

Mycological linkages between renewable energy and agriculture

*1st Arab-American Frontiers of Sciences,
Engineering and Medicine Symposium*

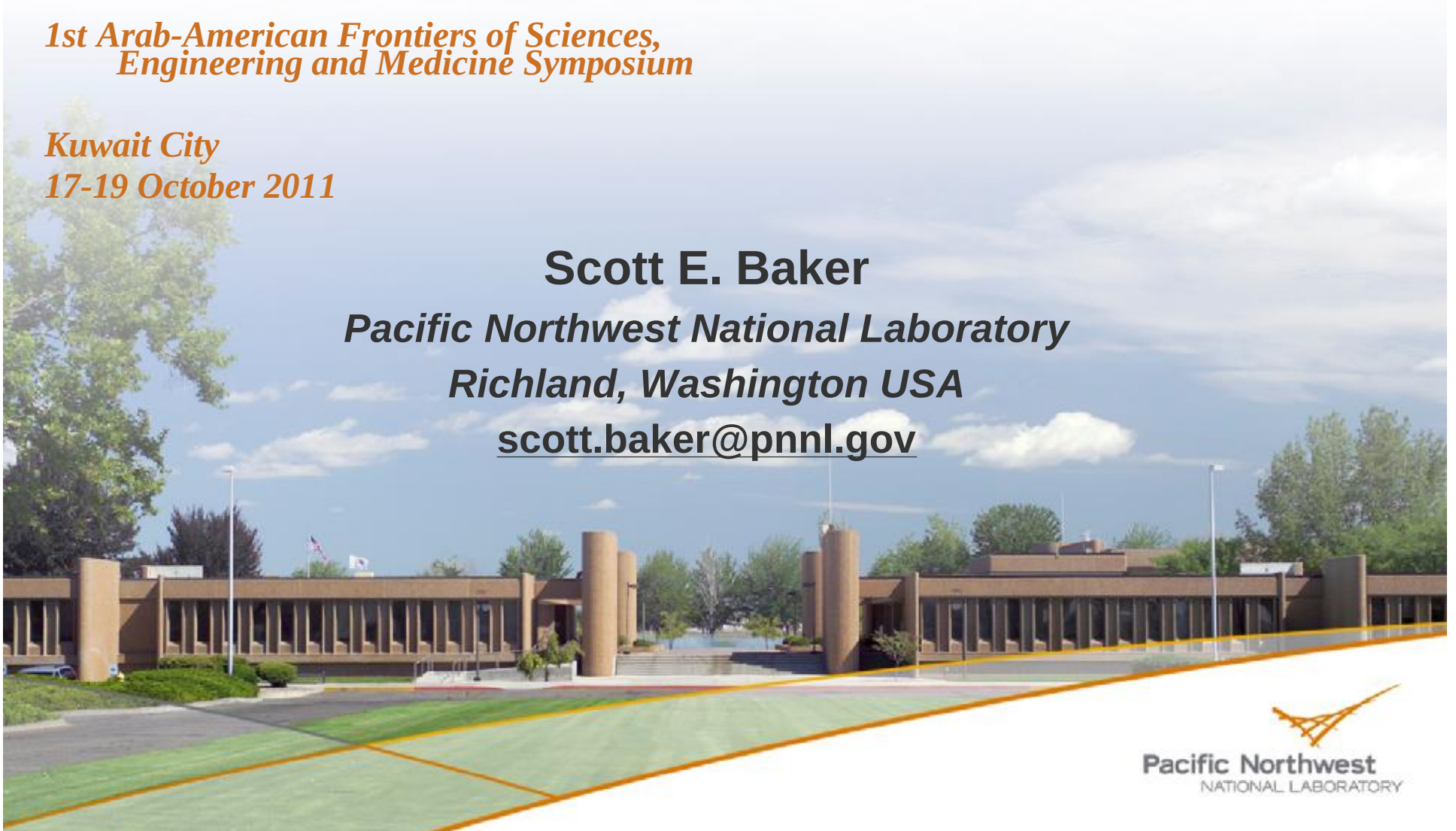
*Kuwait City
17-19 October 2011*

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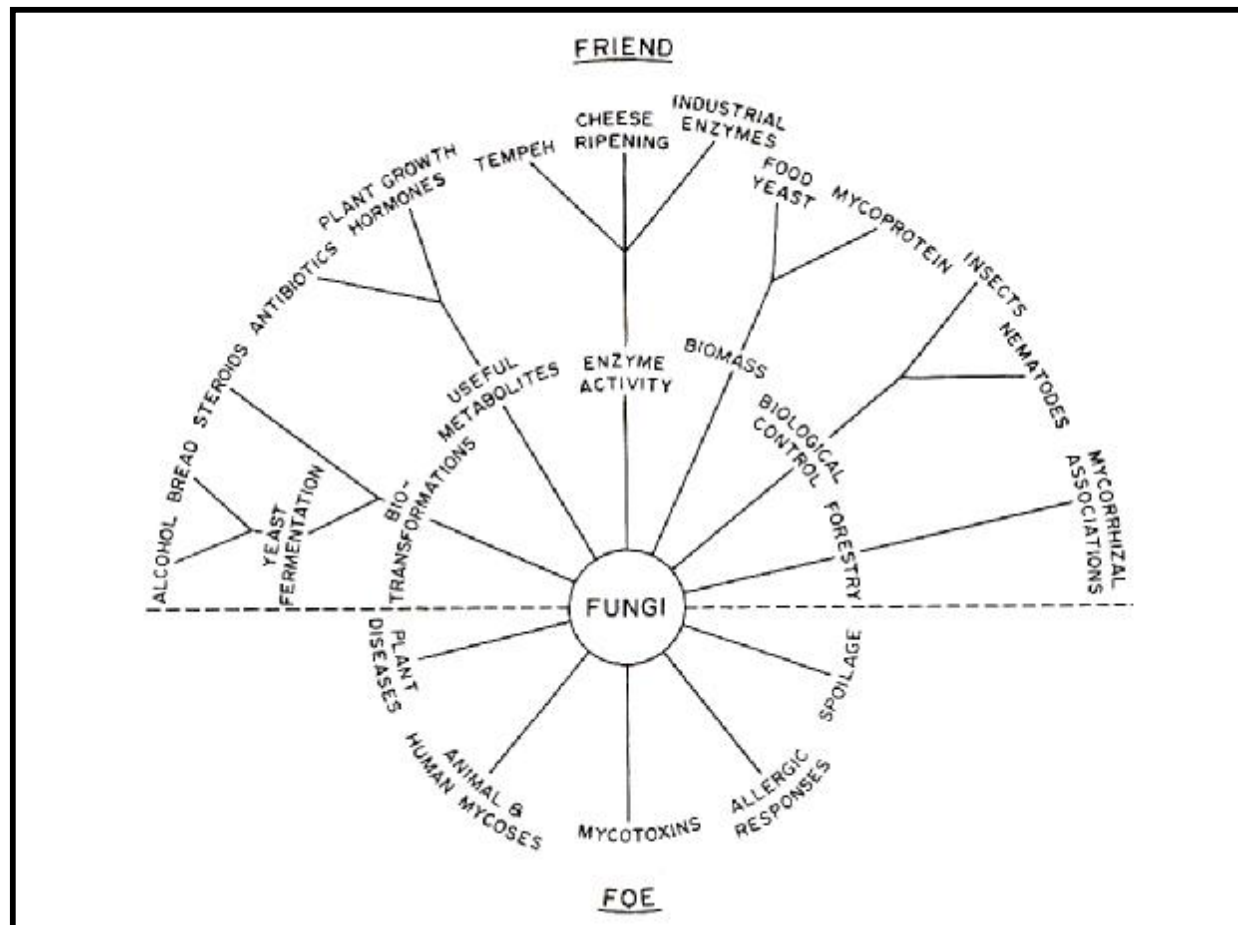
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Pacific Northwest National Laboratory



The world view of a mycologist...



