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Biological funneling of aromatic phenolics from transgenic plants engineered to express the bacterial 3-dehydroshikimate dehydratase (qsuB) gene

Background/Objective

- Plants have been engineered with the qsuB gene to accumulate protocatechuate (DHBA)
- Accumulation of coproducts such as DHBA in biomass can enable higher titers of bioproducts

Approach

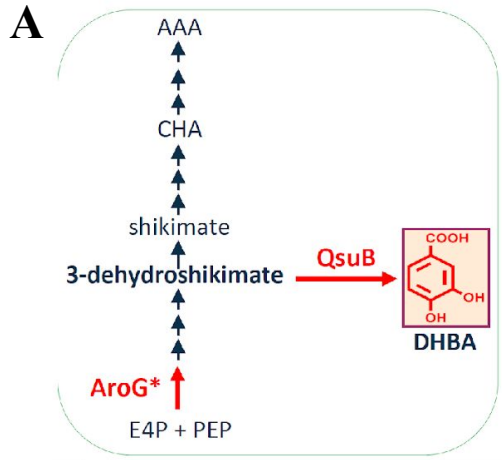
Hydrolysates generated from engineered Arabidopsis, poplar, and sorghum that accumulate DHBA were used to grow a *Novosphingobium aromaticivorans* strain that produces 2-pyrone-4,6-dicarboxylate (PDC)

Results

DHBA and other aromatics derived from plant biomass were biologically upgraded into PDC with *N. aromaticivorans*.
The engineered Arabidopsis, poplar, and sorghum types enabled higher PDC titers compared to their non-modified controls.

Significance/Impacts

DHBA from in engineered crops can be biologically funneled into valuable bioproducts such as PDC. Other upgrading metabolic routes will be tested.



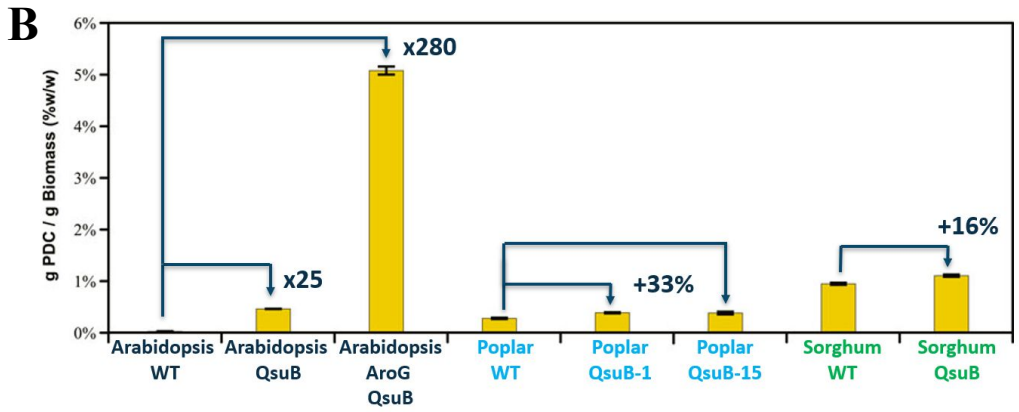


Figure 1: (A) Plant engineering approach to increase DHBA. AroG and QsuB are introduced in plants to modify the shikimate pathway. **(B)** Production of PDC from biomass hydrolysates by *N. aromaticivorans.* WT: control genotypes. QsuB: DHBA-rich genotypes.

Umana G, et. al. Frontiers in Chemical Engineering. doi: 10.3389/fceng.2022.1036084

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Investigation of the Effects of Ternary Deep Eutectic Solvent Composition on Pretreatment of Sorghum Stover

Background/Objective

- Deep eutectic solvents (DESs) are promising solvents for biomass pretreatment
- Sorghum can be engineered to accumulate protocatechuate (DHBA)
- Can DESs be synthesized from DHBA for biomass pretreatment?

Approach

A binary DES, composed of choline chloride (ChCl) and DHBA, and ternary DESs with additional third constituents like water and ethylene glycol were synthesized

Results

Ternary [ChCl-DHBA] DESs exhibited better performances in delignification, fermentable sugar production, and preservation of β-O-4 ether linkage in lignin compared with binary [ChCl-DHBA] DES.
Total glucan conversion was achieved with engineered sorghum vs wildtype.

Significance/Impacts

Bioenergy crops can be engineered to accumulate chemical precursors such as DHBA for the synthesis of efficient biomass pretreatment solvents

Wang Y., et. al. AIChE Journal. doi: 10.1002/aic.18227

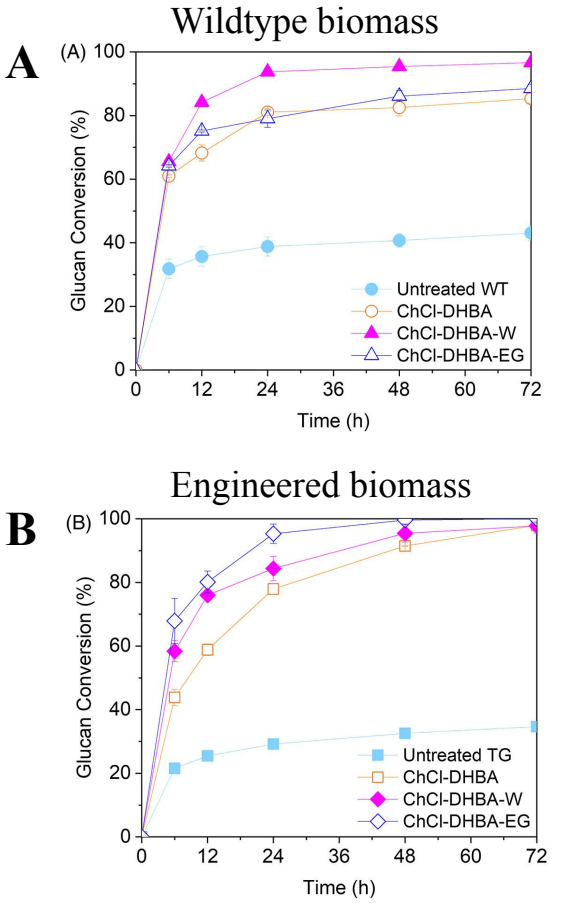

Figure 1: Saccharification yields of sorghum stover biomass pretreated with different DESs. The cellulase Ctec2 was loaded at 20 FPU/g-biomass. (**A**) Wildtype sorghum. (**B**) DHBA-rich sorghum. Water (w) or ethylene glycol (EG) was added to the binary [ChCl-DHBA] DES. Untreated: no DES.

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Deciphering triterpenoid saponin biosynthesis by leveraging transcriptome response to methyl jasmonate elicitation in Saponaria vaccaria

Background/Objective

- Saponins are important defense compounds made by many plants. They can cause inhibition of yeast during biomass conversion. Saponins also have multiple pharmaceutical applications, notably as vaccine adjuvants.
- The biosynthesis of saponins is not well understood.

