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Continuous culture of anaerobic fungi enables growth and metabolic flux tuning without use of genetic tools

Background/Objective

- Anaerobic gut fungi (AGF) excel at lignocellulose breakdown, but no genetic tools are available to manipulate growth and metabolic flux.
- AGF are not yet able to be cultivated in continuous bioreactors.

Approach

Herein, a cost-effective, Arduino-based, continuous-flow anaerobic bioreactor with online optical density control is presented to probe metabolism and predictably tune fluxes in the AGF strain *Caecomyces churrovis*

Results

Varying the *C. churrovis* turbidostat setpoint titer reliably controlled growth rate, metabolic flux, and production rates of acetate, formate, lactate, and ethanol. Bioreactor setpoints to maximize production of each product were identified, and all continuous production rates significantly exceed batch rates.

Significance/Impacts

The DIY bioreactor culture schemes demonstrated here offer tools to tailor AGF fermentations to application-specific hydrolysate product profiles.

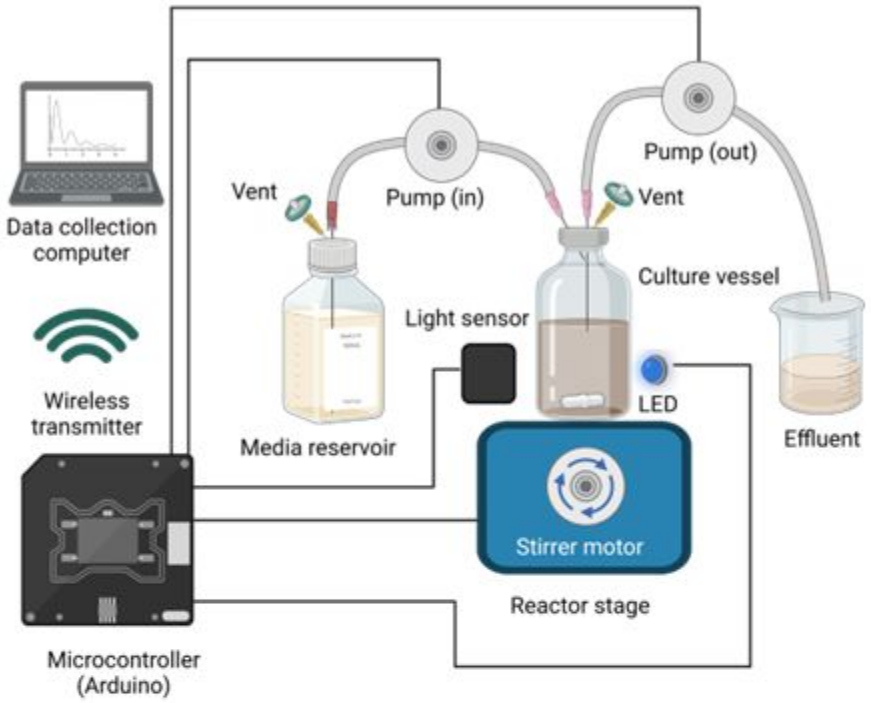

Figure 1: A continuous, high-throughput bioreactor was constructed to enable anaerobic growth and bioreactor setpoint evaluation of anaerobic fungal cultures.

Leggieri P. A., et. al. Bioresource technology. doi: 10.1016/j.biortech.2023.129854

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Simultaneous suppression of lignin, tricin and wall-bound phenolic biosynthesis via the expression of monolignol 4-O-methyltransferases in rice

Background/Objective

Grass crops yield billion tons of non-woody lignocellulosics annually, but their cell walls feature complex compositions and structures, which make grass lignocellulosics difficult to optimize for effective biofuel/bioproduct applications. We assess the efficacy of two engineered monolignol 4-*O*-methyltransferases (MOMTs) that are designed to alter the chemical structure of monolignols in modifying the lignocellulosic properties of rice.

Approach

Two MOMT variants were overexpressed in rice driven by the *C4H* promoter. The resulting transgenic lines were subjected to comprehensive chemical analyses. The biomass was pre-treated with either acid or alkaline solutions and tested for saccharification yield.

