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Integration of genome-scale metabolic model with biorefinery process model reveals market-competitive carbon-negative sustainable aviation fuel utilizing microbial cell mass lipids and biogenic CO_2

Background/Objective

- Producing scalable, economically viable, low-carbon biofuels or biochemicals hinges on more efficient bioconversion processes.
- While microbial conversion can offer robust solutions, the native microbial growth process often redirects a large fraction of carbon to CO2 and cell mass.

Approach

We modeled a biorefinery that makes full use of cell pass and biogenic CO2 to produce a range of fuels and products.

Results

Upgrading microbial lipids or both microbial lipids and CO2 using renewable hydrogen produces carbon-negative bisabolene. On-site electrolytic hydrogen production offers a supply of pure oxygen to use in place of air for bioconversion and fuel combustion in the boiler.

Significance/Impacts

These biorefineries increase carbon utilization efficiency and compete for incentives targeted at very low-GHG fuels.

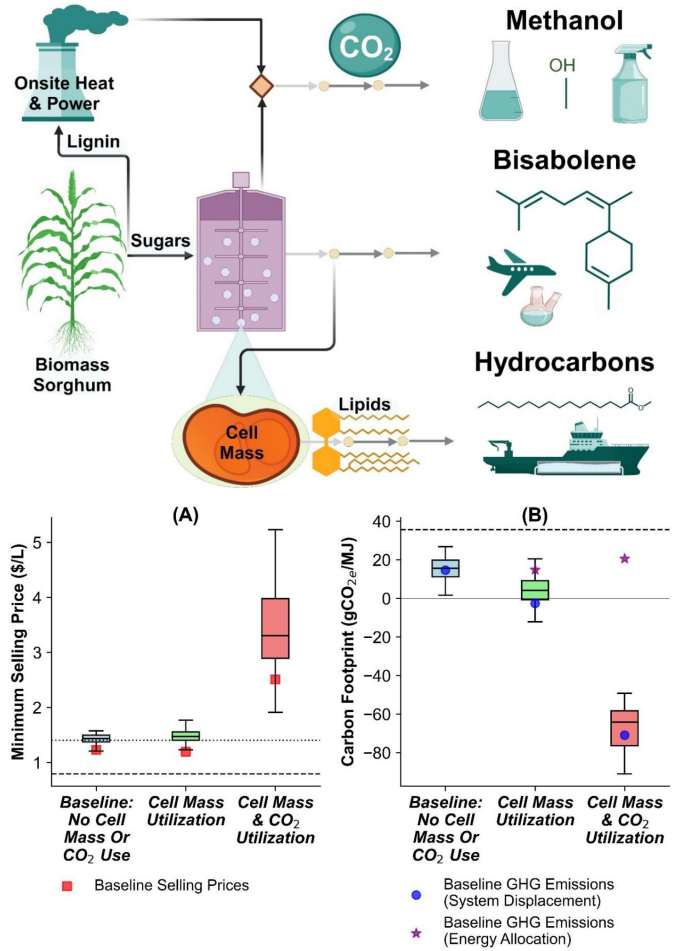

Figure (top) shows a system for converting biomass sorghum to bisabolene and lipid-derived hydrocarbons. Biogenic can be converted to methanol. Figure (bottom) shows minimum selling price and life-cycle GHG footprint results.

Baral N.R., et al. BioResources. doi: 10.15376/biores.19.3.4056-4086

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Ectopic production of 3,4-dihydroxybenzoate in planta affects cellulose structure and organization

Background/Objective

- Transgenic poplar that produces 3,4-dihydroxybenzoic acid (DHBA) is known to have decreased lignin content and altered lignin composition.
- How these modifications affect cellulose is unknown.

Approach

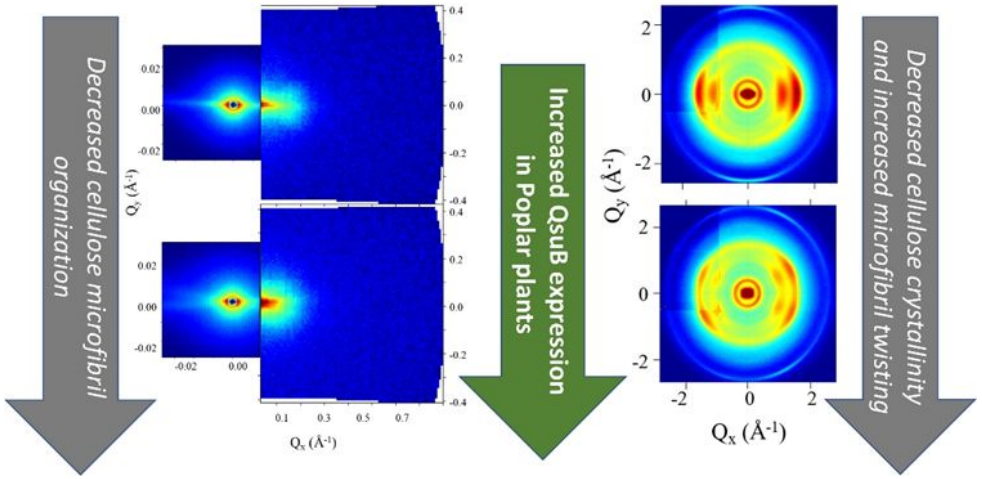
Poplar QsuB transgenic lines that accumulate DHBA were studied. SANS of stem pieces provided nanoscale structural features. WAXS of freeze-dried stems provided atomic level details cellulose structure. Cellulose producing *Acetobacter sp*. cultures grown with DHBA showed its incorporation into cellulose structure.