From *Introductory Mycology*, 4th Edition by Alexopoulos, Mims and Blackwell

Filamentous fungi inside the Bio-refinery



Bio-refinery



**Complex biomass:
Agricultural products and
“waste”**

**Fungal
fermentation
and catalysis**

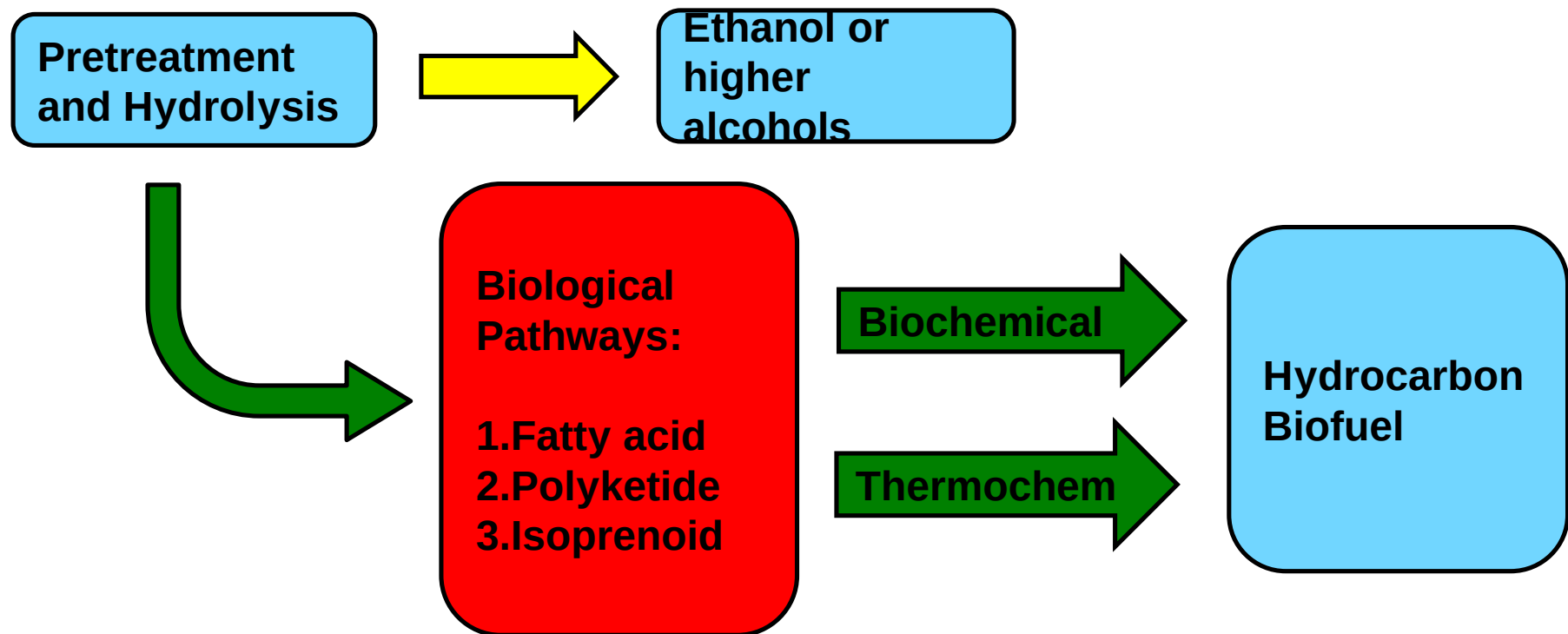
**Simple
sugars**

**Fungal
fermentation
and catalysis**



**Products: Fuels
and chemicals**

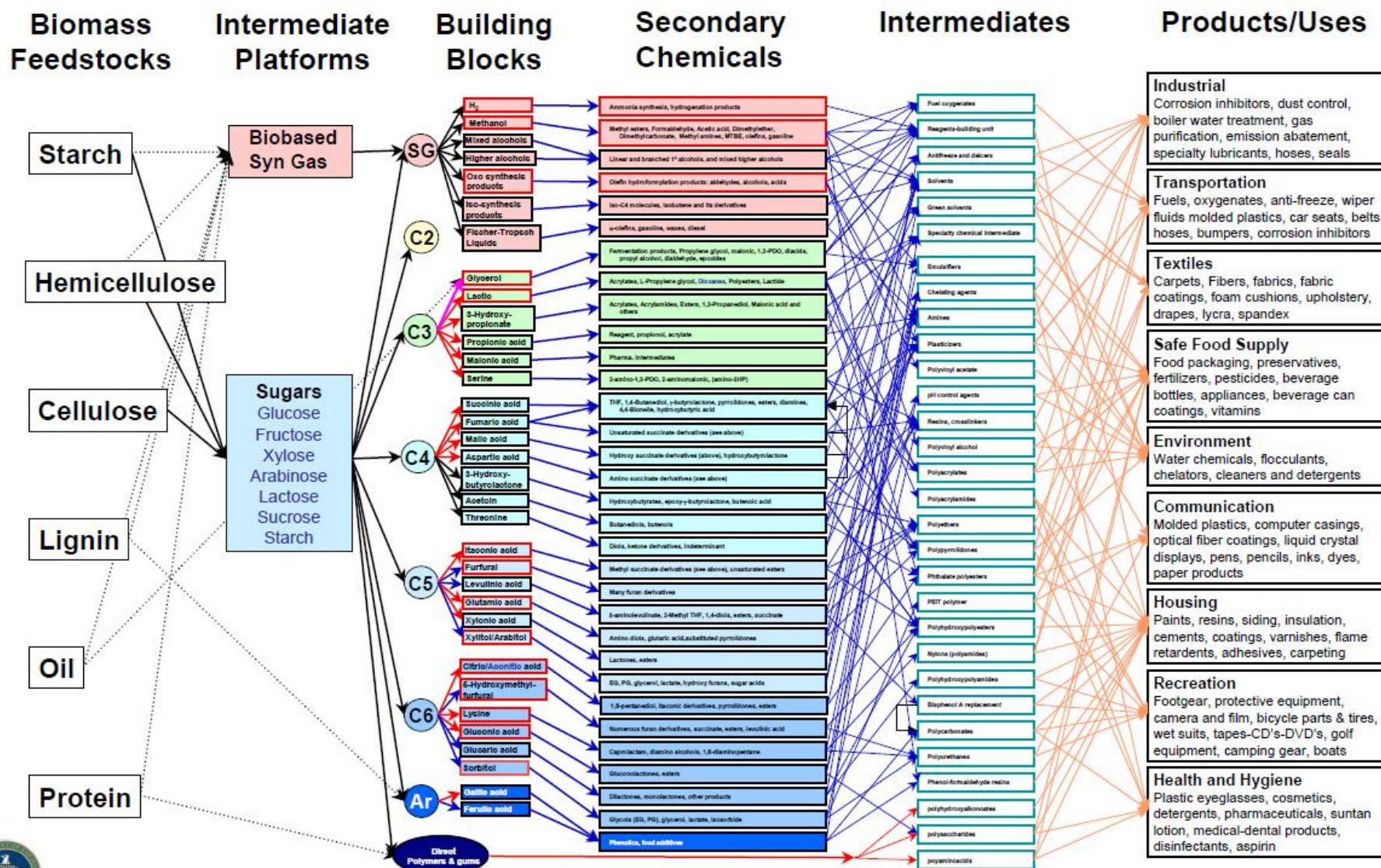
Biofuels of the future: DOE is moving from Ethanol to Hydrocarbon Biofuels



