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Computational Advances in Ionic Liquid Applications for Green Chemistry: A Critical Review of Lignin Processing and Machine Learning Approaches

Background/Objective

- IL-based lignin valorization can be accelerated using computational tools by rapid screening of IL-lignin systems and developing molecular-level insights for process design

Approach

- Performed comprehensive literature review of recent computational methods for lignin processing (since 2022)
- Analyzed and synthesized findings across computational and machine learning studies

Results

- Produced critical review manuscript examining current state of the field
- Identified key knowledge gaps and future research directions for computational lignin processing

Significance/Impacts

- Computational screening methods significantly reduce experimental costs and accelerate discovery of effective ionic liquids for lignin processing
- Review provides strategic roadmap for future R&D of machine learning approaches in lignin processing

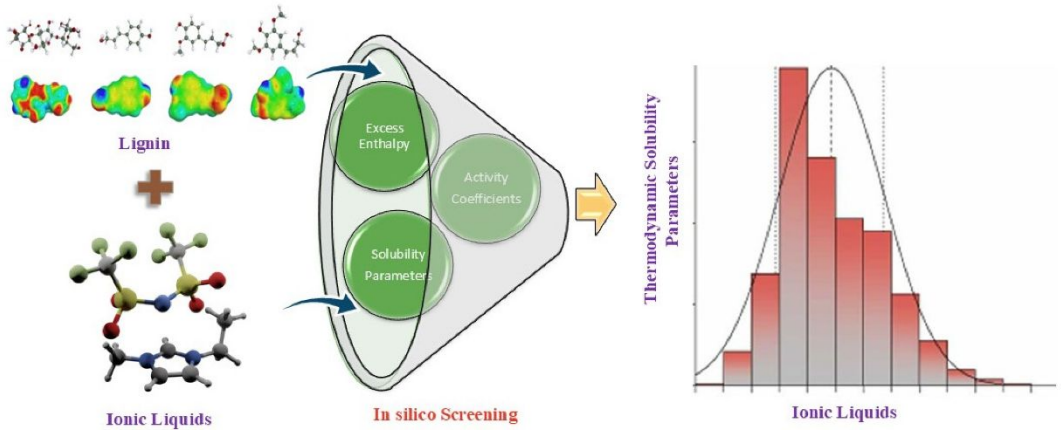

Figure: The schematic diagram for screening of solvents for lignin dissolution.

Taylor B.R., et. al. Molecules. doi: 10.3390/molecules29215073 (JBEI #1197)

The crystal structure of Grindelia robusta 7,13-copalyl diphosphate synthase (GrTPS2) reveals active site features controlling catalytic specificity

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Background/Objective

- Diterpenoid natural products serve critical functions in plant development and ecological adaptation and many diterpenoids have economic value as bioproducts

Approach

- GrTPS2 catalyzes the committed reaction in the biosynthesis of grindelic acid. Grindelic acid has been explored as a potential source for drug leads and biofuel production

Results

- The GrTPS2 crystal structure adopts the conserved three-domain fold of class II diterpene synthases featuring a functional active site in the γβ-domain and a vestigial α-domain

