ABSTRACT

TITLE:	Characterization of Aldolase and Transaldolase Activities in Acidobacterium Capsulatum
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ABSTRACT: Acidobacterium capsulatum, a mildly acidophilic, chemotrophic, aerobe with the ability to grow on glucose, is found in diverse soil and aquatic conditions worldwide, suggesting a significant role in carbon and nutrient cycling in the environment. A. capsulatum has successfully been cultured and its genome has been completed, allowing for genome-directed studies of the organism's glucose metabolizing capabilities. Based on the genomic information, this organism was thought to lack aldolase, but contained two putative transaldolase genes (TA), one of which contains an EF-hand domain (TAEF). However, recent results show there are aldolase and transaldolase activities in cell-free lysate. We are continuing to characterize the aldolase and transaldolase activities in cell-free lysates to better understand how these enzymes play a role in partitioning of carbon from glucose between energy production and biomass formation. The pH dependence of the aldolase and transaldolase activities found to have a narrow optimal activity around pH 7.4 in the crude cell lysate. The two putative transaldolase genes have been PCR amplified, cloned into a pET151 directional TOPO expression vector, and transformed into E. coli. Sequencing of the TAEF and TA genes showed proper insertion into the expression vector. Current work is focusing on expression and purification of the TA and TAEF proteins. Genomic and structural features of the transaldolase genes and proteins are being characterized through bioinformatics tools such as Pymol, NCBI gene searches, and Cn3D. Structural homologs are being used to identify structurally similar features in the A. capsulatum transaldolase enzymes. Conserved active site residues have been identified using these same homologs as model transaldolases. These bioinformatics studies support the hypothesis that the A. capsulatum transaldolases are capable of catalyzing the accepted transaldolase mechanism. Future work will focus on identifying an aldolase gene and characterizing key regulatory points of glucose metabolism. The identification of aldolase will be done through bioinformatics tools as described above, comparing it to hypothetical proteins in the A. capsulatum genome.