Characterizing uptake of lignin breakdwon products into P. chrysosporium and S. cerevisiae

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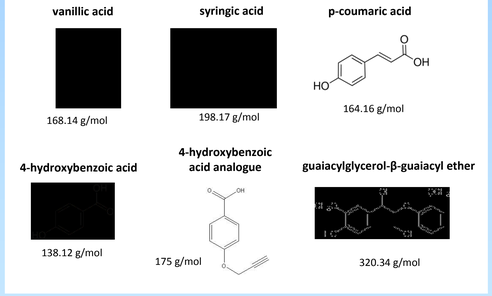
Abstract: Valorization of lignin has the potential to significantly improve the economics of lignocellulosic biorefineries. However, efficient conversion of lignin to useful molecular building blocks has been elusive. Microbial conversion of lignin in nature is efficient, but occurs very slowly. Engineering microbes to produce more efficient and more directed lignin conversion is a promising strategy.[1] One aspect of that engineering effort is to optimize transport. However, very little is currently known about lignin transport into microbes. Indirect evidence such as growth and toxicity studies suggests lignolytic organisms may transport a wide range of mono-, di-, and possibly even higher molecular weight lignin breakdown products across the cellular membrane,[2-6] but direct measurements of the substrate range and specificity are lacking except for a few select studies.[7] To that end we are characterizing the profile of lignin-like substrates internalized by *P. chrysosporium* (*P. chrys*) and *S. cerevisiae* (*S. cer*) using mass spectrometry. *P. chrys.* is a white rot fungus, and a known lignin degrader. *S. cer* can be genetically manipulated to study eukaryotic transporters. We describe the method and report initial results for a range of mono- and di-aryl compounds.

Compounds tested to date:



Methods:

***P. Chrysosporium* (IFO 31249, ATCC 34541)**: The uptake trials were initiated on day 5 after innoculation, growth at T=37 ^{o}C. 200 ul of compound stock solution in the media (Kirk's mineral salts and trace elements, 2 mg/ml microcrystalline cellulose, 20 mM ammonium tartrate) were added to each 1.8 ml culture and contents of the well were mixed underneath the mycelium with a pipette. After the incubation period, the entire contents of culture were centrifuged, the pellet was washed 5 times, then vortexed for 15 min with 1% SDS and lysing matrix D, and filtered through 10 K cutoff spin filter. Filtrate was analyzed by MS.

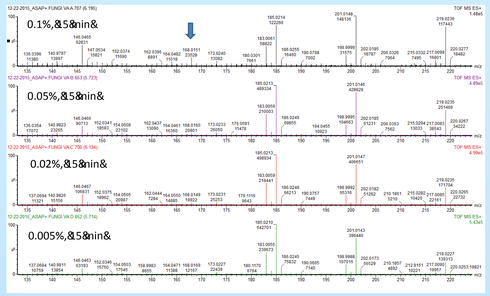
***S. Cerevisiae* (BY4741, ATCC 201388)**: The trial was initiated on day 5 after innoculation, growth at T=30 ^{o}C. 200 ul of compound stock solution in the media (YPD) were added to each 1.8 ml culture. Incubations harvesting, lysing, filtration, and MS protocols were the same as for trials with P. chrysosporium.

Mass spec: Atmospheric Solids Analysis Probe (ASAP) MS and Electrospray ionization (negative mode) with direct injection.

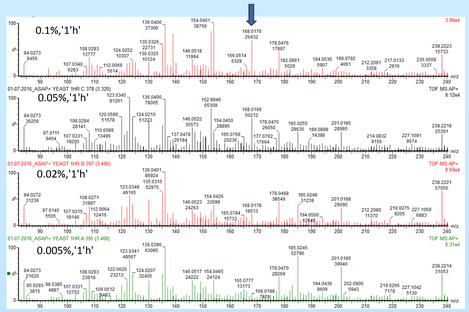
vanillic acid

variation with compound concentration

P. chrysosporium



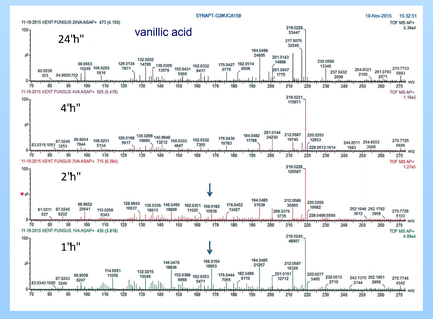
S. cerevisiae



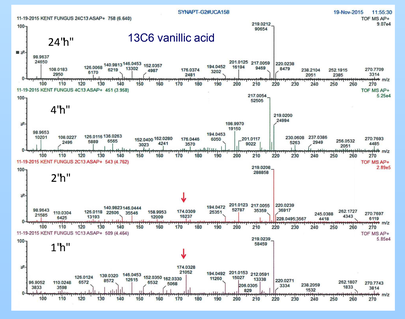
0.05% vanillic acid required for a strong signal

variation with incubation time

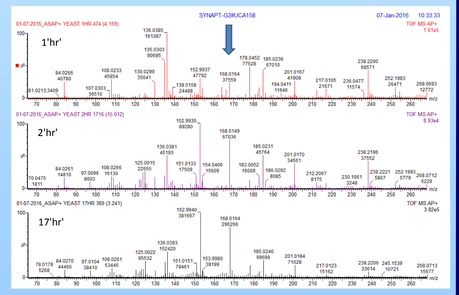
P. chrysosporium



P. chrysosporium



S. cerevisiae



decrease with incubation time likely due to metabolism[8]

increases with incubation time

Summary - all compounds

all tested at 0.1%

compound	P. chrysosporium	S. cerevisiae
vanillic acid	Y	Y
syringic acid	N	Y
p-coumaric acid	N	N
4-hydroxybenzoic acid	N	N
4-hydroxybenzoic acid analogue	Y	N
GGE	N	N

same results for ASAP MS and ESI MS

Surprising results:

Only certain monomeric acids detected for either organism

***P. chrys.* takes in the nonnatural analogue of 4-HBA but not 4-HBA. Or is 4-HBA rapidly metabolized?**

No uptake of GGE was detected

On-going work:

Develop fluorescence imaging capability for studying uptake kinetics (4-HBE analogue)

Develop methods to quantify uptake by mass spec

Compare direct uptake detection (mass spec. or fluorescence) with studies of substrate depletion in media

References:

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