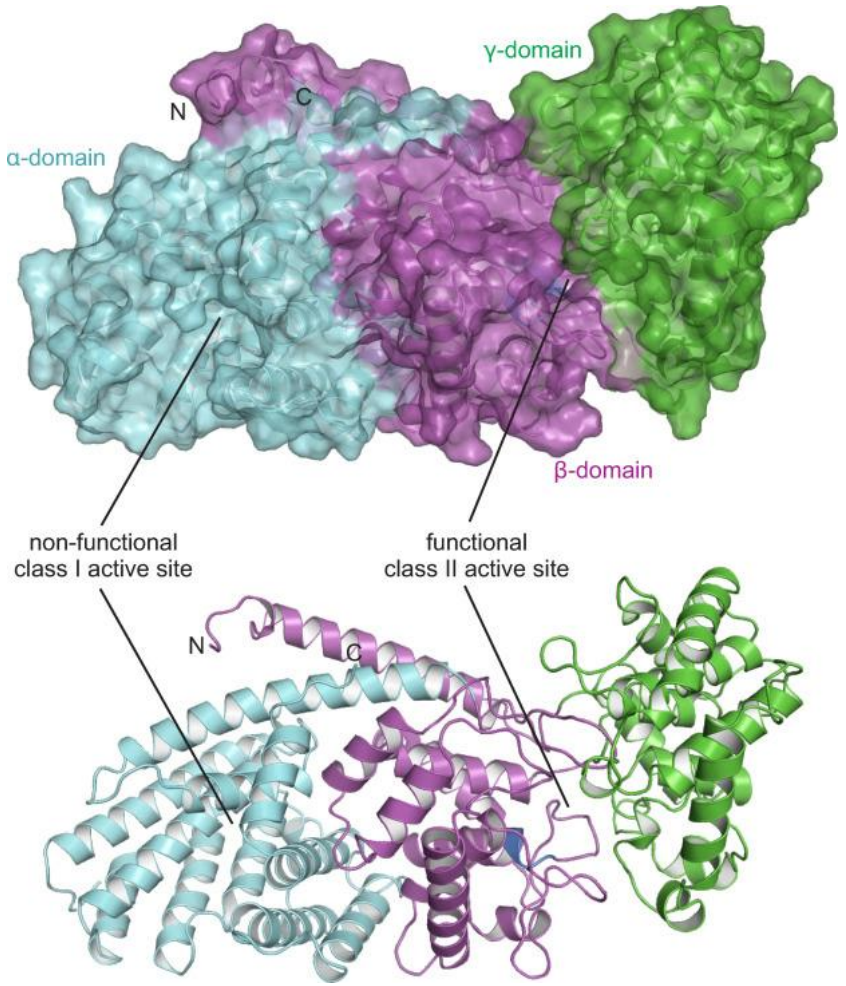

Figure 1: GrTPS2 structure. Surface (top) and cartoon (bottom) representation of the crystal structure of GrTPS2 (PDB ID 9B99) adopting the typical three-domain architecture of class II diterpene synthases.

Cowie A. E., et. al. The Journal of biological chemistry. doi: 10.1016/j.jbc.2024.107921 (JBEI #1198)

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Engineered reduction of *S*-adenosylmethionine alters lignin in sorghum

Lignin impedes biomass deconstruction into simple sugars

- Sorghum is a bioenergy crop that can be genetically transformed
- Reducing lignin in sorghum is an important milestone to improve the economics of biofuels and bioproducts

Heterologous expression of *S*-adenosylmethionine hydrolase (AdoMetase)

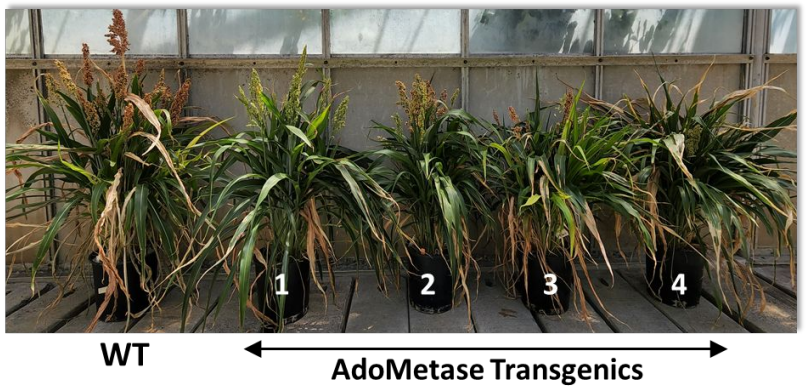
- AdoMetase from coliphage T3 cleaves the coffactor *S*-adenosylmethionine used by *O*-methyltransferases during lignin synthesis
- AdoMetase was introduced in sorghum

