ABSTRACT

TITLE:	Quantification and Purity Assessment of Acidobacterium Capsulatum Using Real-Time Polymerase Chain Reaction to Amplify the 16 S Ribosomal Gene
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ABSTRACT: The bacteria Acidobacterium capsulatum is a gram-negative acidophilic chemoorganotroph found in soils throughout the world. A. capsulatum is a member of the relatively new identified phylum of bacteria, Acidobacterium. Among the unique characteristics of this organism is the absence of the gene coding for the key glycolytic enzyme aldolase. Currently, colleagues within the department are underway examining how A. capsulatum is able to bypass the aldolase enzymatic process. To aid in the common endeavor this project's focus was to develop a method to assess the purity and quantification of experimental A. capsulatum samples through qPCR reaction. The 16 S ribosomal gene was selected for amplification because of its universal expression and virtual omnipresence in all bacteria. Differentiating A. capsulatum from all other bacteria was accomplished using two forward primers: 31F [GATCCTGGCTCAGAATC], specific for the A. capsulatum 16 S gene; the other, 27F [AGAGTTTGATCMTGGCTCAG], is capable of priming all bacterial 16 S genes. A reverse primer, 138AR, with the sequence [TTACTCACCCGTNCG] was explicitly designed to accommodate complementary priming of both forward primers. The resulting amplicons are 107 and 111 base pairs, respectively. To account for all other potential bacterial contaminants, Escherichia coli DNA was used as a general representative of the bacterial domain. Quantification of cells was determined through serial dilution standards of both A. capsulatum and E. coli after primer specificity had been confirmed. The protocol developed here will allow researchers working with A. capsulatum to quickly and confidently verify that their experimental samples are not tainted with unwanted bacteria, plus the relative concentration of sample cells. Additionally, this method may serve as a launching pad for future qPCR experimentation with A. capsulatum.