Results

This work shows that the cellulose organization is disrupted and that cellulose crystalline structure is changed significantly in QsuB poplar.

Significance/Impacts

The nano- and atomic-scale changes in cell wall structure in QsuB poplar show how elevated metabolites that disrupt cell wall formation can be an attractive strategy to reduce biomass recalcitrance for production of biofuels and bioproducts.



Graphics show changes in poplar cell wall structure with increased expression of 3-dehydroshikimate dehydratase (QsuB). **Left panel:** 2D Small angle neutron scattering (SANS) detector images of wild-type (top) and QsuB1 mutant (bottom). **Right panel**: wide angle X-ray scattering (WAXS) images.

Senenayake M., et. al. Biomacromolecules. doi: 10.1021/acs.biomac.4c00187

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amnogalacturonan-II glycosyltransferase identified gh callus gene editing bypasses embryo lethality

Background/Objective

- Biomass (the plant cell wall) is a complex network of polysaccharides and lignin.
- The highly conserved rhamnogalacturonan-II (RG-II) is the most complex of these polymers.
- Alteration to RG-II structure is lethal, so little known about synthesis since challenging to study. Hypothesised to have a role in cell wall adhesion and organisation.

Approach

We developed a new gene editing method that bypassed lethality to generate null lines in candidate RG-II biosynthesis genes. We collaborated with CBI on RG-II structural analysis.

Results

We validated the method, and then we identified the first eukaryotic CMP-Kdo transferase (RCKT1)

Significance/Impacts

- RG-II is a key which unlocks cell wall architecture, so understand how it is synthesised and how it functions is critical
- This method allows us to characterise additional lethal genes in future work

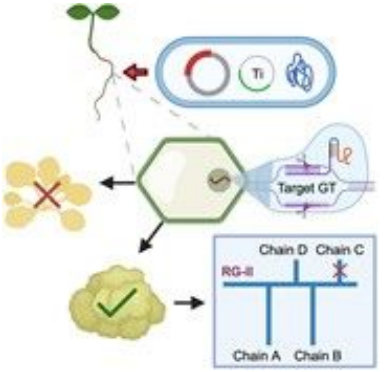
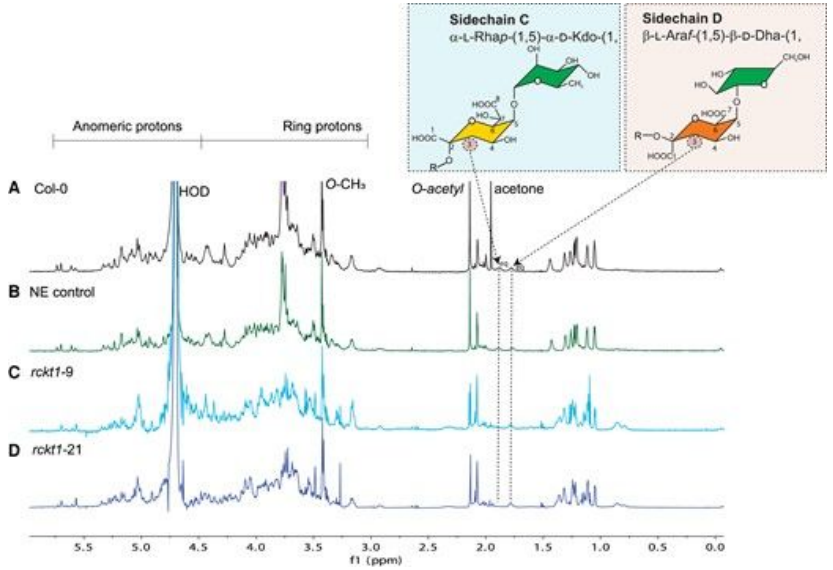


Figure (L): Overview of gene editing approach and Figure (below): 1H-NMR analysis of RG-II. RG-II (dimers) was isolated from the callus cell wall of A) Col-0 wild-type, B) Non-edited control, C) *rckt1-9*, and D) *rckt1-21* and subsequently treated with dilute HCl to generate monomers for analysis. The diagnostic resonance of 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo) is shown in A) (black) and B) (green) but absent from C) (light blue) and D) (dark blue).