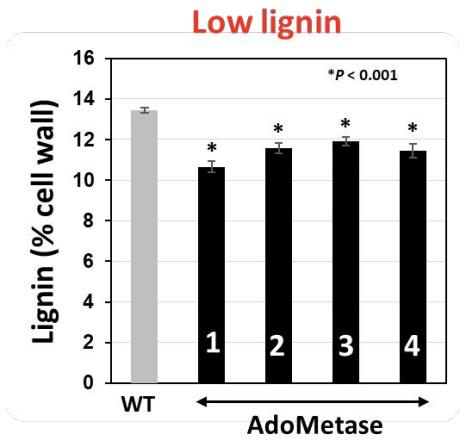
Lignin is reduced in AdoMetase sorghum

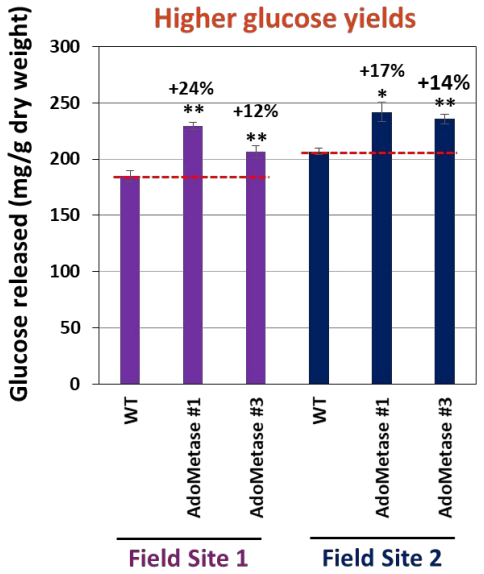
- AdoMetase expression reduces lignin by 18%
- Biomass from AdoMetase sorghum releases 20% more glucose after saccharification

AdoMetase sorghum shows reduced biomass recalcitrance

- Optimum sugar yields from AdoMetase sorghum could be achieved at lower enzyme loadings (cost saving)
- The low lignin trait needs to be introduced in bioenergy sorghum types







Tian Y., et. al. (2024) Biotechnology for Biofuels and Bioproducts. doi: 10.1186/s13068-024-02572-8 (JBEI #1199)

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Energy and nutrient recovery from municipal and industrial waste and wastewater - a perspective

Background/Objective

Reimagining our energy and carbon rich waste streams as a valuable resource can support overcoming current limitations with feedstock supply chains for biorefining approaches

Approach

This perspective highlights the latest advancements in the field of energy and nutrient recovery from municipal and industrial waste and wastewater

Results

The anaerobic digestion process must be rewired to produce carboxylic acids via arrested methanogenesis to enable a carboxylate platform that can make a significant contribution to advanced waste and wastewater treatment

Significance/Impacts

This comprehensive review underlines a multi-dimensional strategy for wastemanagement that harnesses technological innovations across several domains to transform waste into a resource

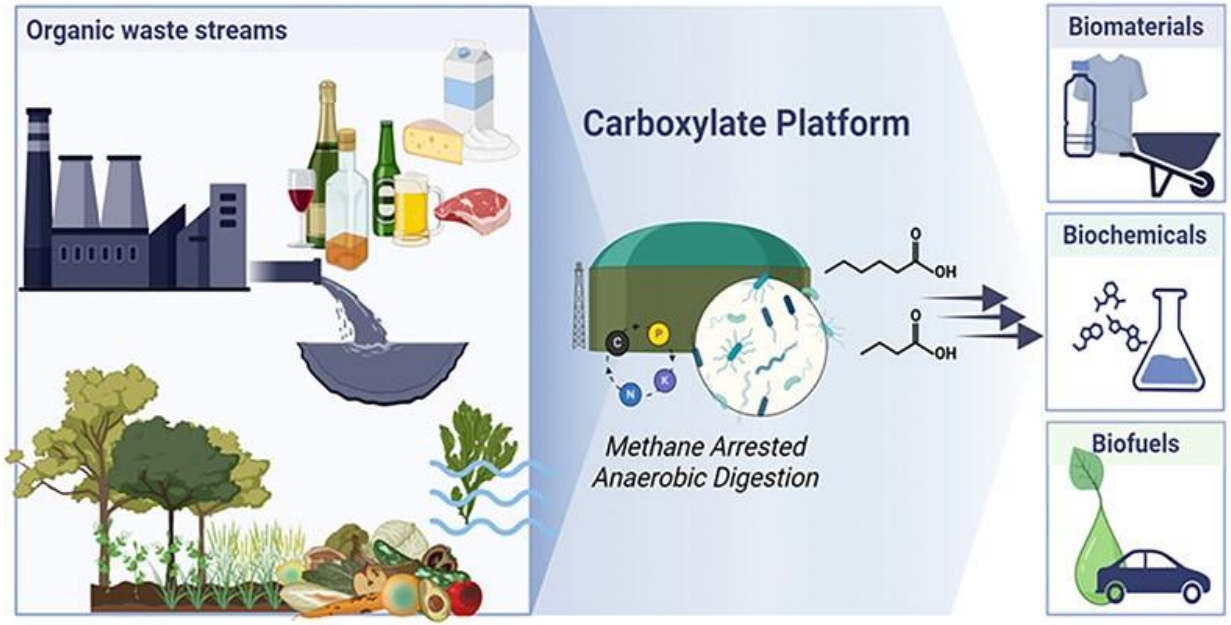

Figure: Schematic depiction of an integrated approach to valorizing waste streams into bioproducts and biofuels.

