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Branched-Chain Higher Alcohols (A book chapter in "Comprehensive Biotechnology", 4th Ed., Elsevier)

Background/Objective

- BCHAs (≥C4 branched alcohols) are valuable as solvents, flavors, fragrances, and advanced biofuels.
- Growing demand makes microbial bioproduction from renewable sugars an attractive target.

Approach

- Reviewed the native Ehrlich pathway in *S. cerevisiae* and its heterologous use in *E. coli* and emerging hosts.
- Surveyed pathway optimization, host engineering, flux control, and other orthogonal routes (fatty acids, polyketides, isoprenoids).

Results

- Engineered hosts achieve high-titer de novo BCHA production from glucose.
- Orthogonal pathways expand the diversity of accessible BCHA products beyond the Ehrlich route.

Significance/Impacts

- Enables sustainable, bio-based production of fuels and chemicals.
- Highlights key challenges (toxicity, yield, scale-up) for industrial deployment.

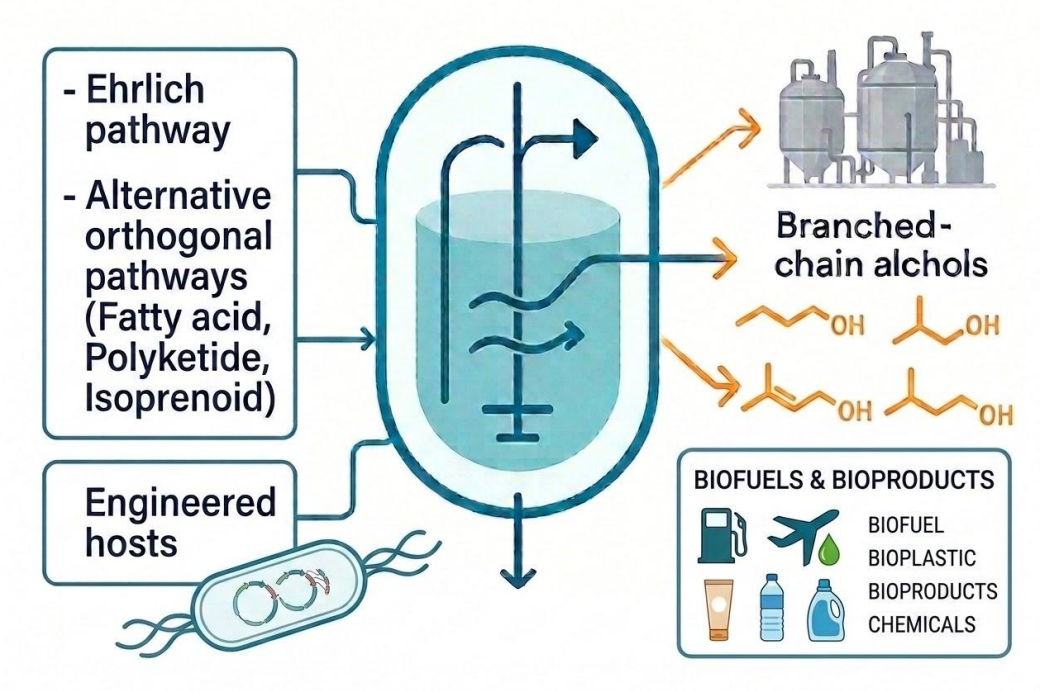

Figure caption: Schematic overview of microbial production of branched-chain higher alcohols (BCHAs). The figure highlights the native Ehrlich pathway in Saccharomyces cerevisiae and other alternative biosynthetic routes derived from fatty acid, polyketide, and isoprenoid metabolism in engineered hosts to produce BCHAs.

Carruthers, D.N., et.al. Comprehensive Biotechnology. doi:10.1016/B978-0-443-24738-5.00080-X (JBEI #1308)

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Multi-strain Analysis of *Pseudomonas Putida* Reveals the Metabolic and Genetic Diversity of the Species

Background/Objective

- *Pseudomonas putida* is a key microbial biotech chassis, but species-level metabolic diversity remains poorly mapped.
- Construct and validate a pan-putida metabolic network and strain-specific genome-scale models (GEMs) to capture species-wide diversity.

Approach

- Performed hybrid whole-genome sequencing of 40 new *P. putida* strains, expanding genomic data by ~8%.
- Profiled 24 strains in-depth using the Biolog phenotypic microarray technology plus 15 aromatics, yielding 4,920 strain-phenotype measurements.