Issues

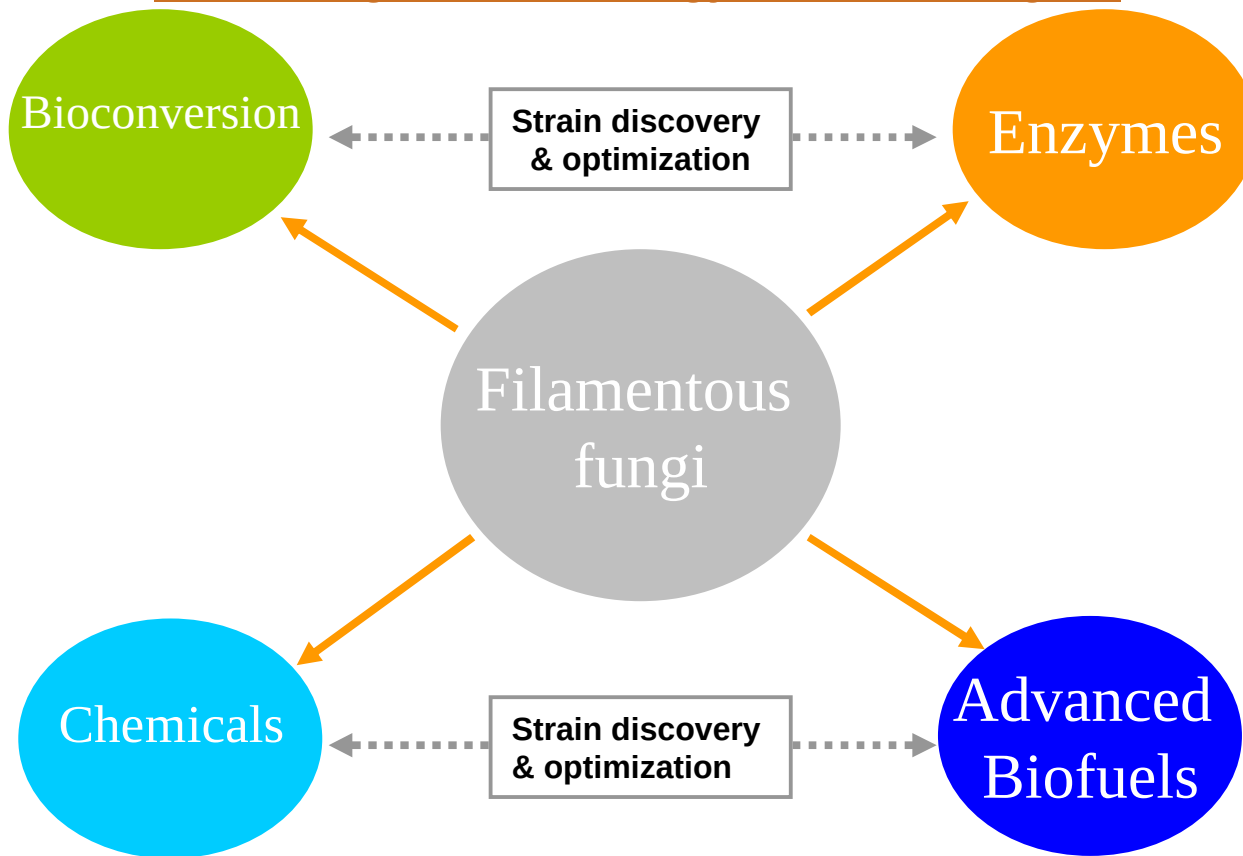
- Moving primary metabolites into secondary metabolites
- Disrupting primary metabolism for secretion of compounds

Organic acids as commodity chemicals – The “Top 10” Report



Filamentous fungi are foundational organisms for the biorefinery

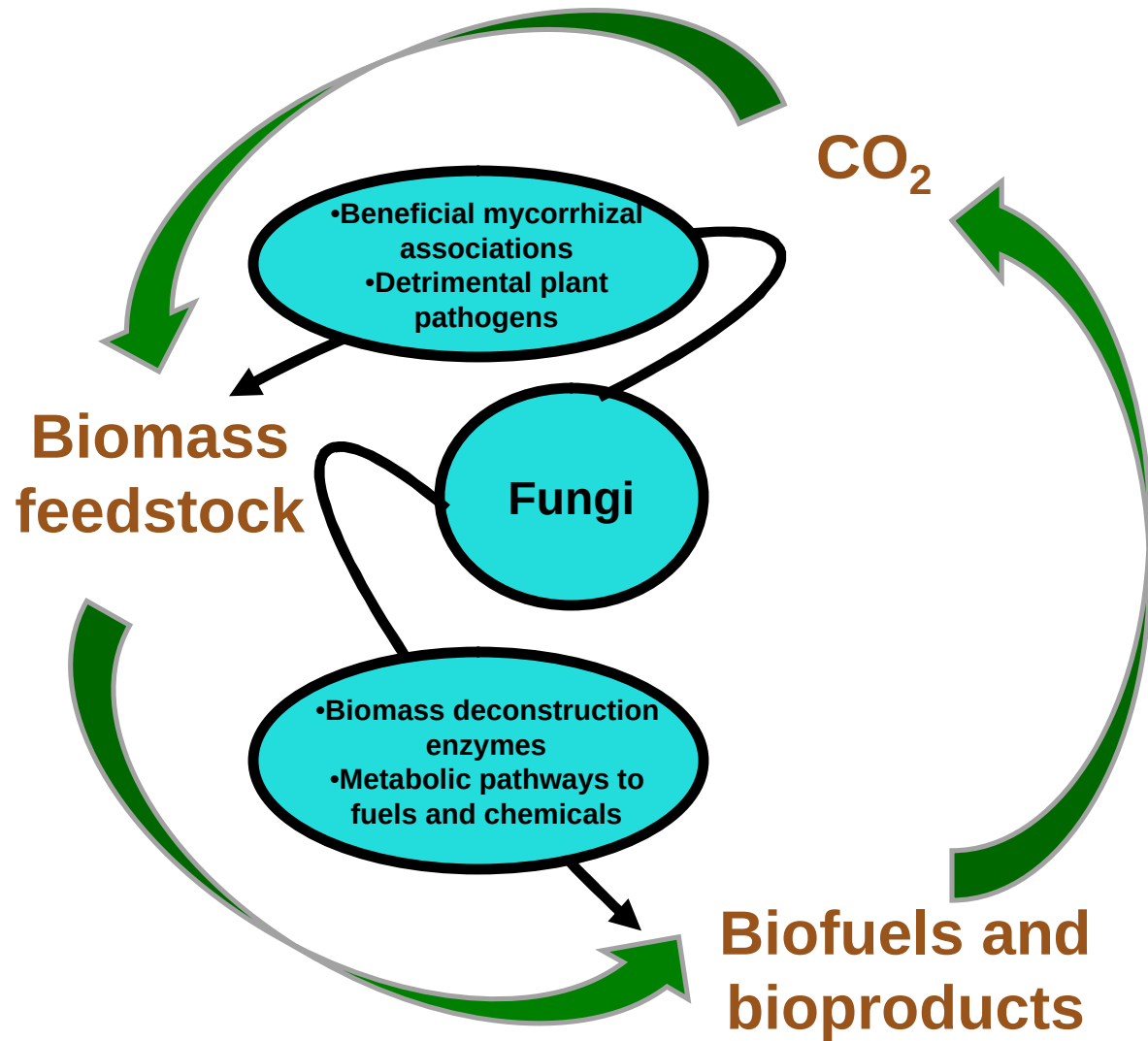
PNNL Fungal Biotechnology Research Program



Why filamentous fungi?

