

Abstract Title: Serendipitous Science: Discovery and Characterization of a possible *E. histolytica* Protein Involved in Regulating Virulence.

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Entamoeba histolytica is a food- and waterborne parasite that causes 50 million cases of amoebiasis annually. Despite such prevalence, little is known about the parasite's divergent genome. One-third of putative genes encode proteins that are unique to *Entamoeba*. Studying such hypothetical proteins may reveal novel drug targets. *E. histolytica* exhibits a 2-stage life cycle that includes the infectious amoeba and environmentally-stable cyst. Due to the difficulty of inducing cyst formation of *E. histolytica in vitro*, many laboratory studies are conducted with a related reptilian pathogen, *E. invadens*, which readily forms cysts *in vitro*. We previously characterized a hypothetical protein in *E. invadens* (EIN_059080) and found that it regulates encystation and phagocytosis. Since these are important virulence functions, the goal of this project is to characterize the *E. histolytica* homolog of this protein (EHI_056700). To isolate the gene, *E. histolytica* genomic DNA was purified and used as a PCR template with gene-specific primers that also added BglIII and XhoI restriction enzyme sites to the ends. The PCR-amplified gene was subcloned into a Trigger plasmid, which facilitates reduced expression of genes by RNA-interference (RNAi) and confers resistance to the antibiotic, G418. DNA sequencing was used to confirm the correctness of the gene in the Trigger plasmid. *E. histolytica* cells were transfected with the Trigger plasmid using the reagent, Lipofectamine 3000 (Thermo Fisher). Transfected cells were selected by adding G418 to the medium. Future studies will include confirming gene knockdown of expression by RT-PCR and characterizing virulence functions in transfected cells.

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