Probing phylloplane microbial community physiology and dynamics in situ using whole-cell yeast biosensors

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Project Goals

- Engineer phyllosphere yeasts to express biosensor proteins/constructs
- Non-destructively observe spatial and temporal variation in phyllosphere environment
- Towards predictive microbial ecology – understand the microenvironments which select specific microbial taxa

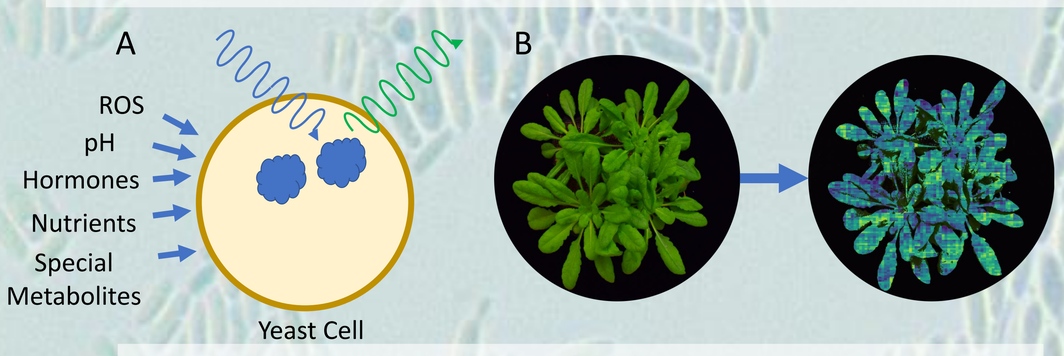


Fig 1. A conceptual image of A) biosensors detecting a cell's environment and B) mapping environmental conditions across the leaf surface using biosensors.

Yeasts in the phyllosphere

- Yeasts are abundant components of the leaf environment
- Phylogenetically and functionally diverse

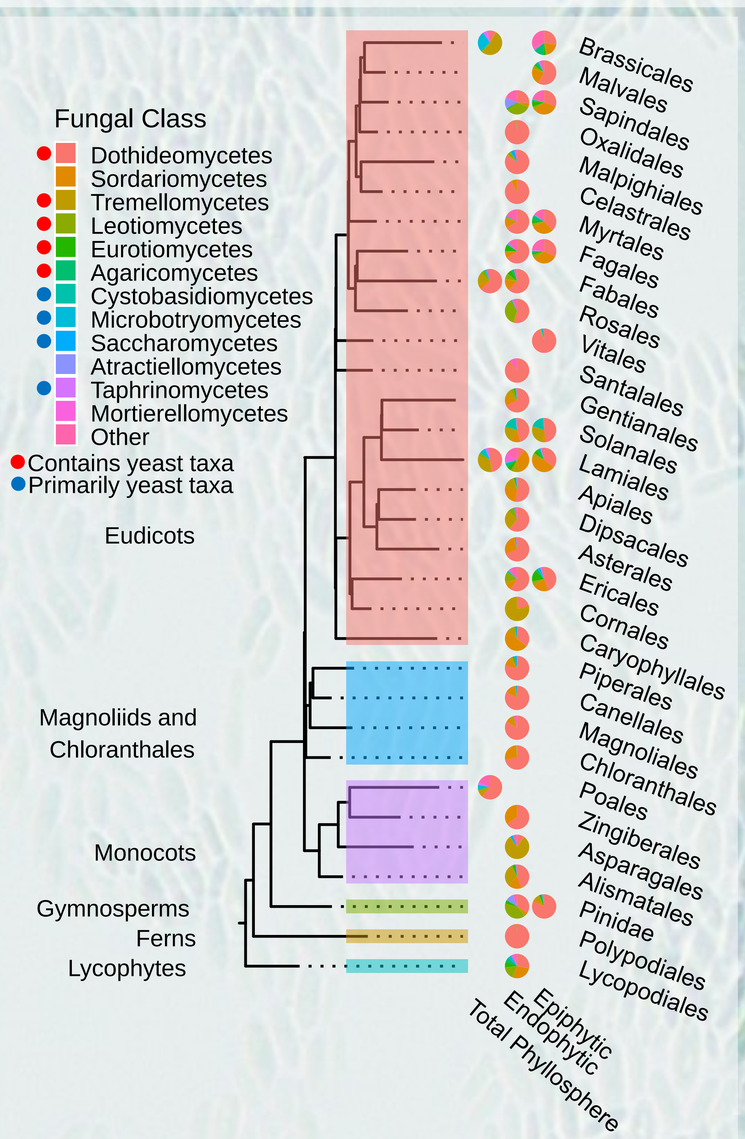


Fig 2. Occurrence of fungal classes in phyllosphere samples across vascular plants, derived from re-analysis of 18 NCBI SRA BioProjects.

Part 1 – Isolate Yeasts

Fig 3. Isolating yeast from the phyllosphere by grinding and plating leaf tissue and via ballistospore-capturing plates.

