

Expression of a mammalian RNA demethylase increases flower number and floral stem branching in *Arabidopsis thaliana*

Background/Objective

- A recent landmark paper reported that transcriptome-wide use of the human RNA demethylase FTO substantially increased the yield of rice and potatoes.

Approach

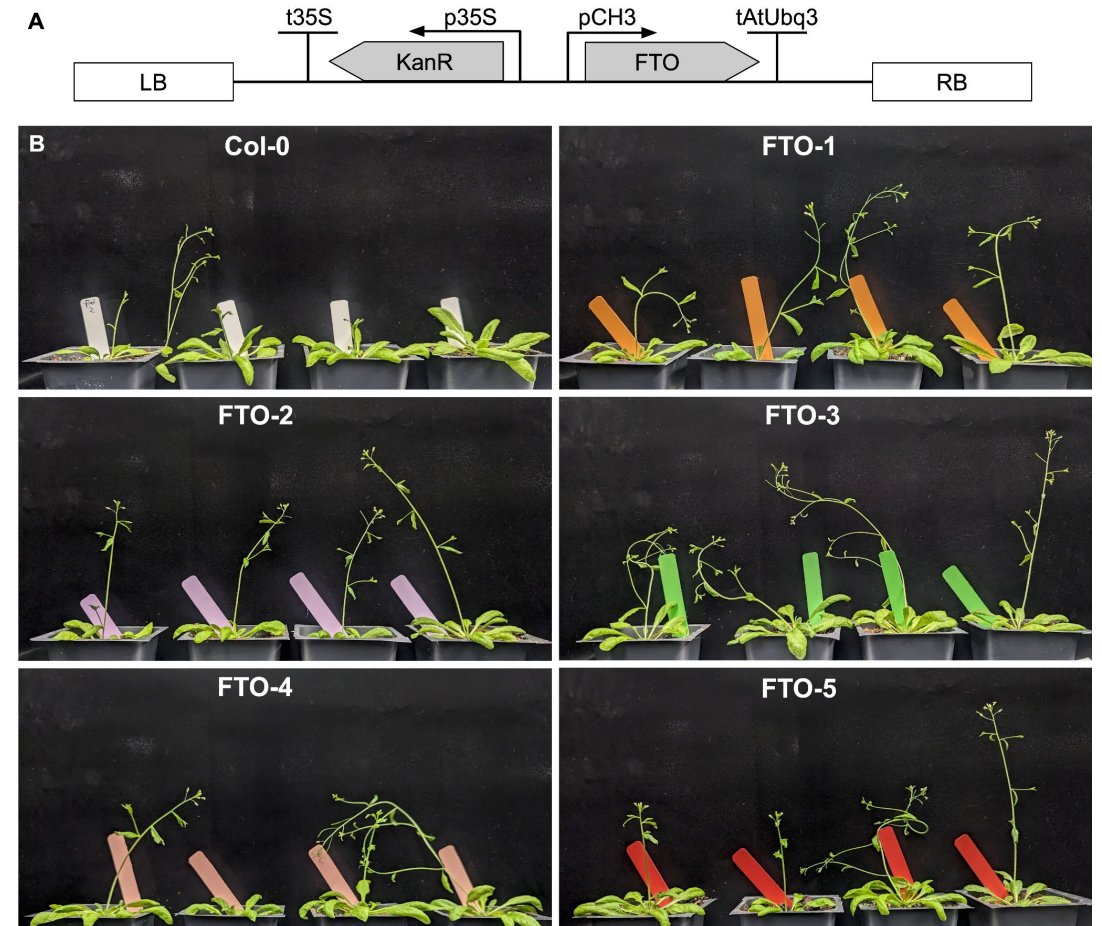
- We have performed the first independent replication of those results and broader transferability of the trait into other plant species.

Results

- Our results demonstrate that increased flower and fruit count in the model species *Arabidopsis thaliana*.

Significance/Impacts

- We present mechanistic hypotheses to explain these surprising results with the goal of spurring more widespread interest in this promising new approach to plant engineering



Markel K, et. al. *Plant Direct*. doi: [10.1002/pld3.70000](https://doi.org/10.1002/pld3.70000) (JBEI #1203)

Binary vector copy number engineering improves Agrobacterium-mediated transformation

Background/Objective

- The copy number of a plasmid is critically linked to its functionality, yet there have been few attempts to systemically modify this property across broad-host range origins.