Rachbauer L., et. al. Journal of industrial microbiology & biotechnology. doi: 10.1093/jimb/kuae040 (JBEI #1200)

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EcoFAB 3.0: a sterile system for studying sorghum that replicates previous field and greenhouse observations

Background

- The bioenergy and biomanufacturing community aims to understand and use beneficial microbes to improve sustainability.
- There is a need for a system to perform studies analogous to model plants in bioenergy crops such as sorghum.

Approach

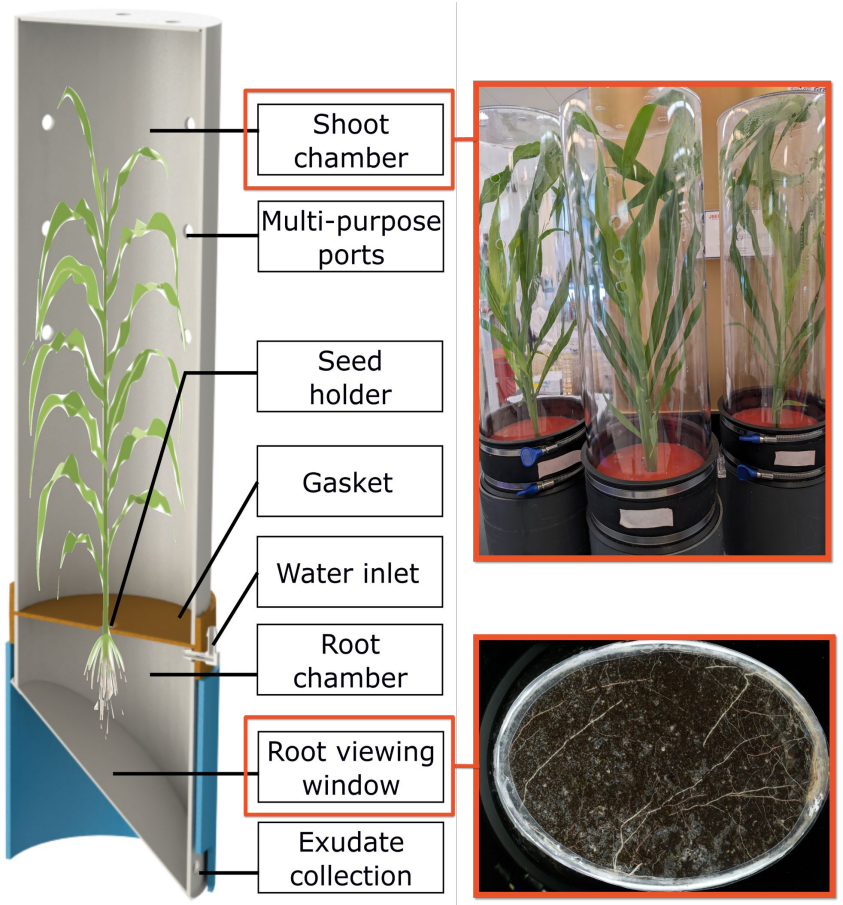
- Develop a system to study sorghum in a sterile and controlled environment.

Results

- EcoFAB 3.0 enables studying sorghum for up to 4 weeks.
- Supports replicable plant growth and 4-hydroxybenzoic acid (4-HBA) production in a JBEI Feedstocks engineered sorghum line.

Significance/Impacts

- Enable studying bioenergy crops and its rhizobiome with a user friendly and reusable device.
- Opens new collaborations with other BRCs and larger research community.