Approach

We used transcriptomics to identify genes induced by methyl jasmonate. Candidate genes were validated by expression in tobacco and yeast.

Results

- Multiple P450 enzymes and glycosyltransferases were identified as part of the pathway for vaccaroside biosynthesis.
- The two enzymes required for synthesis of the rare but widespread UDP-D-fucose were also discovered. L-fucose is the common form in plants.

Significance/Impacts

The identified genes have been used to produce the vaccine adjuvant QS21 in yeast. This could have substantial impact on vaccine development and eliminate the need for extracting saponins from soapbark trees from wild forests.

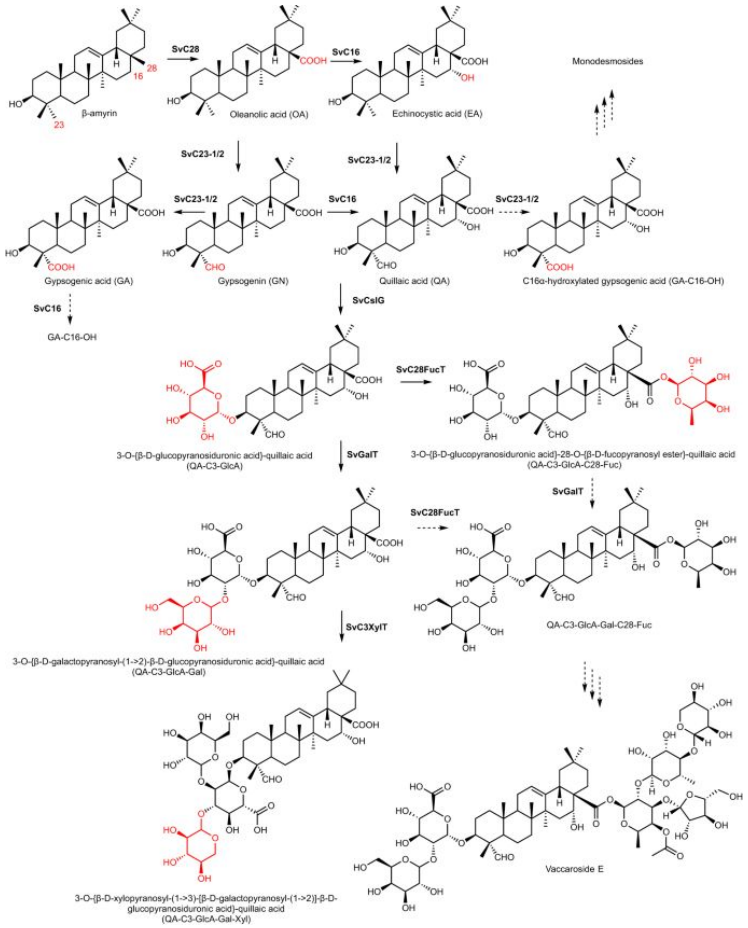

Figure 1: The biosynthesis pathway leading to the production of triterpenoid saponins (e.g., Vaccaroside E) in *S. vaccaria*. Each product of biosynthetic enzyme activity is highlighted in red. Arrows in solid line indicate reactions confirmed in this study, while arrows in dashed lines indicate hypothetical reactions to be established in the future.

Chen, X., *et. al.* Nat Commun. (2023) doi: 10.1038/s41467-023-42877-0

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Enhancing isoprenol production by systematically tuning metabolic pathways using CRISPR interference in E. coli

Background/Objective

- Regulation of metabolic gene expression is crucial to maximize bioproduction TRY, and CRISPRi is a powerful tool for this.
- We harnessed CRISPRi to downregulate genes in *E. coli* strains to improve the titer of isoprenol biosynthesis.

Approach

- 1. Harness CRISPRi methods to downregulate an expanded pool of endogenous genes to improve isoprenol titer via both the original MVA pathway and the IPP-bypass pathway.
- 2. Construct multiplexed gRNA arrays leveraged for enhanced isoprenol biosynthesis.
- 3. Show that the CRISPRi platform is scalable to 2-L bioreactors.

Results

Of the 32 single gRNAs targeting genes associated with isoprenol biosynthesis, a subset was found to vastly improve product titers. Construction of a multiplexed gRNA library based on single gRNA performance enabled simultaneous gene repression and some of them showed 3 to 4.5-fold increase in isoprenol titer.

Significance/Impacts

Our strategy establishes CRISPRi as a powerful tool for tuning metabolic flux and improving the titer with potential for industrial applications.

Kim J, Lee TS, (2023) ***Frontiers in Bioengineering and Biotechnology*****, DOI: 10.3389/fbioe.2023.1296132.**

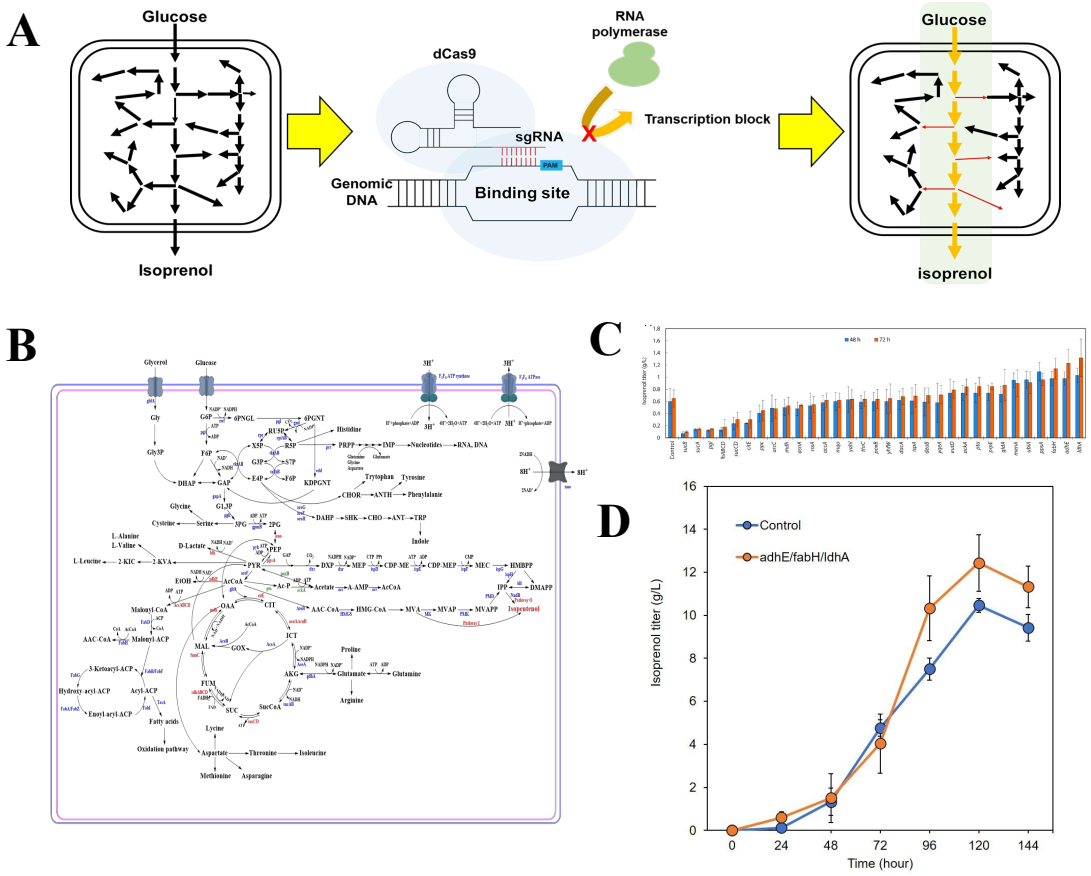

Figure 1: Description in Times 10 (A) Schematic for the improvement of isoprenol biosynthesis by CRISPRi-mediated downregulation of competing pathways. (B) A pathway map of gene knockdown targets for redirecting metabolic flux toward isoprenol production using CRISPRi (C) Isoprenol production of strains harboring single gRNAs and the IPP-bypass pathway. (D) Isoprenol production under fed-batch conditions

