Reducing Batch-to-Batch Variability of *Agrobacterium*-Mediated Transient Protein Expression In Plant Tissue Culture

Jeffrey S. Larsen and Wayne R. Curtis, Chemical Engineering, The Pennsylvania State University, 133 Fenske Laboratory, University Park, PA 16802

Batch-to-batch variations in protein expression levels pose an intractable and often unreported technical hurdle in the development of rapid plant-based heterologous protein expression platforms. Our group is developing protein expression in non-transgenic plant tissue culture which is grown and then co-cultured with *Agrobacterium tumefaciens* to rapidly deliver heterologous transfer DNA (T-DNA) to the nucleus of the plant cells (Curtis 2004). The *Agrobacterium* strain we utilize is an auxotroph that prevents overgrowth within the tissue culture system due to its inability to grow in the absence of a supplied amino acid (Collens, 2004). Since T-DNA transcripts can be rapidly cloned into binary vectors, it is possible to produce kilogram quantities of plant tissue transiently expressing the gene of interest in a matter of days. We have successfully demonstrated this technology at the 50-L pilot scale (O'Neill, 2008) with productivity superior to shake-flask controls. However, even after systematically improving reproducibility via rigor in culture maintenance and synchronization, the concentration of the reporter gene product β-glucuronidase has been observed to vary by orders of magnitude from one experiment to the next. Similar large variations have been observed in plant leaves infiltrated with *Agrobacterium*.

Plant tissue culture-based transient expression provides an ideal platform for controlling variability once the required environmental conditions are identified. We have explored numerous possible physical and physiological sources of batch-to-batch variation in high-level transient protein expression. Some of the important parameters that have been identified and studied as potential contributors to this variation include the organic and inorganic nutrient status of the culture, culture maintenance schedule, gas exchange in shake-flasks, incubator temperature control, and the timing of the nutrient-controlled 'synchronization by starvation' relative to the co-culture of the plant tissues with *A. tumefaciens*.

Achieving reproducible product titers is crucial for developing this protein expression technology for applications ranging from high-throughput screening to commercial-scale production of therapeutic proteins. For large-scale production, predictable and high-level productivity is needed to minimize capital investment as well as develop downstream processing and purification.

Looking forward, we will capitalize on the improved process reliability by utilizing newly developed T-DNA constructs that provide for higher levels of heterologous gene expression (Collens, 2007) and operational strategies that permit implementation of this technology with full gene containment, at a fraction of the cost of traditional pharmaceutical processing. We have developed and patented a low cost (plastic-lined) bioreactor and utilized feed-forward control to grow plant tissues to extremely high density (~40g dry weight / L) at greater than 100 L without the use of expensive pressure-rated vessels, probes or controllers (Curtis, 2004a; 2004b).

References

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