ABSTRACT

TITLE:	Genomic Analysis of Excreted Enzymes in Acidobaterium Capsulatum
AUTHOR(S):	Hemerda, Carsyn; Askeland, Gracie; Wilbanks, Joseph; McKenzie, Jason; Sommerville, Les
PRESENTED AT:	2015 Undergraduate Research and Creative Activities Symposium, Fort Lewis College

ABSTRACT: Acidobacterium capsulatum (A. capsulatum) is a gram-negative, acidophilic, heterotrophic, rod-shaped, bacterium belonging to the phylum Acidobacteria, originally isolated out of acid drainage from the Yanahara pyrite mine in Japan. A. capsulatum is thought to play an important role in carbon and nutrient cycling in soil and aquatic environments. A. capsulatum has a genome size of approximately 4.1 Mbp, 88.3% of the genome codes for RNA or proteins, but only 68% of the protein genes encode for proteins of known function. Two genes encoding excreted cellulase enzymes, endo-1,4-D-glucanase and -glucosidase, were selected for further characterization, cloning and expression. These genes are located at nucleotide positions 109,786-110,952 and 22,187-24,517. BLAST searches were done on the nucleic and amino acid sequences to determine the organism with the highest homology to the gene and protein sequences. Structural homologs in the Protein Data Bank were used to identify conserved catalytic residues in the active sites of these enzymes. Primary sources were identified describing the enzyme assays that will be used to test for enzyme activity in cell-free lysates and to test the activity of the proteins after cloning and expression. Forward and reverse primers of these genes were designed such that the gene can be amplified and inserted directionally into a TOPO TA cloning and expression plasmid. Polymerase chain reaction (PCR) was optimized and utilized to amplify the genes and characterize the inserts in the plasmid. Agarose gel electrophoresis was used to determine the size and purity of the amplicons produced by PCR. The amplicons were quantified by absorbance at 260 nm and cleaned up using a PCR cleanup kit prior to cloning. Directionality of the inserts was confirmed by sequencing the insert using manufacturer's provided primers. These results suggest that these genes do encode the putative enzymes, but that they might have unique characteristics. Future work will include optimizing A. capsulatum growth conditions that can result in expression of these active enzymes.