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BayFlux: A Bayesian method to quantify metabolic Fluxes and their uncertainty at the genome scale

Background/Objective

- Metabolic fluxes are crucial to predict and understand biological systems.
- ^{13}C Metabolic Flux Analysis (^{13}C MFA) is considered to be the gold standard to measure metabolic fluxes.
- The optimization approach used to date in 13C Metabolic Flux Analysis to determine fluxes shows several limitations, particularly in characterizing the full distribution of fluxes compatible with the data.

Approach

BayFlux is a new method that rigorously identifies the full distribution of flux profiles compatible with experimental data for a genome-scale model.

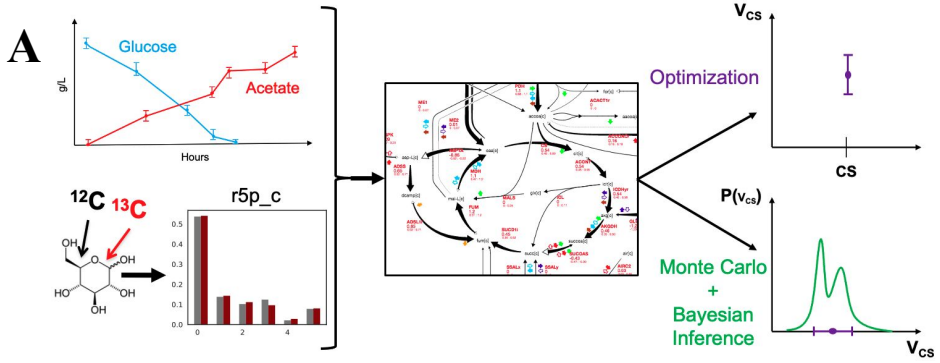
Results

- We find that genome-scale models result in narrower flux distributions than the small core metabolic models that are traditionally used in ^{13}C MFA.
- We develop and evaluate novel methods to predict the results of a gene knockout, that improve on previous methods (FBA-based MOMA and ROOM).

Significance/Impacts

BayFlux provides a rigorous way to find all flux profiles compatible with a given set of ^{13}C experimental data, opening the door to an improved understanding of metabolism and more effective predictions for strain metabolic engineering.

Backman T. W. H., et. al. PLoS Comput Biol 19(11): e1011111. doi: 10.1371/journal.pcbi.1011111



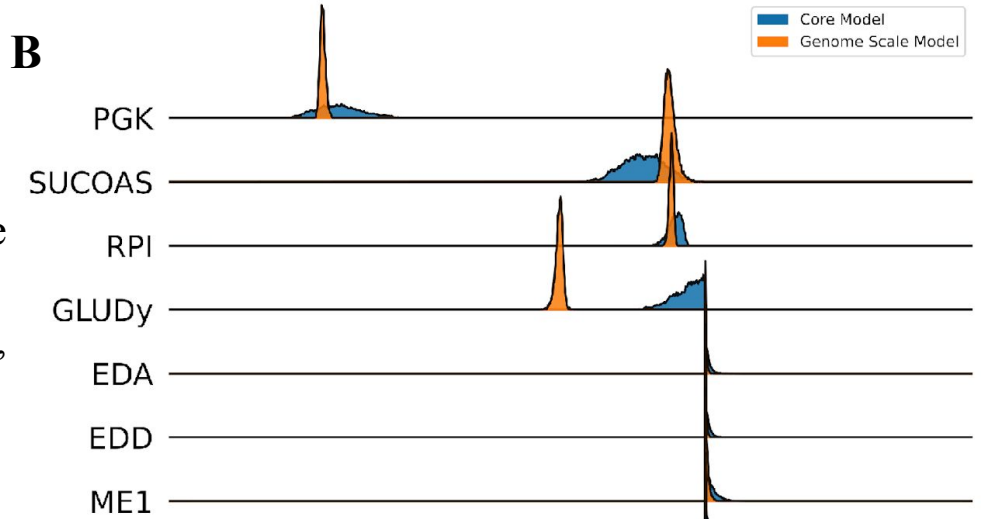


Figure 1: A) BayFlux uses Bayesian Inference and Monte Carlo sampling to provide the full distribution of fluxes compatible with the experimental data rather than an optimization approach. **B)** Using a genome-scale model produces a narrower flux distribution, as informed by a greater amount of biological knowledge encoded in the genome-scale model

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Deep-branching evolutionary intermediates reveal structural origins of form I rubisco

Background/Objective Rubisco is the entry point for all organic carbon in biomass feedstock crops

Approach

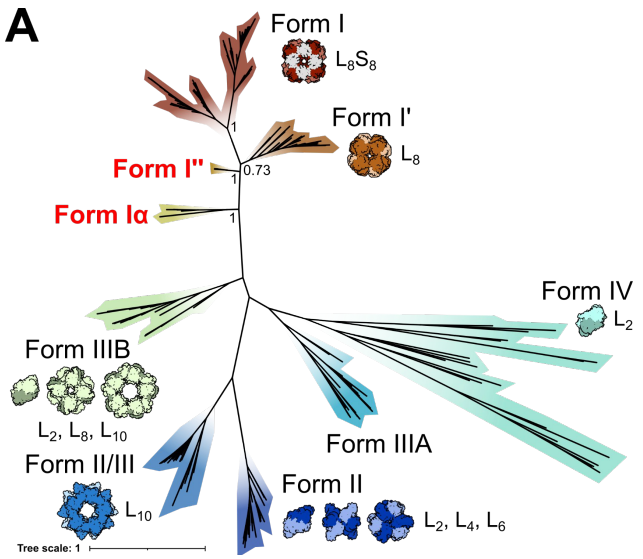
We leverage metagenomics to discover novel rubisco clades that help retrace the complex structural trajectory and evolution of plant-type Form I rubisco.

