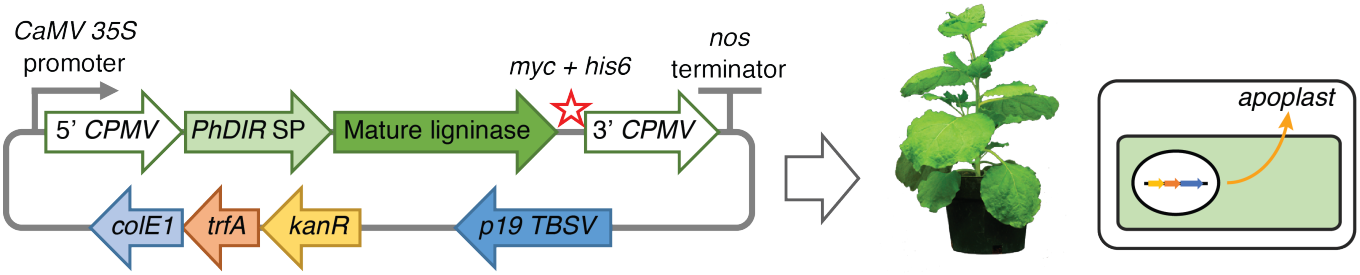
Docket #: S18-418

High-yield production of lignin-degrading enzymes for biocatalysis of renewable chemicals and biofuel

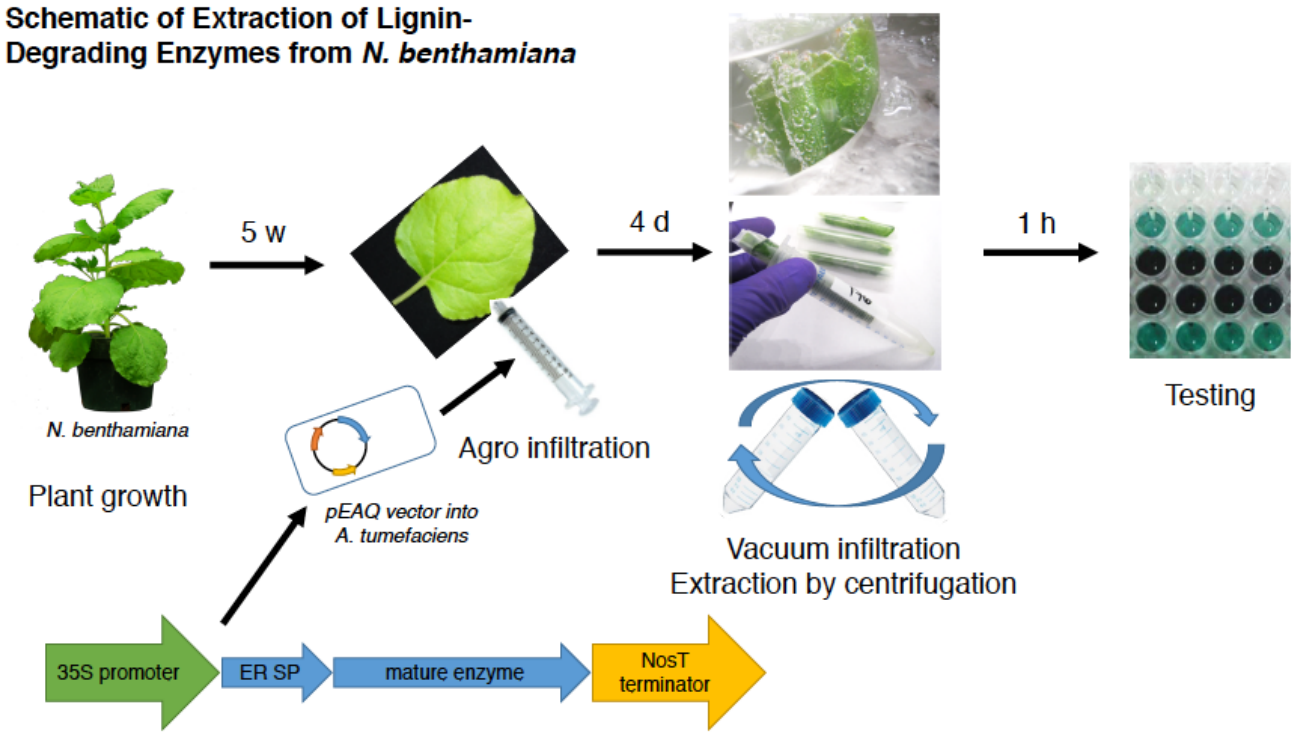
Researchers in Prof. Elizabeth Sattely's laboratory have developed a high-yield, scalable plant-based protein expression system to produce lignin-degrading enzymes for converting waste lignin into useful carbon-based platform chemicals. Lignin is the second most abundant biopolymer, but is underutilized as a renewable source of commodity chemicals because it is difficult to break down chemically. Certain fungal enzymes can be used as biocatalysts to degrade lignin, but traditional expression systems (e.g., in yeast) cannot produce sufficient quantities of those enzymes in a reliable, scalable manner.

