ABSTRACT

TITLE:	Proteomic Characterization and Cloning of Glucokinase and Phosphoenolpyruvate (PEP) Carboxykinase in Acidobacterium Capsulatum.
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PRESENTED AT:	2015 Undergraduate Research and Creative Activities Symposium, Fort Lewis College

ABSTRACT: Acidobacterium capsulatum is an environmental bacterium that is relatively abundant in soil and aquatic environments, prefers pH 3 for optimal growth, and encapsulates itself in a polysaccharide. Little is known about the biochemistry and physiology of this organism, but its genome has been completed and growth conditions have been found that support growth on glucose as a single carbon source. We are focusing on the metabolism of glucose and the proteins that are involved with the first and last steps of glycolysis. These two proteins were chosen because in similar organisms they are important in glucose metabolism. In these studies, PEP carboxykinase and glucokinase were characterized and cloned with the ultimate goal of expressing them and defining their activities. PEP carboxykinase catalyzes the conversion of oxaloacetate to phophoenolpyruvate and carbon dioxide within glycolysis and gluconeogenesis. In the 3 assays we found on PEPCK, they all described conditions for the assay at pH 8. The pI of PEP carboxykinase was found to be 6.09. Glucokinase converts glucose to glucose-6-phosphate in the first step of glycolysis. After searching NCBI for the appropriate assay, it was determined that for glucokinase a glucose-6-phosphate dehydrogenase-coupled reaction would work best at pH 7.5. The pI of glucokinase was found to be 5.48. Because A. capsulatum has optimal growth at pH 3, our hypothesis is that the cytoplasm of these organisms is at a somewhat lower pH than the normal physiological pH of 7.5, which is supported by the calculated pIs for these proteins. The genes that encode for PEP carboxykinase and glucokinase were determined using KEGG. Primers were designed using the primer design function on NCBI. Once the desired primers were created, the genes were amplified using PCR followed by a PCR cleanup to isolate our amplicon. The purified gene was inserted into the TOPO-TA plasmid using a directional TOPO cloning kit. To continue this study we will characterize the gene insert by sequencing, and if glucokinase and PEP carboxykinase are inserted correctly then they will have lengths of 1,000, and 1,500 bp, respectively.