Leveraging Microscopy to Characterize the Morphology and Autofluorescence of Lignocellulose Degrading Microbes

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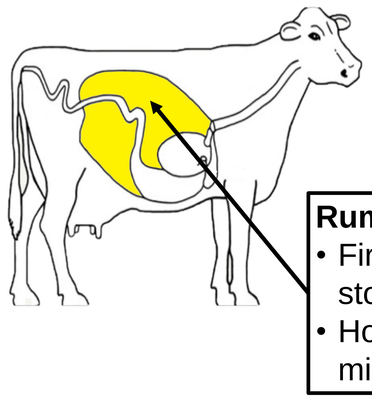
Abstract: Lignocellulose, the woody complex responsible for the strength of plant cell walls, is the most abundant renewable biomass resource available. Its valorization promises to enable sustainable production of value-added products from biofuels to pharmaceuticals; however, lignocellulose is recalcitrant to chemical and biological degradation. Rumenous anaerobic microbial consortia, found in the primary stomach of ruminants (cows, sheep, etc.), naturally evolved to degrade lignocellulose as an energy source. Within the rumen, anaerobic fungi are the primary lignocellulose degraders. Methanogenic archaea consume byproducts of fungal metabolism and are critical to consortium function. Biomass breakdown is spatially dependent in these systems, as fungi bind to biomass and each other, and methanogens associate within the fungal network. To mechanistically understand lignocellulose degradation in anaerobic consortia, we aim to characterize spatial organizations of the constituent microbes. The morphology of a representative anaerobic fungus, *Neocallimastix sp*. (S3), was characterized via brightfield microscopy; morphology of a representative methanogen, *Methanobacterium bryantii*, is already well characterized. To distinguish between constituent microbes in a mixed culture, non-overlapping fluorescence in both species is necessary. Autofluorescence in the fungus was observed with excitation and emission channels spanning virtually the entire visible light spectrum, with limited UV activity. Autofluorescence in methanogens is well-characterized, with UV excitation and blue light emission. Therefore, we developed methods to resolve fungi and methanogens in co-culture without the use of stains, probes, or dyes. Following optimization of cultivation of imageable consortia, the spatial organization of rumenous consortia will be quantified to further our understanding of consortium function.

Lignocellulose can be degraded by microbes isolated from rumenous herbivores

Lignocellulose[1]

Lignocellulose is a complex of lignin, cellulose, and hemicelluloses, that accounts for the majority of plant biomass composition. Lignocellulose is the most abundant renewable resource on earth and can be converted to various valuable commodities, but with one caveat – it is incredibly difficult to degrade. Rumenous microbes, led by anaerobic fungi, have the potential to degrade lignocellulose at an industrial scale.

Rumenous microbes evolved to degrade lignocellulose for as an energy source



Rumen[2]:
- First of multiple stomachs
- Houses numerous microbes

Co-culture of anaerobic fungi and methanogenic archaea to maximize degradation (schematic)

Lignocellulose[1]

Lignocellulose degradation is spatially motivated

The efficiency of lignocellulose degradation depends heavily on the spatial organization of co-culture constituents. To elucidate this spatial organization, S3 morphology was characterized.

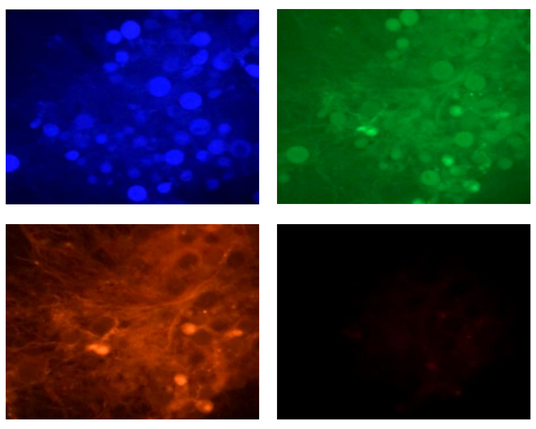
S3 sporangia (mature cell in which spores form) are approximately spherical with branching legs/roots (rhizoids). Rhizoids adhere to and breakdown lignocellulose.

Average Size: 39.41 ± 1.59 µm (diameter)

Via brightfield, 40x

To distinguish between the S3 and *Methanobacterium bryantii* in co-cultures, autofluorescence spectra were characterized for both species. S3 was found to fluoresce nearly everywhere but in the UV and blue light regions. Methanogen fluorescence agreed with literature.

S3 Autofluorescence: No UV/Blue interactions



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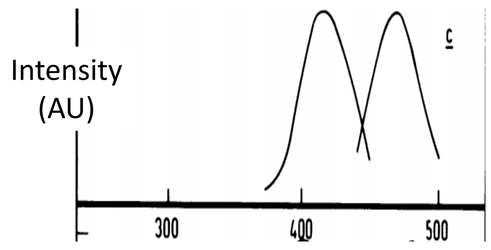
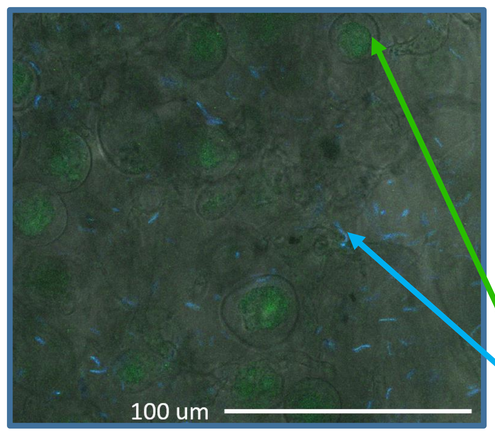
***Methanobacterium bryantii* autofluorescence emission and excitation spectra**



Dodema and Vogels, 1978

Autofluorescence alone can distinguish between S3 and *Methanobacterium bryantii* in a co-culture



Via fluorescent confocal, 20x

Saves us an immense amount of time!
- No need to engineer a genetic transformation
- No need to use fluorescent tagging
- No need to use stains

Green: S3 (λ = 510 nm)
Blue: Methanogen (λ = 460 nm)
(Emission Wavelengths)

Conclusions/Future Research

- Developed tool to distinguish between S3 and methanogens in co-culture
- Characterized sporangia morphology for S3
- Need to characterize zoospore and rhizoid morphology
- Need to develop repeatable method for biofilm development for use in future studies
- Will develop a library of co-culture images

References

1. Sanderson, K. (2011, June 22). Lignocellulose: A chewy problem. Retrieved from https://www.nature.com/articles/474S012a
2. Free Choice Enterprises, Ltd. (1970, January 01). The Rumen. Retrieved from http://www.freechoiceminerals.com/The-Rumen