

# Engineering Auxotrophic *Agrobacterium* Improves Plant Transformation

## Background

- *Agrobacterium tumefaciens* is utilized for both transient and stable plant transformation.
- Antibiotics to control *Agrobacterium* growth are used to prevent the bacterium from overgrowing plant tissue, but this approach is not always successful and can have undesired effects on the plant tissue.

## Approach

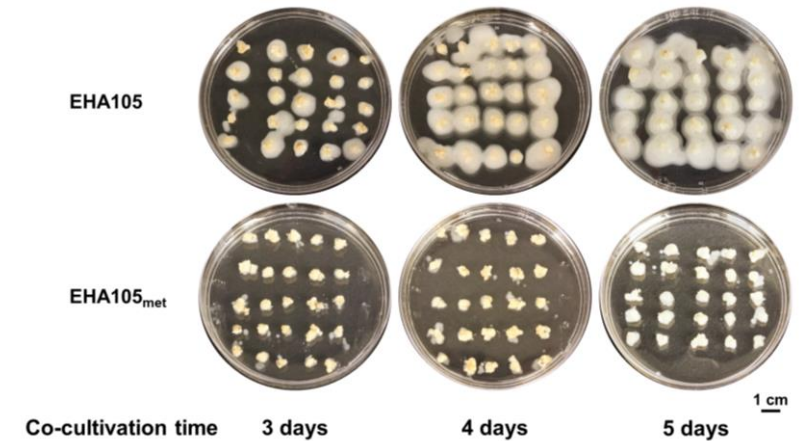
- *Agrobacterium tumefaciens* strains auxotrophic for methionine (EHA105<sub>met</sub> and LBA4404<sub>met</sub>) were engineered via homologous recombination as an alternative counter-selective agent to cure plants of the bacterium.
- Switchgrass transformation, where overgrowth remains a challenge, was chosen to assess the performance of the engineered auxotrophic *Agrobacterium*. Rice transformation was used for comparison.

## Results

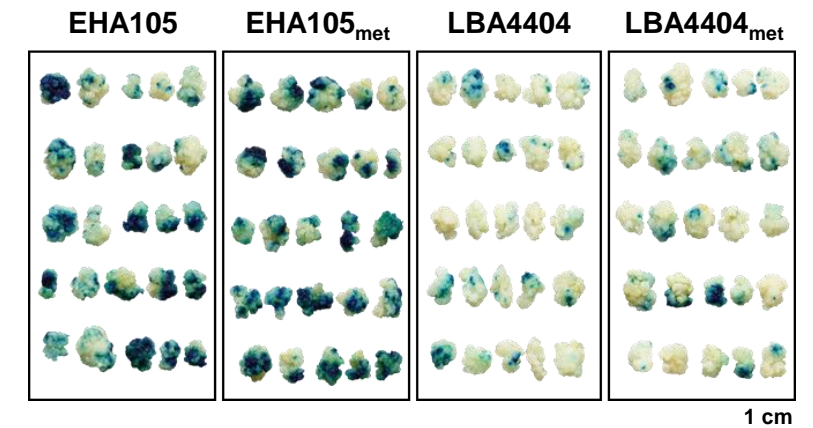
- The use of *A. tumefaciens* auxotrophic strains minimizes the use of antibiotics (Figure 1) and exhibited the same transformation frequency as the parental strains.
- Auxotrophy has no effect on the number of T-DNA copies delivered by any of the *Agrobacterium* strains evaluated.
- Unique to switchgrass transformations, EHA105<sub>met</sub> yielded better outcomes than LBA4404<sub>met</sub>.

## Significance

- Engineering auxotrophic strains eliminates the use of antibiotics while preventing bacterial overgrowth on plants. Furthermore, the auxotrophic *Agrobacterium* and its use in transforming plant cells permits for the biological containment of bacteria carrying transgenes.



*Agrobacterium* growth in co-cultivation with switchgrass tissue.



GUS staining in a switchgrass Performer 7 callus including all *Agrobacterium* strains used in this study at five days of co-cultivation.

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