Monolignol Ferulate Transferase Introduces Chemically Labile Linkages into the Lignin Backbone

**Producing Plants Designed for Deconstruction**


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Literature Seminar

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Lignin degradation is a major issue in industrial processing of biomass
Structure of lignin

- Earth’s second most abundant polymer after cellulose
- 15-40% dry weight in plants
- Found in secondary cell walls of plants
- Provides structural integrity

http://bioenergy.ccrc.uga.edu/Background/background.htm


- Aryl ether bonds are resistant to chemical degradation
- Harsh conditions for depolymerization: 170 °C in 1M NaOH or 200 °C in acid
Lignin biosynthesis

Monolignol biosynthesis → Transport to cell wall → Oxidation → Polymerization

- p-coumaryl alcohol (hydroxyphenyl) "1G"
- coniferyl alcohol (guaiacyl) "1G"
- sinapyl alcohol (syringyl) "1S"

Glycosylated monolignols:

- GlcO
- MeO
- OMe

Oxidation and polymerization reactions:

- β-O-4
- β-β
- β-5
Redesigning lignin for easier depolymerization

1. Monolignol (coniferyl or sinapyl alcohol)
2. Monolignol-Ferulate Conjugate
3. The final Lignin Polymer (with 'zips')
4. Lignin Oligomers (Highly cleaved Polymer)

Steps:
- a) FMT (Transferase)
- b) Monolignols Peroxidase
- c) Continued Lignification
- d) Pretreatment
Expressing the gene in lignifying tissues

A. Transgenic hybrid poplar

B. Non-transgenic control

C. 35S::FMT
Universal FMT expression

D. CesA8::FMT
Expression in xylem only
Determining incorporation of monolignol ferulate conjugates

Derivatization followed by reductive cleavage (DFRC)
Multiple reaction-monitoring (MRM)

MS/MS Operating Mode

- Ionize all peptides
- Mass-select peptide ion
- Fragment peptide ion
- Detect all fragment ions

MRM-MS

- Ionize all peptides
- Mass-select peptide ion
- Fragment peptide ion
- Monitor 3 fragment ions

https://www.broadinstitute.org/scientific-community/science/platforms/proteomics/mrm-multiple-reaction-monitoring
Determining incorporation of monolignol ferulate conjugates

A

DFRC

Derivatization
Followed by
Reductive
Cleavage

8g (CA-FA): 442
8s (SA-FA): 472

Coniferyl dihydroferulate diacetate
(8g, CA-FA)

Sinapyl dihydroferulate diacetate
(8s, SA-FA)

m/z

100 150 200 250 300 350 400 450

103 119 133 137 153 179 193 207 281 358

105 133 149 153 179 207 210 281 341 388

m/z

100 150 200 250 300 350 400 450

B

MRM MS-MS

CA-FA

400→195
trans: 57, 57 (50)
cis: 55, - (50)

400→163
trans: 112, 112 (107)
cis: 111, - (107)

400→131
trans: 100, 100 (100)
cis: 100, - (100)

SA-FA

430→193
trans: 104, 0 (103)
cis: 109, 0 (103)

430→161
trans: 100, 0 (100)
cis: 100, 0 (100)

430→133
trans: 14, 0 (13)
cis: 11, 0 (13)

CesA8::FMT-6
WT

CesA8::FMT-6
WT

CesA8::FMT-6
WT
Determining incorporation of conjugates into lignin backbone

- Use deuterium labelled AcBr to differentiate between free and conjugated phenols
- Use MS to identify per-deuterated species

Free phenols on both ends = conjugate not in lignin backbone
Determining incorporation of conjugates into lignin backbone
Determining incorporation of conjugates into lignin backbone

- 53% of conjugates had deuterium labels
- Conjugates can be incorporated in lignin backbone as well as conventional monolignol
Improvement in saccharification in transgenic plants

- Transgenic plants were digested with 6.25 mM NaOH, 90 °C, 3 h
Conclusion

• Engineering lignin with a more labile backbone presents a solution to facilitating biomass processing

• Preliminary results in the paper shows that glucose yields can be improved

• Other active areas of research: chemical depolymerization to yield high quality lignin products