

Section-level genome sequencing and comparative genomics of *Aspergillus* sections *Cavernicolus* and *Usti*

Background/Objective

- The genus *Aspergillus* is diverse, including species of industrial importance, human pathogens, plant pests, and model organisms

Approach

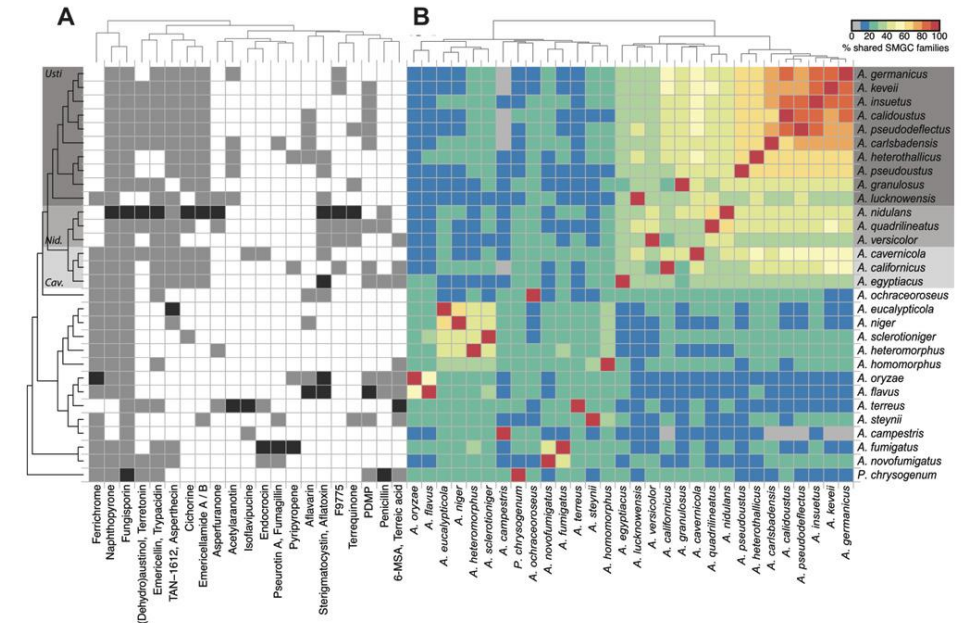
- We compared these genomes with 16 additional species from *Aspergillus* to explore their genetic diversity, based on their genome content, repeat-induced point mutations (RIPs), transposable elements, carbohydrate-active enzyme (CAZyme) profile, growth on plant polysaccharides, and secondary metabolite gene clusters (SMGCs).

Results

- Analyses of genes found only in single species show that these constitute genes which appear to be involved in adaptation to new carbon sources, regulation to fit new niches, and bioactive compounds for competitive advantages, suggesting that these support species differentiation in *Aspergillus* species.

Significance/Impacts

- Sections *Usti* and *Cavernicolus* have mainly unique SMGCs. Section *Usti* contains very large and information-rich genomes, an expansion partially driven by CAZymes, as section *Usti* contains the most CAZyme-rich species seen in genus *Aspergillus*. Section *Usti* is clearly an underutilized source of plant biomass degraders and shows great potential as industrial enzyme producers.



Quantitative dissection of *Agrobacterium* T-DNA expression in single plant cells reveals density-dependent synergy and antagonism

Background/Objective

- *Agrobacterium* pathogenesis, which involves transferring T-DNA into plant cells, is the cornerstone of plant genetic engineering.

Approach

- We examined if a classic Poisson model of interactions between pathogens and host cells holds true for *Agrobacterium* infecting *Nicotiana benthamiana*. Systematically challenging this model revealed antagonistic and synergistic density-dependent interactions between bacteria that do not require quorum sensing. Using various approaches we studied the molecular basis of these interactions.

Results

- To overcome the engineering constraints imposed by antagonism, we created a dual binary vector system termed ‘BiBi’ which can improve the efficiency of a reconstituted complex metabolic pathway in a predictive fashion.

Significance/Impacts

- Our findings illustrate how combining theoretical models with quantitative experiments can reveal new principles of bacterial pathogenesis, impacting both fundamental and applied plant biology.

