

Rethinking the Soil Core Microbiome

Background/Objective

- The concept of a "core microbiome" is applied inconsistently, often conflating statistically selected taxa with biologically conserved ones.
- This study aimed to test for a universally shared taxonomic core and evaluate how analytical choices influence core identification.

Approach

- Researchers analyzed merged 16S rRNA datasets from rhizosphere soils across diverse bioenergy cropping systems.
- They systematically varied sequence clustering thresholds and occurrence cutoffs to assess their impact on core membership.

Results

- No universal taxonomic core was found across bioenergy systems when using fine-resolution amplicon sequence variants (ASVs).
- The identified "core" was highly sensitive to methodological parameters, changing substantially with different thresholds.

Significance/Impacts

- The study advocates for precise terminology, reserving "core microbiome" for demonstrably conserved and functional components.
- It proposes "explanatory subsets" as a more accurate framework for taxa selected via study-specific analytical criteria.

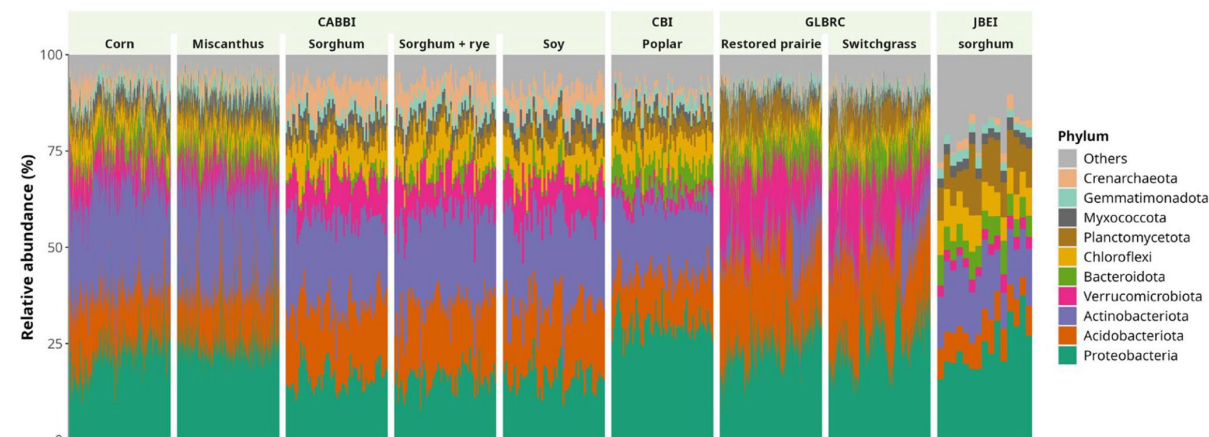


Figure caption: Broad phylum-level composition of soil bacterial communities across bioenergy cropping systems from four US Department of Energy Bioenergy Research Centers (BRCs). Stacked bar plots show relative abundances of dominant bacterial phyla across 1813 soil samples collected from Center for Advanced Bioenergy and Bioproducts Innovation (CABBI), Center for Bioenergy Innovation (CBI), Great Lakes Bioenergy Research Center (GLBRC), and Joint BioEnergy Institute (JBEI) field sites, representing multiple crops, locations, and management contexts. Phyla contributing lower relative abundance were grouped as Others. At coarse taxonomic resolution, similar phylum-level groups are detected across systems, although their relative abundances vary among sites and crops.

Simultaneous Optimization of Lignocellulosic Sugar Catabolism via Systematic Laboratory Evolution Under Complex Selection Pressure

Background/Objective

- Mixed lignocellulosic hexose/pentose sugar utilization remains inefficient.
- Objective: Systematically evolve *P. putida* to optimize co-utilization of glucose, xylose, and arabinose under complex selection pressures.