Results

- Refined the flagship KT2440 model containing 1,480 genes, 2,191 metabolites, and reached 91.2% carbon utilization accuracy prediction.
- Pathways for key aromatics like ferulate, phenol, and cresols varied markedly across *P. putida* strains, while protocatechuate was conserved.

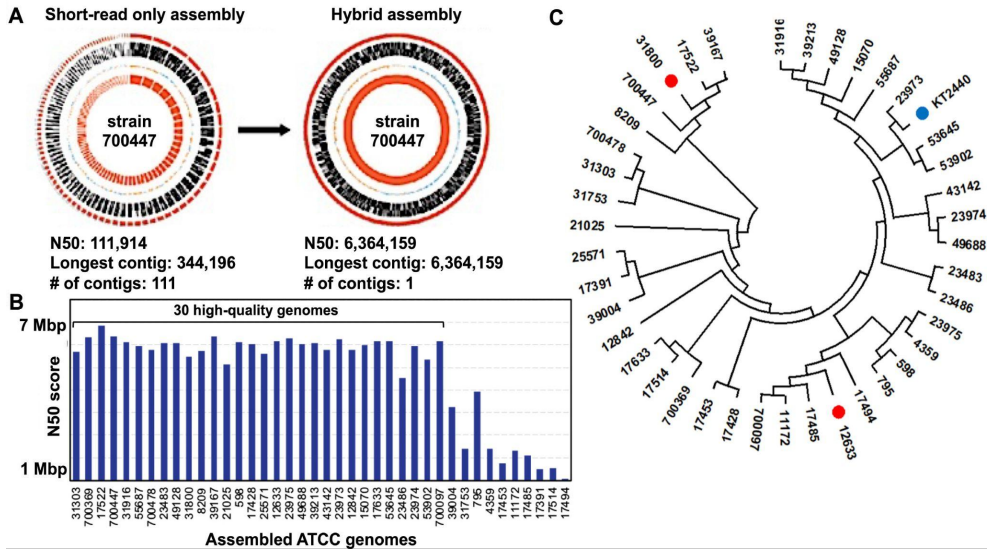

Figure caption: Genome sequencing and phylogenetic analysis of P. putida strains. (A) Comparison of genome completeness between a short-read-only assembly (left) and a hybrid assembly approach with the incorporation of long reads (right) for the ATCC 700447 strain, as a representative example. (B) Comparison of N50 values for assembled genomes of the sequenced strains. Hybrid assembly resulted in significantly higher N50 values that equaled or approached the genome size of assembled high-quality genomes (Table S1). (C) Maximum-likelihood tree of the 40 newly sequenced P. putida strains. KT2440 is denoted with a blue mark. The two strains chosen for initial reconstruction of the pan-putida metabolic network are denoted with red marks.

Significance/Impacts

- Supports BER goals by providing predictive microbial models for bioremediation and aromatic carbon utilization.
- Enables JBEI strain selection for lignin-derived feedstock conversion, advancing bioenergy, and bioeconomy aims.

Mueller, J., et.al. mSystems. doi: 10.1128/msystems.01594-25 (JBEI #1309)

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Influence of Titer, Rate, Yield, and Scale on the Cost and Life-cycle Emissions of Biomanufacturing

Background/Objective

- Develop quantitative, generalizable guidance for titer, rate, and yield (TRY) targets to enable commercialization and scale-up of biomanufacturing.

Approach

- Developed integrated TEA-LCA models to assess the effects of TRY and facility scale on cost and life-cycle emissions for ionic liquid and dilute sulfuric acid deconstruction.
- Applied the framework to three systems: methyl ketones (*Pseudomonas putida*), bisabolene (*Rhodosporidium toruloides*), and ethanol (*Zymomonas mobilis*).

Results

- Microbial biofuels became exponentially more expensive and energy-intensive at product titers below 10 g/L.
- Economically viable production required near-theoretical yield, titer above 130 g/L, and rate over 2 g/L/h.

Significance/Impacts

- Develops scale-aware, science-based TRY benchmarks beyond n^{th}-plant assumptions to guide incremental, cost- and carbon-efficient biomanufacturing scale-up.
- Identifies nonlinear cost-emissions inflection points showing that coordinated TRY improvement and scale selection are essential for viable biofuels and bioproducts.