Schematic and photos showcasing several features of EcoFAB 3.0

Gupta, K., et. al. Frontiers in plant science. doi: 10.3389/fpls.2024.1440728 (JBEI #1201)

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Site Suitability and Air Pollution Impacts of Composting Infrastructure for California's Organic Waste Diversion Law

Background/Objective

- Diverting organic residues, including agricultural residues is critical for achieving decarbonization goals
- Composting plays an important role in almost all bioenergy systems because residual solids must be managed, and can be recycled back to soils

Approach

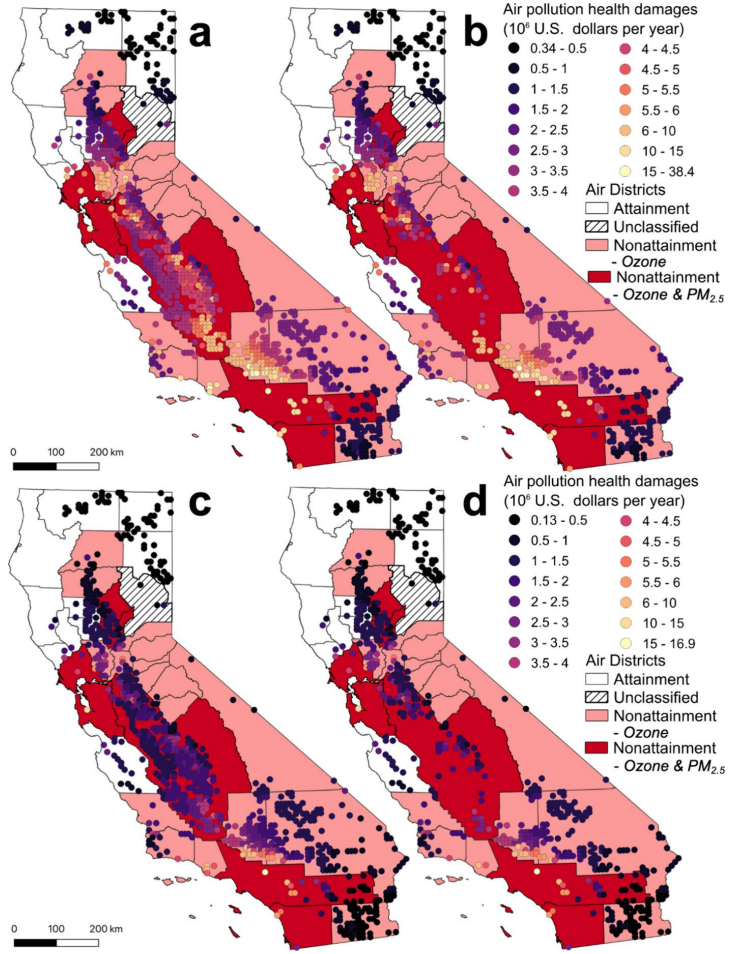
- Built a model for identifying promising sites for composting solids
- Modeled GHG and air pollution impacts of each scenario

Results

- There is far more agricultural land that can benefit from the application of composted solids than there is likely to be supply of that compost. It is possible to scale up while avoiding overburdening disadvantaged communities

Significance/Impacts

- The bioeconomy requires development of both inputs and effective management strategies for outputs, including residual solids
- There is a viable past for managing residual solids



Air pollution health damages ($106 2023 USD yr–1) of each suitable compost facility in the (a) Windrow, (b) No-DAC + Windrow, (c) ASP, and (d) No-DAC + ASP scenarios. Air district attainment status is depicted by color with white for attainment, dashed lines for unclassified, light red for ozone nonattainment, and dark red for ozone and fine particulate matter (PM2.5) nonattainment. No-DAC is no disadvantaged communities, and ASP is aerated static piles.

Harrison B. P., et. al. Environmental science & technology. doi: 10.1021/acs.est.4c06371 (JBEI #1202)

ENABLED PUBLICATIONS

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Optogenetic dissection of transcriptional repression in a multicellular organism

Background/Objective

- The functions of how transcription factors are implemented at the molecular level has remained elusive, particularly in the endogenous context of developing animals

Approach

- We combined optogenetics, single-cell live-imaging, and mathematical modeling to study how a zinc-finger repressor, Knirps, induces switch-like transitions into long-lived quiescent states

Results

- We demonstrated that repression is rapidly reversible (~1 min) and memoryless
- We showed that the repressor acts by decreasing the frequency of transcriptional bursts in a manner consistent with an equilibrium binding model

Significance/Impacts

- Our results provide a quantitative framework for dissecting the in vivo biochemistry of eukaryotic transcriptional regulation.