Results

MOMTs displayed promiscuous activities, leading to complex effects on rice cell walls and growth rates. Expression of MOMTs resulted in the suppression of >50% of guaiacyl lignin and up to 90% of syringyl lignin. The levels of tricin in lignin and wall-bound ferulate were reduced by up to 50%. Concomitantly, the novel 4-*O*-methylated phenolics were accumulated to substantial levels. These changes resulted in up to 30% increase in saccharification yield of rice straw biomass.

Significance/Impacts

The study demonstrates an effective strategy to tailor cell walls of grass to generate improved cellulosic feedstocks for the production of fermentable sugar-based biofuels and bioproducts.

Dwivedi., et. al. *Plant Biotech J*. doi: http://doi.org/10.1111/pbi.14186

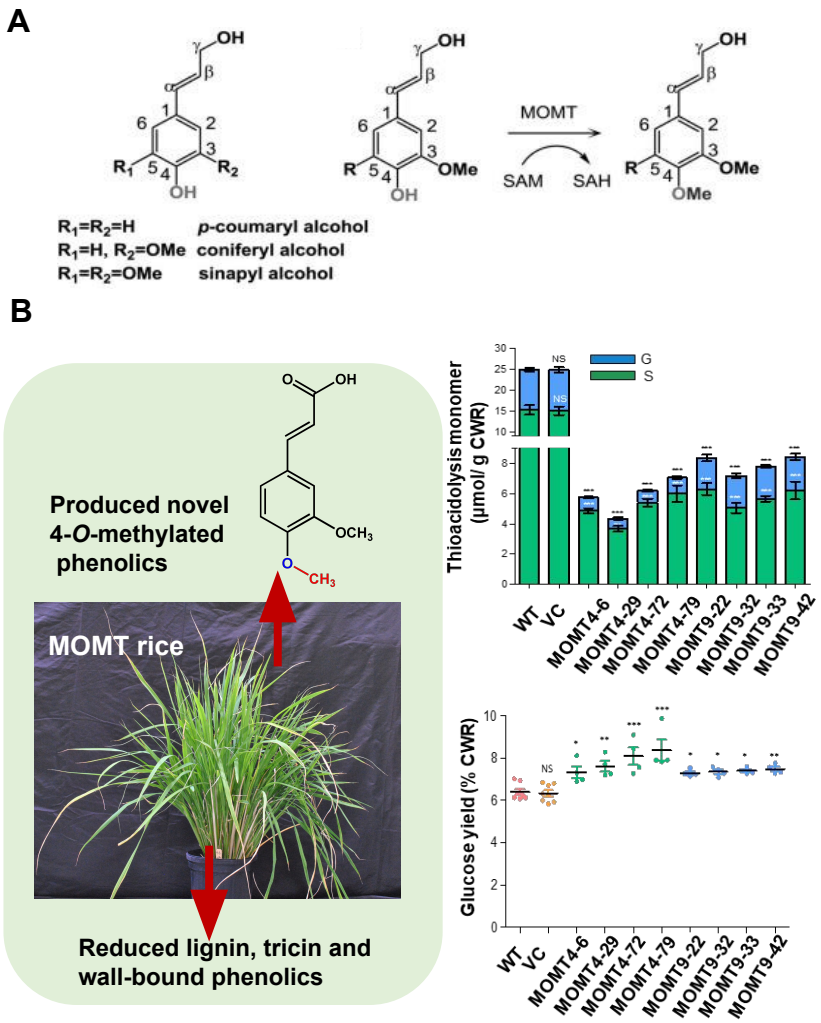

Figure 1. (A) Scheme of MOMT catalyzed reaction. **(B)** Effects of MOMT overexpression in rice and saccharification efficiency of transgenic biomass.