Zhang Y., et. al. Plant physiology. doi: 10.1093/plphys/kiae259

ENABLED PUBLICATIONS

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Agricultural Waste Management by Production of Second-Generation Bioethanol from Sugarcane Bagasse Using Indigenous Yeast Strain

Background/Objective

- The escalating demand of fossil fuels has accelerated depletion of finite energy reservoirs, necessitating urgent exploration of sustainable alternatives.
- Our primary objective was to discover a non-conventional yeast strain capable of growing on multiple C-sources with potential ability for lignocellulose bioconversion.

Approach

- We successfully isolated a yeast strain for bioethanol production, capable of effectively utilizing both hexose and pentose sugars.
- We conducted pre-treatment using organic acids, releasing xylose sugars, which were then fermented into ethanol using Clavispora lusitaniae QG1 (MN592676) strain.

Results

- The highest reducing sugar yield was 1.2% with a solid to liquid ratio of 1:25 (% w/v) and an acid concentration of 1% that was significantly higher yield obtained under similar conditions at 100 C for 1 h.
- Moreover, the newly isolated strain was statistically optimized for fermentation process by Plackett-Burman design to achieve highest ethanol yield.

Table 2 Ethanol titres and percent of theoretical yield achieved through consumption of glucose and xylose by strains QG1 and QG2

Yeast strain	Ethanol titer (% w/v)	Theoretical yield (%)
QG1 (Glucose)	63.1	82.4
QG1 (Xylose)	12.3	80.5
QG2 (Glucose)	38.7	50.5
QG2 (Xylose)	5.1	33.7

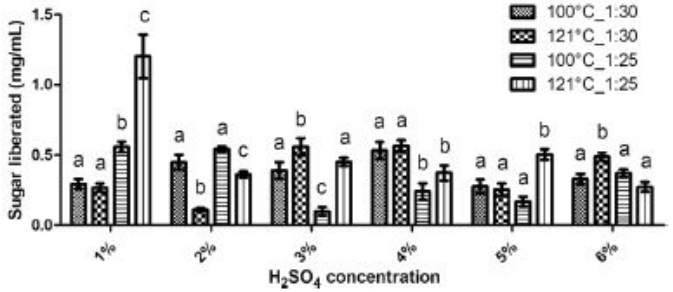

Fig. 2 Sugars liberated at solid to liquid ratio 1:30 and 1:25 and temperature 100 °C (1 h) and 121 °C (15 min). Sugarcane bagasse was pretreated with H_2SO_4 at concentrations (1–6%) for release of sugars. Different lowercase letters on the adjacent bars represent significant ($P<0.05$) difference. The data was presented as mean ± standard deviation (SD). Two-way ANOVA was used followed by Bonferroni's post-test for multiple comparisons. All the reactions were carried out in duplicate

Significance/Impacts

The desirable characteristics depicted by newly isolated strain shows its promising utilization in management of industrial waste like sugarcane bagasse by its conversion into renewable biofuels like ethanol with its future potential for lignocellulose bioconversion.

Ali S., et. al. Curr Microbiol. doi: 10.1007/s00284-024-03668-y

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XA21-mediated resistance to Xanthomonas oryzae pv. oryzae is dose dependent

Background/Objective

The rice receptor kinase XA21 confers broad-spectrum resistance to Xanthomonas oryzae pv. oryzae (Xoo), the causal agent of rice bacterial blight disease.

