, and genetically competent AM563 E. coli were not successful in the uptake of the lipid anchor probe. Specifically, the probe was not fully embedding into the inner membrane of the bacteria which would allow overexpressed enzymes to catalyze reactions. These findings indicate that more studies will be needed to determine under what conditions the bacteria will fully take up the probes. This work provides insight into how E. coli strains respond to lipid anchor probes when subject to varying conditions and set the groundwork for the optimization of this uptake so the probe with a glycan attached can be targeted for isolation.