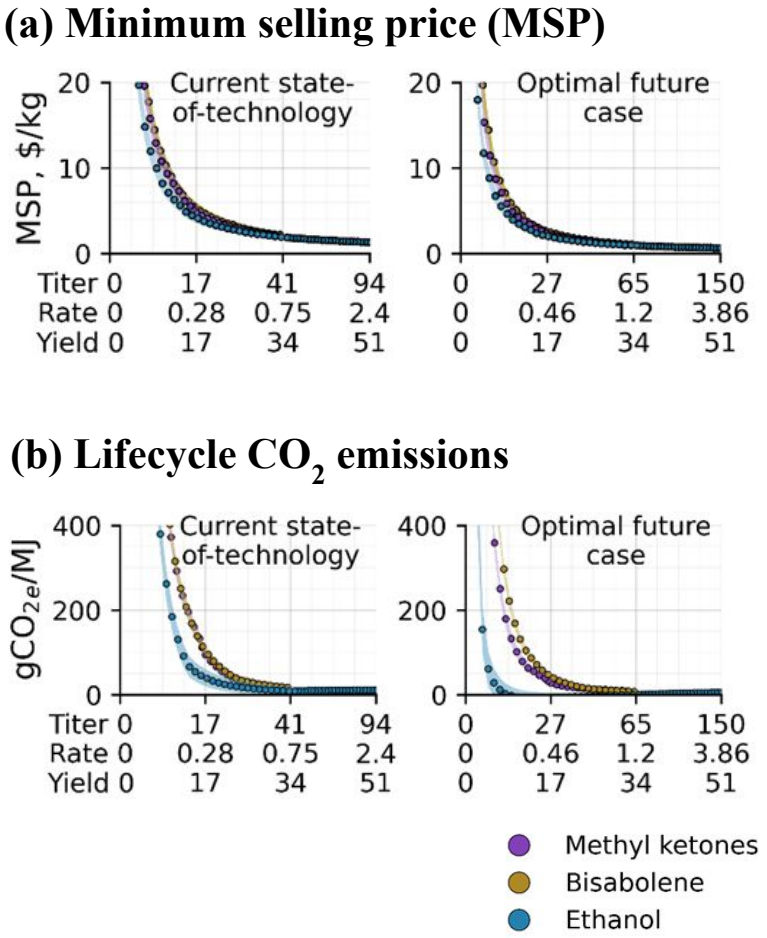

Figure 1: Impacts of titer, rate, and yield on (a) minimum selling price (MSP) and (b) lifecycle CO_2 emissions of biofuels.

Baral, N. R., et.al. Trends in Biotechnology. doi: 10.1016/j.tibtech.2026.03.023 (JBEI #1310)

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Advancing Specialized Biofoundries via Automated Adaptive Laboratory Evolution

Background/Objective

- Outlines how microbial experimentation is labor-intensive and hard to scale in distributed labs, limiting reproducibility.
- Define how specialized biofoundries enable automated science at scale, using adaptive laboratory evolution (ALE) as a test case.

Approach

- Reviewed different automated ALE formats: serial transfer, continuous culture, colony transfer, and microfluidics.
- Outlined design principles integrating robotics, real-time monitoring, omics analytics, and validation to make a bifoundy.

Results

- Auto serial transfer emerged as an effective industrial format due to scalability and robustness for forming an effective biofoundry.
- Parallelized and controllable ALE reveals convergent mutational targets, generating reusable design-ready genetic parts.