Approach

- Leverage parallel adaptive laboratory evolution using an automated ALEbot under six distinct sugar regimes with multiple replicates for each condition.
- Utilize single-substrate and multi-substrate “complex” regimes to drive adaptation of a “generalist” simultaneous sugar consumer.

Results

- Simple and high sugar mixture regimes produced catabolic specialists; a low sugar mixture regime enforcing complete consumption produced metabolic generalists.
- LSM1 isolate achieved a 1.4x higher mixed-sugar growth rate and 1.3x higher total sugar consumption than parental strain.

Significance/Impacts

- The low sugar mixture (LSM) regime forced stable retention and coordinated optimization of multiple heterologous catabolic pathways.
- LSM1 generalist phenotype improved indigoidine titers by 1.5-fold from mixed sugars, validating industrial host potential.

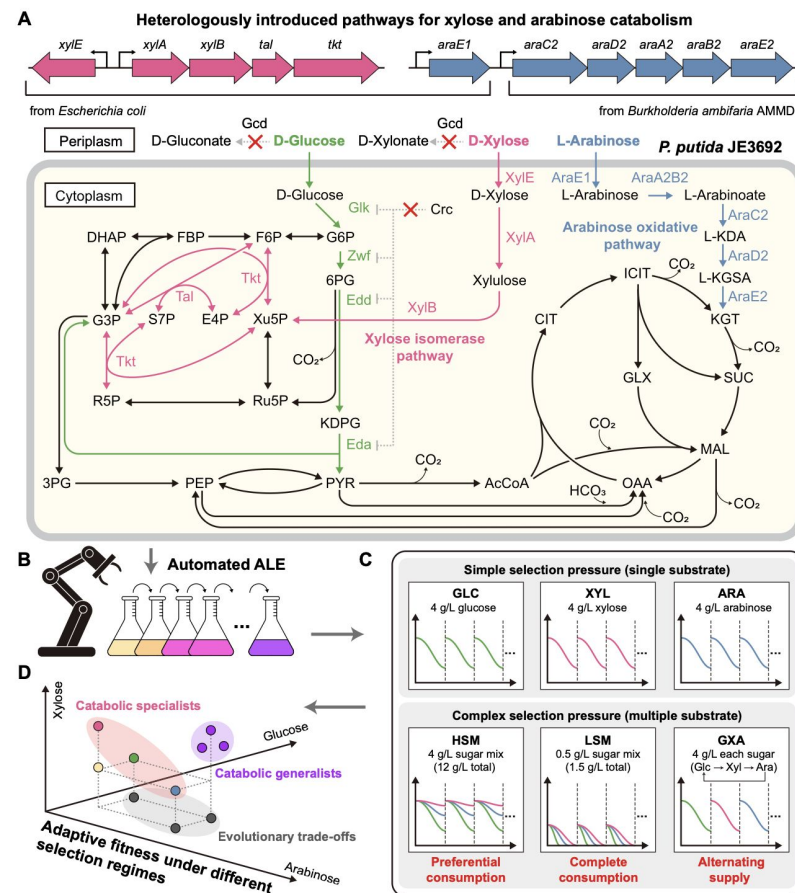


Figure caption: A Catabolic pathways for glucose (green, ED), xylose (pink, isomerase), and arabinose (blue, oxidative) in JE3692, with deleted genes (gcd, crc) indicated. B Automated ALEbot platform for parallel, consistent evolution. C ALE regimes under simple (GLC, XYL, ARA) and complex selection pressures (mixed: HSM, LSM; alternating: GXA; n = 4). D Conceptual fitness landscape illustrating outcomes ranging from catabolic specialists to generalists.

Nitrene Generation and Transfer for Unnatural Biosynthesis in Living Cells

Background/Objective

- Biosynthesis historically limited to nature's narrow set of nitrogen-incorporation reactions.
- Enable intracellular intermolecular nitrene transfer for unnatural, nitrogen-containing pharmaceutical synthesis.