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IMA genome-F18 : The re-identification of Penicillium genomes available in NCBI and draft genomes for Penicillium species from dry cured meat, Penicillium biforme, P. brevicompactum, P. solitum, and P. cvjetkovicii, Pewenomyces kutranfy, Pew. lalenivora, Pew. tapulicola, Pew. kalosus, Teratosphaeria carnegiei, and Trichoderma atroviride SC1

Background/Objective

The fungal genus *Penicillium* is home to tremendous biochemical and enzymatic diversity that contributes to its phenotype and may also have use in biotechnology

Approach

Working with the Joint Genome Institute (JGI) we have sequenced the genome of a variety of *Penicillium* species

Results

We have generated high quality draft genomes of a breadth of *Penicillium* species

Significance/Impacts

The *Penicillium* genomes encode a significant catalog of biomass deconstruction enzymes and complex secondary metabolite biosynthetic pathways that could be used in biotechnology applications.

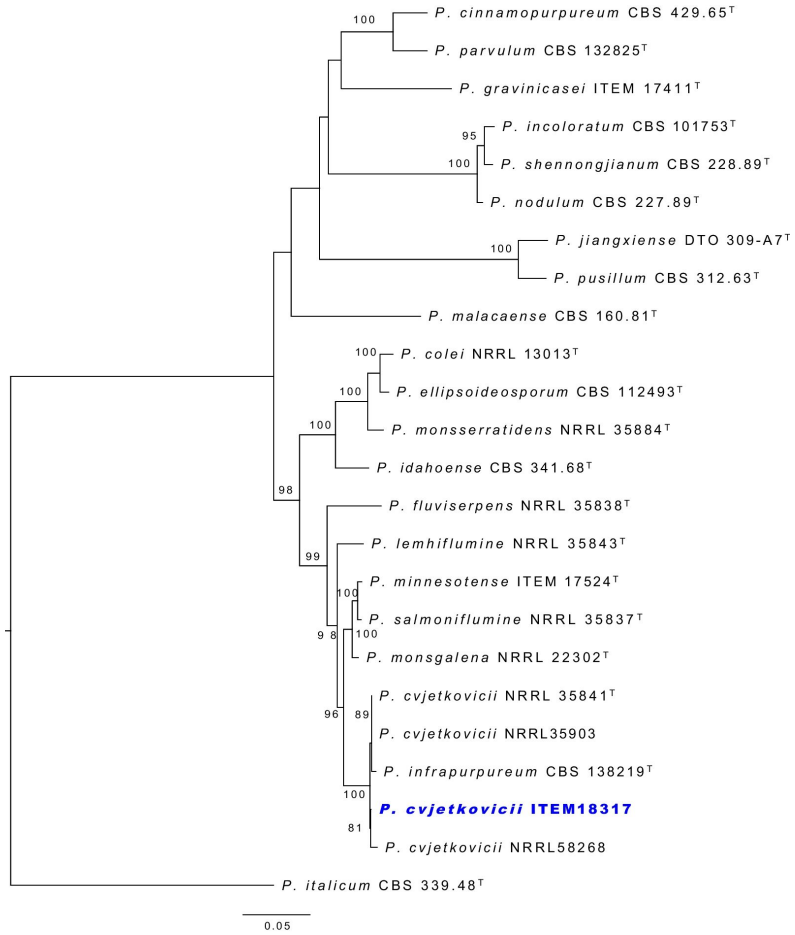

Figure 1: ML phylogenetic tree of *Penicillium* section *Cinnamopurpurea*, indicating the sequenced isolate of *P. cvjetkovicii* ITEM 18317 (in blue), and ultrafast bootstrap support at the nodes

Visagie C. M., et. al. IMA fungus. doi: 10.1186/s43008-023-00121-w

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Module-based polyketide synthase engineering for de novo polyketide biosynthesis

Background/Objective

- Polyketide synthases (PKSs) represent an attractive avenue to the access of truly complex carboxylic molecules.
- This review summarizes methods for engineering PKSs to produce new-to-nature molecules.

Approach

Retrobiosynthesis can be used to choose which PKS modules and domains to produce nearly any desired organic molecule.

Results

Mixing and matching modules from natural PKSs is one of the routes to produce these molecules. Recent advances in structural modeling and synthetic biology present an opportunity to accelerate PKS engineering by re-evaluating insights gained from previous engineering efforts with cutting edge tools

Significance/Impacts

PKS collinearity presents an interesting opportunity for machine-learning scientists to test modern protein design strategies on a system with a reduced design space.