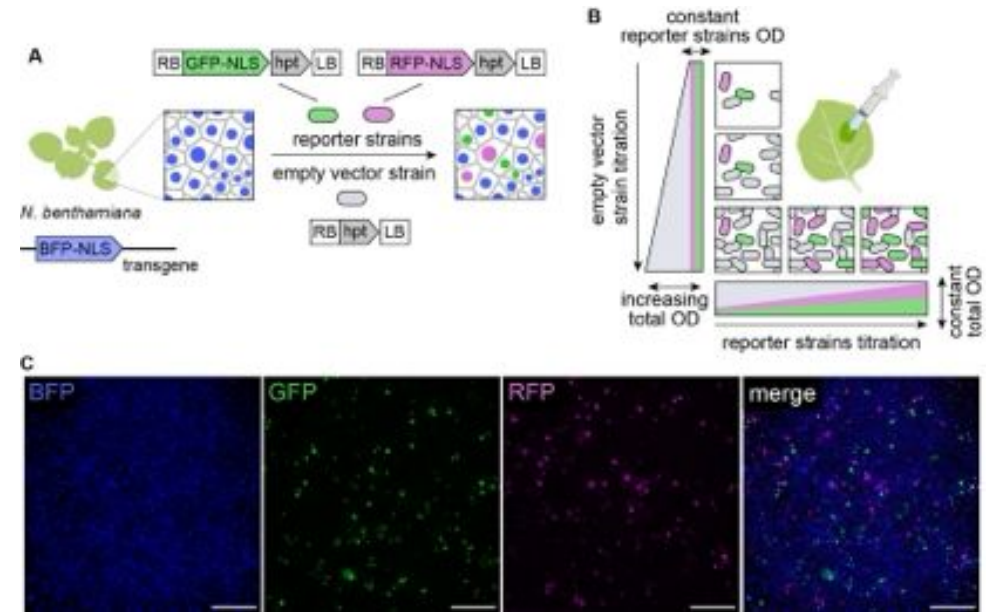


Figure caption: Experimental setup to quantify *Agrobacterium* T-DNA expression as a function of bacterial density and test the Poisson model of infection.

Evolution-guided tolerance engineering of *Pseudomonas putida* KT2440 for production of the aviation fuel precursor isoprenol

Background/Objective

- Current titers, rates, and yields of the biofuel, isoprenol in engineered *Pseudomonas putida* KT2440 are not commercially viable due to product toxicity.
- To enhance isoprenol tolerance and production in *P. putida* through evolution-guided engineering strategies.

Approach

- Adaptive laboratory evolution (ALE) followed by rational host engineering to develop *P. putida* strains with increased isoprenol tolerance

Results

- Developed isoprenol-tolerant (upto 8 g/L isoprenol) *P. putida* strains by ALE.
- Identified *gnuR*, *ttgB-PP_1394*, phage island, and *mxtR* as key tolerance loci.
- Validated the genetic basis of improved tolerance traits by reverse engineering.
- Proteomics revealed membrane, stress, and metabolism changes after evolution.
- Rational editing restored isoprenol production yields in evolved tolerant strains.

Significance/Impacts

- This study advances the development of robust and resilient microbial platforms for sustainable biomanufacturing by integrating evolutionary and rational engineering approaches.

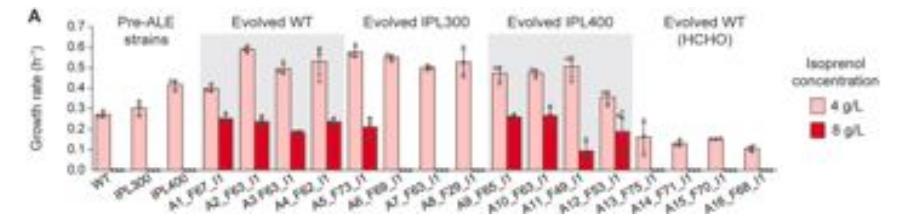


Figure caption 1: Enhanced growth rates of evolved *P. putida* in the presence of isoprenol (4 and 8 g/L)

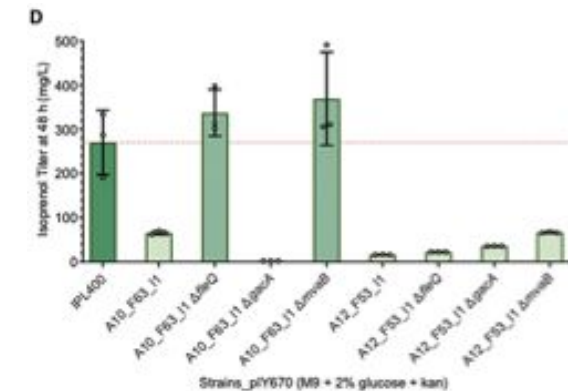


Figure caption 2: Restored isoprenol production in tolerized *P. putida* via rational strain engineering