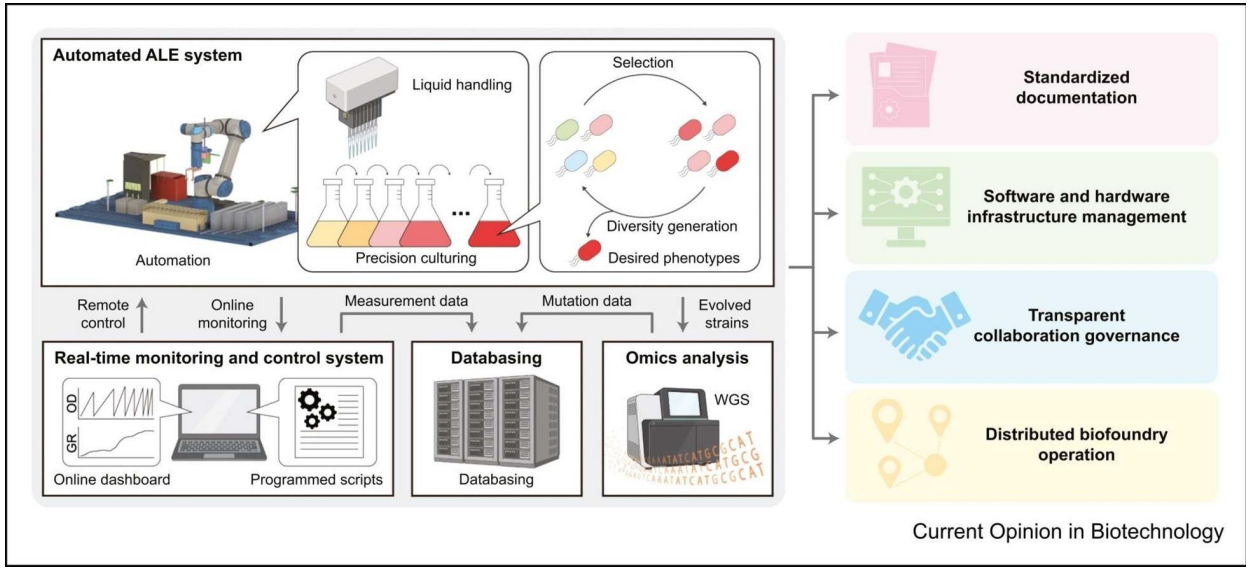

Figure caption: Advancing ALE through Biofoundry Specialization. A conceptual overview of how automated platforms, real-time data integration, and standardized operational protocols transform labor-intensive evolutionary engineering into a scalable, AI-compatible discovery engine.

Significance/Impacts

- Aligns with BER mission by accelerating microbial strain development for energy and environmental applications.
- Supports JBEI bioenergy goals by enabling AI-guided strain engineering for bioeconomy-relevant phenotypes.

Feist, A. M., et.al. Current Opinion in Biotechnology. doi: 10.1016/j.copbio.2026.103496 (JBEI #1311)

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Off-gas Capture: A Promising Strategy for Removal and Recovery of Toxic Bioproducts in Aerobic Fermentation

Background/Objective

- Aerobic fermentation titers are limited by toxic accumulation and volatilization of hydrophobic bioproducts.
- Assess off-gas capture as an in situ recovery method for volatile aviation fuel precursors.

Approach

- Compared liquid-liquid extraction and direct off-gas recovery for isoprene and DMCO jet fuel intermediates.
- Evaluated product/solvent toxicity, partitioning, and aeration effects, then optimized chilled-solvent capture.

Results

- Off-gas condensation in chilled solvent achieved 84% capture efficiency without an internal organic overlay.
- Continuous off-gas removal enabled record isoprenol titers of 20.4 g/L, the highest reported to date and 94% higher than conventional L-L extraction.