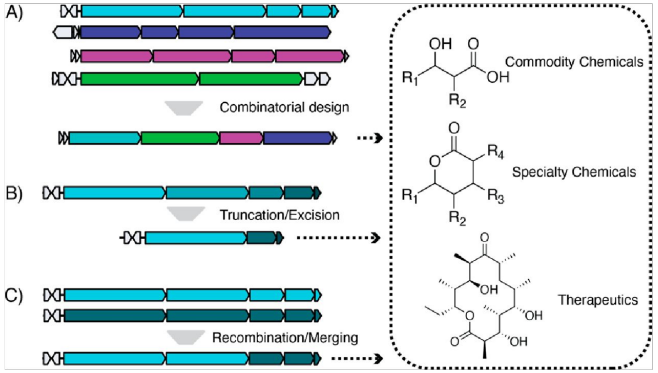

Figure 1: Module-based PKS engineering for commodity chemicals, specialty chemicals and therapeutics.

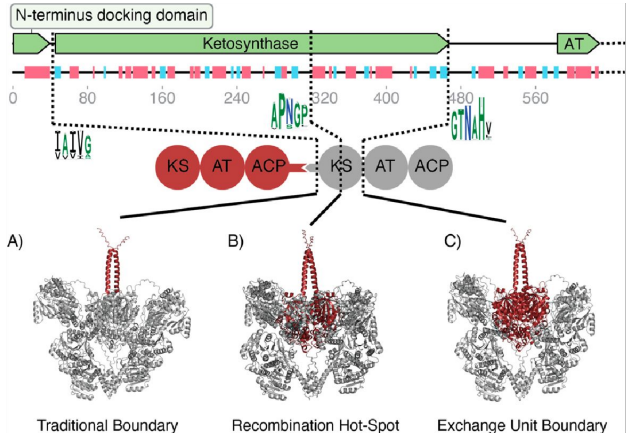

Figure 2: PKS module junction selected is critical to the solubility and activity of engineered PKSs.

Nava A., et. al. ACS Synthetic Biology. doi: 10.1021/acssynbio.3c00282

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Maximizing Heterologous Expression of Engineered Type I Polyketide Synthases: Investigating Codon Optimization Strategies

Background/Objective

- Systematic analysis of codon optimization strategies in industrial hosts
- Improve heterologous expression of engineered type I PKSs

Approach

Three different codon optimization algorithms were applied to an engineered PKS, targeting expression in *C. glutamicum*, *E. coli* and *P. putida*. Analysis of resulting transcript, protein and product amount determined best strategy.

Results

- Established potential high-throughput strain build process using serine-assisted genomic engineering (SAGE)
- Identified most suitable codon optimization strategy for targeted hosts
- Developed a web application for ease of use with up-to-date codon usage tables (https://basebuddy.lbl.gov)

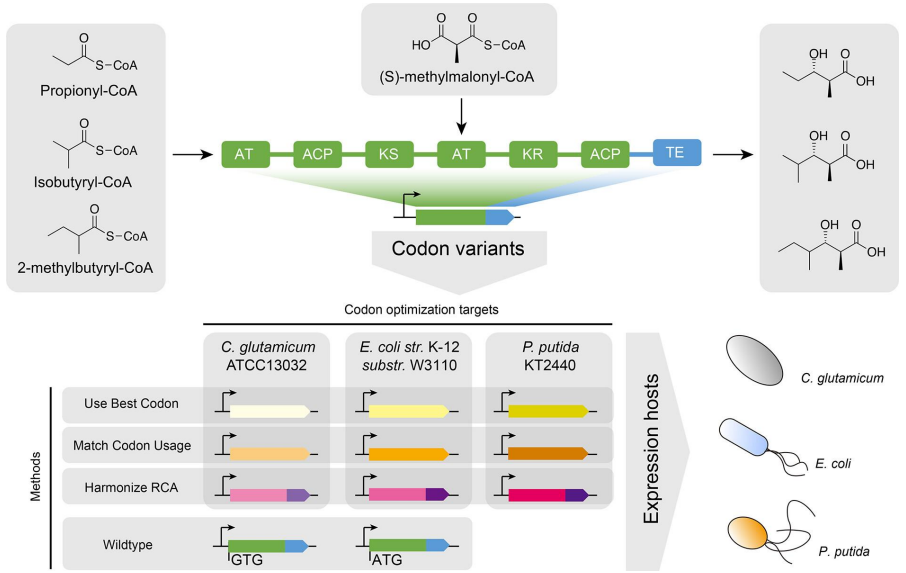

Figure 1: Engineered polyketide synthase with applied codon optimization strategies and targeted heterologous hosts.

