**Phenolic amides are potent inhibitors of *de novo* nucleotide biosynthesis**

Lignocellulose-derived hydrolysates contain several different inhibitors (collectively called lignotoxins or LTs) that arise during pre-treatment of biomass. Determining the mechanisms by which yeast or bacteria are adversely affected by LTs is a key step toward improving the efficiency of fermentation and bioconversion. Prior work has established that LTs present in ammonia pretreated corn stover hydrolysates inhibit growth and sugar utilization in *Escherichia coli*. Researchers at GLBRC have now keyed in on two of the LTs, feruloyl amide (FA) and coumaroyl amide (CA), which are able to recapitulate the inhibitory effects of LTs. Analysis of the metabolome in untreated vs. treated cells indicated that the phenolic amides cause rapid accumulation of 5-phosphoribosyl-1-pyrophosphate (PRPP), a key precursor in nucleotide biosynthesis. Moreover, isotopic tracer studies using 13C-sugars and 15N-ammonia confirmed that carbon and nitrogen flux into nucleotides is inhibited by the amides, suggesting that they act as inhibitors of purine and pyrimidine biosynthetic pathways. Biochemical studies showed that the amides directly inhibit glutamine amidotransferases, with FA acting as a competitive inhibitor of the *E. coli* enzyme PurF, which catalyzes the first committed step in *de novo* purine biosynthesis. Supplementation of cultures with nucleosides was sufficient to reverse the effect of the amides, suggesting the ability to bypass the block in *de novo* nucleotide biosynthesis via salvage pathways. Collectively these results provide a direct mechanism for the inhibitory effects of phenolic amides, knowledge that will inform future design of biocatalysts for improved bioconversion.

**References:** Pisithkul, T., Jacobson, T.B., O'Brien, T.J., Stevenson, D.M., Amador-Noguez, D., 2015. Phenolic amides are potent inhibitors of *de novo* nucleotide biosynthesis. Applied and Environmental Microbiology 10.1128/AEM.01324-15.

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