ABSTRACT

TITLE:	Development of a Cloning Procedure for Triose Phosphate Isomerase, Phosphofructokinase, and Transaldolase Genes from Acidobacterium Capsulatum
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ABSTRACT: Acidobacterium capsulatum was discovered in 1991 as the first species of the phylum Acidobacteria. It is a bacterium found in soils with an uncanny ability to withstand extremely polluted and low pH environments. It is ubiquitous in soil and aquatic environments, suggesting that A. capsulatum serves an important role in these ecosystems. Interestingly the annotated genome of A. capsulatum suggests that the organism is missing aldolase, an enzyme known to play a crucial role in glycolysis. To better understand the way A. capsulatum utilizes glucose, we began the process of developing cloning and expression vectors for three key enzymes involved in glucose metabolism. These enzymes are triose phosphate isomerase (TIM), phosphofructokinase (PFK), and transaldolase (TA). These three enzymes were chosen because they are the most likely candidates for catalyzing alternate routes for glucose utilization by A. capsulatum. Phosphofructokinase is an enzyme normally involved in regulating standard glycolytic pathways. It phosphorylates fructose-6-phosphate to fructose-1,6-bisphosphate, which takes place immediately before aldolase splits fructose-1,6-bisphosphate into two three-carbon molecules. Triose phosphate isomerase interconverts between glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), the two three-carbon molecules produced by aldolase. Transaldolase catalyzes the aldol condensation of fructose-1,6-bisphosphate and erythrose-4-phosphate (E4P) into sedoheptulose-7-phosphate (S7P) and GAP. They were examined to see how their metabolic functions compared to those of well-understood organisms with fully functioning glycolytic pathways. To further study this pathway, cloning experiments were designed using a TOPO Cloning Kit. The genes that encompass these three enzymes were amplified using PCR. The amplification of these genes will lead to future cloning experiments that will help determine the role and regulation of these enzymes in A. capsulatum.