Metabolic effects and toxicity mechanisms of lignocellulose-derived inhibitors

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Project Goals: The mission of the Great Lakes Bioenergy Research Center is to perform the basic research that generates technology to convert cellulosic biomass to ethanol and other advanced biofuels. In alignment with this goal, we have made use of time-resolved, quantitative metabolic flux analyses (MFA) to gain insight into the mechanisms by which lignotoxins disrupt metabolism and inhibit sugar (i.e. xylose) conversion to biofuels. Understanding the mechanisms behind these deleterious effects is of great value for devising metabolic engineering strategies to overcome them.

An outstanding challenge toward efficient production of biofuels and value-added chemicals from plant biomass is the impact that lignocellulose-derived inhibitors have on microbial fermentations. Using *E. coli* and *Z. mobilis* as model systems, we investigated the metabolic effects and toxicity mechanisms of feruloyl amide and coumaroyl amide, the predominant phenolic compounds in ammonia-pretreated biomass hydrolysates.

Using metabolomics, isotope tracers, and biochemical assays, we discovered that these two phenolic amides act as potent and fast-acting inhibitors of purine and pyrimidine biosynthetic pathways in *E. coli* (1). Feruloyl or coumaroyl amide exposure leads to (i) a rapid buildup of 5-phosphoribosyl-1-pyrophosphate (PRPP), a key precursor in nucleotide biosynthesis, (ii) a rapid decrease in the levels of pyrimidine biosynthetic intermediates, and (iii) a long-term generalized decrease in nucleotide and deoxynucleotide levels. Tracer experiments using 13C-labeled sugars and 15N-ammonia demonstrated that carbon and nitrogen fluxes into nucleotides and deoxynucleotides are inhibited by these phenolic amides. We found that these effects are mediated via direct inhibition of glutamine amidotransferases that participate in nucleotide biosynthetic pathways. In particular, feruloyl amide is a competitive inhibitor of glutamine PRPP amidotransferase (PurF), which catalyzes the first committed step in de novo purine biosynthesis.

Similarly to *E. coli*, exposure to feruloyl amide in *Z. mobilis* results in a large accumulation of the biosynthetic intermediate PRPP, suggesting that this lignotoxin also affects nucleotide biosynthetic pathways in this biofuel producer. However, while PRPP accumulation was accompanied by decreased nucleotide levels in *E. coli,* nucleotide and deoxynucleotide levels were actually elevated in *Z. mobilis* after feruloyl amide-treatment. To examine whether this increase in nucleotide levels was due to active biosynthesis or to DNA/RNA degradation, we performed isotopic tracer experiments using 13C-labeled glucose. We found that feruloyl amide did not block *Z. mobilis* nucleotide biosynthesis: 13C-carbons were incorporated into ATP, GTP, UTP, and CTP in the feruroyl amide-treated cells similarly to control cells. It is currently unclear how *Z.*

mobilis recovers from PRPP accumulation and the decrease in levels of dihydroorotate and orotate, but these unexpected observations point to the possibility that *Z. mobilis* may have additional nucleotide biosynthetic routes or mechanisms to overcome inhibition of glutamine amidotransferases by feruloyl amide. We are currently investigating these questions.

References

1. **Pisithkul T**, **Jacobson TB**, **O'Brien TJ**, **Stevenson DM**, **Amador-Noguez D**. 2015. Phenolic Amides Are Potent Inhibitors of *De Novo* Nucleotide Biosynthesis. Appl. Environ. Microbiol. **81**:5761–5772.

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