- Digest biomass
- Utilize C5 and C6 sugars
- Grow at low pH
- Produce enzymes & organic acids
- Can produce ethanol
- Molecular tools available
- Genome sequences available

Fungal relevance to DOE bioenergy and carbon cycle mission areas

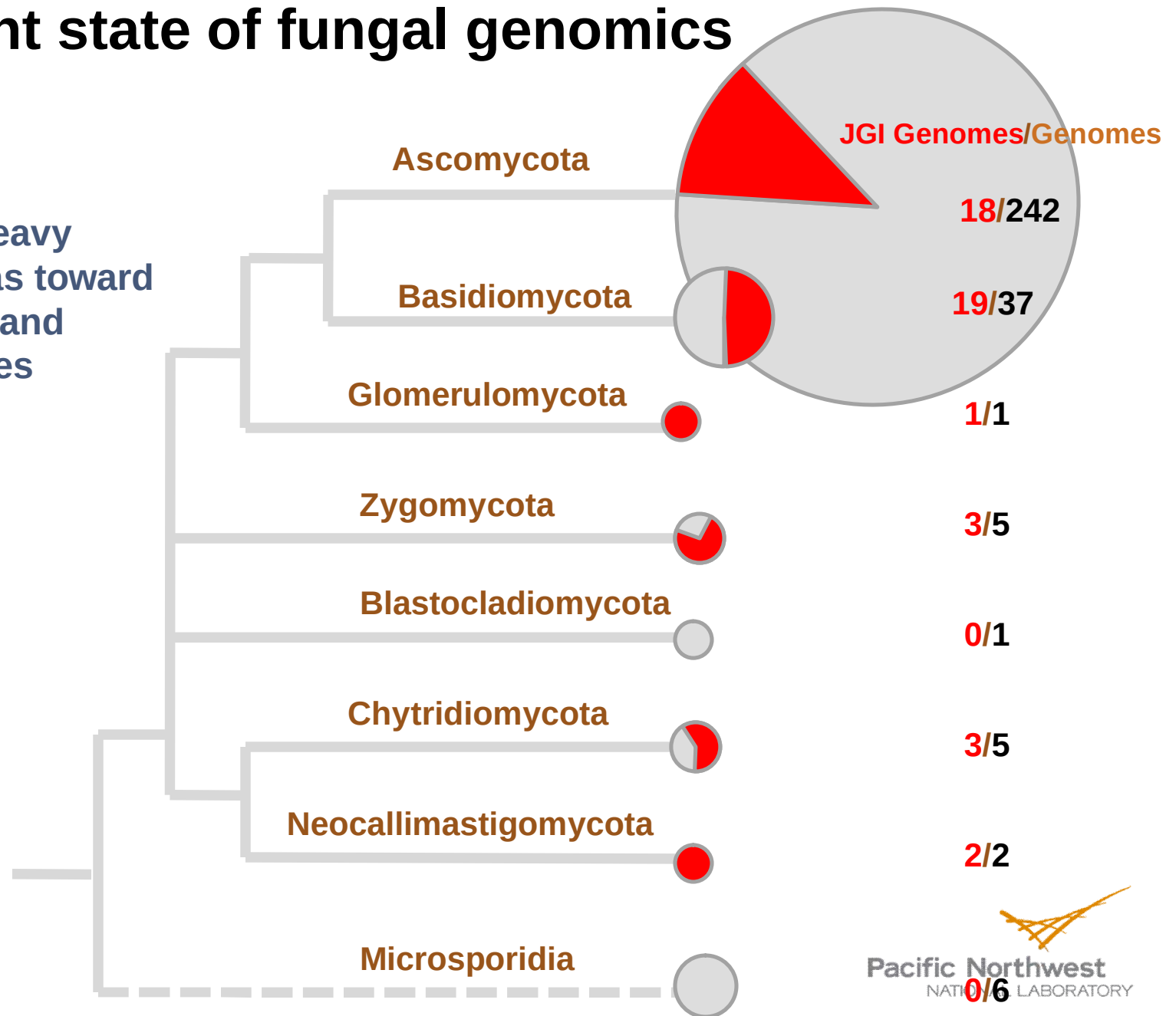


Why Fungal Genomics?

- ▶ Genome sequence paints a high level picture of organism biology
- ▶ Genome sequence is a platform for discovery
- ▶ Genome sequence enables other high throughput discovery tools (proteomics, transcriptomics, etc)
- ▶ A genome project can unite/revive a research community
- ▶ Tremendous metabolic and enzymatic diversity still undiscovered

The current state of fungal genomics

Ø There is a heavy taxonomic bias toward ascomycetes and basidiomycetes

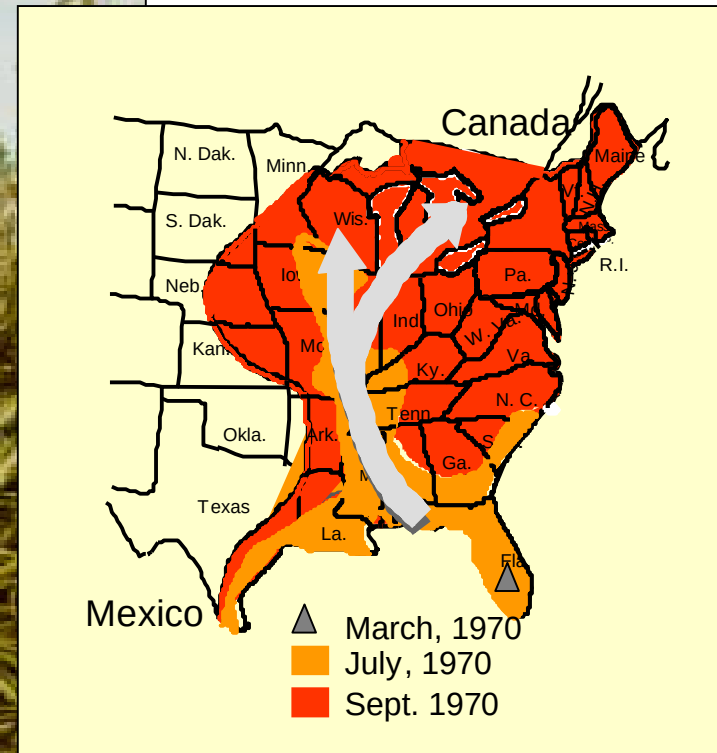


from a genome sequence

[illegible][illegible]

enotype

***Cochliobolus heterostrophus* and Southern Corn Leaf Blight**



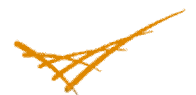
The 1970 outbreak of SCLB in the United States

