34. Metabolic Engineering of Increased 2-Phenylethanol Production in Plants

Joseph H Lynch[1], John Morgan[2*] (jamorgan@purdue.edu), Rachel M McCoy[1], Peng Wang[1], Rohit Jaini[2], Clint Chapple[1], Natalia Dudareva[1]

[1]Department of Biochemistry, 2School of Chemical Engineering, Purdue University, West Lafayette, IN 47907

Project Goals: We seek to test a metabolic engineering strategy for the overproduction of 2-phenylethanol, a potential biofuel. Our approach is to 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 results will be incorporated into kinetic models which will be used for identifying targets of future metabolic engineering strategies for optimized biofuel production.

2-Phenylethanol is a naturally occurring organic volatile with a characteristic rose scent. Currently, 2-phenylethanol from both natural and artificial sources is utilized for flavoring and fragrance. Although the properties of 2-phenylethanol make it a potential biofuel which can be used as a substitute for petroleum-derived gasoline, there is currently no economical large scale production platform available. In the synthesis of 2-phenylethanol, phenylalanine is first deaminated and decarboxylated by a single enzyme, phenylacetaldehyde synthase, to form phenylacetaldehyde. Subsequent reduction by phenylacetaldehyde reductase forms 2- phenylethanol. We are engineering Arabidopsis as a model system for increased endogenous synthesis of this compound. We have generated gene cassettes to allow for simultaneous expression of combinations of the following genes: AAS, the Arabidopsis phenylacetaldehyde synthase; PAAS, the phenylacetaldehyde synthase from petunia that produces hydrogen peroxide as a byproduct; PAR1, the phenylacetaldehyde reductase from tomato; and KatE, an E. coli catalase which is only utilized in conjunction with PAAS. In each cassette, separate genes are linked via a synthetic 2A linker, which causes ribosome skipping, allowing expression of multiple distinct proteins from a single open reading frame without proteolytic cleavage. Gene cassettes were placed under control of either the CMV 35S promoter for constitutive overexpression, or the Arabidopsis C4H promoter to target expression specifically to lignifying cells. Arabidopsis plants from the T1 generation have been shown by quantitative real-time PCR to overexpress the genes. Analysis of these plants by GC-MS confirmed elevated levels of 2- phenylethanol. Homozygous progeny for the transgenic lines are currently being obtained by self-pollination. The homozygous lines will be used for precise determination of levels of phenylacetaldehyde, 2-phenylethanol, and glycosylated 2-phenylethanol derivatives in both leaves and stems, and comparison of the different cassettes will reveal the most effective gene combination. To analyze whether substrate availability is limiting, the lines with highest production will be crossed with Arabidopsis engineered for increased phenylalanine content, and the progeny will be analyzed to determine how this improves 2-phenylethanol production. The results will be used to refine our kinetic model currently being developed to describe flux toward lignin production.

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