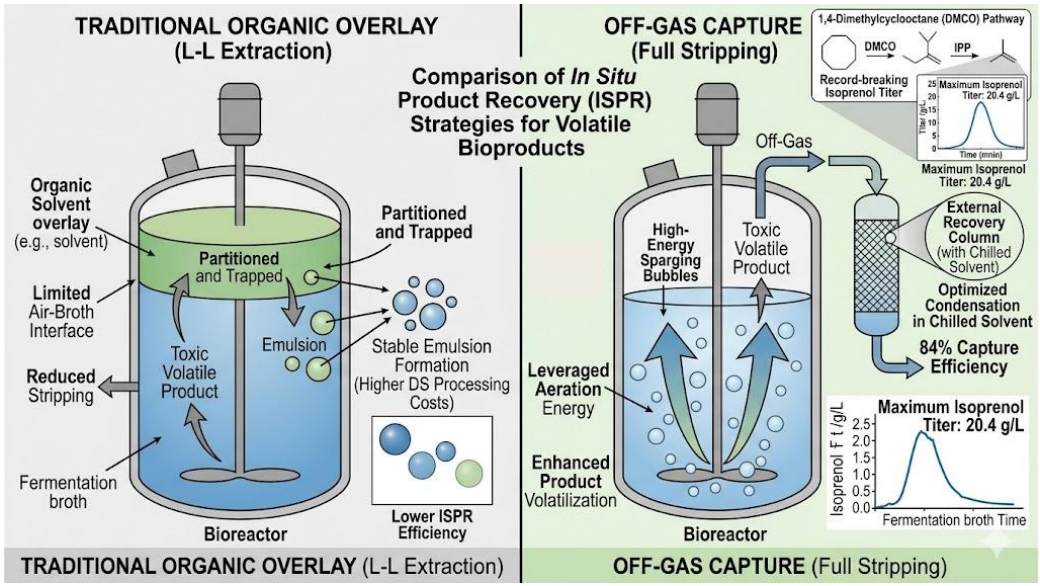

Figure caption: Comparative analysis of In Situ Product Recovery (ISPR) strategies for volatile bioproducts. > The diagram contrasts the traditional Liquid-Liquid (L-L) Extraction method (left), which utilizes an organic solvent overlay, with the optimized Off-Gas Capture strategy (right). Key advantages of the off-gas system include the elimination of stable emulsion formation and the leveraging of aeration energy to achieve a record-breaking isoprenol titer of 20.4 g/L with an 84% capture efficiency via an external chilled solvent column.

Significance/Impacts

- Advances BER bioenergy aims by improving downstream recovery of toxic biofuel intermediates from fermentation.
- Supports JBEI sustainable aviation fuel pipeline, simplifying recovery for isoprene-based drop-in fuels.

Araujo Barcelos, C., et.al. Biotechnology for Biofuels and Bioproducts. doi: 10.1186/s13068-026-02764-4 (JBEI #1312)

Enabled Publications

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Energy Emissions Accounting Methods Can Determine Whether Direct Air Capture with Storage Achieves Net Removal

Background/Objective

- The impact of carbon conversion/management systems hinges on how energy is sourced and accounted for. JBEI modeling capabilities enabled this work.
- Compared five electricity emissions accounting methods for sorbent and solvent direct air capture and storage (DACS) facilities.

Approach

- Simulated hourly DACS operation in California, Louisiana, Texas, and Wyoming for 1 MMt CO_2/year facilities.
- Used the ReEDS capacity expansion model and Cambium hourly model to compute average and marginal factors.

Results

- The electricity accounting method varied calculated DACS net removal from −1049% to +108% across scenarios.
- All other factors combined introduced at most ±14% variation in calculated net CO_2e removal.

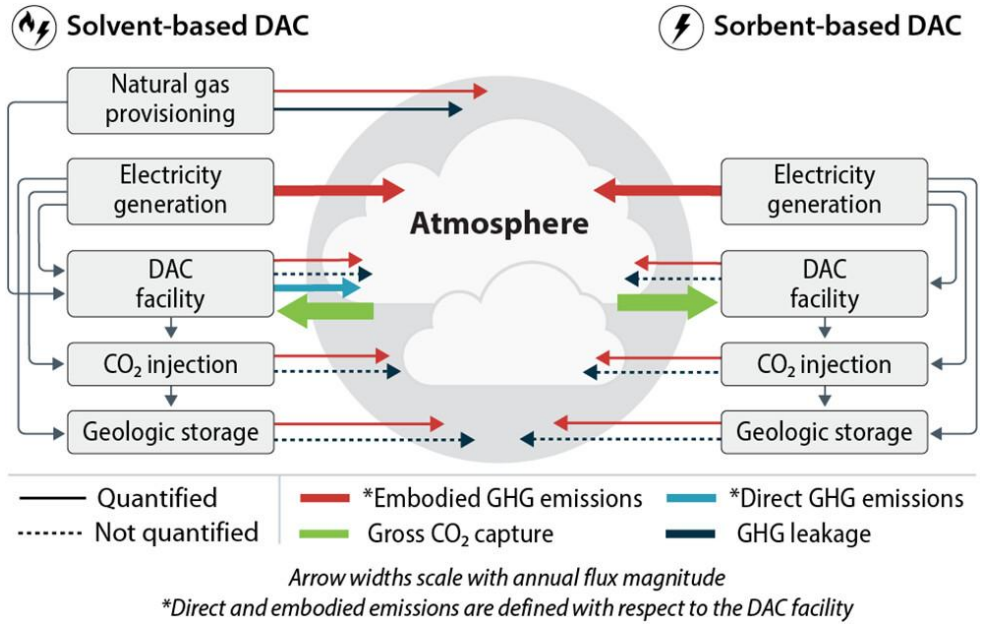

Figure caption: Greenhouse gas fluxes associated with solvent- and sorbent-based DACS. Solid lines represent emissions or removals quantified in this paper, with arrow width representing approximate magnitude. Dashed lines represent fluxes not quantified in this paper, which either are anticipated to be small or lack established quantification methods.