-- the worst plant disease epidemic on record

- More than 30 states affected
- 15% of corn crop destroyed
- \$1 billion lost

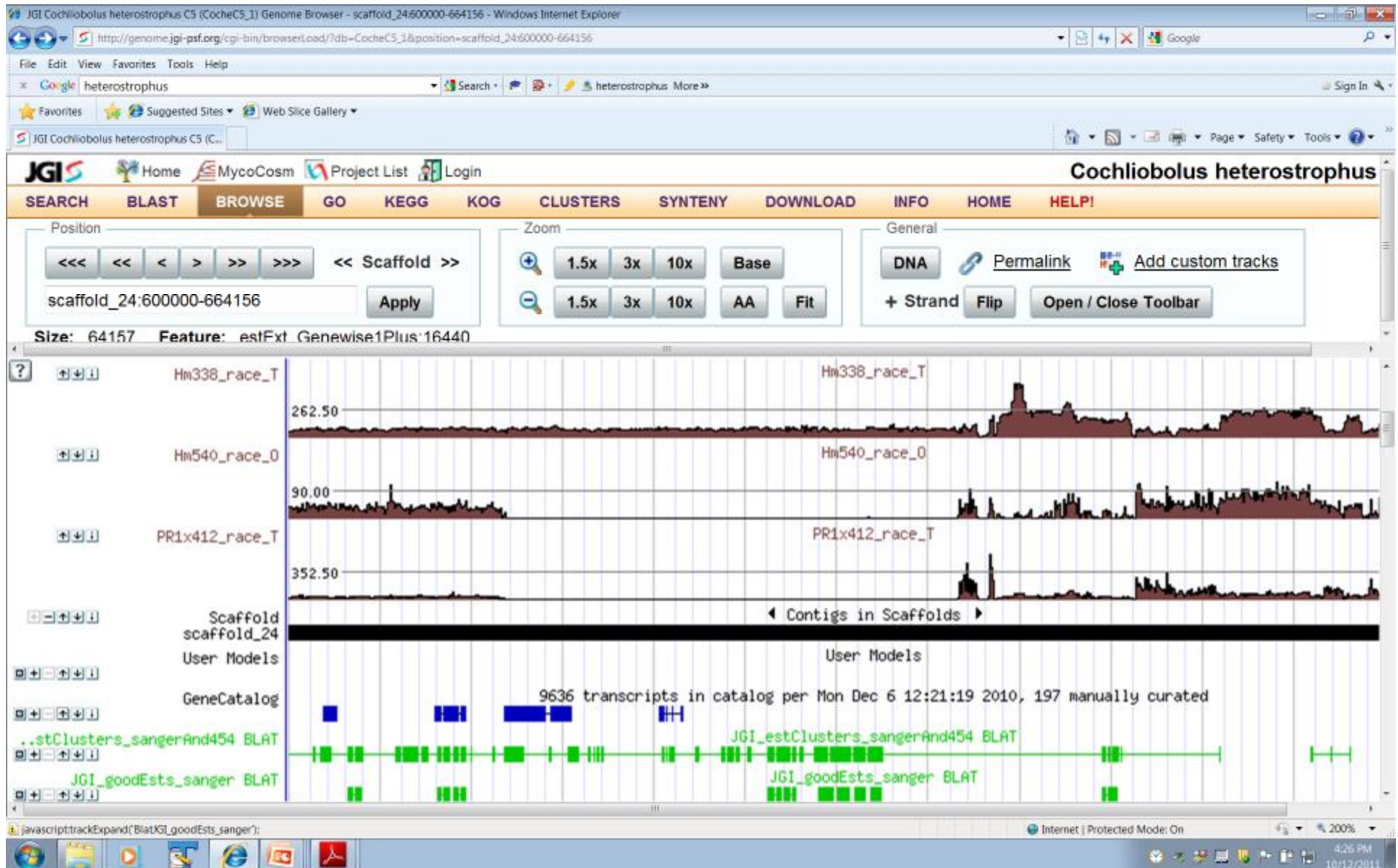


T-toxin (C₄₁)



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Cochliobolus heterostrophus: From Southern Corn Leaf Blight to genome sequence



Trichoderma: Plant health and enzyme production

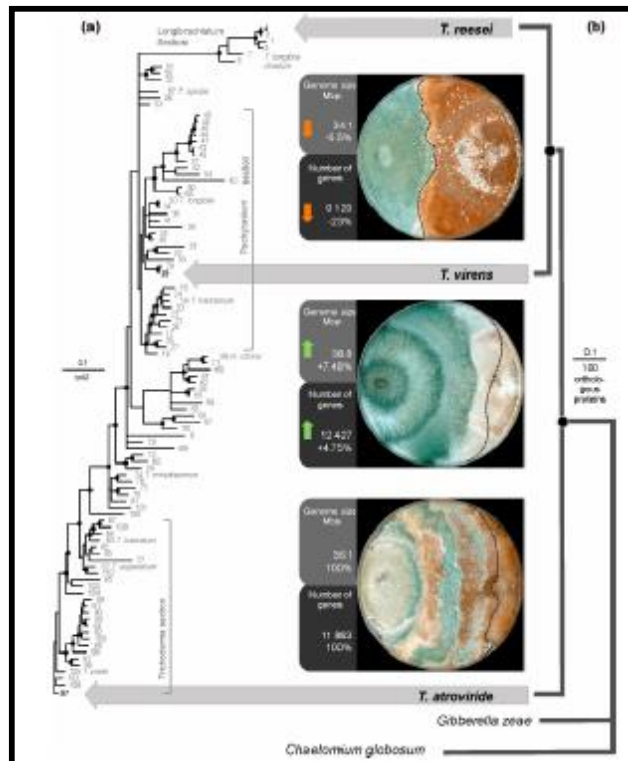
Kubicek et al. *Genome Biology* 2011, 12:R40
<http://genomebiology.com/2011/12/2/R40>



RESEARCH

Open Access

Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*

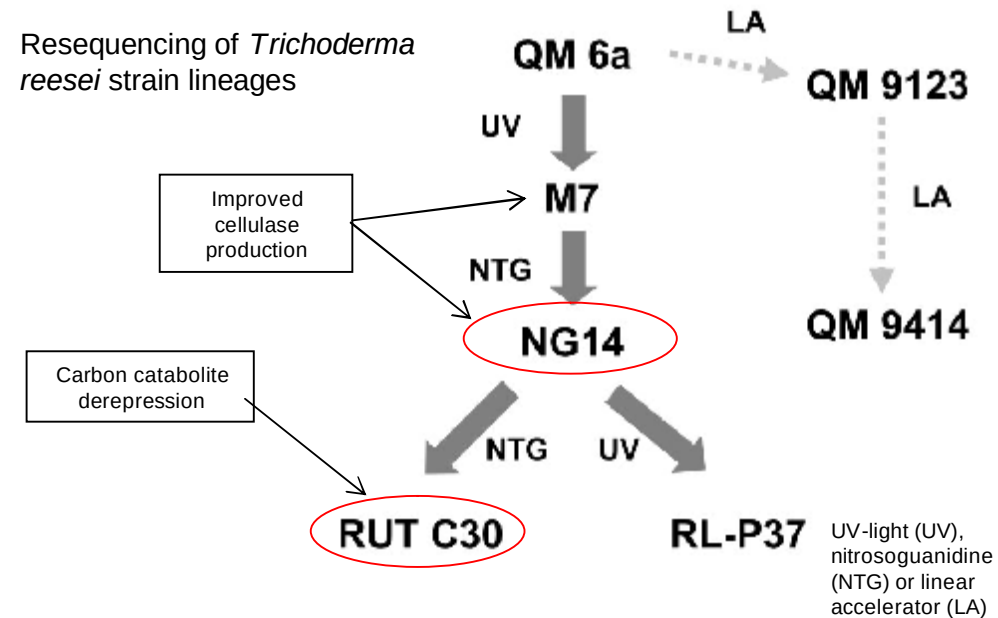


- *Trichoderma*, is an important biocontrol genus
- *Trichoderma reesei*, isolated during WWII by the US Army is the major producer of plant cell wall degrading enzymes
- Seminal research on the *T. reesei* cellulase system was performed at the US Army Natick Research Laboratory in Natick, MA led by Mandels and Reese
- A number of high enzyme producing mutant *T. reesei* strains have been generated

Trichoderma reesei genomics

Use genomics information to better understand hyperproductivity in industrial organisms

- ▶ Fungi are the primary source of most enzymes involved in industrial biomass degradation and they are the cell factories that produce them
- ▶ Currently, most of the enzymes produced for biomass degradation are produced by *Trichoderma reesei*
- ▶ **Increased production host enzyme output leads to decreased cost of enzymes**



Tracking the roots of cellulase hyperproduction by the fungus *Trichoderma reesei* using massively parallel DNA sequencing

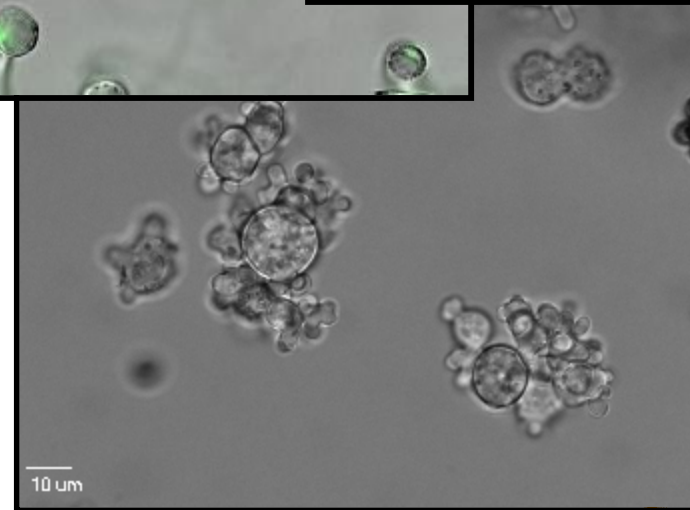
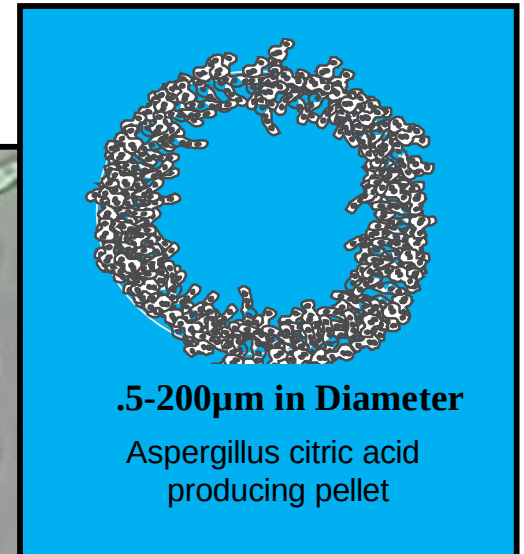
Stéphane Le Crom^{a,b,c,1}, Wendy Schackwitz^{d,1}, Len Pennacchio^d, Jon K. Magnuson^e, David E. Culley^e, James R. Collett^e, Joel Martin^d, Irina S. Druzhinina^f, Hugues Mathis^g, Frédéric Monot^g, Bernhard Seiboth^f, Barbara Cherry^h, Michael Rey^h, Randy Berka^h, Christian P. Kubicekⁱ, Scott E. Baker^{d,e,2}, and Antoine Margeot^{a,2}