Approach

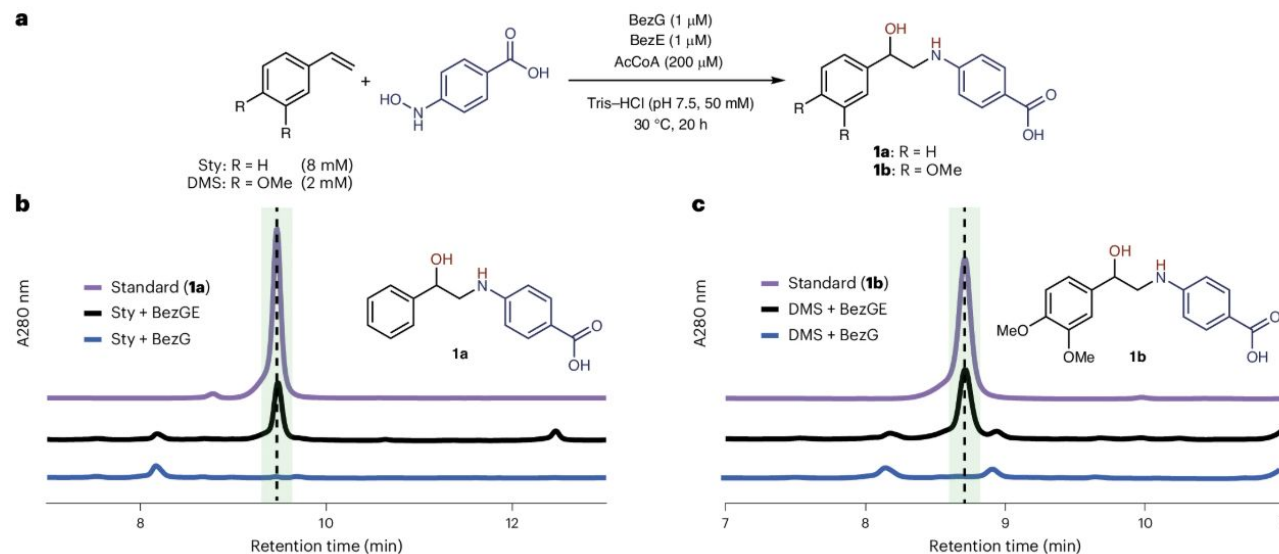
- Constructed *E. coli* pathway combining PABA-based nitrene precursor biosynthesis with vinylarene generation.
- Enzyme Screening: Tested various arylamine oxidases and P450 homologues for optimal in vivo nitrene transfer catalysis.

Results

- P450 enzyme catalyzed formal nitrene insertion into aryl C–H bonds, producing diarylamines from 4-vinylphenol.
- Engineered Performance: BezE^{Ure} A87H_T231S_Y339F triple mutant increased 3-methoxystyrene product 1c enantioselectivity to 91% e.e.

Significance/Impacts

- Revealed P450-catalyzed aryl C–H amination and hydroamination reactions not previously reported for this enzyme class.
- Scalable Bioproduction: Pathway functions in minimal media from glucose, enabling future large-scale, sustainable production of pharmaceutically relevant amines.



Unlocking Microbial Access to Amino-substituted 4-Hydroxycoumarin Derivatives via Enzymatic Promiscuity and Metabolic Engineering

Background/Objective

- Microbial biosynthesis of amino-substituted 4-hydroxycoumarins and their precursor 5-aminosalicylic acid (5ASA) remains underexplored.
- Establish a de novo microbial route in *Pseudomonas putida* to produce 6-amino-4-hydroxycoumarin (6A4HC) and its pharmaceutical precursor, 5ASA.

Approach

- Leveraged substrate flexibility of the type III polyketide synthase PqsD with promiscuous CoA ligase MdpB2 to convert substituted salicylic acids.
- Assembled a pathway combining PctV for 3ABA synthesis and 3HB6H for hydroxylation, then coupled it to the MdpB2-PqsD module for cyclization.

