TITLE: Cloning and initial characterization of a likely heme binding protein from Group B Streptococci

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Introduction: Group B streptococcus (GBS), also known as *S. agalactiae*, is a beta-hemolytic opportunistic pathogen that is part of the normal flora in the gastrointestinal tract. Like many other streptococcal pathogens, it requires iron from the host for growth and to establish virulence. Several heme binding and acquisition proteins have been identified that have provided significant insight into the heme metabolism in other *streptococcus spp*. A previous microarray analysis Group A streptococcus (GAS) showed up-regulation of specific genes in the presence of iron. Further bioinformatic analysis suggested a homologue in GBS. In addition, sequence analysis showed that the gene of interest lies near an iron uptake system. In the present study, we aim to successfully clone the gene in an inducible vector system for recombinant protein expression. In the future, this recombinant protein will be evaluated for its iron and heme binding abilities.

Methods: The gene for the putative heme-binding protein (orfX) was cloned from the GBS chromosome and inserted into an inducible pET101 plasmid vector, and transformed into Top 10 competent *E. coli* cells. The plasmids were extracted by mini-prep to be transformed into BL21 star *E. coli* for expression and purification.

Results: So far, gene specific primers have been designed that supported amplification of GBS DNA encoding the heme-binding protein by PCR. An agarose gel showed a concentration of 75 ng/μL of the purified PCR product. The PCR product was cloned into the pET101 vector and transformed into Top 10 chemically competent cells. The transformation was confirmed by PCR colony screening for the recombinant construct pDJD-01. The plasmid was extracted from a successful transformant with a 30 ng/μL concentration.

Conclusion: Cloning of the GBS open reading frame has led to the creation of a successful recombinant construct (pDJD-01). The newly constructed plasmid will be transformed into *E. coli* and expressed for biochemical and molecular analyses, to evaluate the heme binding capabilities of the protein.