Isoforms of 3-Methylcrotonyl-CoA Carboxylase of Arabidopsis thaliana

Christina Trueba¹, Bryon Upton², Basil J. Nikolau²
¹Los Lunas High School, Los Lunas, NM 87031
²Center for Biorenewable Chemicals (CBiRC), Iowa State University, Ames, IA 50010

Introduction

3-Methylcrotonyl-CoA Carboxylase (MCCase) is an enzyme found in many organisms. Arabidopsis thaliana has two forms of MCCase. Roughly eighty percent of the time Arabidopsis has the long version of the gene. Twenty percent of the time it has the short version of the gene. The short version is missing the 6th exon (figure 1). This exon is 60 nucleotides in length and encodes for 20 amino acids which form an α-helix on the backside of the enzyme (yellow arrows, figure 2).

The original project was to optimize for soluble MCCA-L purified, and quantified. Points are collected and soluble proteins are extracted in inoculate large cultures which are induced with IPTG. Time colonies picked into 10ml LB cultures. These are used to transformation the cells are spread onto plates, single cells with a vector containing E. coli and MCCA-S. This is done by transforming BL21(DE3) and MCCA-S. This is done by transforming BL21(DE3)

The project had a rough start. The 6XHis tag was missing in the pDEST17 Gateway™ vector. 4 (green letters, figure 4) The next step is to reclone the mcca-l/s into pENTR™D-TOPO®. PCR is used to amplify the mcca genes. PCR products are then ran through an agarose gel, bands removed (blue boxes) and purified via a spin column (Qiagen ®) (Figure 5).

The third variable changed was the incubation periods increased varied ratios with the concentration of the samples (see figure 6). For the past four weeks I have been trying to reclone the mcca-l and mcca-s with no positive results. A new variable had been added to the TOPO reaction which allowed the enzyme to work. Protein analysis of homologous proteins

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Results and Discussion

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