Results

- Engineered *P. putida* strain PP-CZ5 produced 7.56 mg/L 6A4HC from glucose, representing the first reported microbial biosynthesis.
- Host engineering (e.g., Δ quiC, PLP cofactor supplementation) increased 3ABA accumulation 21-fold (to 7.5 mg/L) and enabled detectable 6A4HC production.

Significance/Impacts

- Provides the first biological route to 5ASA (mesalamine), an FDA-approved drug with annual sales exceeding \$1 billion.
- Expanded Enzyme Toolbox: Demonstrated broad substrate scope for MdpB2-PqsD cascade and experimentally validated seven putative PqsD homologs for coumarin production.

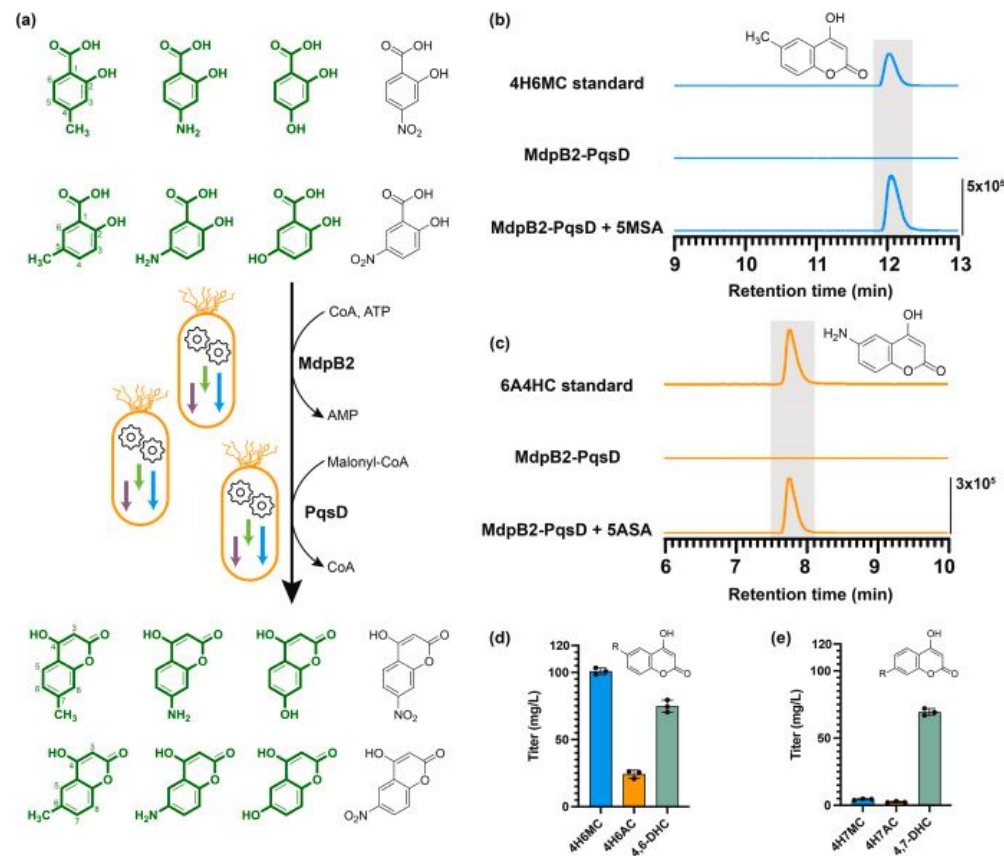


Figure caption: Substrate scope of the MdpB2-PqsD cascade for substituted coumarin biosynthesis. (a) Schematic of tested salicylic acid derivatives (green = accepted; grey = unaccepted). (b, c) Representative LCMS traces for 4H6MC (from 5MSA) and 6A4HC (from 5ASA) production. (d, e) Production titers from C5- and C4-substituted salicylic acids, showing higher yields for C5-methyl/amino analogs over C4 equivalents. Error bars represent SD ($n = 3$).