This technology solves the enzyme-production problem by engineering tobacco plants (*N. benthamiana*) with an inducible Agrobacterium-mediated platform. These transgenic plants can produce significant quantities of a large panel of soluble ligninases. A crude leaf extract can then be used to degrade lignin in vitro. The basic platform can be expanded to produce other enzymes of interest, providing a robust, reliable, versatile system to produce biocatalysts. This system could capitalize on the massive lignin waste stream to produce biofuels and industrial chemicals.

Stage of Research
The inventors have demonstrated that multiple enzymes from major fungal lignin-modifying enzyme families can be produced at high titers using with this tobacco expression system. Half of all ligninases tested from 4 major families were successfully expressed. Furthermore, the inventors used the tobacco-produced enzymes to catalyze cleavage of a model lignin dimer in vitro.



Overview of heterologous production of lignin-degrading enzymes in N. benthamiana. Production of enzymes in N. benthamiana was driven by a 35S promoter on a pEAQ expression vector and exported to the plant apoplast via fusion to the signal peptide of dirigent protein from Sinopodophyllum hexandrum (PhDIR SP). The pEAQ vector includes 5' and 3' untranslated regions from cowpea mosaic virus (5' CPMV and 3' CPMV) to enhance expression levels. Hemaaglutinin (HA), c-myc (myc), and hexahistidine (his6) affinity tags were included as N- and/or C-terminal fusions where indicated.



Applications

- **Biocatalyst production** - plant-based production platform for lignin-modifying enzymes, with end-user applications in:
  - converting lignocellulosic biomass to valuable chemicals or biofuel
  - cellulose biorefineries - improve cellulose valorization through efficient lignin removal
- **Transgenic plants** - inducible agrobacterium expression system could potentially be utilized in host plants to deconstruct lignin in situ in an effort to improve their own biomass conversion
- **Research** - expression system for studying fungal enzymes

Advantages

- **High-yield** - expression in tobacco substantially improved production of fungal lignin-degrading enzymes compared to other hosts:
  - up to 3000-fold greater volume of extract than with yeast expression (*S. cerevisiae*)
  - particularly pronounced improvement for heme peroxidases
- **Robust and scalable:**
  - enzyme production can be increased by using a larger number of plants
  - reliable expression between batches
  - agrobacterium-mediated expression is inducible, allowing plants to mature and avoid side effects before ligninases are produced
- **Versatile** - enables production of a greater collection of isoforms than existing platforms:
  - about half of isoforms from 4 major families of ligninases tested to date could be produced with high yields
  - minimal optimization
  - can rapidly produce and test various members of the lignin family
- **Soluble and homogeneous enzymes:**
  - can be used directly from crude extract without in vitro refolding from inclusion bodies
  - single, well-defined glycosylation form (unlike heterogenous enzymes produced in yeast)

Publications

- N.A. Khystov, Y. Yoshikuni, S. Deutsch, E.S. Sattely A plant host enables the heterologous production and combinatorial study of fungal lignin-degrading enzymes *bioRxiv* posted October 7, 2019.

Patents

- Published Application: 20200095291
- Issued: 11,312,753 (USA)

Innovators

- Nikita Khlystov
- Elizabeth Sattely

Licensing Contact

Evan Elder

Senior Licensing Associate

Email