Approach

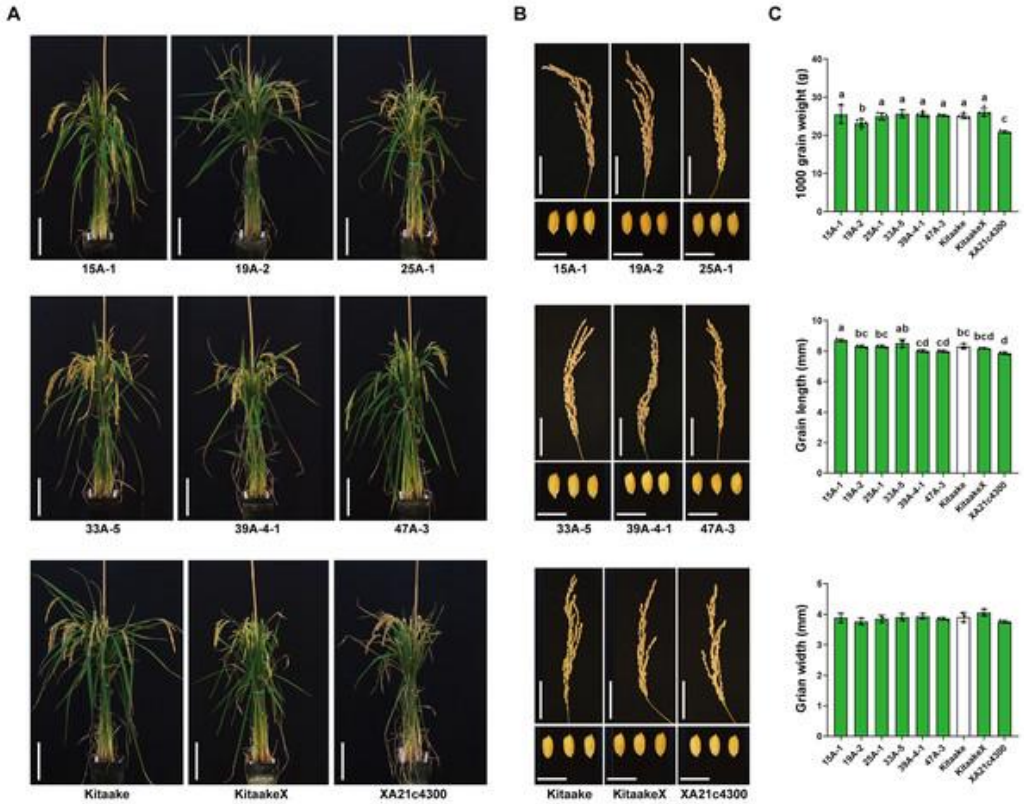
To investigate the relationship between the expression level of XA21 and resulting resistance, we generated independent HA-XA21 transgenic rice lines accumulating the XA21 immune receptor fused with an HA epitope tag.

Results

- XA21-mediated resistance is dose dependent.
- Yield is unlikely to be affected by the expression level of HA-XA21.

Significance/Impacts

The results add to the known genomic landing pads in the rice genome that can be targeted for gene insertion without compromising yield.



Agronomic trait analysis of the homozygous HA-XA21 transformation events.
(A) Morphology of homozygous HA-XA21 transgenic plants ten weeks after transplanted to soil. Each pot contains three plants of the indicated line. All plants were grown in greenhouses under similar conditions. Kitaake and transgenic Kitaake lines expressing XA21 under the maize Ubi-1 promoter (KitaakeX) or the native XA21 promoter (XA21c4300) were included as controls. Bars represent 20 cm. (B). Upper: pictures of panicles from the plants in (A). Bars represent 5 cm. Lower: picture of grains from the plants in (A). Bars represent one cm. (C). 1000-grain weight, grain length, and grain width of plants in (A). Green bars correspond to plants harboring the XA21 transgene while the white bar represents the Kitaake control. Values are means ± SD. Different letters indicate significant differences ranked by pairwise multiple comparison followed by Tukey's test ($P < 0.05$).

Zhang N., et. al. PeerJ. 10.7717/peerj.17323

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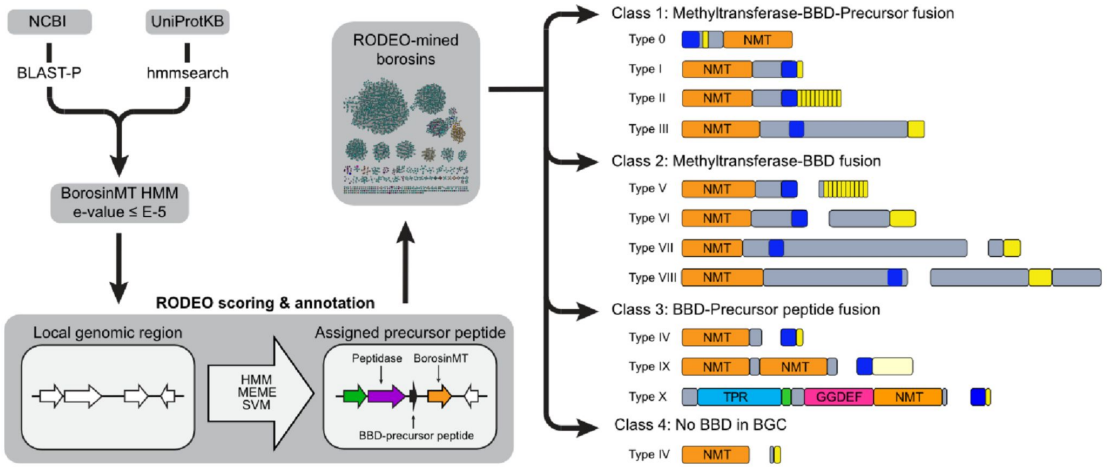
Discovery of Borosin Catalytic Strategies and Function through Bioinformatic Profiling

Background/Objective

- Borosins are a recently elucidated class of ribosomally synthesized and post-translationally modified (RiPP) natural product bearing backbone *N*-methylation and featuring anti-parasitic bioactivity.
- The distribution of these biosynthetic gene clusters remained unexplored.