Approach

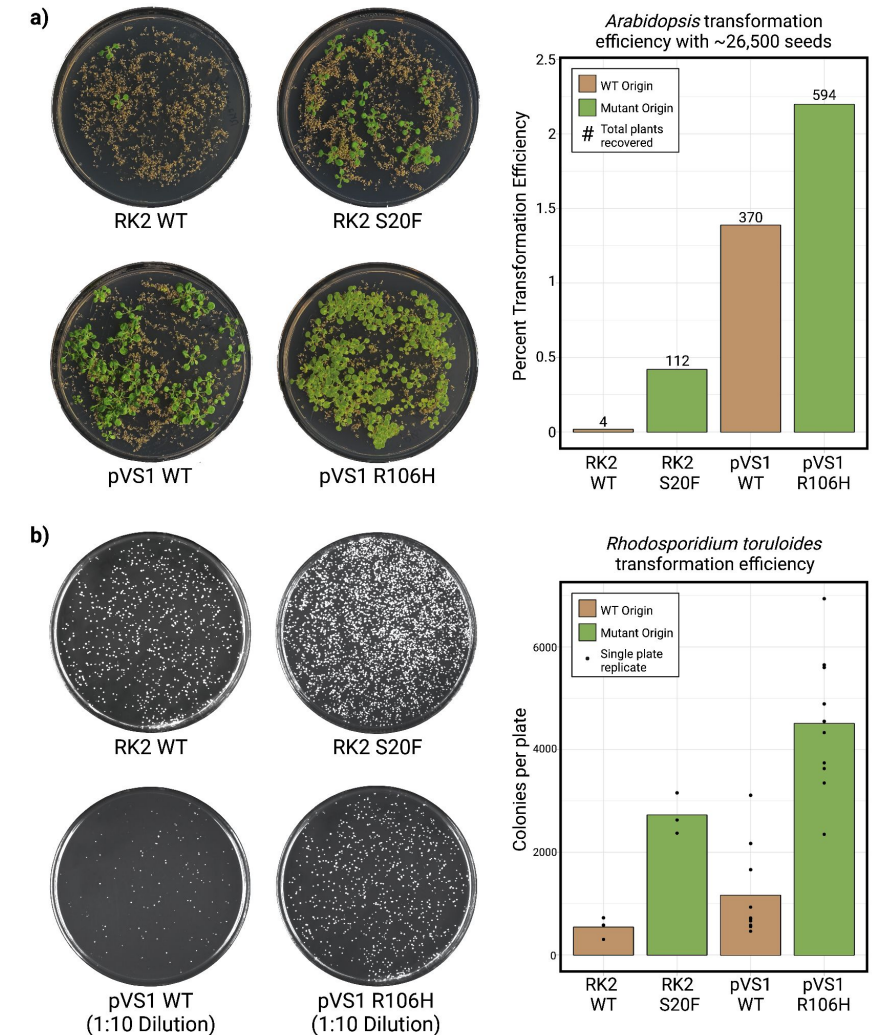
- Using a directed evolution approach, we generated copy number variants of four commonly used broad-host range origins.

Results

- When these mutations were introduced into binary vectors used for Agrobacterium-mediated transformation (AMT), we increased stable transformation efficiencies by 60-100% in *Arabidopsis thaliana* and 390% in the oleaginous yeast *Rhodospiridium toruloides*.

Significance/Impacts

- Our work provides an easily deployable framework to generate plasmid copy variants that will enable greater precision in prokaryotic genetic engineering, in addition to improving AMT efficiency.



Szarzanowicz, M. J., et. al. *Nature biotechnology*. doi: [10.1038/s41587-024-02462-2](https://doi.org/10.1038/s41587-024-02462-2) (JBEI #1204)

Genetic modification of the shikimate pathway to reduce lignin content in switchgrass (*Panicum virgatum* L.) significantly impacts plant microbiomes

Background/Objective

- Switchgrass has been engineered to reduce lignin
- The effect of modifying lignin on the switchgrass microbiome is unknown

Approach

- Switchgrass-associated fungal and bacterial communities were investigated using high-throughput illumina MiSeq amplicon sequencing of ITS and 16S rDNA

Results

- Engineered switchgrass host different microbial communities in roots, rhizosphere, and leaves
- Arbuscular mycorrhizal fungi (AMF) and *Actinobacteriota* are less abundant in engineered switchgrass

Significance/Impacts

- Changes in plant metabolism impact both AMF and *Actinobacteriota* or potential interactions between them
- This study provides some knowledge on plant-microbiome interactions in low lignin engineered switchgrass

