Developing DNA transformation methods for non-model anaerobic gut fungi

Nikola Malinov
University of California Santa Barbara
Department of Chemical Engineering
P.I.: Michelle O'Malley
Mentors: Igor Podolsky and Candice Swift
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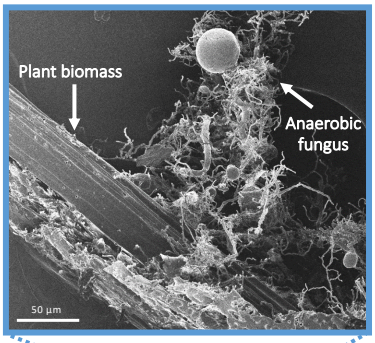
Anaerobic gut fungi (AGF) lack reliable methods for genetic manipulation

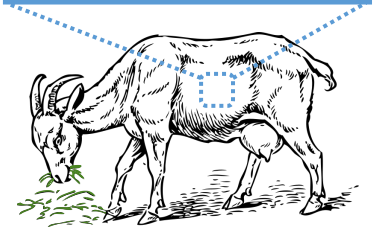
Colonizers and degraders of ingested biomass [1,2]

AGF enables biomanufacturing directly from waste biomass

Unable to engineer tolerance to industrial conditions

Approach: Optimize a yeast DNA transformation technique for AGF





[1] Mountfort, D. O. & Orpin, C. G. (1994).
[2] Henske, J.K. *et al.* (2018). *Biotechnol. Bioeng.*

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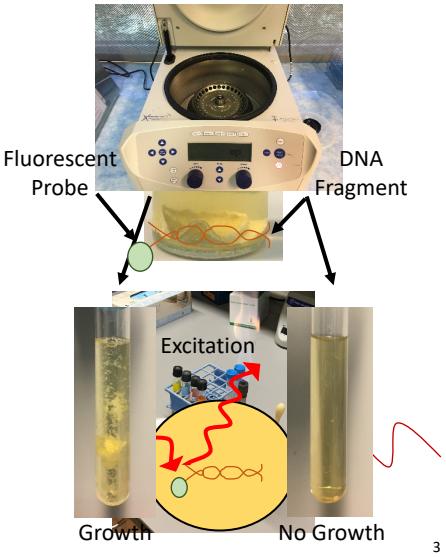
Project Goals

1. Gain familiarity with culturing non-model AGF

2. Screen effect of protocol parameters on cell survival

3. Utilize fluorescent probe to observe DNA uptake



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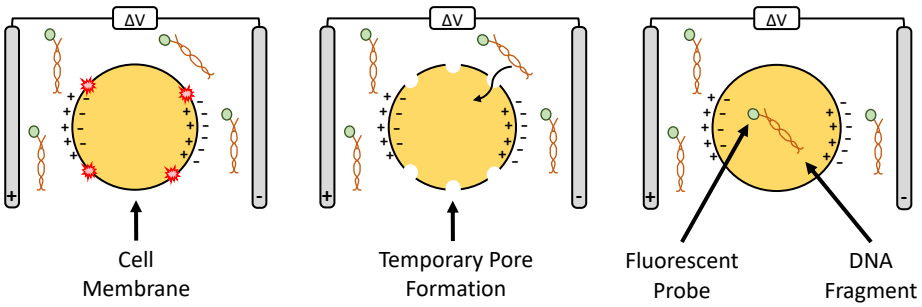
Approach: transformation by electroporation



Electric pulse induces voltage across cell membrane

Temporary pores form along the cell membrane

DNA uptake

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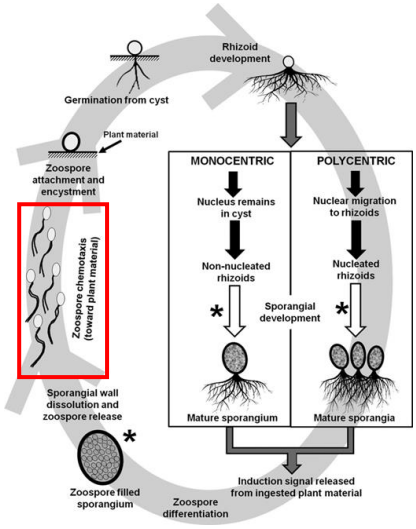
Approach: isolate of zoospore life-stage

AGF have a complex life cycle

Cell wall absent during zoospore life-stage

Optimizes chance of successful electroporation



Gruninger, Robert J. et al. (2014) *FEMS microbiology ecology* 90 (1-17.)