Enabled Publications

Black Yeasts are Efficient Heterologous Hosts for the Production of a Wide Range of Fungal Polyketides

Background/Objective

- Current fungal hosts for polyketide biosynthesis are either metabolically limited (Saccharomycotina) or complex and slow (filamentous Eurotiomycetes).
- Establish robust, tractable black yeasts (*Exophiala viscosa*, *Knufia petricola*) as efficient heterologous hosts for diverse fungal polyketides.

Approach

- Engineered genetic tools (CRISPR–Cas9, AMA1 plasmids, oligo-mediated editing) and established in vivo assembly methods for these yeasts.
- Produced six polyketides with distinct architectures (6-MSA, YWA1, monocillin II, farinosones, monacolin J) across the hosts.

Results

- Black yeasts outperformed *S. cerevisiae* POP4 for complex PKSs (e.g., YWA1 titer >1,300 mg L⁻¹ in *E. viscosa*).
- Black yeasts grew robustly on second-generation feedstocks like xylose and beechwood hydrolysates.

Significance/Impacts

- Provides a unicellular, metabolically robust alternative with simpler background metabolism than *Aspergillus*.
- Enables systematic expression and pathway elucidation of complex fungal natural products (e.g., 2-pyridone YNP1).

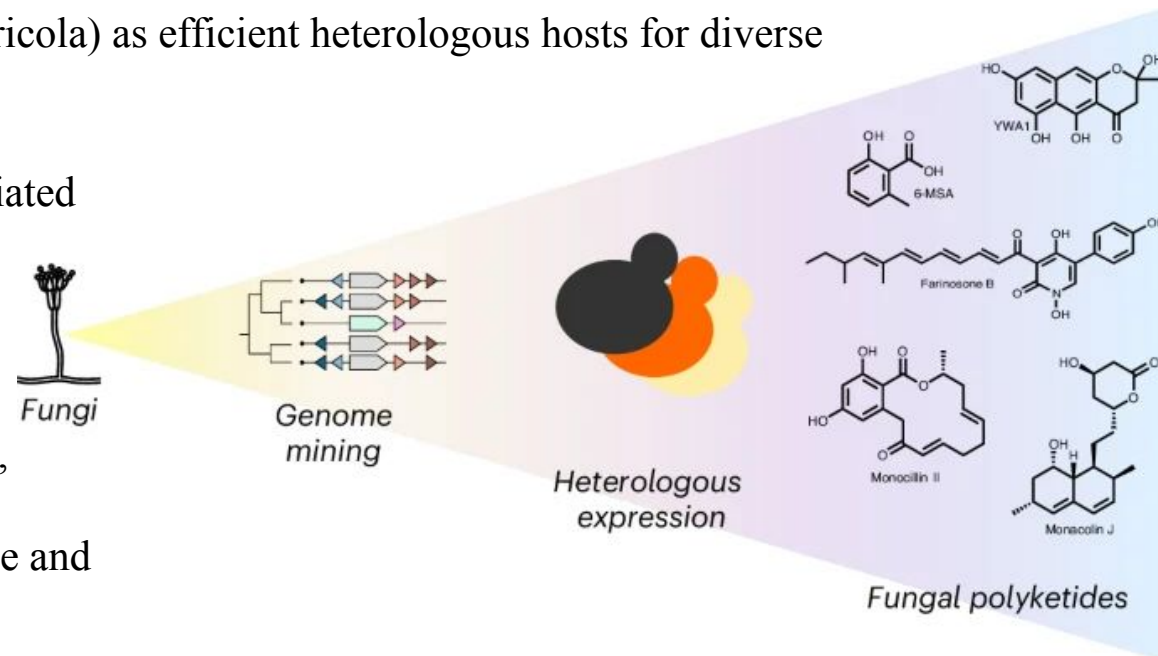


Figure caption: Heterologous polyketide production using black yeast hosts. *Exophiala* and *Knufia* species combine genetic tractability with robust metabolic backgrounds to efficiently express complex fungal polyketide synthases and produce diverse bioactive molecules, including 6-methylsalicylic acid, YWA1, monocillin II, farinosone B, and monacolin J.