Approach

The bioinformatics program Rapid ORF and Description & Evaluation Online (RODEO) was expanded in functionality to enable the genome mining of borosin gene clusters in sequenced organisms.



The bioinformatics workflow used to identify borosin biosynthetic gene clusters in sequenced organisms in NCBI and UniProt (left). This bioinformatics survey led to the discovery of many novel cluster architectures (right).

Results

~3000 borosin clusters were identified in bacteria, archaea, and fungi with many featuring diverse precursor peptides and novel biosynthetic architectures, which can be rapidly identified by RODEO.

Significance/Impacts

Discovering novel borosins may lead to useful novel bioactive RiPPs and furthermore the biosynthetic enzymes may be repurposed to create engineered *N*-methylated peptides with enhanced protease resistance and/or membrane permeability/bioavailability.

Lee A.R., et. al. ACS chemical biology. doi: 10.1021/acschembio.4c00066

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Complete biosynthesis of QS-21 in engineered yeast

Background/Objective

- QS-21 is a potent vaccine adjuvant that has been approved by the FDA.
- Sourcing QS-21 remains difficult due to the laborious chemical synthesis or unsustainable harvesting from and extraction of *Quillaja saponaria*.

Approach

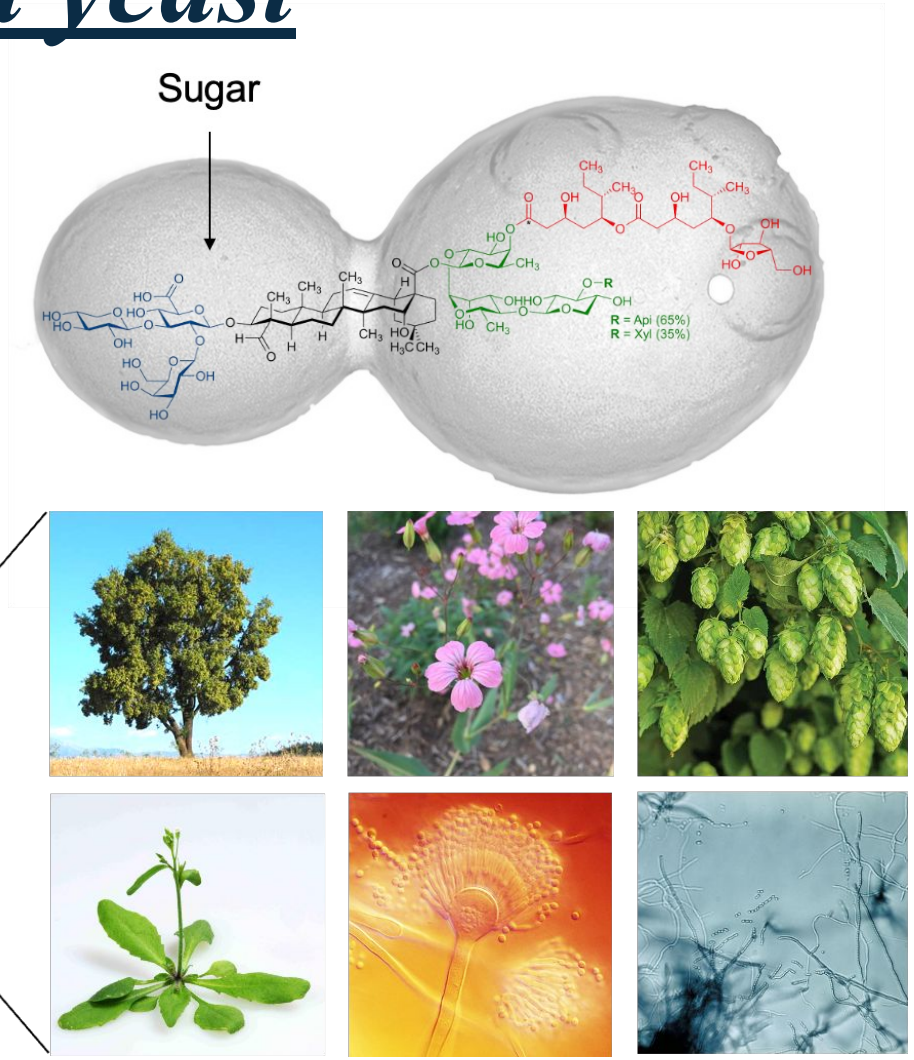
- Gene discovery and integration of 38 heterologous genes, sourced from six different organisms, in engineered *Saccharomyces cerevisiae.*
- While balancing the native metabolism of the host itself.

