Model-Guided Metabolic Engineering of Increased 2-Phenylethanol Production in Plants

Joseph Lynch[1], Shaunak Ray*[2], **Clint Chapple[1]**, Natalia Dudareva[1], John A. Morgan[1,2]

[1]*Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907*

[2]*School of Chemical Engineering, Purdue University, West Lafayette, Indiana 47907*

Project Goals: We are testing a metabolic engineering strategy for the overproduction of 2-phenylethanol, a potential biofuel. Our approach is to first overexpress the enzymes catalyzing the multiple biosynthetic steps from phenylalanine to 2-phenylethanol in *Arabidopsis thaliana*. The strategy utilizes a single gene cassette to simultaneously express all proteins in near stoichiometric amounts under the control of a single promoter. The second part of the strategy is to increase the amount of the precursor, phenyalanine. Results from transgenic plants will be incorporated into kinetic models which will be used for identifying targets of future metabolic engineering strategies for optimized biofuel production.

2-Phenylethanol (2-PE) is a naturally occurring volatile organic compound with properties that make it a candidate oxygenate for petroleum-derived gasoline. However, its use for this purpose is limited by a lack of economically viable large scale production. 2-PE is produced naturally in some plant tissues via the sequential deamination/decarboxylation and reduction of phenylalnine. This pathway competes with the phenylpropanoid pathway for the common precursor phenylalanine. The phenylpropanoid pathway directs approximately 30% of carbon flux towards the biosynthesis of lignin, a major constituent of plant cell walls that impedes the process of cellulosic biofuel production. Therefore, we propose a genetic engineering strategy at the phenylalanine branch point, whereby a portion of the carbon flux towards lignin biosynthesis is diverted towards the production of an economically valuable product, 2-PE. Transgenic *Arabidopsis thaliana* were generated to introduce the pathway for production of 2-PE through overexpression of an aromatic aldehyde synthase (AAS) in tandem with phenylacetaldehyde reductase (PAR), which successfully increased production of 2-phenylethanol. However, as was previously reported for similar strategies, the *in planta* accumulation observed remains far lower than desired. To assess metabolic bottlenecks to further accumulation, excised 5-week-old stems and rosette leaves were exogenously fed different concentrations of $^{13}C_6$-ring labeled Phe. Both the amount and isotopic enrichment of downstream intermediates was quantified using LC-MS/MS at multiple time points after feeding. A kinetic model of the phenylpropanoid network was constructed, and the parameters were identified through non-linear optimization with training datasets, and validated with data from an independent experiment. *In silico* analysis of the results from our model predicted that the endogenous cytosolic Phe pools limit the 2-PE production in these transgenic plants. This prediction was tested by combining overexpression of PAR and PAAS with overexpression of a feedback-insensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase, the latter of which has been previously shown to have hyper-induced phenylalanine biosynthesis in Arabidopsis. Additionally, increased substrate availability was also tested in the *pal1*/*pal2* double mutant background combined with the overexpression of PAR and PAAS. These transformations led to a significantly increased accumulation of 2-PE in transgenic

Arabidopsis. The use of kinetic modeling combined with time-course *in vivo* metabolite profiling is shown to be a promising approach to rationally engineer plants that accumulate high-value commodity chemicals.

This research is supported by the Office of Biological and Environmental Research in the US Department of Energy.