Part 2 – Characterize Yeasts

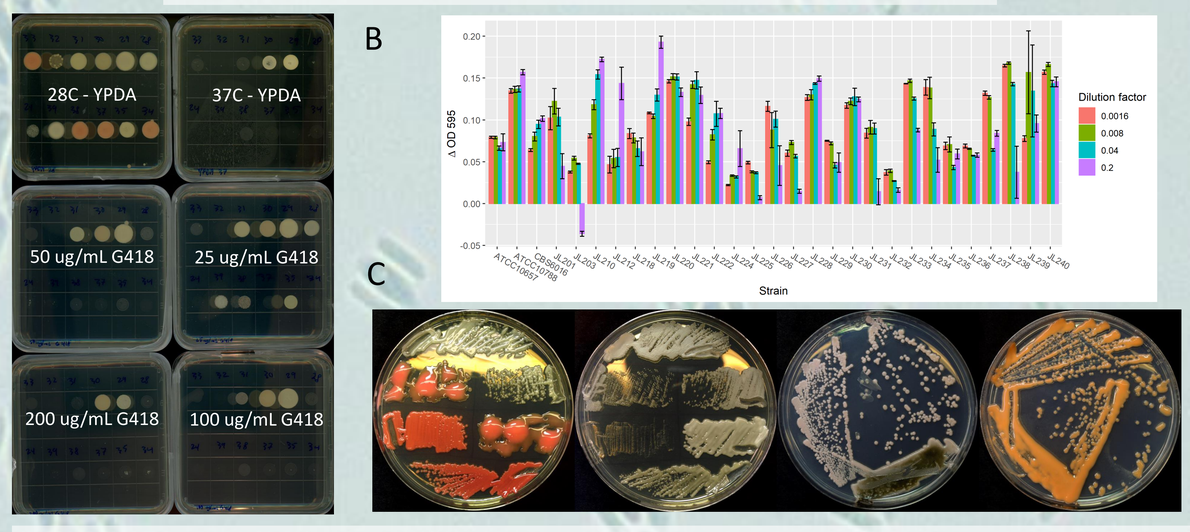


Fig 4. Characterizing phyllosphere isolated yeasts A) Temperature and antimicrobial susceptibility testing on plates for a panel of yeasts. B) Growth rates of yeasts. C) Morphological comparisons.

Part 3 – Develop Transformation Method

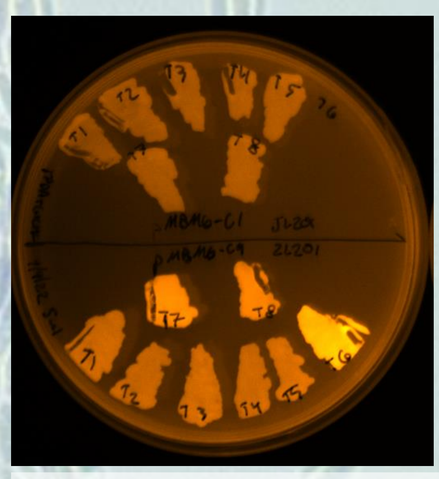


Fig 5. Transformed Microbotryomycete sp. JL201 showing expression of LSSmOrange.

Option 1: *Agrobacterium*

Pros
- Constructs do not require homologous regions
- Available vectors
- Broad range vectors

Cons
- Long protocol length (8+ days)

So far: Success!

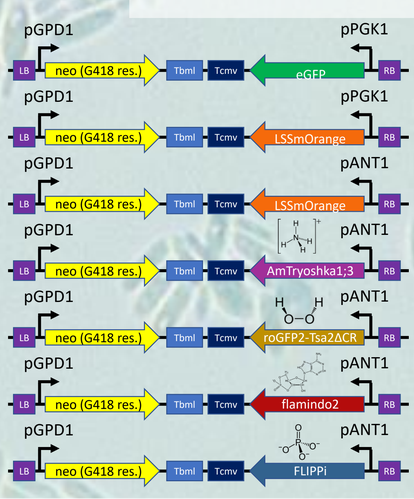
Option 2: Electroporation

Pros
- Shorter protocol length
- Often higher efficiency
- Targeted gene knockout
- Smaller constructs

Cons
- Requires homologous regions
- Unique vector for each strain

So far: Sequencing genome(s) first

Parts 4+ – Optimize expression system, design constructs, apply to plants



- Characterize high-expression endogenous promoters with favorable expression profiles
- Clone and test biosensors for a variety of environmental conditions
- Test methods for applying to multiple plant species and measure persistence
- Focus on strains with high persistence on leaves

Fig 6. Binary vector cassettes for expression of fluorescent proteins and biosensors in Microbotryomycete yeasts.

Applications

- Use spatial/temporal variation in metabolites/hormones to predict microbial communities
- Novel detection-response systems to biotic and abiotic stresses
- Induce changes in community composition using specific metabolites – test the importance of environmental filtering