eFormate-mediated CO₂ bioconversion: A Modular Electrochemical-microbial Cascade Compared Across a Diverse set of Microorganisms

Background/Objective

- Integrated CO₂ electrolysis–bioconversion systems rely on limited microbial hosts.
- Establish a modular eFormate-mediated abiotic–biotic cascade platform for systematic microbial screening.

Approach

- Temporal decoupling of SnO₂-catalyzed electrochemical formate production (300 mA cm⁻²) from biological upgrading.
- Evaluated 13 formatotrophic strains across three metabolic pathways (Wood–Ljungdahl, Calvin–Benson–Bassham, serine–TCA/reductive glycine).

Results

- Achieved 0.87 M formate in 4 h with 84% Faradaic efficiency; PTFE addition enhanced electrode stability.
- 10 of 13 strains utilized eFormate, producing acetate, ethanol, lactate, pyruvate, succinate, and PHB.

Significance/Impacts

- Provides a unified biocompatible feedstock (eFormate) for direct cross-strain comparison and selection.
- Enables systematic identification of CO₂-to-C_n pathways across diverse microbial metabolisms and trophic requirements.

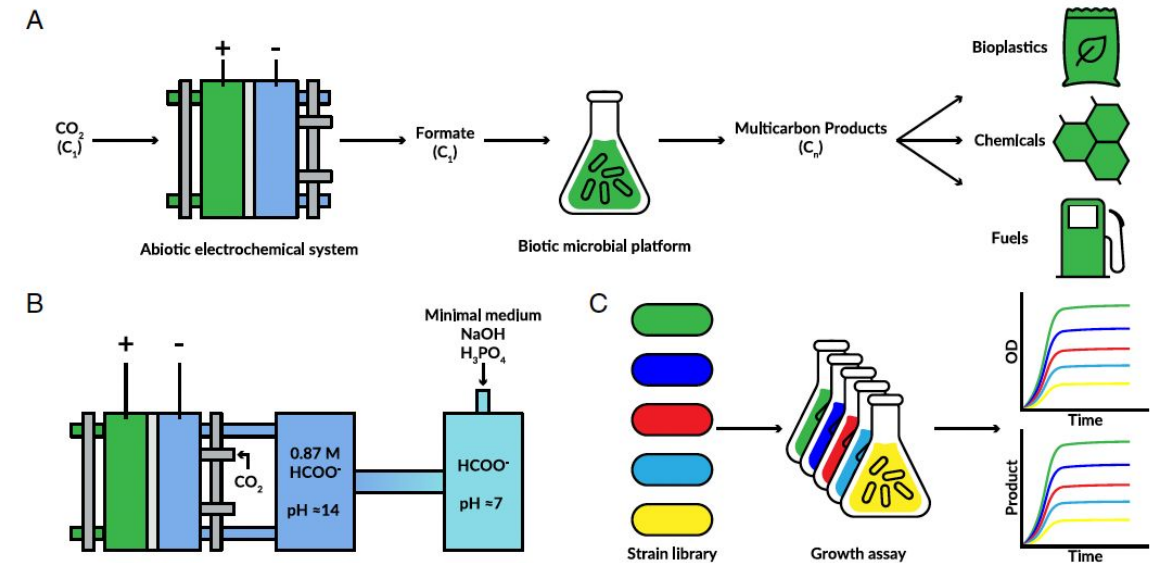


Figure caption: Schematic overview of integrated abiotic–biotic system for CO₂ conversion. (A) CO₂ can be electrochemically reduced to formate, which can then be biochemically converted into multicarbon products using engineered microbes. (B) CO₂ is electrochemically reduced to formate using a SnO₂ nanoparticle catalyst in a flow cell system. The formate- enriched solution is subsequently pH- tuned and diluted in a biocompatible medium to enable microbial upgrading. (C) Electrochemically synthesized formate is directly harnessed as a biofeedstock by genetically amenable microbial platforms. 13 bacterial strains were assessed for growth and product formation on formate solution.