Impact

Highlighted the significance of codon optimization and laid out the foundation for high-throughput assembly and characterization of PKS pathways in alternative hosts

Schmidt M., et. al. ACS synthetic biology. doi: 10.1021/acssynbio.3c00367

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Advances in genome-scale metabolic models of industrially important fungi

Background/Objective

- Fungi are used for industrial production of biofuels and bioproducts
- Genome-scale metabolic models (GEMs) provide a mathematical framework to gain a systems-level understanding of metabolism

Approach

Recently developed or updated GEMs have been reviewed for several industrially important fungi. Advances in constraint-based methods and machine learning approaches for GEMs have been investigated.

Results

Enzyme-constrained GEMs improve predictions of cellular states. Machine learning methods facilitate the construction of enzyme-constrained GEMs. Fluxes predictions from GEMs can be used as features in machine learning approaches in hybrid frameworks.

Significance/Impacts

GEMs play a pivotal role in iterative design–build–test–learn cycles (Figure 1), ultimately advancing the field of fungal biomanufacturing.

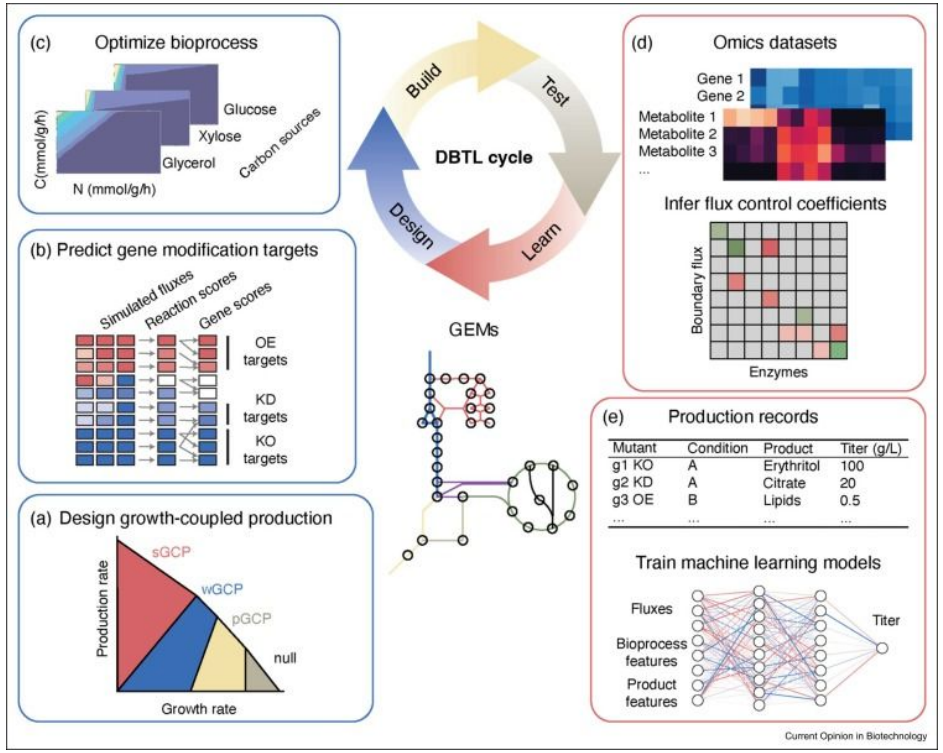

Figure 1: Representative applications of fungal GEMs in biomanufacturing. Applications in the 'design' and 'learn' stages are respectively surrounded by a blue line and a red line

Han Y., et. al. Current opinion in biotechnology. doi: 10.1016/j.copbio.2023.103005

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Towards intrinsically flame-retardant, bioenabled nitrogen aromatic nylon 6,6 comonomers

Background/Objective

- JBEI Target Council identified flame retardant polymers as product of interest
- Trying to find biological target knowing aromatic carbon and nitrogen content potentially important

Approach

Project focused on using a biologically produced molecule as the basis for a synthetic library that was subsequently screened for flammability performance. The biological molecules selected as th starting point was muconic acid.

