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Molecular and Population Level Approaches to Understand Taxus Metabolism in Cell Suspension Cultures

Rohan Anil Patil, *University of Massachusetts - Amherst*

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Date of Award
2-2013

Document Type
Open Access Dissertation

Degree Name
Doctor of Philosophy (PhD)

Degree Program
Chemical Engineering

First Advisor
Susan C. Roberts

Second Advisor
Elsbeth L. Walker

Third Advisor
Neil S. Forbes

Keywords
Cellular Engineering, Gene expression, Paclitaxel, Plant Cell culture, Secondary metabolite

Subject Categories
Chemical Engineering

Abstract
Plant cell culture is an attractive platform technology for production and supply of important plant derived medicinals. A unique characteristic of plant cells is the ability to grow as multicellular aggregates in suspension. The presence of these non-uniform aggregates results in creation of distinct microenvironments, which can induce variations in cellular metabolism (e.g., growth, oxygen consumption and secondary metabolite synthesis). This heterogeneity can lead to unpredictable and suboptimal performance in large scale bioprocesses. One example is the Taxus cell culture system, which produces a widely used chemotherapeutic drug - paclitaxel (Taxol ®). Despite extensive process engineering efforts which have led to increased yields of paclitaxel, Taxus cells exhibit variability in productivity that is poorly understood. Elicitation of Taxus cultures with methyl jasmonate (MeJA) induces the accumulation of paclitaxel, but to varying extents in culture. A significant negative correlation was observed

between paclitaxel level and mean aggregate size of the culture, demonstrating the relevance of measuring, and potentially controlling aggregate size during long term subculture.

Understanding the regulation of gene expression can provide rational engineering strategies to control variability and optimize performance of Taxus cell cultures. Biosynthetic pathway gene analyses revealed upregulation of genes upon elicitation with MeJA; results also suggested additional molecular regulatory points outside of the biosynthetic pathway. In order to fully understand Taxus molecular regulation and the relationship to paclitaxel production variability, a transcriptome-wide analysis using next generation sequencing (454 and Illumina) methods was performed. Several pathways outside of paclitaxel biosynthesis were found active upon MeJA elicitation. Global comparison of gene expression amongst cultures accumulating different levels of paclitaxel is being performed to completely understand the interactions amongst the paclitaxel biosynthetic pathway and other complimentary and competing pathways to suggest effective targets for metabolic engineering. This work collectively represents the first molecular studies to understand metabolic regulation in Taxus cell cultures.

Apart from inducing paclitaxel biosynthesis, MeJA decreases cell growth in Taxus cell cultures. The MeJA-mediated repression of cell growth was shown to correlate with inhibition of cell cycle progression as evident both at the culture level through flow cytometric analyses and at the transcriptional level by repression of key cell cycle-associated genes. Results from this study provide valuable insight into the mechanisms governing MeJA perception and subsequent events leading to repression of Taxus cell growth.