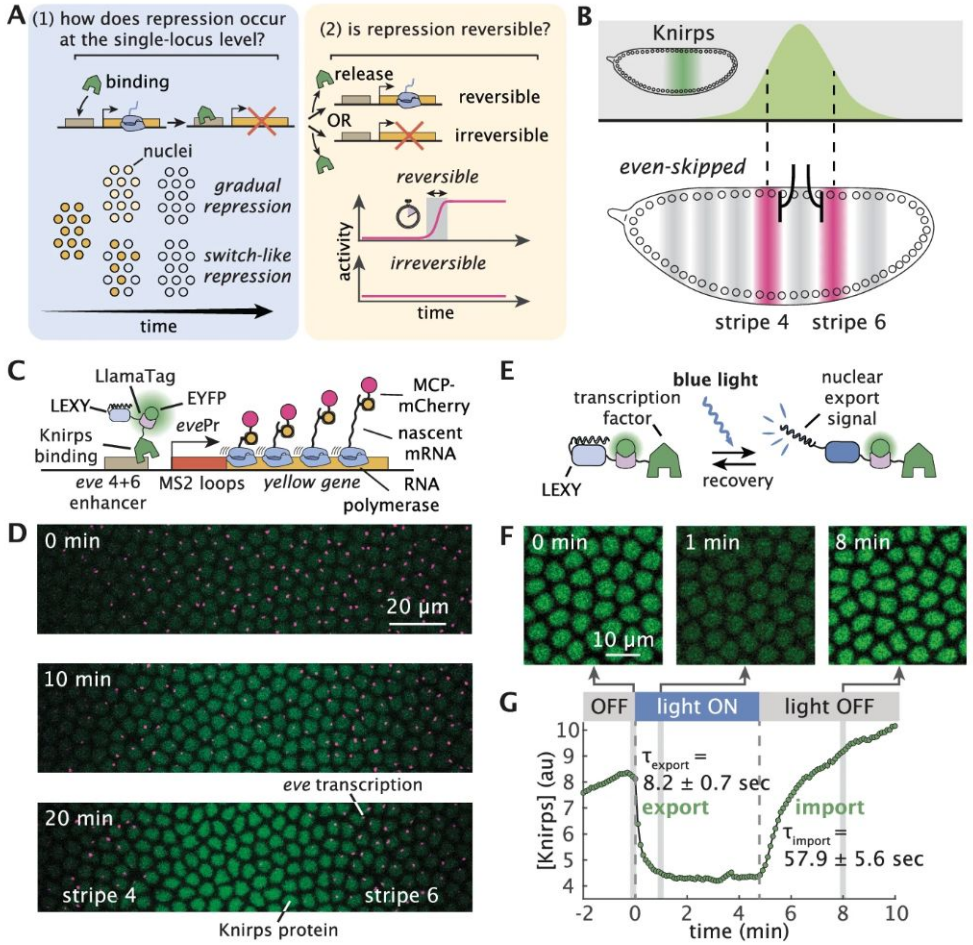

Figure: Combining optogenetics and live imaging enables dissection of single-cell repression dynamics in a developing animal.

Zhao J., et. al. Nature communications. doi: 10.1038/s41467-024-53539-0 (JBEI #E102)

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Methane to bioproducts: unraveling the potential of methanotrophs for biomanufacturing

Background/Objective

- CH_4 is a flammable, greenhouse gas, which is abundantly released as a waste from natural and anthropogenic sources
- CH_4 consuming microbes– methanotrophs, can be engineered and leveraged for enabling CH_4 based C_1 biomanufacturing technologies

Approach

- Performed an extensive survey of literature on sources of CH_4, methanotrophic metabolic pathways, engineered platforms and proof of concept technologies developed in these microbes

Results

- Presented a critical review of the past and current practices in bioengineering, scale-up, product titer produced by methanotrophic bacteria
- Identified and discussed the technology challenges and potential solutions

Significance/Impacts

- The review provides a strategic overview of C_1 biomanufacturing current state of the field and its potential in the future to contribute to a circular economy