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Edited by Joan Wennstrom Bennett, Rutgers University, New Brunswick, NJ, and approved July 27, 2009 (received for review May 28, 2009)

Aspergillus niger and citric acid

- 1917 – Citric production from *Aspergillus niger* first patented by James Currie at USDA in Peoria
- 1917 – James Currie hired at Pfizer. Pfizer begins development of the citric acid process in response to limited citrus fruit supplies during WWI
- 1924 – First commercial process
- 1933 – Lowered feedstock cost with development of *A. niger* citric from molasses process
- Currently – The citric acid process is the most productive fungal bioprocess currently employed



Cell factory genomics: A tale of two *Aspergillus niger* strains

- ▶ *Aspergillus niger* genome project published in *Genome Research*

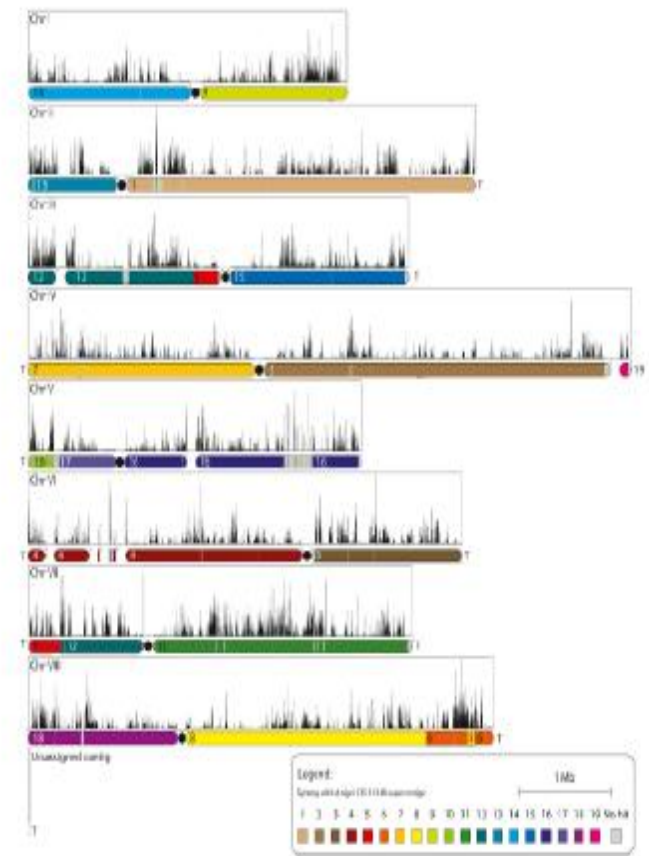
Research

Comparative genomics of citric-acid-producing *Aspergillus niger* ATCC 1015 versus enzyme-producing CBS 513.88

213000-000 © 2011 by Cold Spring Harbor Laboratory Press; ISSN 1088-9051/11; www.genome.org

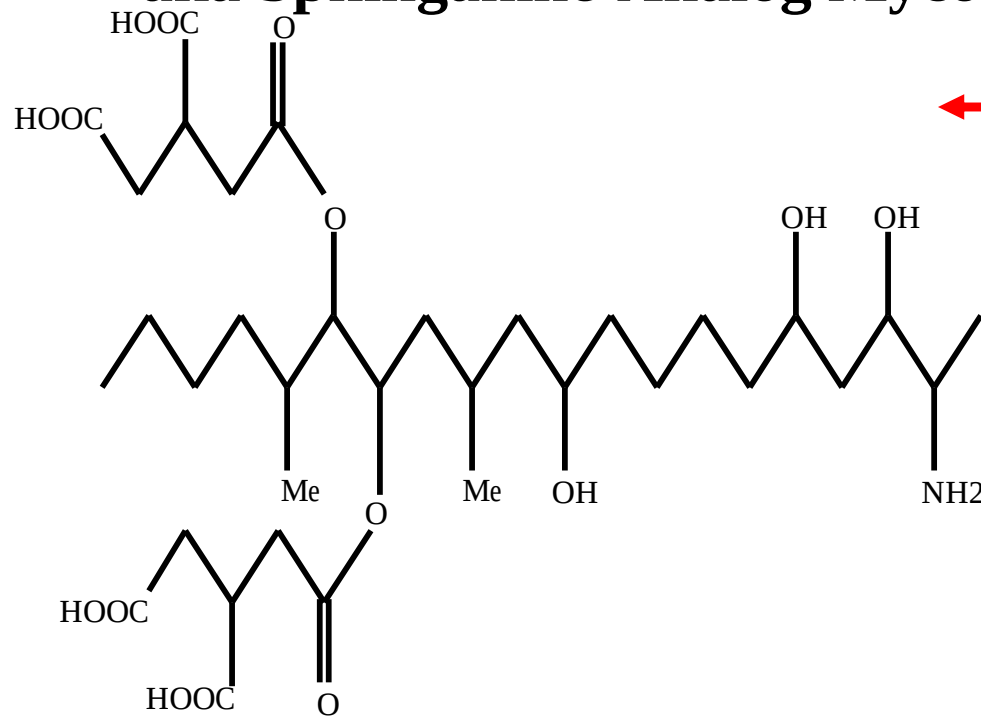
Genome Research
www.genome.org

- Comparison of an *Aspergillus niger* enzyme producing strain (CBS 513.88) with an *A. niger* citric acid strain (ATCC 1015)
- Enzyme production strain has extra copies of gluco-amylase encoding gene
- Chromosomal rearrangements were evident in comparison of strains
- High diversity detected within the *Aspergillus niger* species complex leads to versatility in phenotype (i.e. enzyme or acid production)



SAMs

aka Sphinganine Analog Mycotoxins



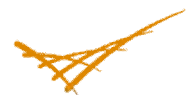
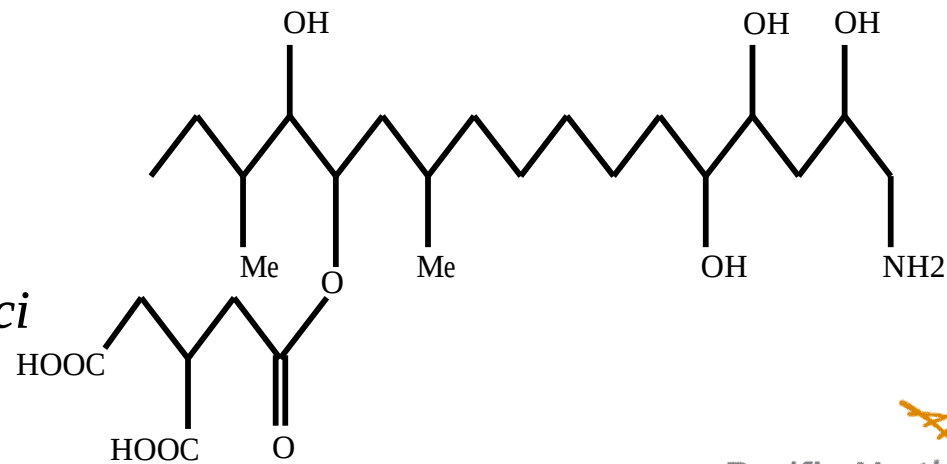
Fumonisin B1

