Cloning and Expression of Cellulose Synthase (*CesA*) in *Pichia pastoris* for Analyzing Cellulose Production

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Abstract

Cellular production of polysaccharides from sugar molecules is critical in many metabolic processes to ensure survival of living organisms such as plants, bacteria and even humans. Thus, cell wall polysaccharide synthesis is particularly of interest for biomass and bioenergy applications.There are three main objectives to complete the focus of the project critical for the next step in further research. Firstly, sequence and ligation independent cloning (SLIC) is performed for fusing cellulose synthase *(CesA5 and CesA8* from Poplar plant) with fluorescence proteins (Skylan-S and moxMaple3) and is transformed into *E.cloni* competent cells. This construct is then cloned into a species of yeast named *Pichia pastoris* for expression. Lastly, the expressed protein is purified using affinity chromatography to get the proteins responsible for polysaccharide synthesis. In terms of further research, the next step is to image these proteins through a proposed multimodal single-molecule optical imaging instrument to study the key aspects of *in planta* polysaccharide synthesis and their turnover dynamics. The constructs developed here will act as model systems to study the *in planta* polysaccharide synthesis.

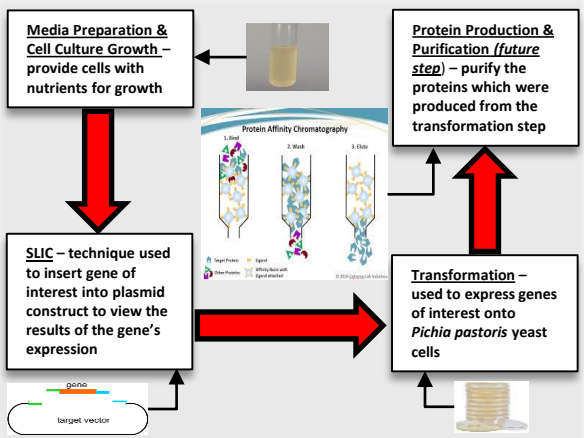
Background

-Cellular production of polysaccharides from sugar molecules is critical in many metabolic processes to ensure survival of every living organism such as us humans
-Cell wall polysaccharide synthesis is particularly of interest for biomass and bioenergy applications such as transportation, providing heat and energy and even charging one's phone
-However, general understanding of *in planta* cell wall synthesis is far from complete due to a lack of technology for *in vivo* plant cell imaging
-We will be examining cellulose, the most abundant natural polymer, and extracting the genes (*CesA5 & CesA8*) responsible for cellulose synthase production, which is an enzyme that produces cellulose.

Objectives

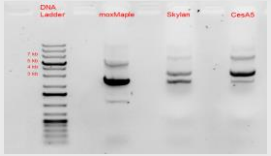
-Use (SLIC) to fuse cellulose synthase with fluorescence proteins (Skylan-S & moxMaple3) and then transform into competent cells
-Clone into vector (pPICZA) in order to transform into yeast for protein expression
-Collect and then purify these proteins using affinity chromatography

Methods

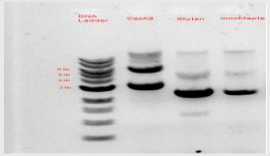


Results/Discussion

-*CesA* genes have been cloned successfully into *Pichia pastoris* and were confirmed through Sanger sequencing
-Optimized growth conditions were used to produce cellulose synthase enzymes
-The expressed enzymes were detected using a dot blot
-These results will be included in setting up the multimodal imaging platform designed for studying/analyzing cellulose synthesis

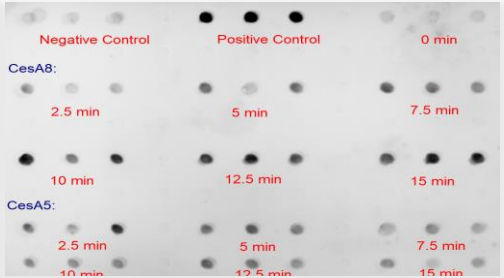


0.7% agarose gel of *CesA5*, Skylan-S & moxMaple3



0.7% agarose gel of *CesA8*, Skylan-S & moxMaple3

Results/Discussion Cont.



Dot blot results of CesA5 & CesA8 proteins which were collected every 2.5 minutes after sonication which lysed the cells

Conclusions & Future Directions

Moving forward, we plan to study and analyze other varying reasons for how cellulose is synthesized by creating this multimodal imaging platform. We can move on to analyze other genes involved that are responsible for this process. Moreover, we will study how all of this can be used in biomass and bioenergy applications, which is the end goal of this project.

Acknowledgements

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