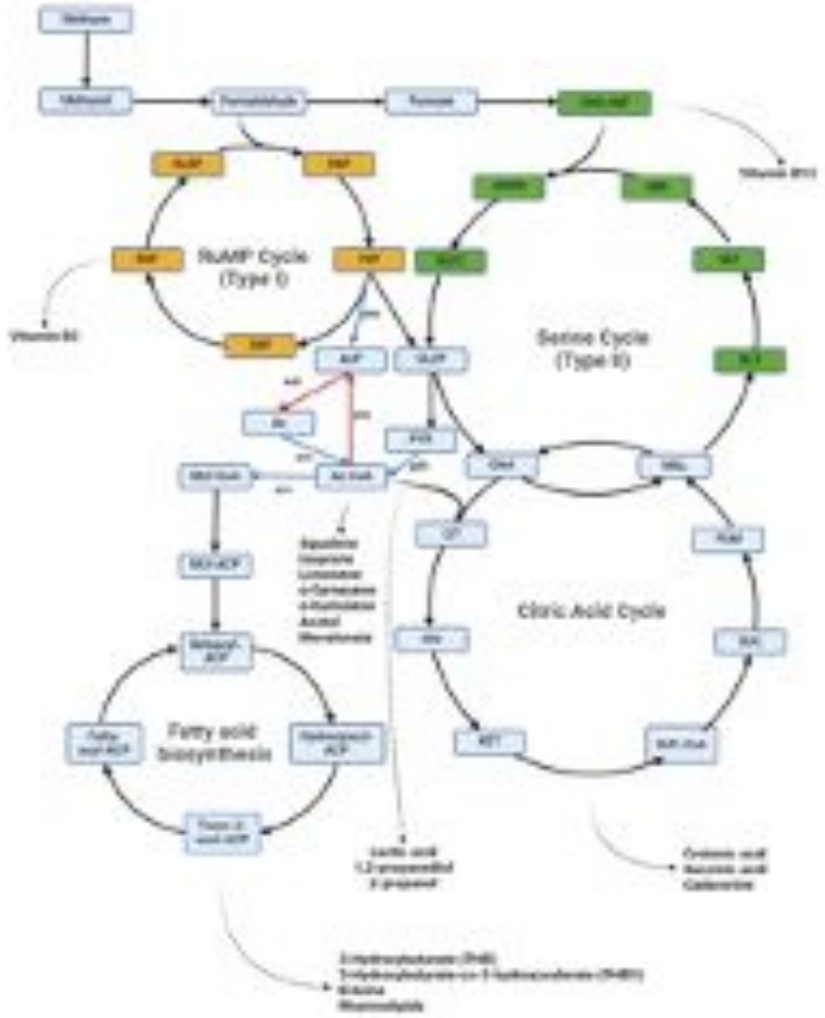

Figure: Metabolic pathways found in methanotrophs-Type I and II, targeted for engineering.

Tan J. N., et. al. Current opinion in biotechnology. doi: 10.1016/j.copbio.2024.103210 (JBEI #E103)

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Metabolic engineering of yeast for de novo production of kratom monoterpene indole alkaloids

Background/Objective

- Monoterpene indole alkaloids from *Mitragyna speciosa*, such as mitragynine and speciogynine, are promising natural products

Approach

- We engineered *Saccharomyces cerevisiae* to produce mitragynine and speciogynine by reconstructing the five-step synthetic pathway from strictosidine comprising fungal tryptamine 4-monooxygenase to bypass an unknown kratom hydroxylase

Results

- The engineered yeast produced ~290 µg/L kratom MIAs from glucose
- By feeding fluorinated tryptamine and expressing a human tailoring enzyme, the engineered yeast produced fluorinated and hydroxylated mitragynine derivatives

Significance/Impacts

- This study introduces a yeast cell factory platform for the biomanufacturing of complex natural and new-to-nature kratom MIAs derivatives with therapeutic potential