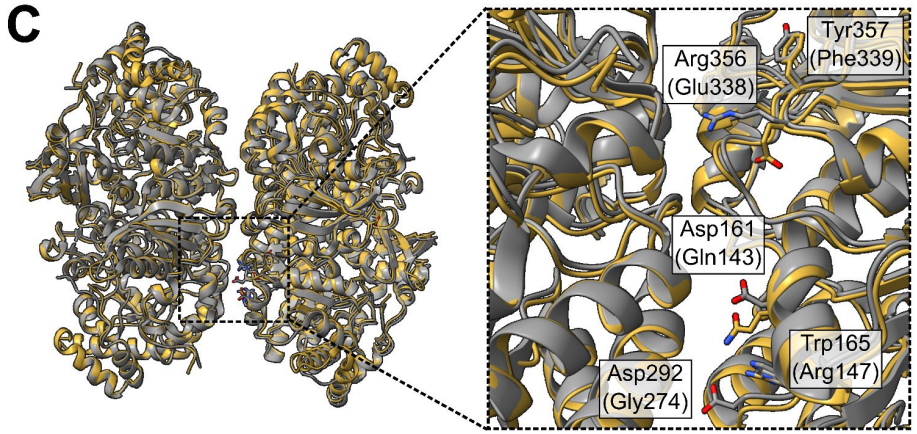
Results

We solve the structure of deep-branching form Iα and I″ rubiscos recently discovered from metagenomes, which represent key evolutionary intermediates preceding the form I clade, revealing the molecular determinants that likely primed the enzyme core for the transition from a homo-oligomer to a hetero-oligomer.

Significance/Impacts

Understanding the structural constraints and evolutionary origins of the enzyme may reveal new paths to improving and engineering rubisco





Liu, et al. (2023) *Current Biology* DOI: 10.1016/j.cub.2023.10.053

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Matching diverse feedstocks to conversion processes for the future bioeconomy

Background/Objective

- The bioeconomy must use a diverse set of feedstocks, including dedicated crops, agricultural residues, and other wastes
- There has been no systematic review to explore good feedstock-conversion matches

Approach

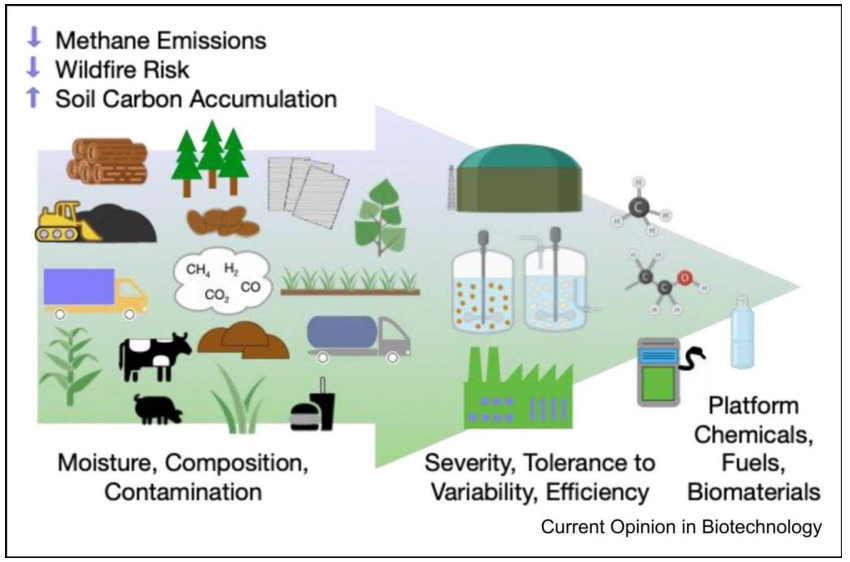
We assembled data on composition of different feedstocks and the ease with which different conversion processes handle this variation.

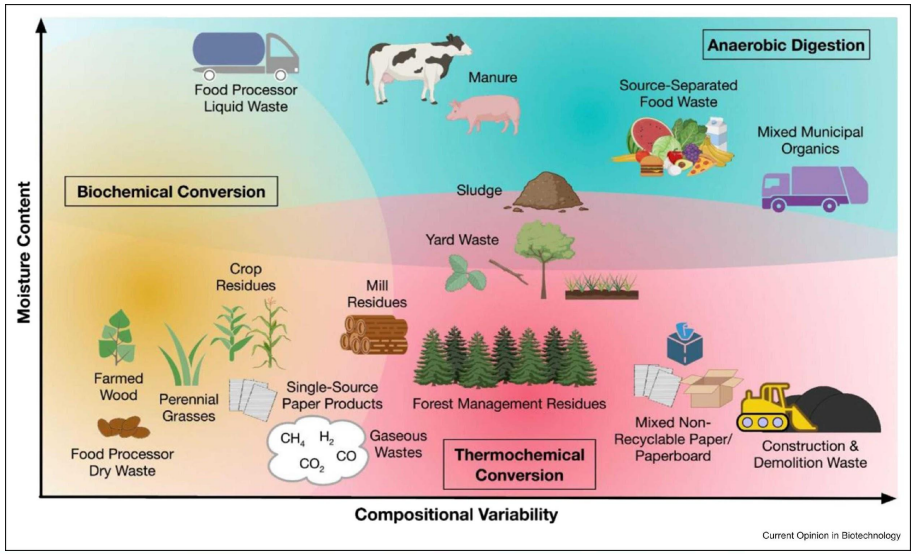
Results

- Low-variability feedstocks are well-suited for biochemical conversion.
- Contaminated or variable feedstocks may be easier to gasify or pyrolyze.
- Cost analysis studies likely underestimate costs of handling contaminated feedstocks.

Significance/Impacts

The study maps feedstock types against different conversion routes, offering guidance for how to best use diverse materials for biofuel and bioproduct production.





Scown C. D., et al. Current opinion in biotechnology. doi: 10.1016/j.copbio.2023.103017

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Automated platform for the plasmid construction process

Background/Objective

- There is a growing need for applications capable of handling large synthesis biology experiments.
- At the core of synthetic biology is the process of cloning and manipulating DNA as plasmids.

Approach

Here, we report the development of an application named DNAda capable of writing automation instructions for any given DNA construct design generated by the J5 DNA assembly program.

Results

The pipeline is particularly useful for the construction of combinatorial DNA assemblies. We demonstrate the platform by constructing a library of polyketide synthase parts, which includes 120 plasmids ranging in size from 7 to 14 kb from 4 to 7 DNA fragments.

Significance/Impacts

This platform will increase the speed and throughput of constructing plasmids for various synthetic biology applications.