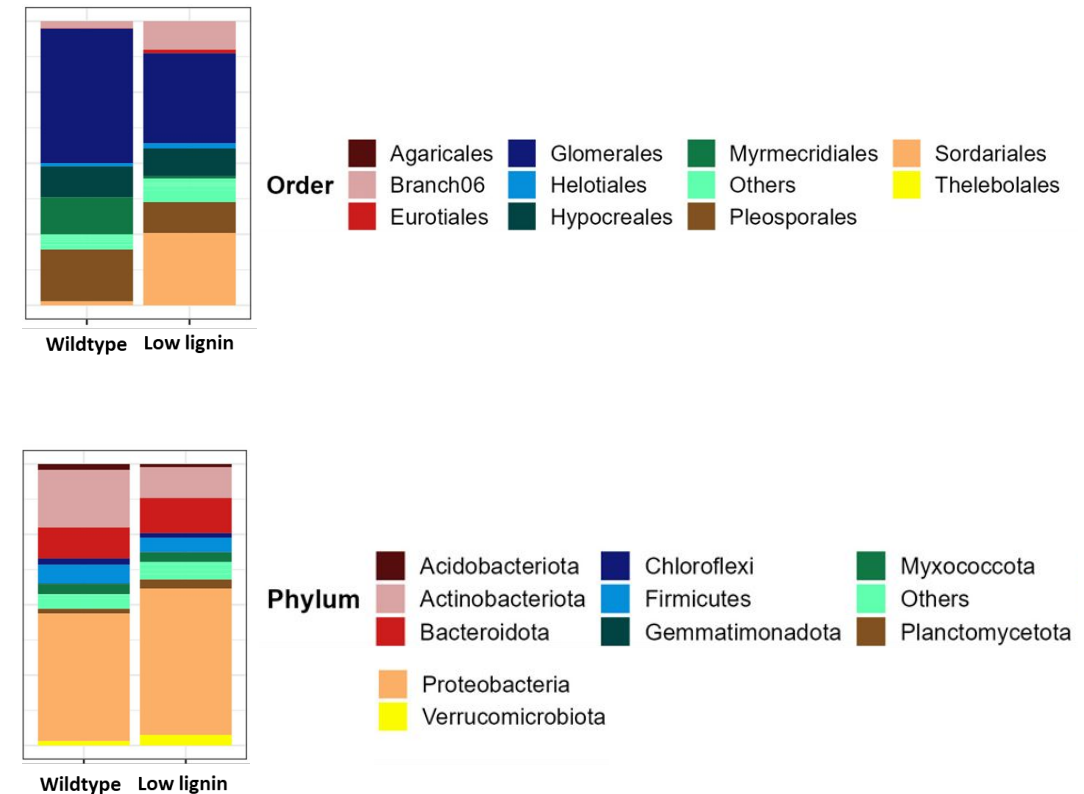


Figure: (Top) Order-level fungal taxonomic distribution in roots (Bottom) Lineage-level bacterial taxonomic distribution in roots

Liu, S., et. al. *Microbiology spectrum*. doi: [10.1128/spectrum.01546-24](https://doi.org/10.1128/spectrum.01546-24) (JBEI #1205)

Analysis of novel bacterial metagenome-assembled genomes from lignin-degrading microbial consortia

Background/Objective

- Despite recent progress, bacterial degradation of lignin is not completely understood
- Goal: Identify the mechanisms that bacteria from unknown taxonomic groups use to perform lignin-monomer degradation

Approach

- Conduct functional analysis of bacterial metagenome-assembled genomes from soil-derived consortia enriched for microorganisms capable of degrading lignin

Results

- Actinobacteria_BY 70 had the highest number of enzymes and pathways for degradation of monolignols

Significance/Impacts

- This analysis has identified novel lignin degrading bacteria that have the potential to enable biological lignin valorization