Results

Replacement of 25% of the adipic acid with the best bioenabled, functionalized monomer from the library increased the charring of the resulting nylon 6,6 by more than 100%.

Significance/Impacts

Biobased molecules can be used to introduce improved performance in end use products by exploiting their unique chemistry.

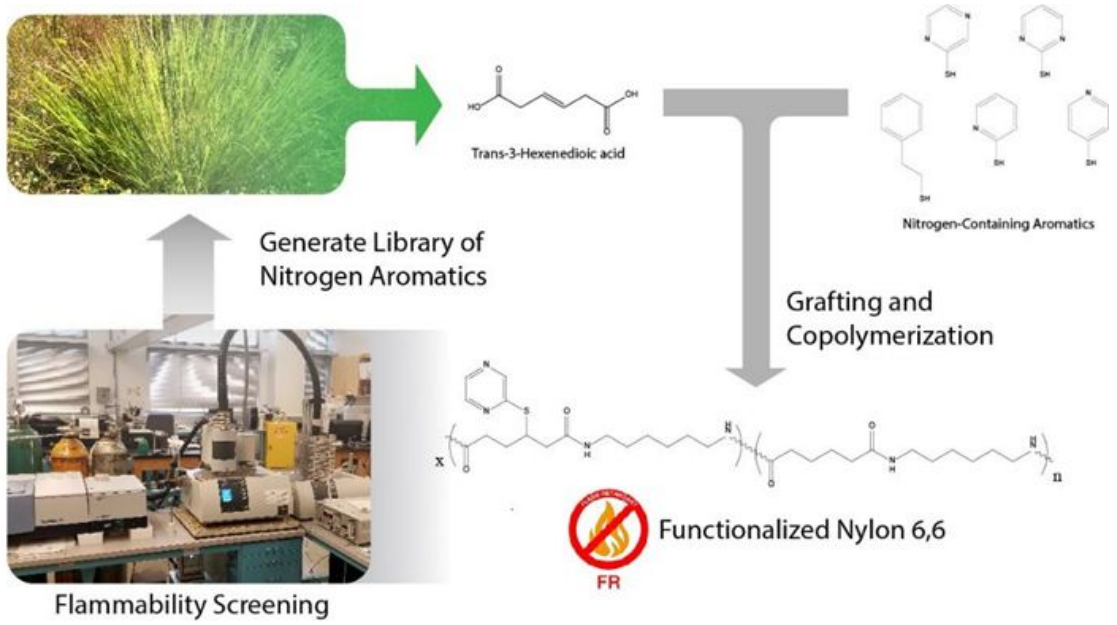

Figure 1: Process to generate property information for future synthesis from comonomer functionalization with nitrogen containing aromatics and copolymerization into nylon 6,6.

Hadel J., et. al. In Chang H.N., Gross R.A. (eds) Sustainable Green Chemistry in Polymer Research. Volume 2. Sustainable Polymers and Applications. ACS Publications. doi: 10.1021/bk-2023-1451.ch008

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Coupling chemistry and biology for the synthesis of advanced bioproducts

Background/Objective

In this opinion paper, we put forth the concept that by carefully considering the merits and drawbacks of synthetic biology and chemistry as well as one's own use case, there exist many opportunities for coupling chemical and biological syntheses

Approach

We discuss the advancements that have been made in bio-based manufacturing and chemical synthesis, examples that have merged methods from both fields, and computer-aided synthesis planning (CASP) tools to accelerate these efforts

Results

- To maximize efficiency and reduce costs, the use of CASP tools is highly encouraged to streamline pathway discovery
- Regardless of the degree of hybridization, all synthesis pathways can begin with biomass

Significance/Impacts

By embracing the merger of biological and chemical syntheses, we are poised to usher in the next generation of advanced bioproducts while prioritizing environmental stewardship