Nava A., et al. ACS Synthetic Biology. doi: 10.1021/acssynbio.3c00292

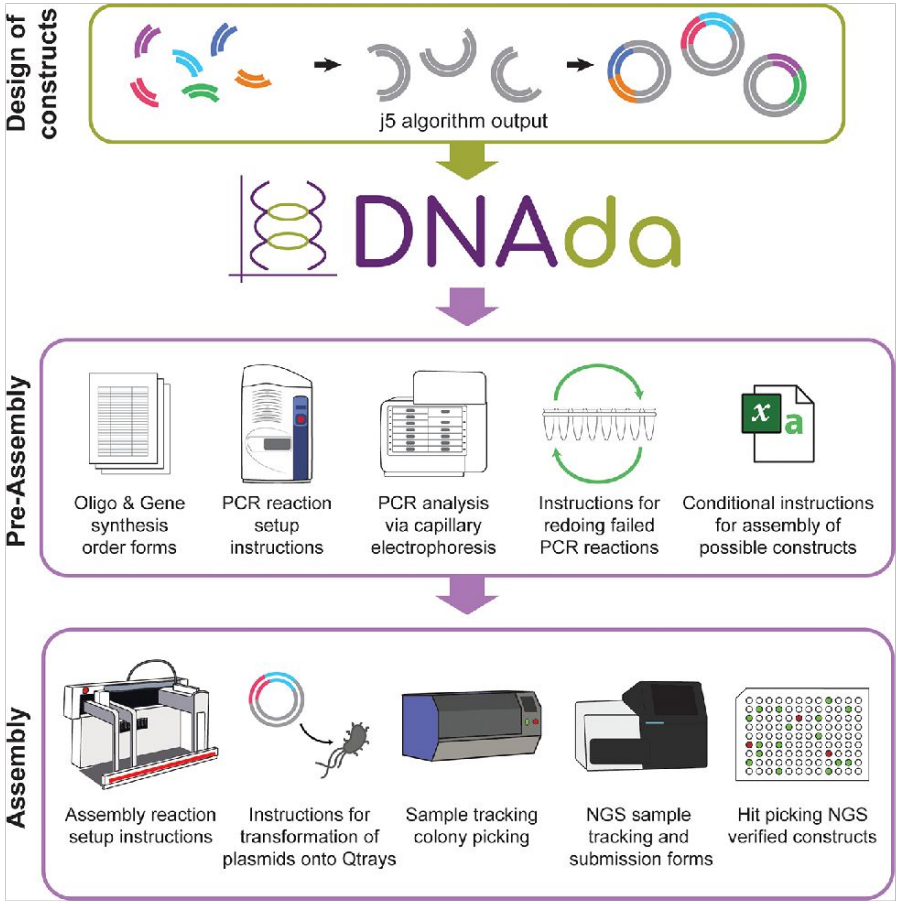

Figure 1: DNAda build workflow. DNA constructs are designed using the computer-aided design tool DeviceEditor, and build instructions are generated through the j5 DNA assembly algorithm. Those build instructions are then translated into step-by-step instructions for automated liquid handlers by DNAda.

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Improving microbial bioproduction under low-oxygen conditions

Background/Objective

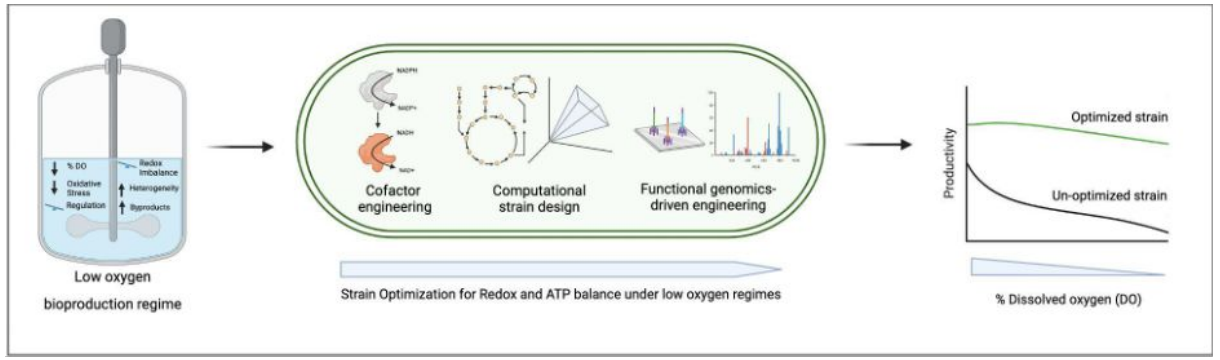
In this review we explore existing research and approaches used to lower oxygen requirement in biomanufacturing workflows.

Review topics

- Rewiring cofactor utilization in metabolism can reduce cellular oxygen demand
- Genome-scale and kinetic models of metabolism can assist microbial strain design for robust bioproduction under low oxygen conditions
- Functional genomics can reveal the role of regulatory and non-metabolic gene targets that are critical to robust phenotypes under low oxygen

Significance/Impacts

Low oxygen bioproduction may be necessary to expand the range of industrially biomanufactured chemicals



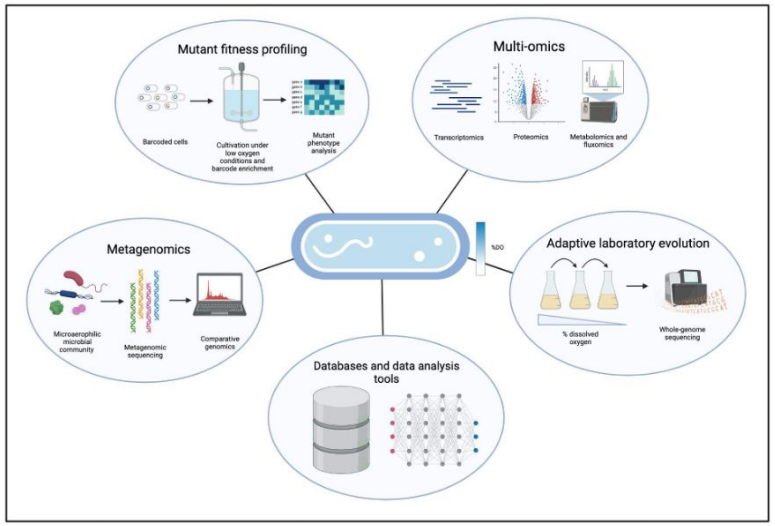


Figure 1: Functional genomics methods relevant for the investigation of microbial physiology under low oxygen

Kulakowski S., et al. Current opinion in biotechnology. doi: 10.1016/j.copbio.2023.103016

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Carbene chemistry for unnatural biosynthesis

Background/Objective

- The power of biomanufacturing is restricted by the narrow scope of chemical reactions in biological systems.
- Bringing unnatural carbene transfer reactions into biosynthesis can enhance our ability to produce chemicals through biological processes.

Approach

Based on our knowledge, understanding and thoughts on this field, an insight for this field was provided.

Results

- The background, major solved obstacles and important progresses in this field were summarized and discussed.
- Some future challenges and developing directions to facilitate the application of carbene chemistry for unnatural biosynthesis were proposed.

Significance/Impacts

This insight provides people an overview of the development of carbene chemistry for unnatural biosynthesis and the future directions.