Results

- We were able to achieve the complete biosynthesis of QS-21-Xyl and QS-21-Api, as well as their structural derivatives in engineered yeast.

Significance/Impacts

- 1000-times higher space-time yield
- Immediate on-demand scalability
- Sustainable and portable production scheme



Liu Y., et. al. Complete biosynthesis of QS-21 in engineered yeast." Nature. doi: 10.1038/s41586-024-07345-9

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Data assimilation converges global soil organic carbon simulations of structurally different models

Background/Objective

To investigate roles of model structure versus parameters in generating the inter-model uncertainty while predicting global soil organic carbon (SOC) dynamics.

Approach

We used global soil profile observations, data of environmental factors, and two process-based models CLM5 and COMPAS.

Results

Convergence in global SOC simulations resulted from similar simulations of key model components such as carbon transfer efficiency, baseline decomposition rate, and the impact of environmental factors on carbon fluxes after data assimilation.

Significance/Impacts

Data assimilation can inform new model process development and constrain model uncertainties.

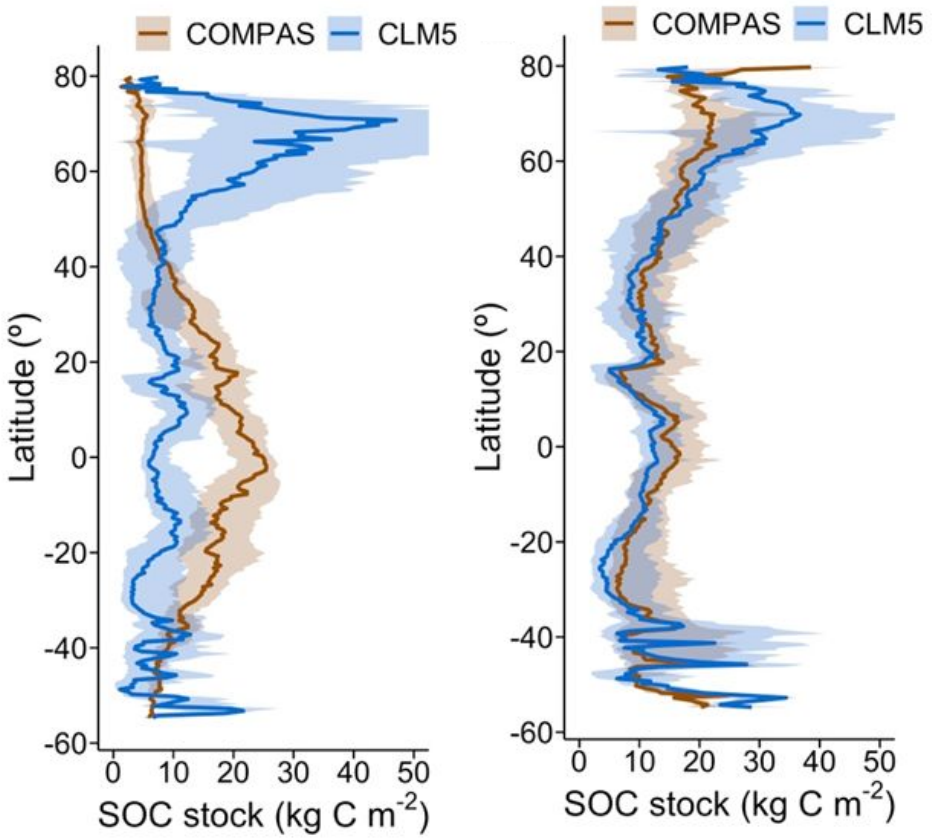

Figure: Global SOC stocks simulations from two structurally different models (CLM5 & COMPAS) before (left) and after (right) data assimilation.

Tao F., et. al. Global change biology. doi: 10.1111/gcb.17297

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Advances in Engineering Nucleotide Sugar Metabolism for Natural Product Glycosylation in Saccharomyces cerevisiae

Background/Objective

Glycosylation is a ubiquitous modification present across all of biology, affecting many things such as physicochemical properties, cellular recognition, subcellular localization, and immunogenicity.

Approach

Saccharomyces cerevisiae is a potentially powerful platform for producing glycosylated biomolecules, but it lacks nucleotide sugar diversity.

Results

Nucleotide sugar metabolism is complex, and understanding how to engineer it will be necessary to both access and study heterologous glycosylations found across biology.

Significance/Impacts

This review overviews the potential challenges with engineering nucleotide sugar metabolism in yeast.

