Towards New Degradable Lignin Types

Investigators
Internal

Wout Boerjan, Professor, Group Leader
Kris Morreel, Post-doctoral researcher
Joanna Cross, Post-doctoral researcher
Chiarina Darrah, Post-doctoral researcher
Oana Dima, PhD student
Ruben Vanholme, PhD student
Geert Goeminne, Engineer
Alain Goossens, Group Leader
Marnik Vuylsteke, Group Leader
Véronique Storme, Statistician
Roeland Merks, Group Leader
Address: VIB Department of Plant Systems Biology; UGent Department of Plant Biotechnology and Genetics, Technologiepark 927, 9052 Gent, Belgium

External

Claire Halpin (GCEP PI)
John Ralph (GCEP PI)

Abstract

Lignin is an aromatic heteropolymer abundantly present in secondarily-thickened plant cell walls. It is a major limiting factor in the conversion of lignocellulosic biomass to liquid biofuels. In dicots, the lignin polymer is built up by the combinatorial radical coupling of mainly coniferyl and sinapyl alcohol, although a range of minor units are also present in the polymer. Radical coupling results in a variety of chemical bonds, the frequency of which depends on the relative abundance of the various monomers, on the chemical characteristics of the monomers, and the local environment in the cell wall. For applications such as the conversion of lignocellulosic biomass to fermentable sugars in the process of bioethanol production, cell walls would ideally contain less lignin, and lignin rich in bonds that are easily cleaved.

The goal of the project is to identify natural products (called “target molecules”) that can be biosynthesized in energy crops, translocated through the plasma membrane and cross-coupled with lignin units such that the final lignin polymer is more susceptible to chemical cleavage, or is more hydrophilic, or is less cross-linked with hemicelluloses. Ideally, the structures of the target molecules are very similar to traditional monolignols so that they can be exported to the wall using the same transport system.

The project is divided into four tasks. Task 1 aims at defining which molecules are good targets for engineering and targeting to plant cell walls. We have made a comprehensive list of target molecules. We also have published a new mass-spectrometry based method to sequence small lignin polymers, a method that can be used to determine how new lignin monomer substitutes couple into the growing lignin polymer. In addition, we have published a mathematical model of lignin polymerization that shows which factors determine the chemical degradability of the lignin polymer. Task 2 aims at cloning biosynthetic genes for target molecules and overexpressing these in bioenergy crops. We have identified two Arabidopsis genes that, when knocked out, steer the flux through the phenylpropanoid pathway into the biosynthesis of ferulate-derivatives. This is interesting, because incorporation of ferulate into the wall gives rise to labile acetal bonds. NMR analysis, however, did not reveal any incorporation of ferulate in the lignin polymer, indicating that engineered re-routing these ferulate derivatives to the cell wall will be needed. One of these mutants has an increased incorporation of *p*-coumaryl alcohol units (H-units) in the cell wall, and has improved saccharification potential. Task 3 aims at rerouting a selected number of target molecules, of which the biosynthetic pathway and subcellular localization are already partially known, to the cell wall in transgenic plants. Because several of the proposed target molecules are thought to be located in the vacuole, we have identified putative transporters that are involved in translocation/retention of several target molecules into the vacuole. Arabidopsis knock-out mutants in these putative transporters genes are currently being analyzed by vacuolar metabolomics. This technique, developed for the first time, has also provided insight into the subcellular localization of particular classes of monolignol-derived compounds in vacuoles. Task 4 aims at identifying biosynthetic pathways for promising target molecules by a combination of genetics and metabolomics. To this end, we have metabolically profiled 250 natural Arabidopsis accessions and identified several of our target molecules in this model system. We have used association genetics to identify SNPs in genes that influence the abundance of compounds, and that may be involved in their biosynthesis.

References

1. Vanholme, R., Van Acker, R., and Boerjan, W., Potential of Arabidopsis systems biology to advance the biofuel field. *Trends in biotechnology* **28**, 543-7, 2010.
2. van Parijs, F.R., Morreel, K., Ralph, J., Boerjan, W. and Merks, R.M. Modeling lignin polymerization. I. Simulation model of dehydrogenation polymers. *Plant Physiol.* **153**, 1332-1344, 2010.
3. Morreel, K., Dima, O., Kim, H., Lu, F., Niculaes, C., Vanholme, R., Dauwe, R., Goeminne, G., Inzé, D., Messens, E., Ralph, J. and Boerjan, W. Mass spectrometry-based sequencing of lignin oligomers. *Plant Physiol.* **153**, 1464-1478, 2010.
4. Vanholme, R., Ralph, J., Akiyama, T., Pazo, JR., Kim, H., Christensen, JH., Van Reusel, B., Storme, V., De Reycke, R., Rohde, A., Morreel K., BoerjanW (2010). Engineering traditional monolignols out of lignin by concomitant up-regulation of F5H1 and down-regulation of COMT in Arabidopsis. *Plant Journal* **64**(6):885-97.
5. Vanholme, R., Demedts, B., Morreel, K., Ralph, J. and Boerjan W. (2010) Lignin biosynthesis and structure. *Plant Physiol.* **153**, 895-905.
6. Morreel, K., Kim, H., Lu, F., Dima, O., Akiyama, T., Vanholme, R., Niculaes, C., Goeminne, G., Inzé, D., Messens, E., Ralph, J. and Boerjan, W. Mass spectrometry-based fragmentation as an identification tool in lignomics. *Anal. Chem.* **82**, 8095-8105, 2010.

Contacts
Wout Boerjan: woboe@psb.vib-ugent.be
Kris Morreel: krmor@psb.vib-ugent.be
Chiarina Darrah: chdar@psb.vib-ugent.be
Joanna Cross: jocro@psb.vib-ugent.be
Oana Dima: oadim@psb.vib-ugent.be
Ruben Vanholme: ruhol@psb.vib-ugent.be
Geert Goeminne: gegoe@psb.vib-ugent.be
Alain Goossens: algoo@psb-vib-ugent.be
Marnik Vuylsteke: mavuy@psb.vib-ugent.be
Véronique Storme: vesto@psb.vib-ugent.be
Roeland Merks: roeland.merks@cwi.nl

External
Claire Halpin (GCEP PI): c.halpin@dundee.ac.uk
John Ralph (GCEP PI): jralph@wisc.edu