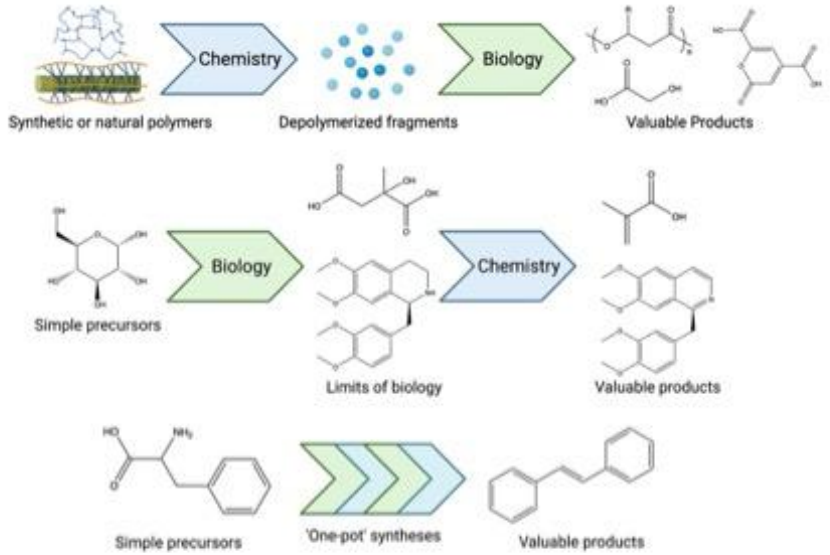

Figure 1: Highlighted potential use-cases of coupling biological and chemical cascades. Chemical catalysis is effective in rapidly breaking down synthetic or natural polymers into fragments that can subsequently be biologically valorized into useful products, such as monomers, monomer precursors or polymers. When beginning from simpler precursors, such as glucose instead, enzymatic transformations are useful until a toxic intermediate is formed or a transformation is not possible biologically. In such scenarios, chemical catalysis can be incorporated to reach the final product.

Chainani Y., et. al. Current Opinion in Biotechnology. doi: 10.1016/j.copbio.2023.102992

JBEI Enabled Papers

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Novel genetic tools improve Penicillium expansum patulin synthase production in Aspergillus niger

Background/Objective

- The CRISPR-Cas9 and disrupted Ku-dependent nonhomologous end-joining (NHEJ) pathway are two current genetic tools for *A. niger*.
- Novel genetic tools enable enhancement of protein and chemical production in this industrial filamentous fungus.

Approach

The NHEJ pathway was initially disrupted and then multiple of glucoamylase landing sites (GLS) were created via CRISPR-Cas9 knockout and homologous knock-in. finally, the expression cassettes were introduced into the multiple GLS.

Results

The *P. expansum* patulin synthase production in *A. niger* was 8-fold improvement as compared to the traditional genetic engineering method.

Significance/Impacts

This is a novel strategy for augmenting the productions of selected proteins or chemicals in the industrial filamentous fungi.