Machine learning based reduced-order models to predict spatiotemporal dynamics of soil carbon and biomass yield of different bioenergy crops

Background/Objective

- Computational & data requirements of biogeochemical models limit their scalability across multiple scenarios and timescales
- Objective: develop ML-based models to enable uncertainty & scenario analysis

Approach

- Combined ML model with DayCent to project baseline and future (2021–2100) biomass yields and SOC changes for three bioenergy crops: Miscanthus, sorghum, and switchgrass on U.S. ag lands

Results

- Sorghum and Miscanthus projected to see an increase in economically viable land area of 29 % and 10 %, respectively.
- The most significant increases for sorghum are expected at higher latitudes

Significance/Impacts

- Demonstrates the potential of ML-based reduced order models to provide accurate predictions
- Allows us to do more robust uncertainty and scenario analysis

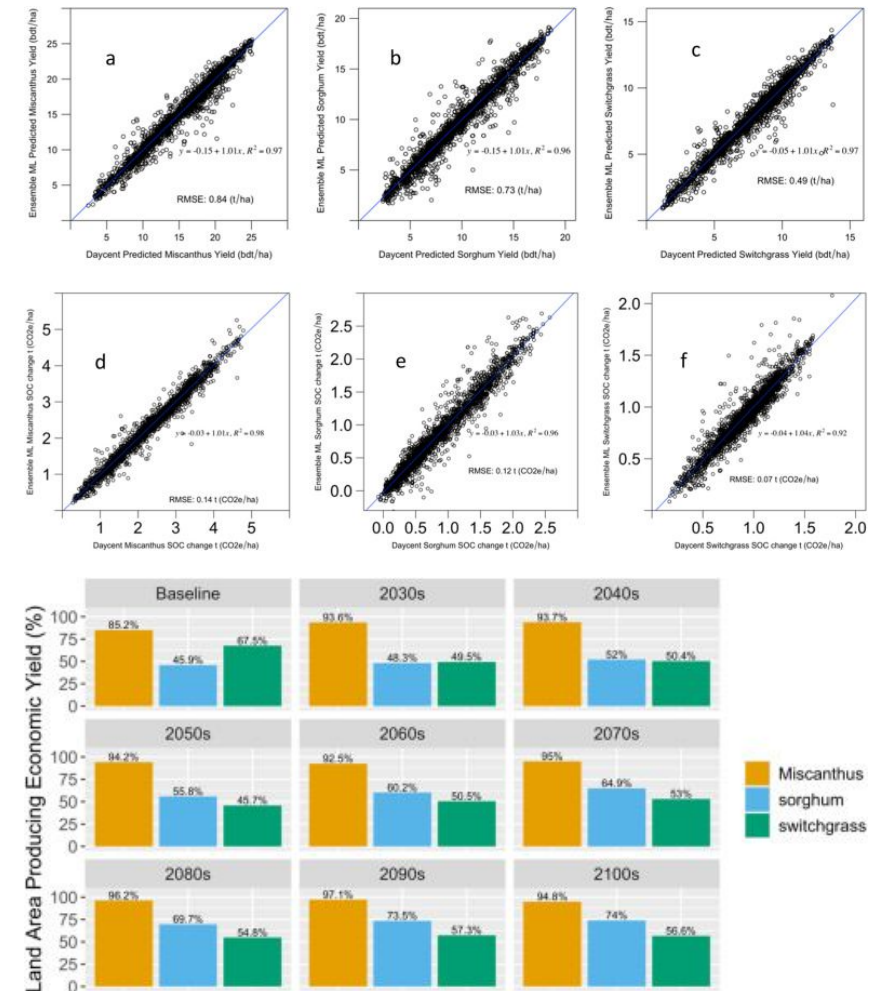


Figure 1: Validation plots comparing the ML and DAYCENT baseline

Figure 2: Changes in cropland area producing >10 bone-dry tonne/ha biomass yield for sorghum and Miscanthus and 7.5 bone-dry tonne/ha for switchgrass across the baseline and future decades

Enabling malic acid production from corn-stover hydrolysate in *Lipomyces starkeyi* via metabolic engineering and bioprocess optimization

Background/Objective

- *Lipomyces starkeyi* is an oleaginous yeast with a native metabolism well-suited for production of lipids and biofuels from complex lignocellulosic feedstocks.
- However, the feasibility of redirecting *L. starkeyi* lipid flux away from lipids and towards other products remains relatively unexplored.

