Leveraging Microscopy To Characterize Morphology And Autofluorescence Of Lignocellulose Degrading Microbes

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Lignocellulose is the Most Abundant Renewable Resource

Lignocellulose
- Dry woody complex in plants
- Most abundant renewable resource

Rumen:
- First of multiple stomachs
- Houses numerous microbes
Using A Co-Culture To Maximize Degradation and Methane Production

1. **Lignocellulose**
   - **Degrade**
   - **Produce** Hydrogen

2. **Fungi**
   - **Produce** Hydrogen

3. **Methanogen (Methanobacterium bryantii)**
   - **Produce** Methane

4. **Archaea**
   - **Produce** Methane
   - **Consume**

5. **S3 (Neocallimastix sp.)**
   - **Produce** Hydrogen

6. **Further Processing**
   - Drugs, Cosmetics, Fuels, Other Commodity Chemicals
Investigating S3: Non-Model Fungal Organisms

Why are these fungi “non-model” organisms?
• Little known about their biological functions
• Very few established methods for analysis

What we need to investigate:
• Morphology (shape and size) - to elucidate spatial organization
• Autofluorescence - to distinguish in mixed culture of methanogens and fungi**

S3 Fungal Cells (Brightfield Microscope)
Autofluorescence: The emission of light from molecules within a biological sample that have been excited by some light source.

- Can be used to easily distinguish constituents in a sample or culture
Characterizing S3 Shape, Size, and Autofluorescence

- Approximately spherical
- Branching legs/roots (rhizoids)
- **Average Size:** 39.41 +/- 1.59 um (diameter)
  
  ~2/5 of a human hair!

**Autofluorescence:**

- DAPI Filter
- GFP Filter
- Cy3 Filter
- Cy5 Filter

Leggieri et al. - In preparation
Characterizing Methanogen Shape, Size, and Autofluorescence

- Approximately cylindrical (rod-like)
- **Average Size:** To Be Determined

Excitation - Emission Spectra for Methanogenic Autofluorescence

*Dodema and Vogels, 1978*
We Can Distinguish Fungi From Methanogens Using Autofluorescence

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

Blue = Methanogens (Wavelength: ~460 nm)
Green = Fungi (Wavelength: ~510 nm)
We Need Thin Films Before We Can Begin Investigating Co-Cultures

S3 Fungal Biofilm

- **Want:** Single-layer film
  - Thicker films = noisy images
- **Parameters:** Substrate volume and concentration, film adhesion to surface

**Major Complication!**

**Need to develop:**
- Repeatable method for forming biofilms
- Monolayer formation <100 um

![Diagram showing incoming light and monolayer formation]
Thin Fungal Biofilms Will Enable Further Analysis

- Begin co-culture monolayer film analysis

- Determine what causes the cells to autofluorescing (Proteins? Carbohydrates? Etc.)

- Quantitative characterization of autofluorescence parameters:
  - Fluorescence lifetime
  - Fluorescence emission intensities
  - Fluorescence bleaching
References

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