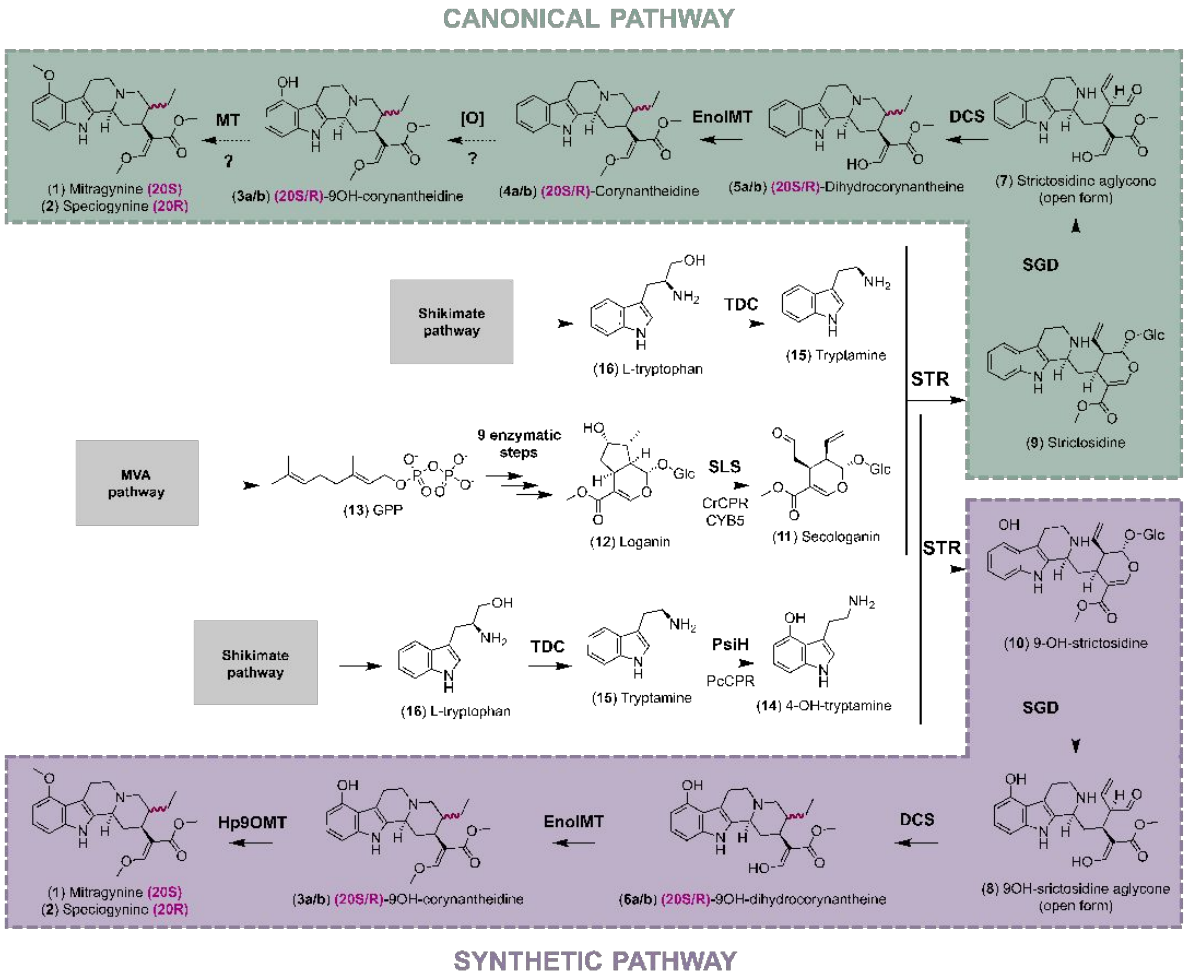

Figure. Biosynthetic pathways for production of mitragynine and speciogynine in yeast

Holtz M., et. al. Metabolic engineering. doi: 10.1016/j.ymben.2024.09.011 (JBEI #E104)

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Corn Stover variability drives differences in bisabolene production by engineered *Rhodotorula toruloides*

Background/Objective

- Less is known about the impact of feedstock heterogeneity on microbial conversion of lignocellulosic biomass
- Investigate the effects of corn stover variability on bisabolene production by *Rhodotorula toruloides*

Approach

- Evaluate different corn stover batches, anatomical fractions, and storage conditions
- Fermentation using AMBR250 and global proteomics

Results

- Different sources of feedstock heterogeneity influence microbial growth and product titer in counterintuitive ways as revealed through proteomics analysis

Significance/Impacts

- Corn stover is an important model for lignocellulosic biomass-to-ethanol processing and SAF conversion
- Importance of understanding feedstock variability to enhance bioprocess efficiency and economic outcomes

Figure: Biomass conversion processes. (A) Characterized corn stover biomass. (B) Pretreatment method (C) Fermentation by *R. toruloides* using AMBR250 bioreactor.

Okonkwo O., et. al. Journal of Industrial Microbiology and Biotechnology. doi: 10.1093/jimb/kuae034 (JBEI #E105)