Developing DNA transformation methods for non-model anaerobic gut fungi

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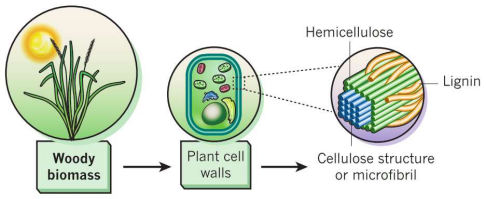
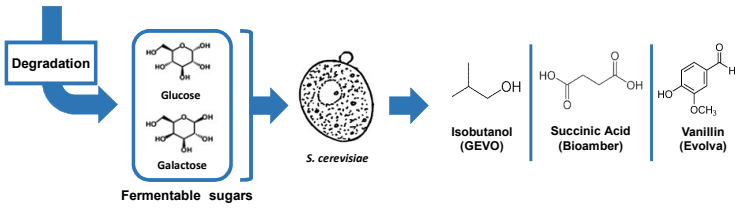
Abstract: Herbivores utilize fungal microbes in their rumen or hindgut to degrade complex lignocellulosic biomass, such as grasses, into constitutive sugars the animal can digest. Harnessing the lignocellulose-degrading capability of these anaerobic gut fungi (AGF) would enable sustainable bio-manufacturing of biofuels and chemicals directly from waste biomass. Currently, we lack reliable genetic methods for these non-model microbes that would otherwise allow us to actively engineer robustness and other useful phenotypes. This study sought to adapt established electroporation methods that have been used for both model and non-model fungi. First, a method was developed to isolate and concentrate the zoospore life-stage, lacking a cell wall, through simultaneous release from agar media. Subsequently, several electroporation workflows were assessed using cell viability as the primary requirement. The spores were washed of salts using centrifugation and observed to tolerate centrifugal forces up to 3000 xg. The protocol was systematically shortened by cutting wash steps that minimally reduced salt content. The electroporation waveform type, the electric field strength, and the buffer temperature were varied in an effort to maintain cell viability. To date, viability after this step has not been observed under any assayed conditions. Future work will further test additional electroporation parameters to ensure cell viability and pursue an alternative, chemical transformation methodology.

Abundant lignocellulosic biomass waste is a sustainable platform for a future bio-economy



Each year, more than 1.5 billion tons of waste biomass is produced on the global scale [1]

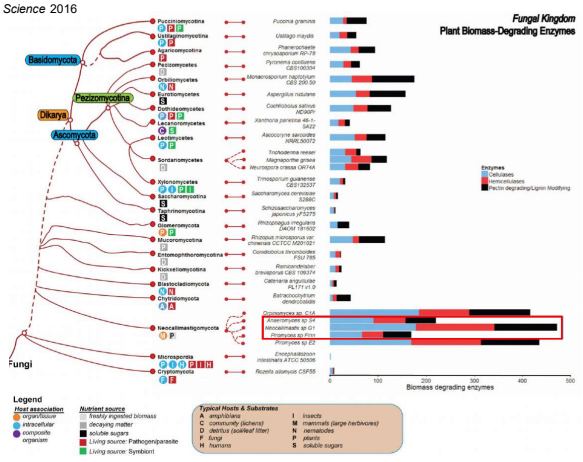
Rigid biopolymers (cellulose & hemicellulose), shielded by lignin, resist degradation into constitutive, simple sugars



Sugars released from lignocellulosic biomass can be fermented by model microbes into value-added chemicals at industrial scales

Anaerobic gut fungi (AGF) produce a large array of enzymes adept at degrading biomass

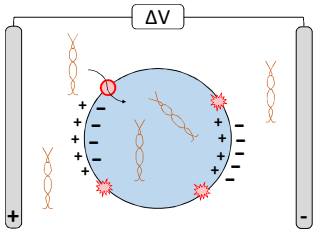
Solomon et al. *Science* 2016



AGF implementation is limited by poor tolerance of temperature variation and oxidative stress

To date, AGF lack reliable genetic manipulation methods necessary to engineer these microbes for industrial applications

Adapt electroporation methods developed for both model and non-model fungi

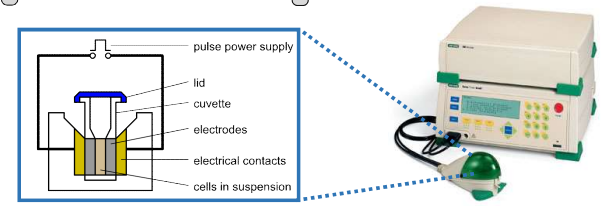


Electroporation induces an electric gradient across the cell membrane [2]

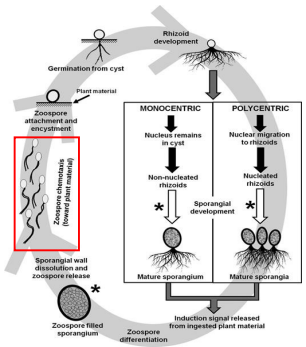
Subsequent temporary pore formation enables DNA uptake [2]

Multitude of parameters to optimize the process

Gene Pulser Xcell™ Electroporation System - Bio-Rad



Preferentially isolate zoospores through simultaneous release in solution



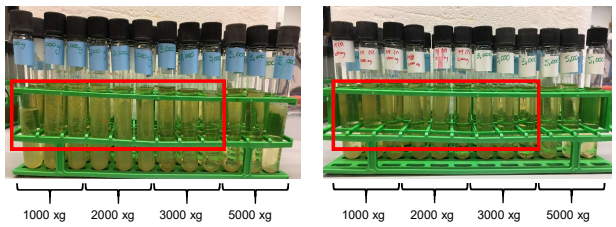
Gruninger, Robert J. et al. (2014) *FEMS microbiology ecology.*

The fungi lack a cell wall during the zoospore stage of their complex life cycle [3]

Electroporation at this life-stage increases the chance of successful DNA transformation

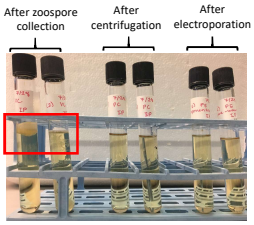
Zoospores were harvested after six days of growth on solid agar media and concentrated to ~3 x 10^6 spores/mL

AGF tolerate range of centrifugal forces but fail to survive varied electroporation conditions



Centrifugation is necessary to wash away excess salts in solution and concentrate the zoospores

Baseline range of centrifugal forces establishes limit for cell viability at 3000 xg



Varied Parameters			
Waveform	Exponential Decay (ΔV, Time (ms))	Square Wave (ΔV, Time (ms))	
ΔV	0.5 kV/mm	0.75 kV/mm	1.25 kV/mm
Solution Temperature	4 C	39 C	

Changing the waveform type, field strength, and temperature failed to preserve viability

Conclusions and future work

Simultaneous release of spore into solution generated high spore yields (~10^6 spores/mL)

Established the upper limit of tolerable forces for the AGF zoospores at ~3000 xg

Future experiments will assess the feasibility of "gentler", chemical transformation methods

References

1. Dashtban, Mehdi, Heidi Schraft, and Wensheng Qin. "Fungal bioconversion of lignocellulosic residues; opportunities & perspectives." *International journal of biological sciences* 5.6 (2009): 578.
2. Weaver, James C., and Yu A. Chizmadzhev. "Theory of electroporation: a review." *Bioelectrochemistry and bioenergetics* 41.2 (1996): 135-160.
3. Mountfort, D. O. & Orpin, C. G. *Anaerobic fungi : biology, ecology, and function.* (M. Dekker, 1994).

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