Huang J., et. al. Science China. Life sciences. doi: /10.1007/s11427-023-2470-5

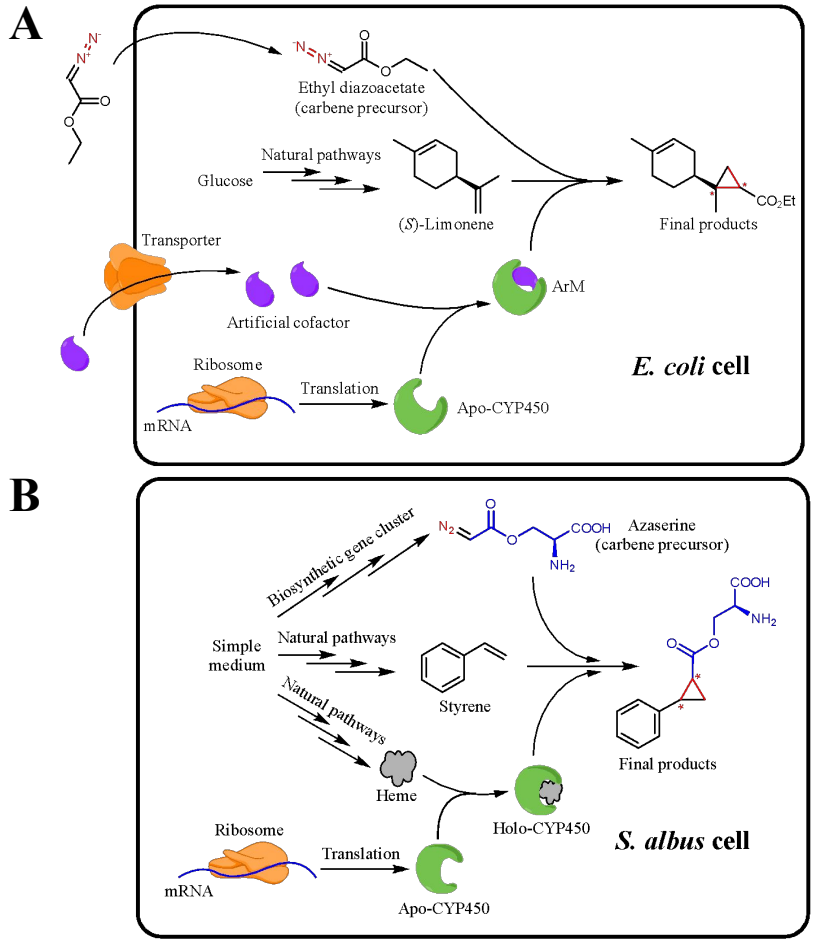

Figure 1: Bringing carbene chemistry for biosynthesis. (A) Interfacing an ArM catalyzed carbene reaction with a natural biosynthetic pathway. (B) Complete integration of carbene chemistry into cellular metabolism.

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A hybrid chemical-biological approach can upcycle mixed plastic waste with reduced cost and carbon footprint

Background/Objective

- The mixture of PLA and PET plastics poses a cross-contamination threat in existing recycling facilities.
- The study aims to upcycle mixed PLA/PET without extra separation.

Approach

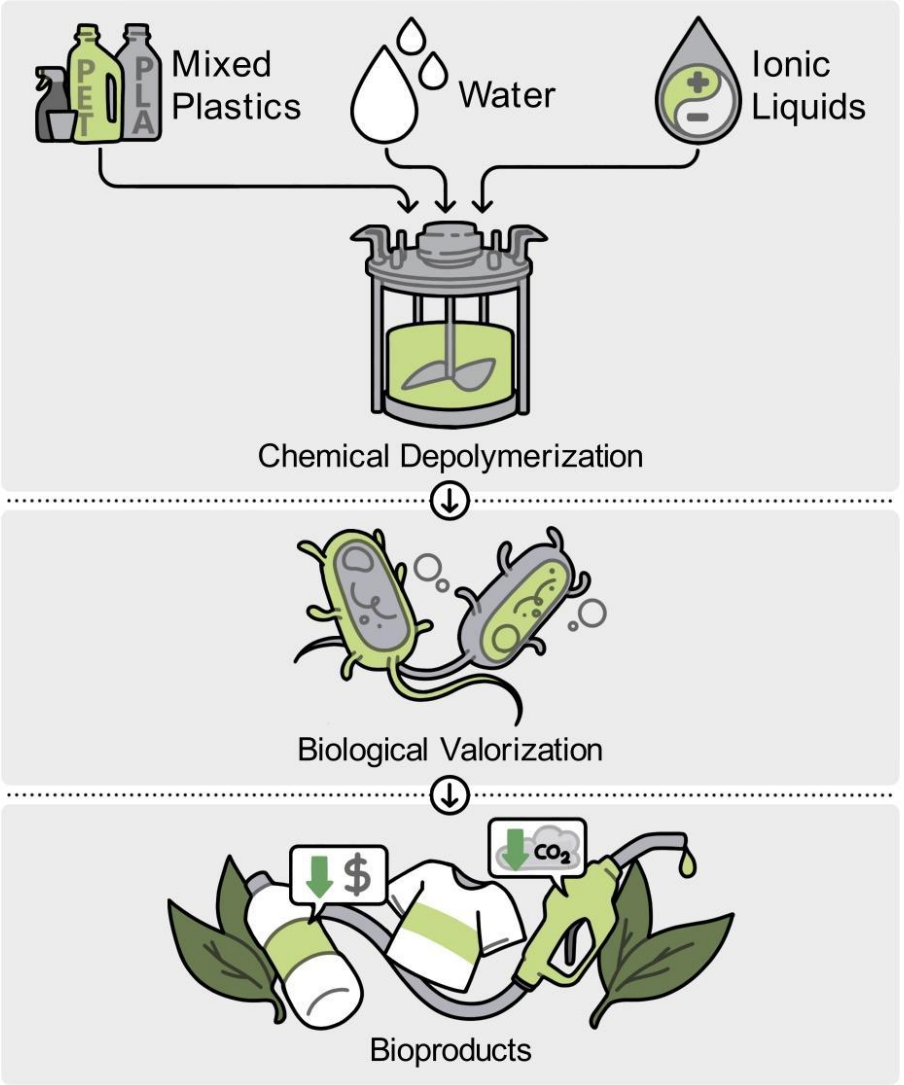
- Depolymerize mixed plastics into monomers using bio-derived IL.
- Convert depolymerized stream via biological upgrading.
- Evaluate production cost and environment impact via TEA and LCA.

Results

- Over 95% of mixed PET/PLA depolymerized into the monomers.
- The depolymerized mixed PET/PLA can serve as the sole carbon source for P. putida, producing biodegradable PHA.
- The optimal production cost and carbon footprint are reduced by 62% and 29% compared to convectional commercial PHAs.

Significance/Impacts

- Demonstrate a hybrid pathway to upcycle mixed plastics with reduced cost and carbon footprint.



Dou C., et al. One Earth. doi: 10.1016/j.oneear.2023.10.015

JBEI Enabled Papers

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High-quality RNA extraction and the regulation of genes encoding cellulosomes are correlated with growth stage in anaerobic fungi

Background/Objective

- Anaerobic fungi produce biomass-degrading enzymes and natural products that are important for biotechnology
- Existing approaches for nucleic acid extraction are limited to fungi in exponential growth phase only

Approach

Here, we developed a systematic method to extract high-quality RNA across the life stage of anaerobic fungi in monoculture and in co-culture with methanogens.