- Mycotoxin
- Produced by *Fusarium* spp.
- Ceramide synthesis inhibitor

AAL toxin

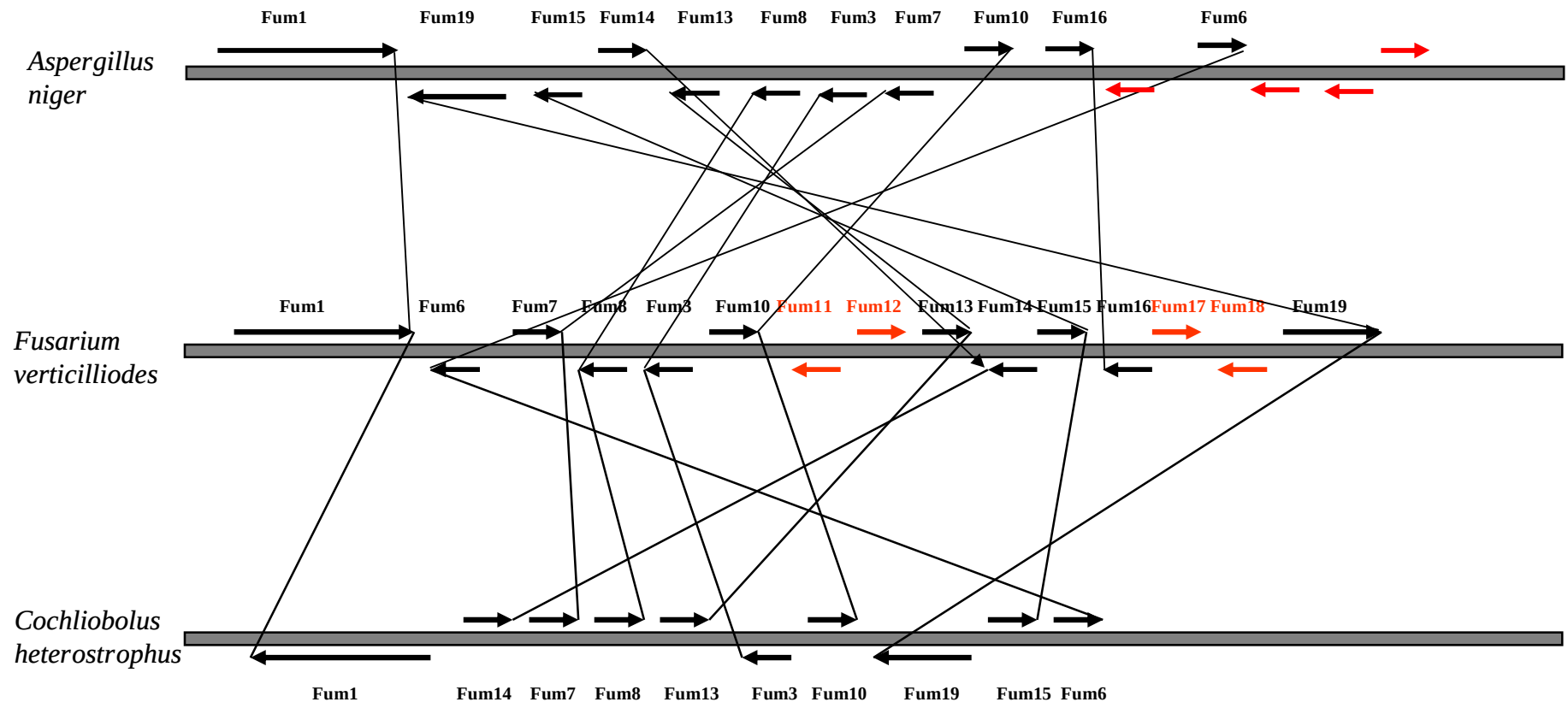


- Virulence factor
- Produced by *A. alternata lycopersici*
- Ceramide synthesis inhibitor

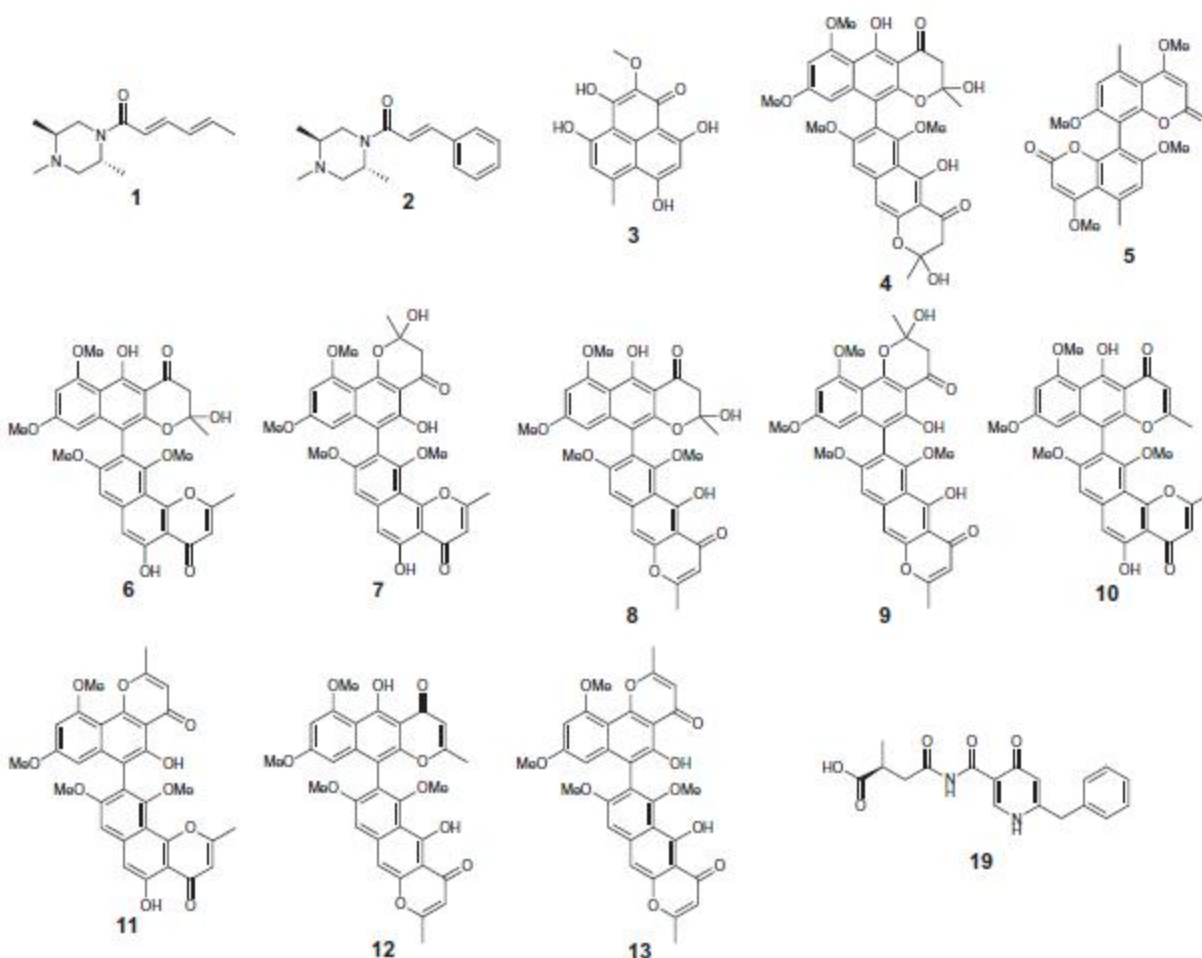
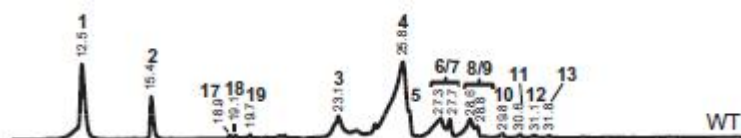