Significance/Impacts

- Highlights BER-relevant need for high-resolution grid emissions data to validate carbon removal technologies.
- Informs carbon accounting underpinning JBEI modeling for fuel and chemical production pathways.

Hanes, R. J., et.al. Environmental Science & Technology. doi: 10.1021/acs.est.5c13494 (JBEI #134)

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A Biosynthetic Survey of Hypocrealean Biocontrol Fungi

Background/Objective

- Pesticide overuse drives resistance and harm; pests cause up to 40% of global crop losses each year.
- Catalog the biosynthetic repertoire of hypocrealean biocontrol fungi to find sustainable pesticides.

Approach

- Combined phylogenomics, metabolomics (UHPLC-MS/MS), and heterologous expression on 87 Hypocreales strains.
- Cultivated strains on PDA, MEA, CMA, PDB, and MEB media using OSMAC strategy across 450+ experiments.

Results

- AntiSMASH mining identified 5,221 biosynthetic gene clusters; ~80% encode unknown natural products.
- Characterized 4 NRPS-like synthetases and linked 104 annotated compounds, including pyridones and efrapeptin.

Significance/Impacts

- Supports BER goals on plant-microbe interactions and sustainable agriculture relevant to bioenergy crops.
- Strengthens JBEI bioeconomy via fungal natural product discovery for biopesticides and crop protection.

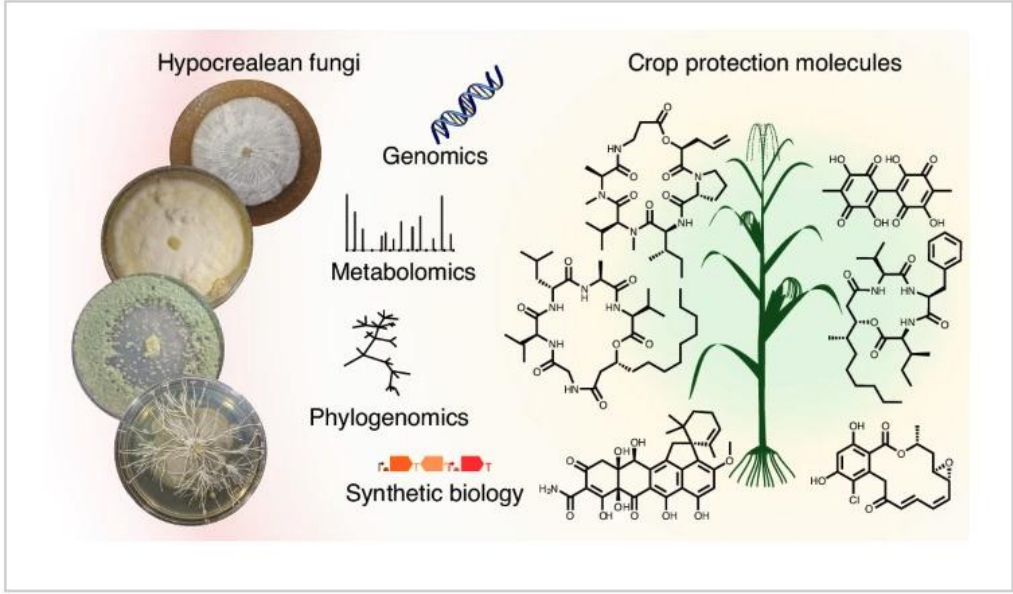

Figure caption: A Multi-Omics Approach to Crop Protection: Integrating genomics, metabolomics, and phylogenomics reveals unique biosynthetic gene clusters (BGCs) in hypocrealean fungi that produce natural molecules, which are valuable for sustainable agricultural biocontrol.

Calheiros de Carvalho, A., et.al. Nature Chemical Biology. doi: 10.1038/s41589-026-02201-5 (JBEI #135)

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Leveraging a Synthetic Biology Approach to Enhance BCG-mediated Expansion of Vγ9Vδ2 T cells

Background/Objective

- Current BCG tuberculosis vaccine fails to prevent pulmonary infection in adults, leaving urgent vaccine gap.
- Engineer BCG to overproduce HMBPP via the MEP pathway to expand protective Vγ9Vδ2 T cells

Approach

- Synteny analysis of 353 mycobacterial genomes guided design of synthetic MEP loci expressed in BCG strains.
- Engineered strains tested via in vitro Vγ9Vδ2 T cell expansion assays and CRISPRi gene silencing.