Results

- The optimal window of time to harvest high-quality RNA is between 2-5 days post sub-culture
- Within this time window, co-culture with methanogens was associated with upregulation of fungal cellulosomes

Significance/Impacts

This approach enables the exploration of non-exponential growth stages to guide in CAZyme and cellulosome secretion strategies.

Brown J. L., et al. Frontiers in fungal biology. doi: 10.3389/ffunb.2023.1171100

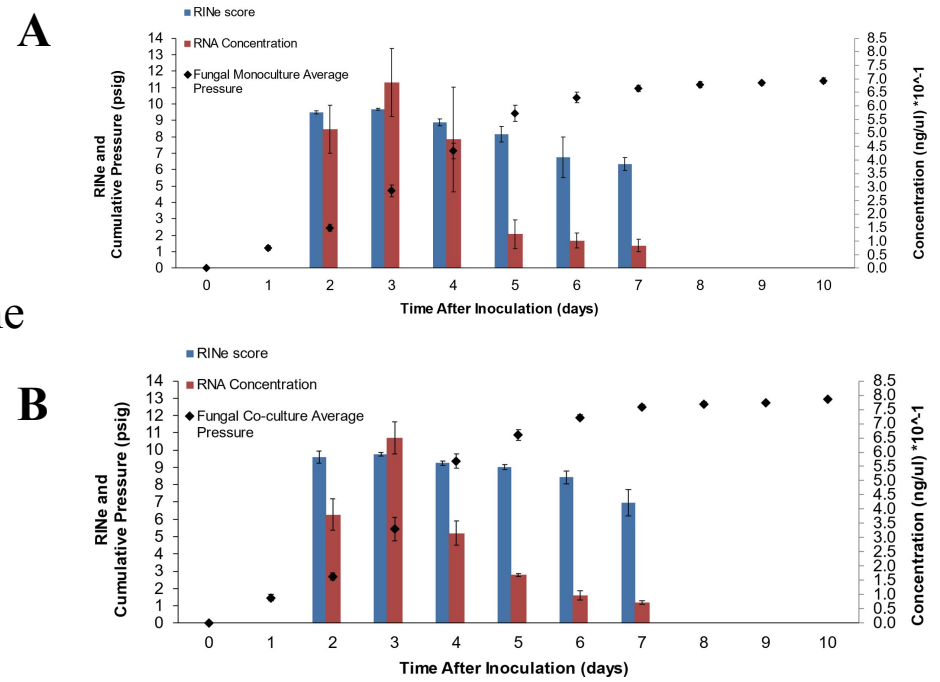

Figure 1: RNA concentrations and RINe scores for cultures harvested over 7 days of growth post-inoculation for fungal monocultures of A. robustus (A) and fungal-methanogen co-cultures of *A. robustus* and *M. bryantii* (B) both grown on a cellulose substrate (Whatman filter paper). RNA was extracted from cultures harvested on days 2-7 using a liquid nitrogen grinding lysis method. Samples were sequenced from both conditions on days 2-5 (co- culture samples from day 6 were also successfully sequenced). RNA degradation was more pronounced and RNA concentration decreased in cultures harvested on days 6 and 7, likely leading to the failure to sequence monoculture samples collected on day 6 and both monoculture and co- culture samples on day 7. The mean value is plotted for each set of replicates and error bars indicate standard deviation.

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Biosynthesis of natural and halogenated plant monoterpene indole alkaloids in yeast

Background/Objective

- Monoterpenoid indole alkaloids represent a large class of plant natural products with marketed pharmaceutical activities.
- Halogenated MIAs have shown improved pharmaceutical properties, but their synthesis is challenging.

Approach

We developed a platform for de novo biosynthesis of two MIAs, serpentine and alstonine, in baker's yeast *Saccharomyces cerevisiae* and deploy it to systematically explore the biocatalytic potential of refactored MIA pathways for production of halogenated MIAs.

Results

We demonstrate conversion of individual haloindole derivatives to a total of 19 different new-to-nature haloserpentine and haloalstonine analogs. By heterologous expression of a modified halogenase, we document de novo halogenation and biosynthesis of chloroalstonine.

Significance/Impacts

This microbial platform enables enzymatic exploration and production of complex natural and new-to-nature MIAs with therapeutic potential.

Bradley S. A., et al. Nature chemical biology. doi: 10.1038/s41589-023-01430-2

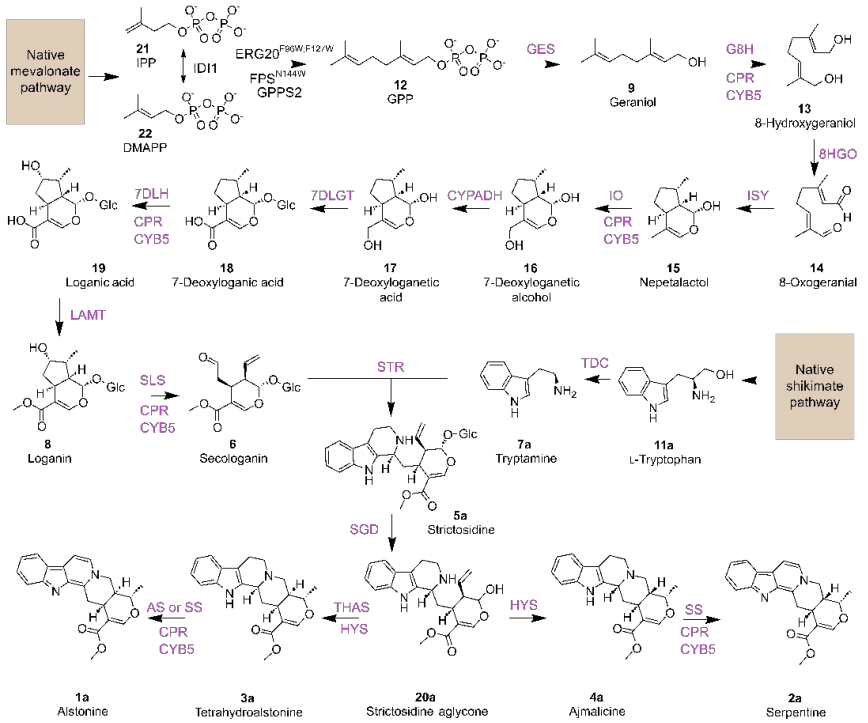

Figure 1: De novo alstonine and serpentine production in yeast. Integration of plant biosynthetic pathways with native yeast metabolic pathways to produce alstonine and serpentine.

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Complementary roles for mechanical and solvent-based recycling in low-carbon, circular polypropylene

Background/Objective

Key to creating a circular economy for carbon-based products such as polymers is the ability to efficiently recycle them to high-quality post-consumer resins

Approach

We obtained industry data on polypropylene recycling processes to quantify how conventional mechanical recycling compares to solvent-assisted "advanced" recycling on a life-cycle GHG basis.