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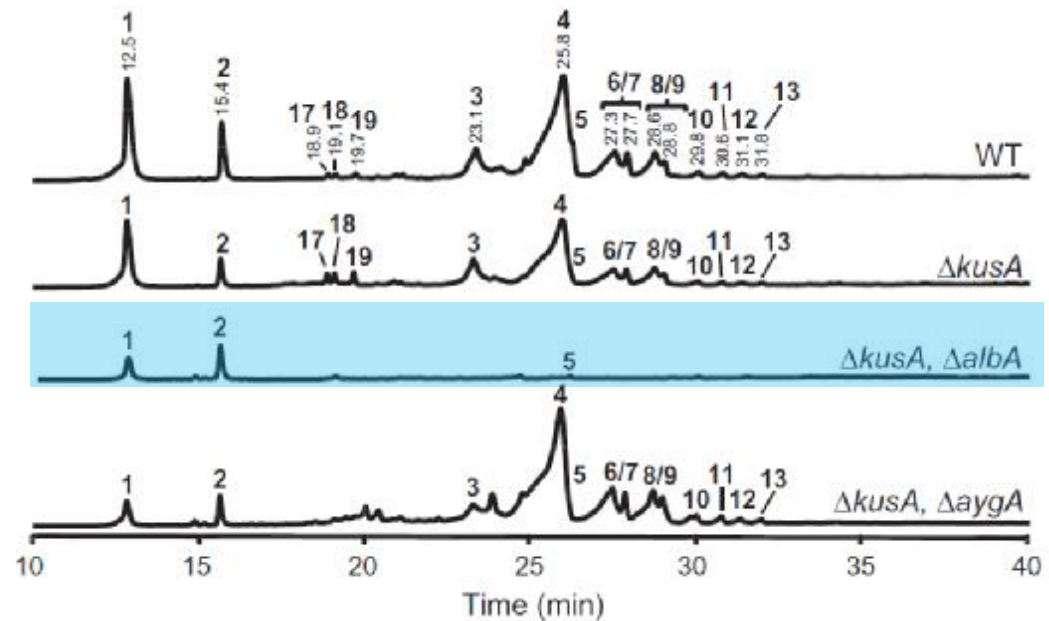
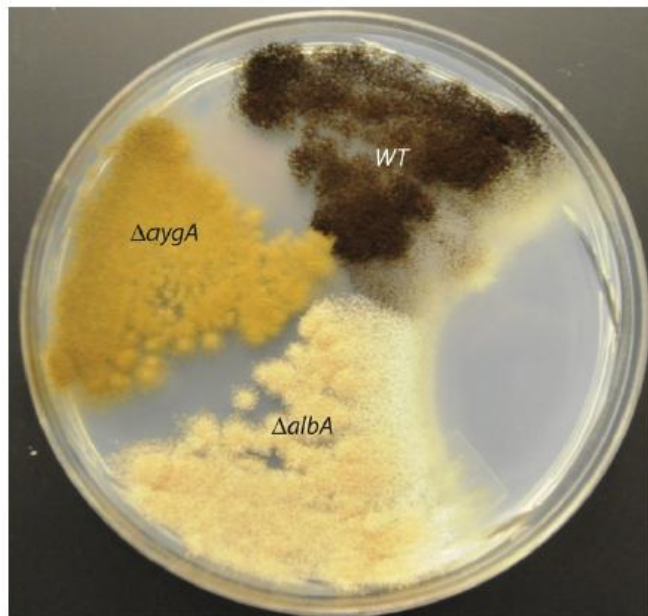
Conserved fumonisin-like gene clusters



Compounds synthesized by *Aspergillus niger* ATCC 11414

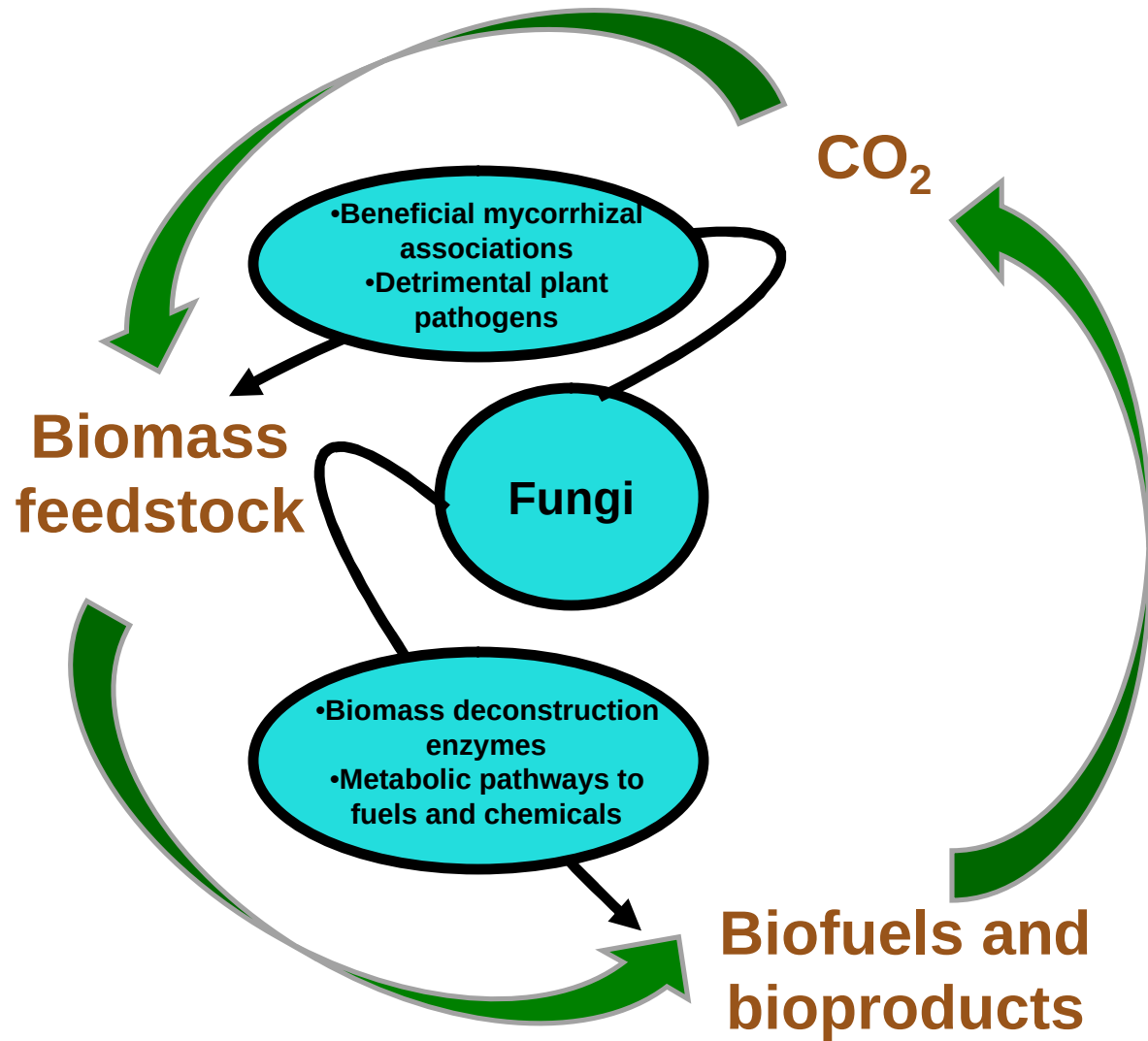


Phenotype: Melanin and naphtho- γ -pyrone production



Single gene deletion generates strain with potential for high selectivity of product production

Fungal relevance to DOE bioenergy and carbon cycle mission areas



Acknowledgments:

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A growing (and incomplete) list of collaborators
at...

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