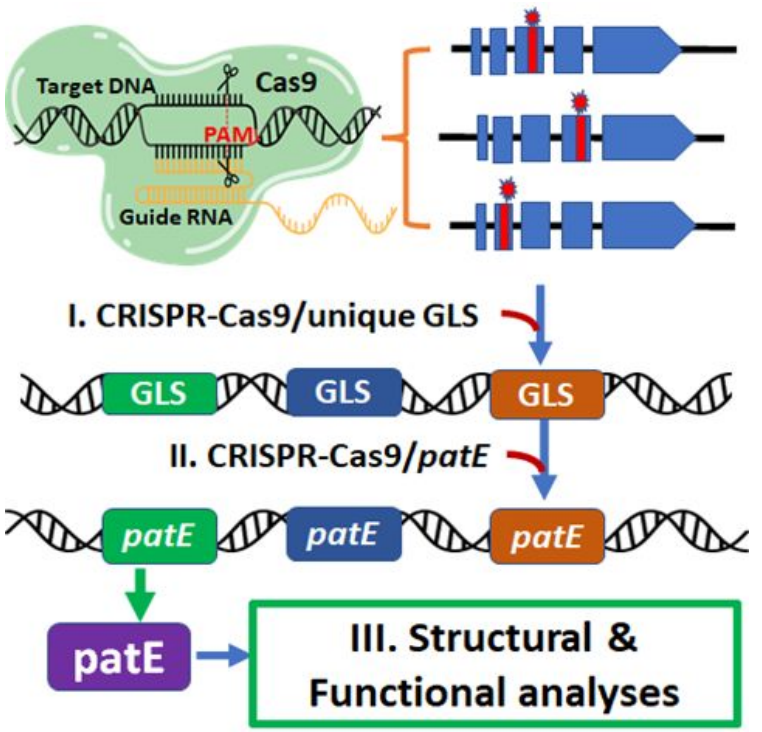

Figure 1: Novel strategy of multiple gene integration in *Aspergillus niger*.

Dai Z. The FEBS journal. doi: 10.1111/febs.16956

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COG-imposed Golgi functional integrity determines the onset of dark-induced senescence

Background/Objective

- Understanding how plants respond to stress under a changing environment is crucial to developing robust crops.

Approach

- In this work, led by researchers at UC Riverside, we used a mutant screen to look for plants which were "constituitvely stressed" and identified that a role for the conserved oligomeric Golgi (COG) complex

Results

- the mutant had accelerated dark-induced carbon deprivation/senescence
- Multiomics and biochemical analyses revealed accelerated induction of protein ubiquitination and autophagy, and a counterintuitive increased protein N-glycosylation in senescencing cog7 relative to wild-type

Significance/Impacts

These findings identify COG-imposed Golgi functional integrity as a main player in ensuring cellular survival under energy-limiting conditions.

Choi H. S., et. al. Nature plants doi: 10.1038/s41477-023-01545-3

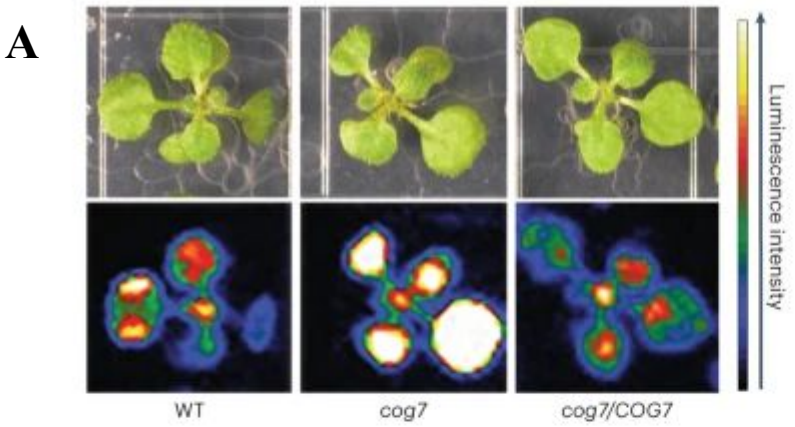

Figure A: Representative images of 2-week-old WT, *cog7* and the complemented *cog7/COG7* seedlings grown in long days (16 h light/8 h dark) and the corresponding dark-field images (lower) showing constitutively active LUC driven by stress-response *cis*-element *RSRE*, exclusively in the *cog7* mutant. The colour-coded bar shows the intensity of LUC activity

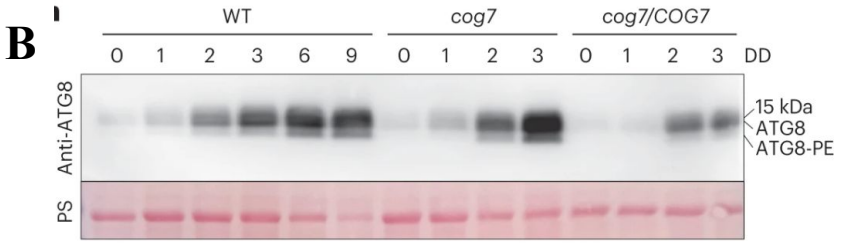

Figure B:, Immunoblot analyses of the autophagy marker protein ATG8/ATG8–PE using anti-ATG8 antibody.