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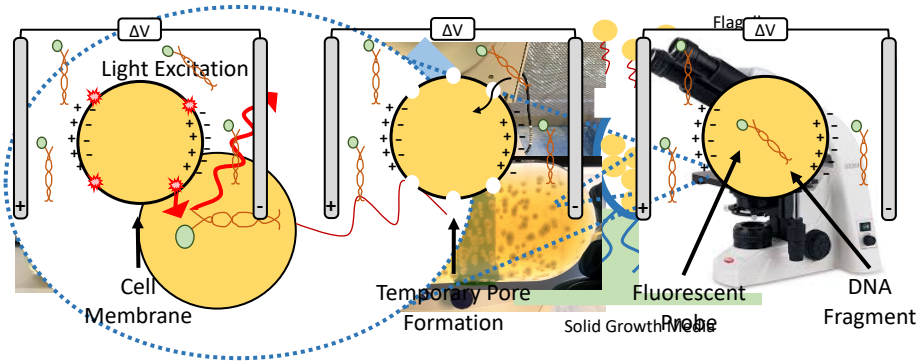
Electroporation workflow

Zoospore Collection → Wash Steps Through Centrifugation → Electroporation → Microscopy



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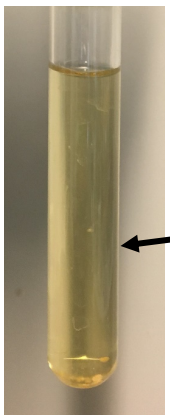
Gas production and cellular debris is indicative of fungal growth

Hydrogen bubbles produced as part of fungal metabolism

Fungi grow on soluble carbon source and form fungal mat

Growth



No fungal mat present

No Growth

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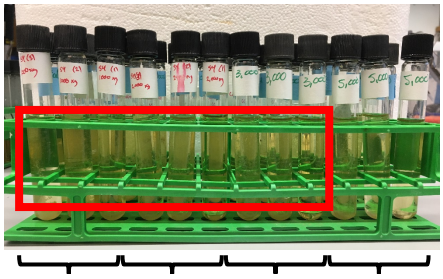
Centrifugation is necessary to clean and concentrate zoospores

Goat Isolate (G1)

1000 xg 2000 xg 3000 xg 5000 xg

Sheep Isolate (S4)



1000 xg 2000 xg 3000 xg 5000 xg

Anaerobic gut fungi strains tolerate centrifugal forces up to 3000 xg

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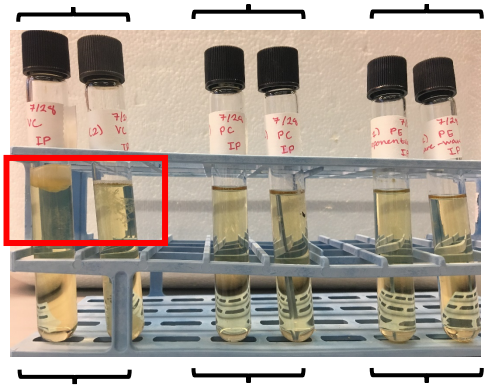
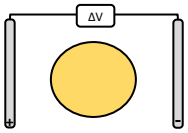
Zoospores were intolerant of varied electroporation parameters

After zoospore collection | After centrifugation | After electroporation



Growth | No Growth* | No Growth



Varied Parameters			
Waveform			
ΔV	0.5 kV/mm	0.75 kV/mm	1.25 kV/mm
Solution Temperature	4 C		39 C

* Centrifuged at upper end of assay; 5000 xg.

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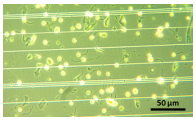
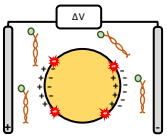
Conclusions and future work

Developed method of viable zoospore collection



Centrifugation assay established upper limit of tolerable forces; 3000 xg

Test additional electroporation parameters



Conduct an alternative, chemical DNA transformation protocol

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