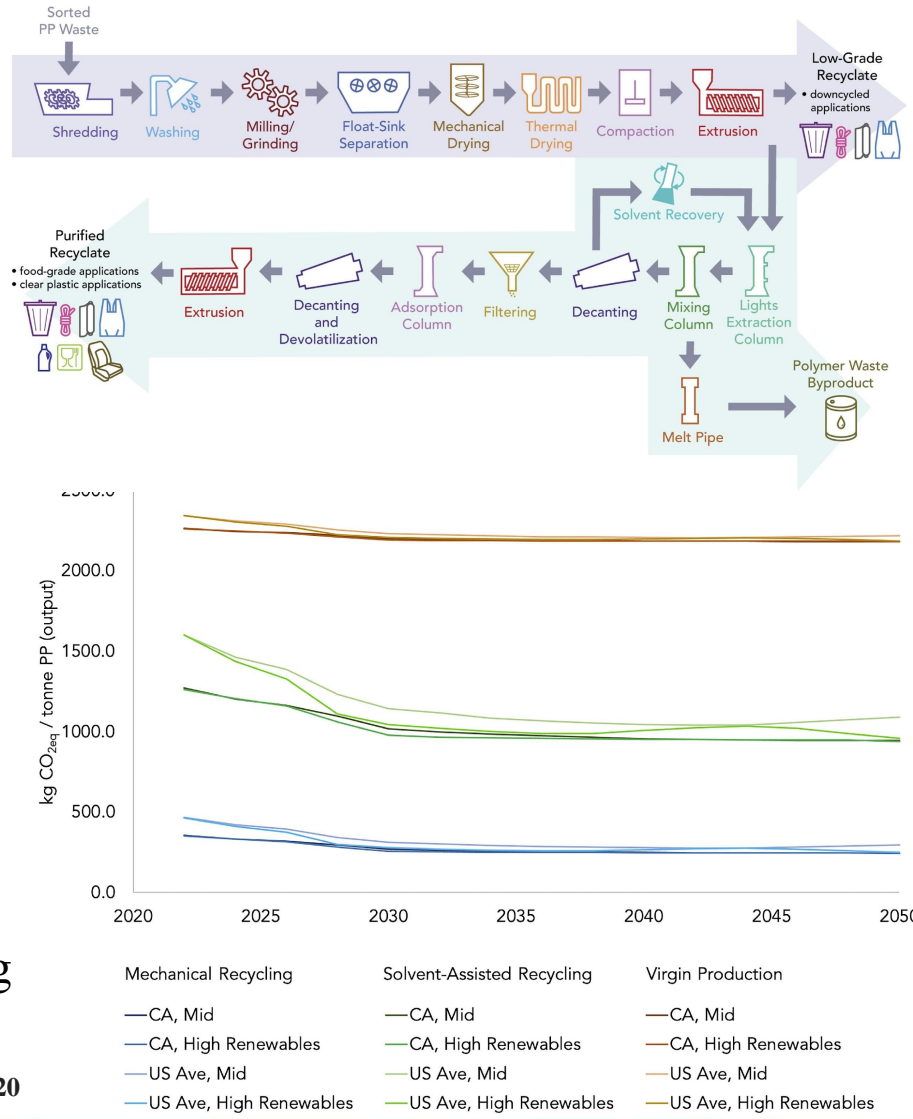
Results

Many of the processes involved in mechanical recycling are required as a pre-processing step for solvent-assisted recycling and other advanced processing. Solvent-based processes can reduce GHG and satisfy markets that require a quality that cannot be achieved through mechanical recycling.

Significance/Impacts

The suggests a shift in paradigm, with mechanical recycling processes serving as a default first step, with some resins being routed to additional upgrading.

Nordahl, S. L., et al. Proceedings of the National Academy of Sciences of the United States of America. doi: 10.1073/pnas.2306902120



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From lab to table: Expanding gastronomic possibilities with fermentation using the edible fungus Neurospora intermedia

Background/Objective

- Fermentation is a powerful tool for producing new flavors and textures for sustainable foods
- We sought to characterize the edible fungus *Neurospora intermedia* and its potential for fermented foods

Approach

Working with two Michelin-star restaurant Alchemist in Copenhagen, we explored the culinary potential of *Neurospora intermedia* by growing it on different plant-based substrates and analyzing the flavor and sensory profiles

Results

We uncovered that *Neurospora intermedia* can be used for the bioconversion of diverse plant-based substrates into palatable and delicious foods positively perceived by consumers, including meat substitutes and a new grain-based beverage naturally sweetened by fungal enzymes

Significance/Impacts

Adds to the microbial toolkit available for bioconversion of plant-based substrates into food; these results inspired a new dish available at Alchemist

Rekdal V.M., et al. Intl J Gastronomy and Food Science. doi: 10.1016/j.ijgfs.2023.100826

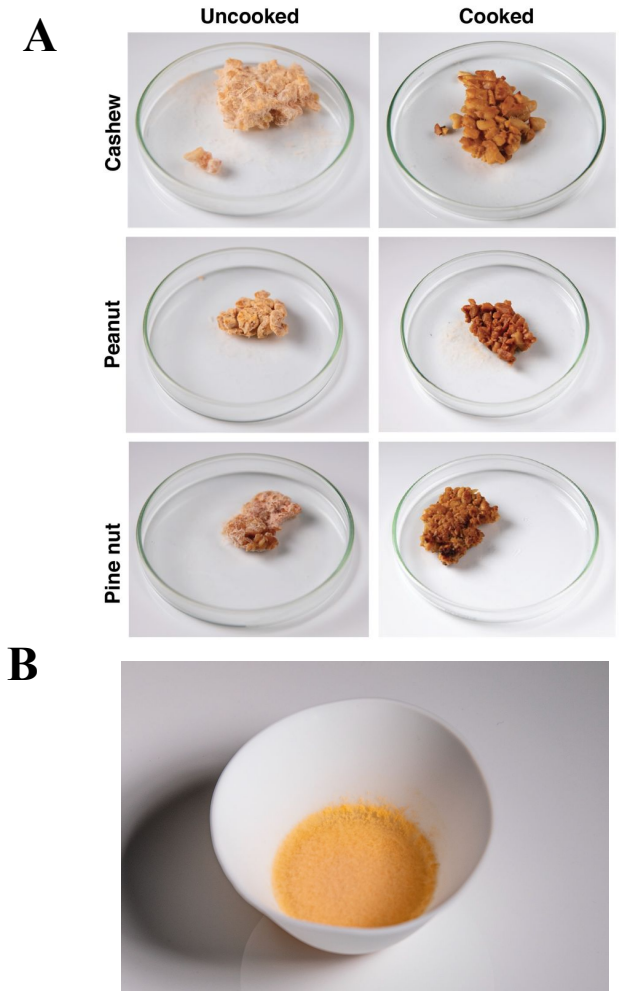

Figure 1: A) growth of *Neurospora intermedia* on different substrates, in the raw and cooked form. b) new dish on menu of Alchemist restaurant