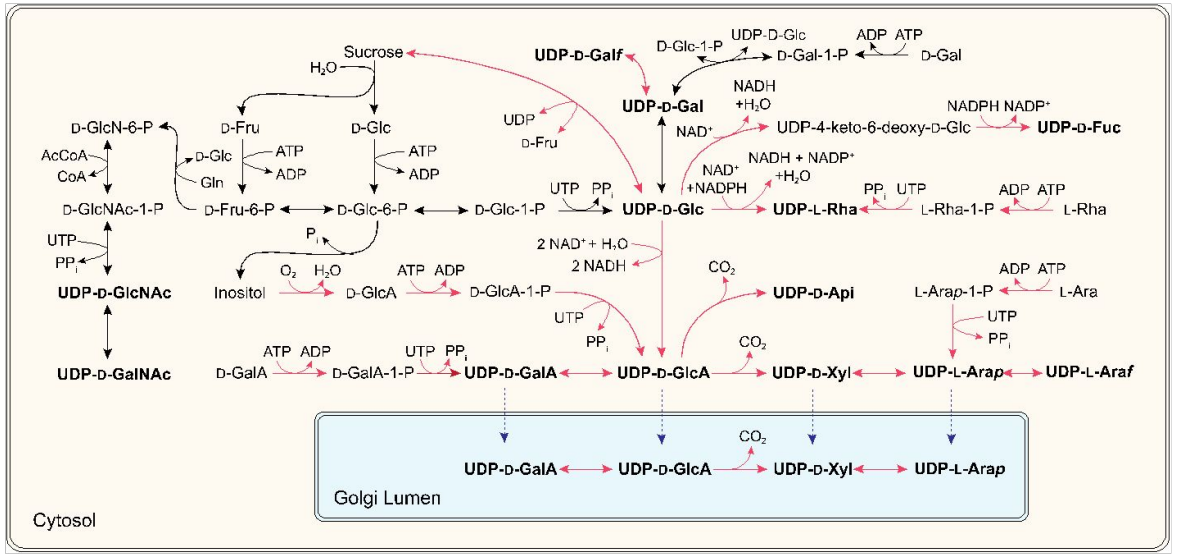

Figure 1. Nucleotide sugar biosynthesis pathways.

Crowe S. A., et. al. ACS synthetic biology. doi: 10.1021/acssynbio.3c00737

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Biocomposite thermoplastic polyurethanes containing evolved bacterial spores as living fillers to facilitate polymer disintegration

Background/Objective

- We Have a Serious Environmental Problem with Plastic
- >70% of plastics end up as waste primarily in landfills/land
- Plastic wastes decomposition is important (in short term at min.)

Approach

We asked what if we integrate spore-forming TPU-degrading bacteria into TPU (Thermoplastic polyurethane)?

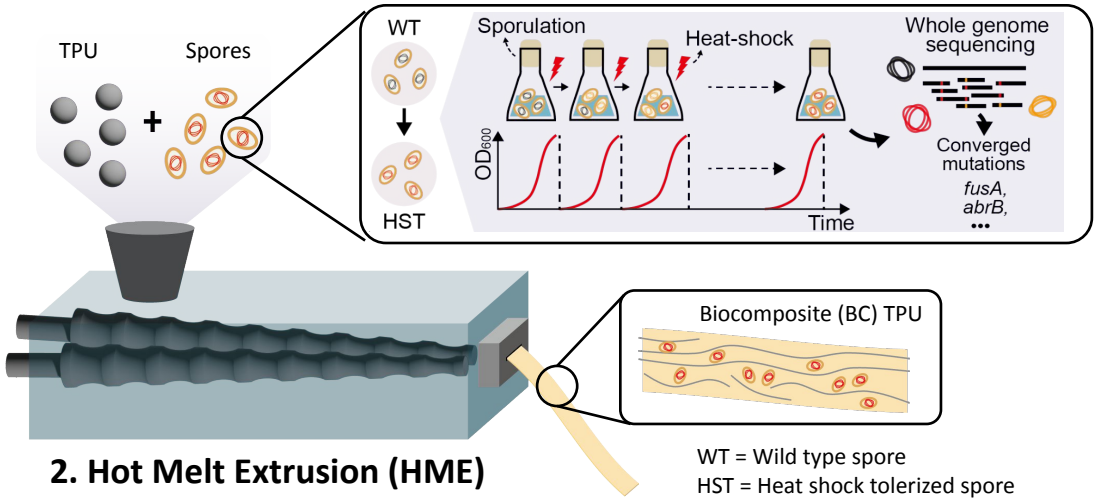
Results

- ALE improved the heat-shock tolerance of B. subtilis spore by up to 17.7-fold and spores retained ~100% viability
- Toughness of TPU was increased by up to 37% by HST spore addition