Approach

- Here, we engineer the native metabolism to produce malic acid by introducing the reductive TCA pathway and a C4-dicarboxylic acid transporter to the yeast.

Results

- Heterogeneous expression of two genes and overexpression of a third one allowed titers to reach 10 g/L during shaking flasks cultivations.
- Controlled bioreactor fermentations on the real hydrolysate produced 26.5 g/L of malic acid.
- Machine learning based medium optimization improved production dynamics by 18% on mock hydrolysate.

Significance/Impacts

- This work demonstrated the ability to produce organic acids in *L. starkeyi* with minimal byproducts, and provides potential targets for overexpression for future *L. starkeyi* engineering work.

Czajka, J. J., et al. Microbial Cell Factories. doi: 10.1186/s12934-025-02705-0 (JBEI #1235)

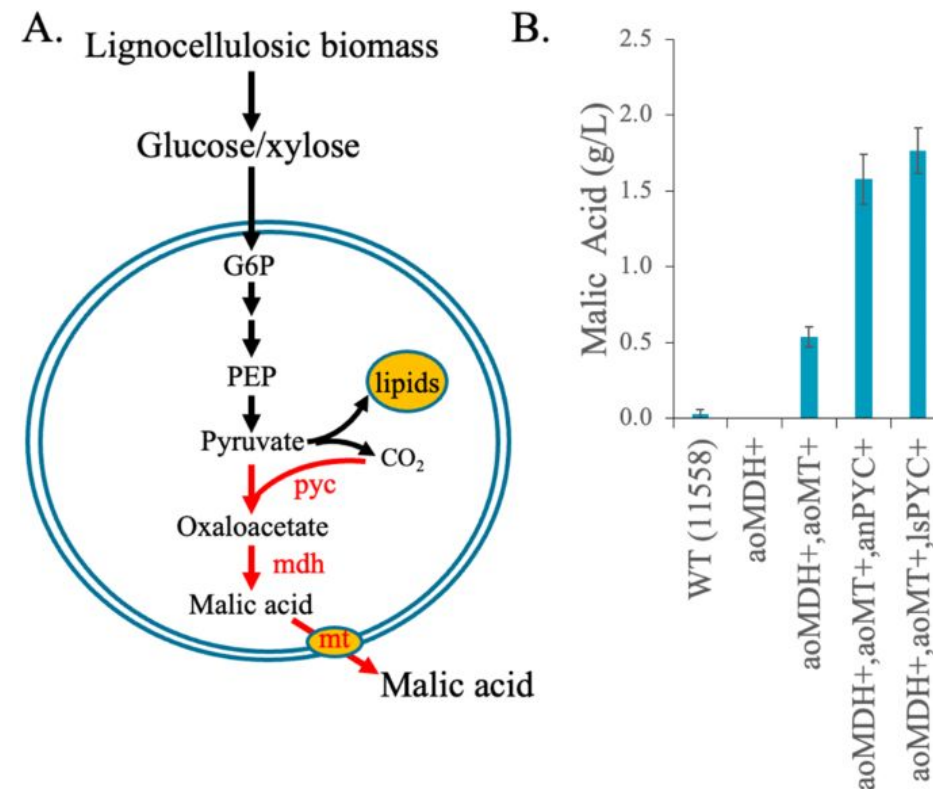


Figure 1: Engineered malic acid biosynthetic pathway and production comparison among mutants. (A). The engineered rTCA pathway and the C4-dicarboxylic acid transporter in *L. starkeyi*. (B). Malic acid titers among different engineered *L. starkeyi* strains grown in glucose containing minimal media.

Enabled Publications

Spatiotemporal development of expanding bacterial colonies driven by emergent mechanical constraints and nutrient gradients

Background/Objective

- Bacterial colonies growing on solid surfaces can exhibit robust expansion kinetics, with constant radial growth and saturating vertical expansion, suggesting a common developmental program.
- Here, we study this process for *Escherichia coli* cells using a combination of modeling and experiments.

Approach

- The growth and maturation of *Escherichia coli* cells colonies were measured over the course of several days, well beyond the establishment phase of linear radial and vertical colony growth.
- Numerical simulations based on an agent-based and metabolic model were used to capture overall colony expansion dynamics.