Results

- BCG carrying a synthetic MEP locus significantly enhanced Vγ9Vδ2 T cell expansion over wild-type BCG.
- GcpE (HMBPP synthase) overexpression alone potently induced T-cell expansion without pathway downregulation.

Significance/Impacts

- Demonstrates synthetic biology and isoprenoid pathway engineering relevant to BER microbial systems research.
- Leverages JBEI MEP pathway expertise, extending bioenergy isoprenoid tools to broader bioeconomy uses.

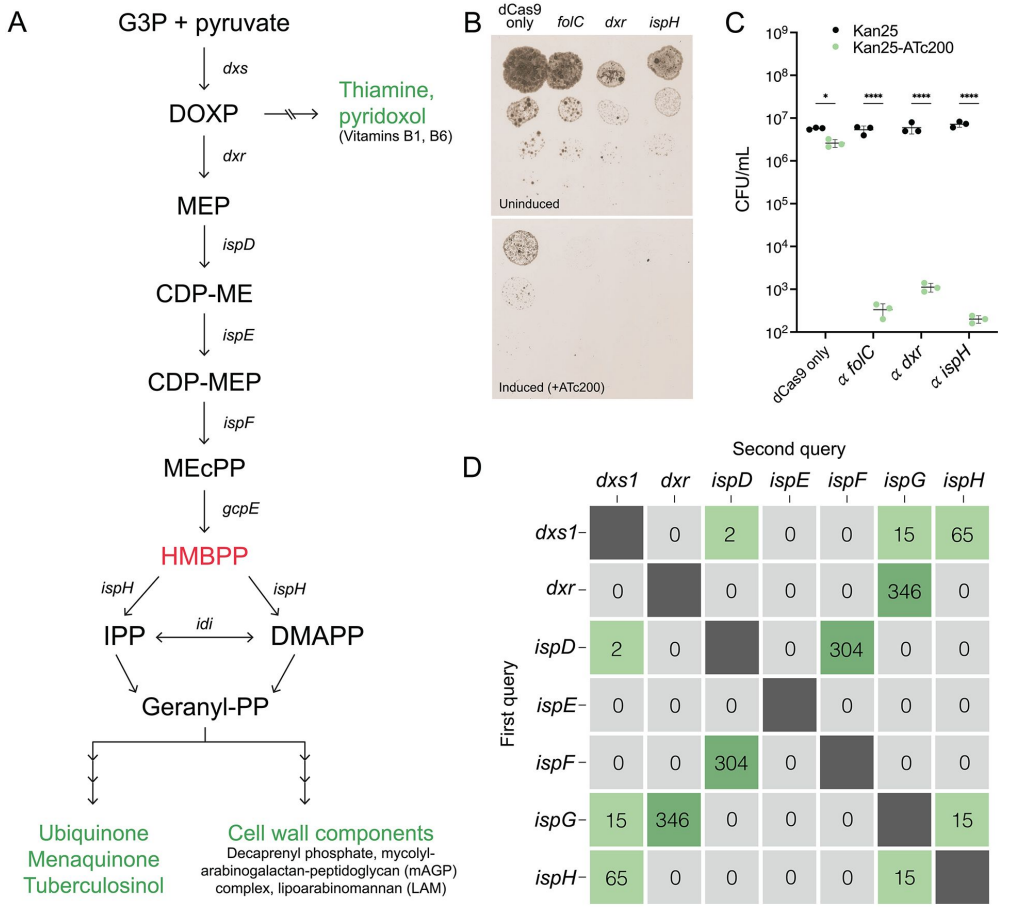

Figure caption: Validating the MEP Pathway as a Synthetic Biology Target. Pathway: Identifies key enzymes responsible for producing T-cell activating HMBPP. Lethality: Silencing *dxr* or *ispH* proves these genes are essential for BCG survival.Design: Conserved gene clusters across 353 genomes reveal natural "blueprints" for the synthetic platform.

Qabar, C. M., et.al. PLoS One. doi: 10.1371/journal.pone.0343925 (JBEI #136)