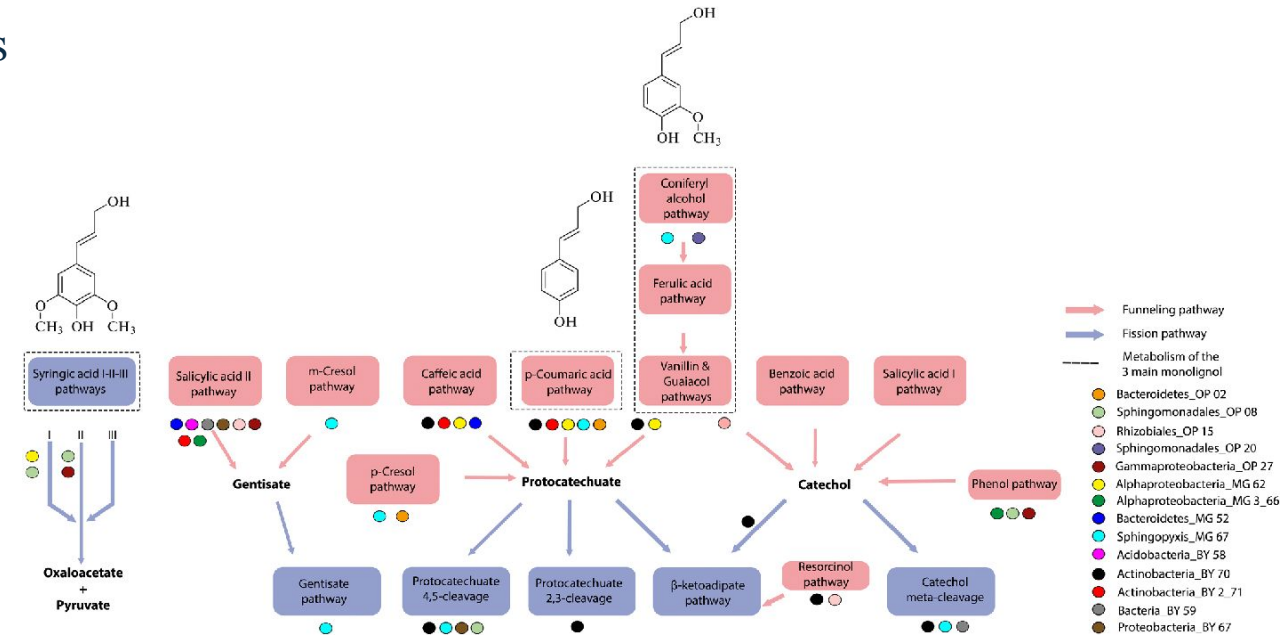


Figure: Lignin monomer degradation pathways for 14 soil bacterium

ENABLED PUBLICATIONS

Cyanobacteria from marine oxygen-deficient zones encode both form I and form II Rubiscos

Background/Objective

- Rubisco is the source of nearly all organic carbon in the biosphere. To date, all studied cyanobacterial lineages encode carbon fixation machinery relying upon form I Rubiscos within a CO₂-concentrating carboxysome.

Approach

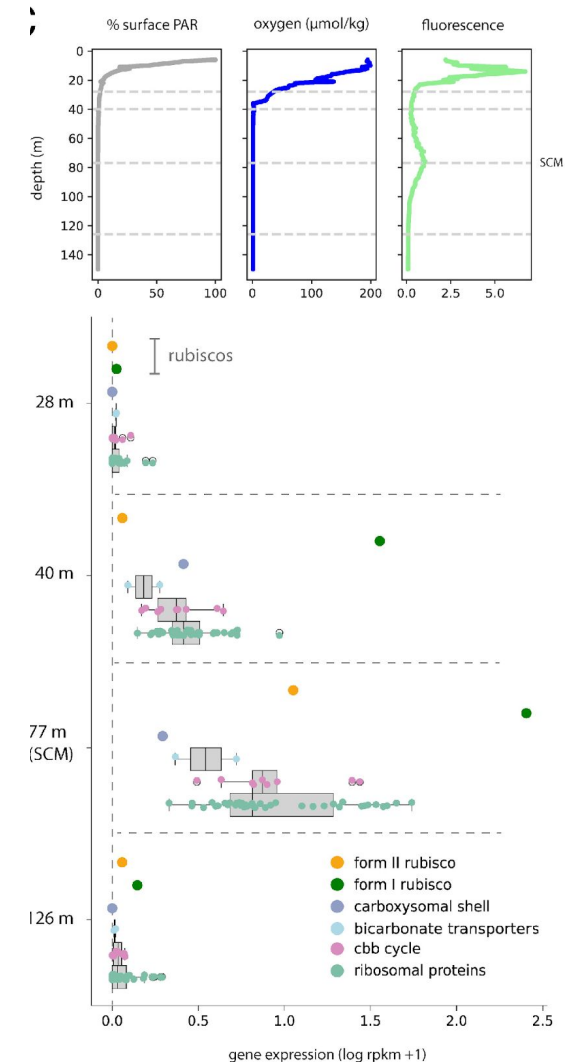
- Here, we report that some cyanobacteria from pelagic oxygen-deficient zones (ODZs) harbors both form I and form II Rubiscos, the latter of which possess biochemical properties tuned toward low-oxygen environments.