Significance/Impacts

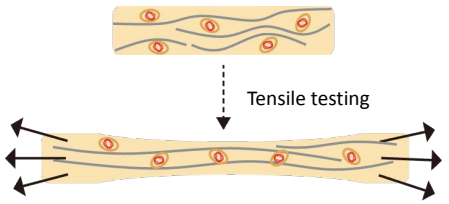
- HST spores enhanced the biodegradation rate of TPU by ~2x
- Spores could be germinated into vegetative cells post extrusion and encode biological functions
- This method could be applied to many more plastics and polymers

1. Heat Shock Tolerization (HST) ALE Optimized Strains

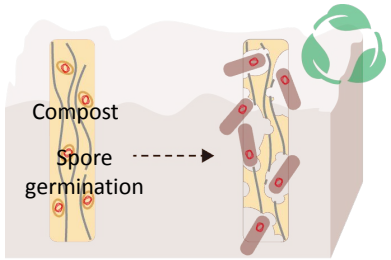


2. Hot Melt Extrusion (HME)

3. Advanced Mechanical Properties



4. Programmable & Faster disintegration



Kim H.S., et. al. Nat Commun. doi: 10.1038/s41467-024-47132-8

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Optimization of Electroporation Method and Promoter Evaluation for Type-1 Methanotroph, Methylotuvimicrobium alcaliphilum

Background/Objective

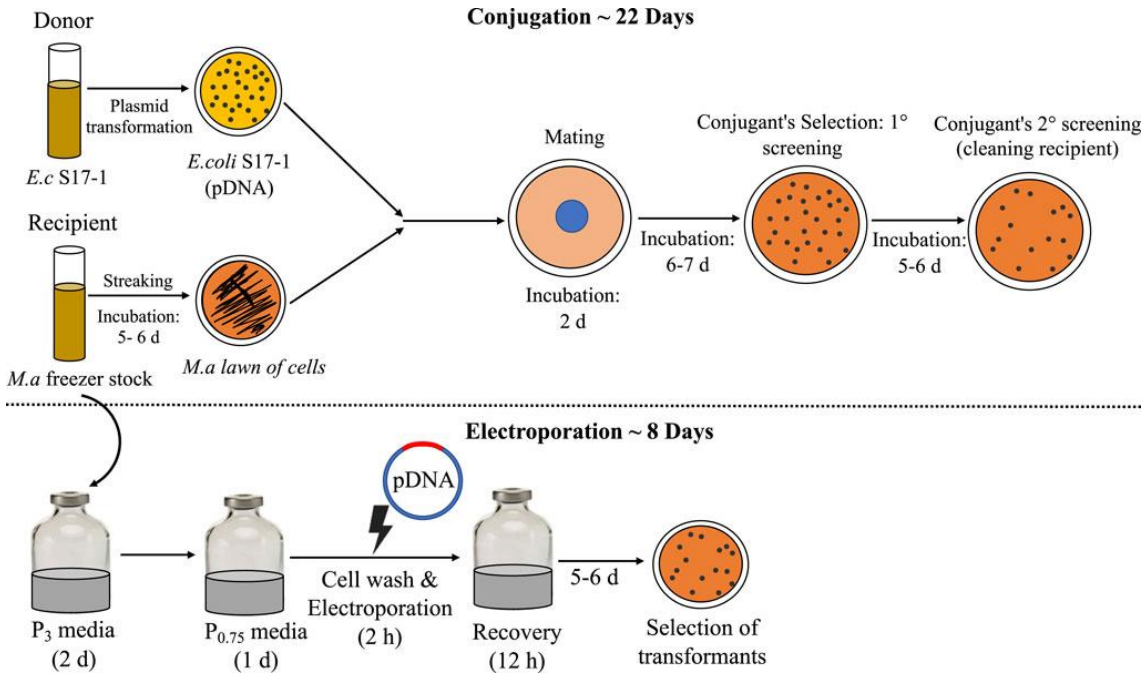
- Methanotrophic bacteria are promising hosts for methane bioconversion to biochemicals or bioproducts.
- Methanotrophs are currently not employed for bioconversion technologies due to long time-lines and lack of tools.

Approach

A rapid and reproducible electroporation protocol was developed for type 1 methanotroph, Methylotuvimicrobium alcaliphilum using common laboratory solutions, analyzing optimal electroshock voltages and post-shock cell recovery time.

Results

A ~ 3-fold decrease in time is reported with use of electroporation protocol developed here, compared to conjugation, which is the traditionally employed approach.



Comparison of conjugation and electroporation: the steps involved and timeline. E.c, E. coli; M.a, M. alcaliphilum

Significance/Impacts

An inducible (3-methyl benzoate) and a constitutive (sucrose phosphate synthase) promoter was characterized for their strength in driving gene expression.

Goswami S., et. al. Frontiers in Bioengineering and Biotechnology. doi: 10.3389/fbioe.2024.1412410