Results

- Linear radial colony expansion is set by the verticalization of interior cells due to mechanical constraints rather than radial nutrient gradients as commonly assumed.
- Vertical expansion slows down due to limitation of cell growth caused by vertical nutrient gradients.

Significance/Impacts

- The intricate dynamics of such emergent behavior can be understood quantitatively from an interplay of mechanical constraints and nutrient gradients arising from obligatory metabolic processes.

Kannan, H., et al. Nature Communications. doi: 10.1038/s41467-025-60004-z (JBEI #113)

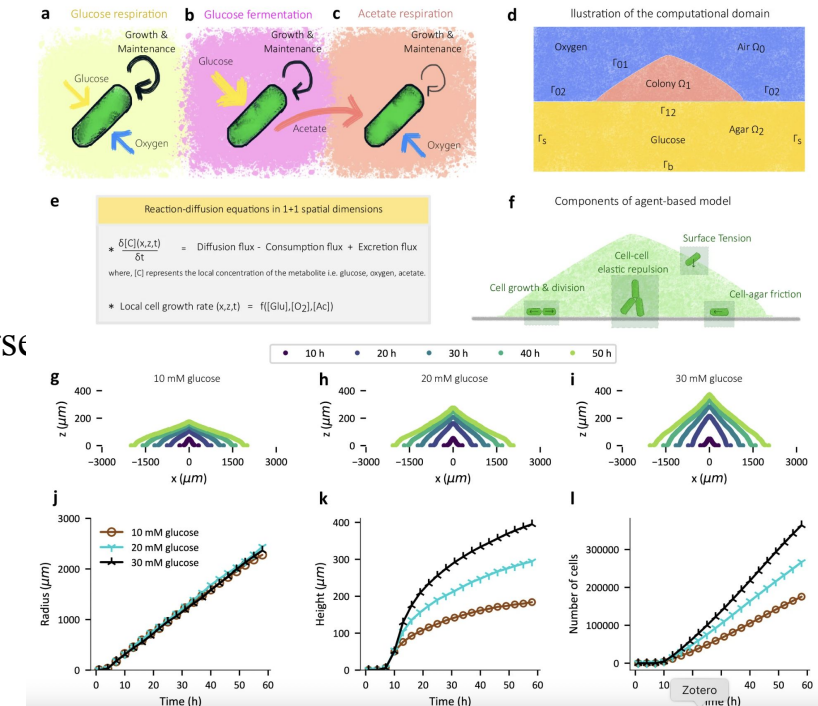


Figure caption: Simulations based on an agent-based and metabolic model capture overall colony expansion dynamics.

Chemical Characterization, Antioxidant and Enzyme-Inhibitory Activities of Different Extracts from Three Phlomis Species

Background/Objective

- Explore the beneficial health potential of Phlomis species (*P. fruticosa*, *P. herba-venti*, and *P. kurdica*)

Approach

- Antioxidant activity based on their capacity to scavenge free radicals, chelate and reduce metal ions, and their ability to inhibit enzymes implicated in diabetes, skin hyperpigmentation, and Alzheimer's diseases were evaluated

Results

- Three Phlomis species are a rich source of biologically active compounds with potential for future development of phytopharmaceuticals targeting specific oxidative stress-linked diseases.

Significance/Impacts

- Key enzyme inhibition is involved in treating global health problems such as Alzheimer's disease, diabetes, and obesity