Disarming a Pathogen with Programmable Phage-derived Particles

Background/Objective

- This invited Spotlight examines Galtier et al.'s strategy to kill Shiga toxin-producing *E. coli* (STEC) while disabling the toxin gene.
- We discuss the study's implications for programmable DNA delivery and the field of precision microbiome editing.

Approach

- The Spotlight examines how the phagemid platform combines programmable receptor recognition, genetic cargo, and CRISPR-Cas12a targeting.
- We compare related microbiome-editing approaches and discuss delivery constraints that limit expansion to additional bacterial taxa.

Results

- Galtier et al. reported targeted killing across clinical STEC isolates, reduced bacterial burden in mice, and improved disease outcomes in infant rabbits.
- Our Spotlight highlights how coupling pathogen killing with toxin-gene disruption could inform broader applications of precision microbiome editing.

Significance/Impacts

- The Spotlight synthesizes implications of independently programmable delivery and editing for targeted genetic manipulation within microbial communities.
- It identifies host-range expansion and development of delivery platforms for additional bacterial taxa as priorities for broader application.

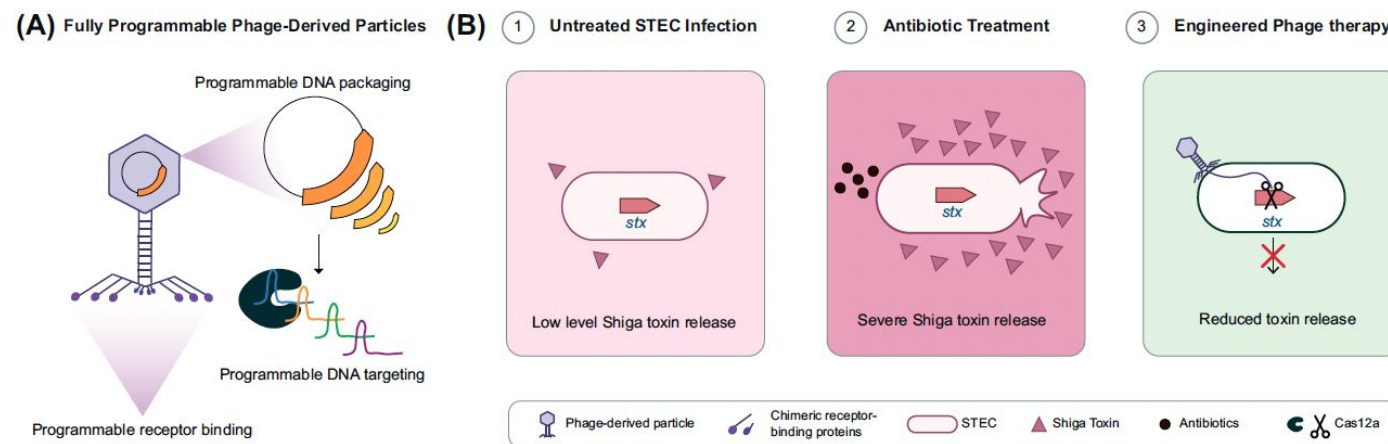


Figure caption: Programmable phage-derived particle strategy developed by Galtier et al., as discussed in the Spotlight by Yang and Cress. The platform combines targeted delivery with CRISPR-mediated disruption of the Shiga toxin gene. Schematic from Yang and Cress (2026).

Yang, S., et.al. Trends in Microbiology. doi: 10.1016/j.tim.2026.07.013 (JBEI #142)