Results

- Both forms of Rubisco are expressed at depths where oxygen and light are low, possibly as a mechanism to increase the efficiency of photoautotrophy under energy limitation.

Significance/Impacts

- Our findings expand the diversity of carbon fixation configurations in the microbial world and may have implications for carbon sequestration in natural and engineered systems.



Jaffe, A. L., et. al. Proceedings of the National Academy of Sciences of the United States of America. doi: 10.1073/pnas.2418345121 (JBEI #E104)

DORA-XGB: an improved enzymatic reaction feasibility classifier trained using a novel synthetic data approach

Background & objective:

- Developed a machine learning (ML) classification model to predict feasibility of enzymatic reactions.
- Building any ML classifier requires both positive and negative data, but failed reactions are seldom reported.

Approach:

- Synthetically generated negative reactions by considering alternate reactions sites on known substrates.
- Built a gradient-boosted tree model with the resulting training data and optimized its hyperparameters further with Bayesian methods.

Results:

- Our gradient-boosted classifier, DORA-XGB, outperforms existing deep-learning based classifiers.

Significance/Impacts:

- DORA-XGB can accelerate design of novel biosynthetic pathways when used with computational tools (DORAnet, Biosynth Pipeline)

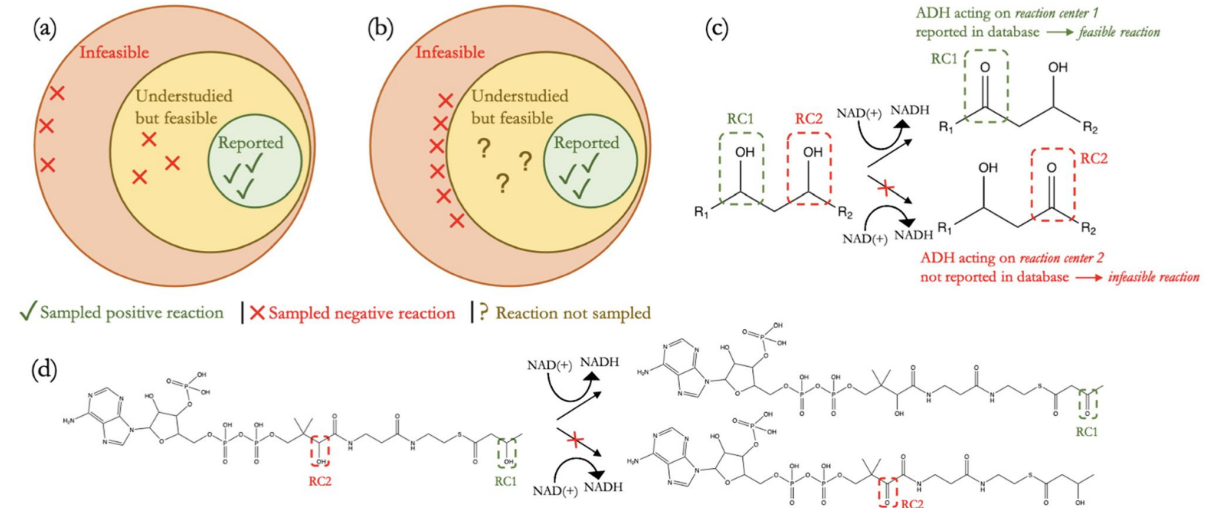


Figure: (a) It is common to assume that all unreported reactions are infeasible but this may lead to sampling negative reactions that are too dissimilar from reported reactions or mislabeling understudied reactions as infeasible; (b) ideally, any training set should not comprise false negatives while true negatives should be as close to true positives as possible; (c) here, we propose the “alternate reaction center” assumption to strategically infer infeasible reactions from reported reactions. Under this assumption, if a reaction has two or more identical functional groups but only one of these groups is known to undergo catalysis, then the transformation of the remaining group/s represents an infeasible reaction; (d) shown here is an example of an infeasible alcohol dehydrogenase (AdH) reaction with 3-hydroxybutyryl-coenzyme A in which the generalized AdH transformation is observed only outside of the CoA group but not within the CoA group.