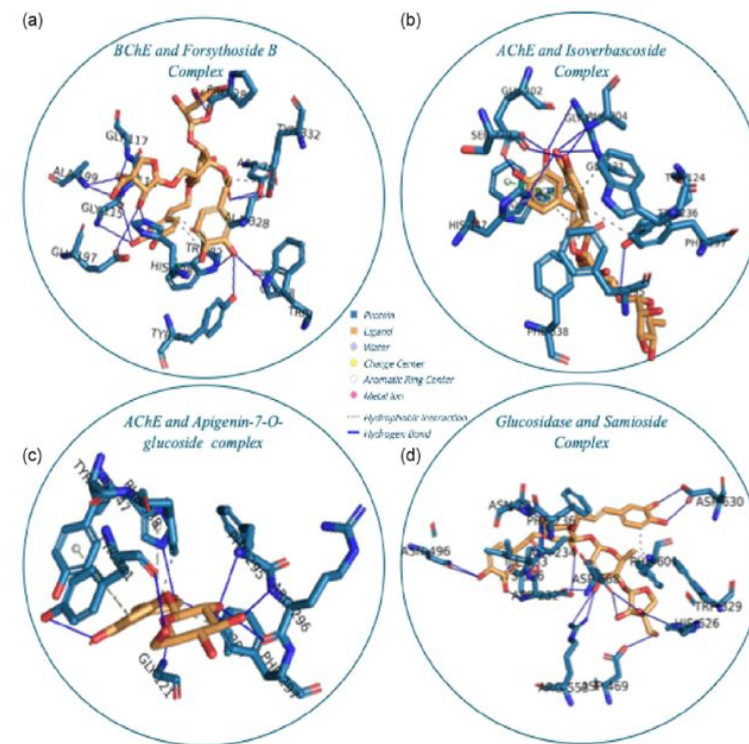


Figure caption: Binding interactions of enzymes with compounds showing the best binding energy; a) Interaction between BChE and Forsythoside B. b) Interaction AChE and Isoverbascoside. c) Interaction between AChE and Apigenin-7-O-glucoside. d) Interaction between Glucosidase and Samioside.

Unveiling Feedstock Variability: Insights into Corn Stover Conversion

Part I: Physicochemical Properties and Self-Degradation

Background/Objective

- Background: The inherent variability in the composition and quality of agricultural waste introduces uncertainties in the conversion efficiency and poses challenges in process development.
- Objective: Measure effects of variability in self-degraded feedstock chemical composition on convertibility.

Approach

- Measured chemical composition of feedstocks that exhibited four different levels of self-heating.
- Measured convertibility with and without hot water pretreatment and correlated for each self-heated sample.

Results

- Self-degradation due to microbial activity during storage decreases the carbohydrate content of corn stover but enhances sugar yields.
- Inhibitory effect of lignin diminishes in self-degraded samples likely due to the disrupted cell wall structure.
- Hot water pretreatment under mild conditions mitigates inherent variability, improving the sugar yield from corn stover by up to 50%.

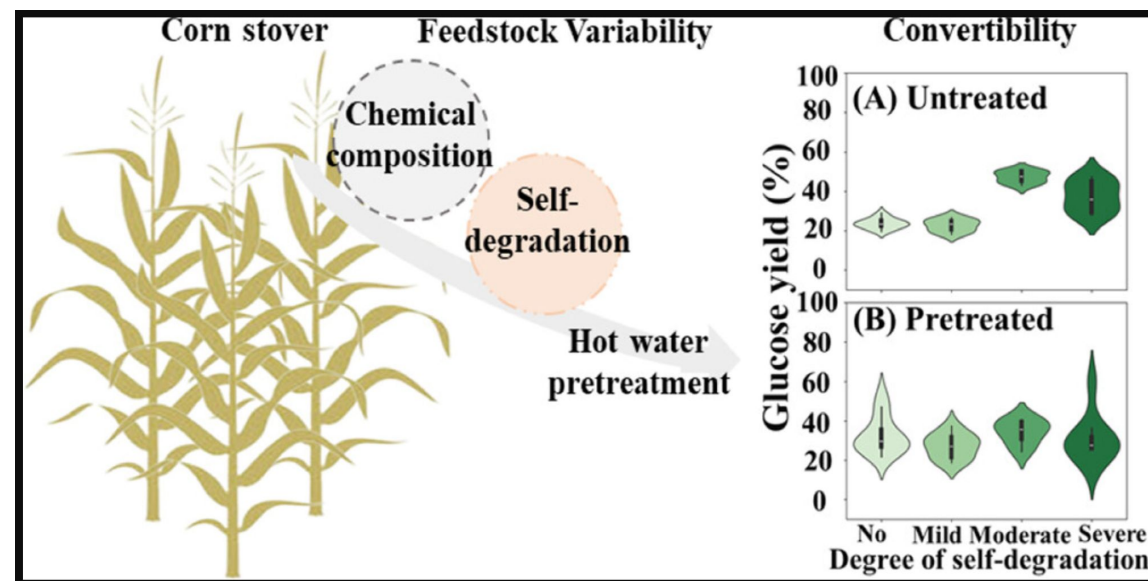


Figure Caption: Glucose yields for hot untreated and hot-water pretreated corn stover samples with varying degree of self-degradation

Significance/Impacts

- By elucidating the feedstock variability and its impact on convertibility, these findings offer valuable insights into appropriate feedstock handling and management, highlighting potential strategies to address variability challenges.

Kang, X., et.al. ACS Sustainable Chemistry & Engineering. <https://doi.org/10.1021/acssuschemeng.5c00233> (JBEI #115)