Exploiting Anaerobes for Biomass Breakdown and Sustainable Chemical Production

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Recalcitrant Biomass Hinders Production of Valuable Products from Cellulose





What can we learn from nature?



- Several microbiomes degrade biomass
- Anaerobes are promising understudied microbes
 - They populate the guts of animals, regulating immune function, nutrition, and other functions
 - Each microbe has a unique "job" within the community
 - New culture, sequencing, and proteomics tools allow us to understand and engineer these systems



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Objectives



Overall Goal: Accelerate the development of renewable lignocellulosic biofuels and value-added products

<u>Approach</u>: Learn from nature to engineer cellulases and anaerobes with novel functions

- Isolate gut microbes from their native microbiomes and integrate 'omics'-based tools to understand function
- Engineer anaerobic consortia for bioprocessing

Finding Fungi in the Muck...





John Henske

Fungal Isolates Exhibit Substrate Preference in Batch Culture



- > Fungi were extracted from a horse named "Finn" from Verrill Farm (Concord, MA)
- Fungal isolate renamed Piromyces sp finn and supported on reed canary grass
 - \succ Full growth cycle is seen in 5 6 days
 - Active growth is characterized by fermentation gas accumulation
 - Pressure accumulation allows substrate preference to be determined



(courtesy of Verrill Farm)

Famous Gut Fungi (Be careful what you wish for...)



Los Angeles Times

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BREAKING	G	PHOTOS	VIDEO	CRIME	OBITUARIES	WEATH	ER TR	AFFIC	CROSSWOR	DS SUDC	KU	HOROSCOPES	APPS

Horse manure yields secret to ethanol fuel

April 11, 2013 | By Geoffrey Mohan



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Michelle O'Malley knows good horse poop when she sees it. While at MIT, the chemical engineer scooped up some manure from Finn, a grass-fed horse at a sustainable farm in Concord, Mass.

That offal has led to a potential breakthrough in turning grasses and nonfood crops into an alternative fuel in attempts to wean motorists from fossil fuels and stem manmade climate change.



Enzyme Discovery: Why Sequencing a Genome is Not Enough





In order to identify the most powerful enzymes, it is critical to examine the transcriptome from a growing organism.



<u>Hypothesis</u>: Cellulosic substrates activate transcription of cellulolytic enzymes and cellulolytic complexes (cellulosomes) while sugars repress transcription

Piromyces sp finn is Rich in Several GH Enzymes and Dockerin-tagged Proteins





JGINT GENOME INSTITUTE

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Anaerobic Syntrophy: Controlling H2 and CH4 Production







- Metabolism of anaerobic fungi and methanogens is linked
- Products depend on the microbes present in a culture
- Fungi acetate, CO2, H2
- Fungi+Methanogens CO2, methane

Accelerating Biomass Breakdown through Anaerobic Co-culture







- Fungal/methanogen co-cultures accelerate biomass breakdown
- Metabolic underpinnings of the interaction are unknown
- Potential applications:
 - Synthetic systems for biogas production
 - Microbiome engineering to control carbon cycling

Jessica Sexton

Where else can we go from here?





"Compartmentalize" Biomass Breakdown and Product Conversion with Two Microbes



Goal: Metabolically link a model facultative anaerobe to released fungal sugars.



✓ Gut fungi produce more sugars than they need to grow and divide

- ✓ Model microbes like yeast and E. coli are easy to metabolically engineer
- ✓ "One pot" strategy to convert hydrolyzed sugars into value-added chemicals

"Compartmentalize" Biomass Breakdown and Product Conversion with Two Microbes





- Up to 5-7 g/L glucose accumulates in spent fungal cultures
- Both E. coli and S. cerevisiae can grow and divide in spent cultures

Current Targets:

- Develop an anaerobic fluorescent protein to confirm "hand-off" of carbon to the model microbe
 - Flavin-based Mononucleotide Fluorescent Protein (FbFP)



Measure production of n-butanol in an anaerobic co-culture process 1

John Henske



✓ Understudied anaerobes are powerful degraders of crude, non-food lignocellulosic materials

✓ Advances in sequencing technologies make it possible to pinpoint nature's key enzyme cocktails

✓ Natural partnerships between anaerobes can accelerate crude biomass processing

✓ By leveraging this understanding, we can engineer novel, stable microbial consortia for targeted value-added production



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