Sustainable carbon solutions by the aid of wood decay fungi

LIGNOCELLULOSE WASTE BIOCONVERSIONS OPENED WITH COMPARATIVE GENOMICS AND ECOPHYSIOLOGY

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Background: terms and facts

- **Sustainability** = capacity for the biosphere and human civilization to coexist on Earth (Wikipedia)
- Climate change = atmospheric warming and its consequences
- Global warming = human caused, release of excess
- Greenhouse gases GHG: CO$_2$, CH$_4$, N$_2$O
- Circular economy = re-use of wastes as raw materials
- Biomass = plant and animal materials for energy and use
- **Renewable natural resources** = plant, animal and microbial biomasses and products, carbon capturing and re-cyclable
- -> alternatives for fossil fuels and resources
Climate change & global warming effects

The University of Manchester, UK, 2016, report
<table>
<thead>
<tr>
<th>Sector</th>
<th>Globe 2018 vs 1990 (CO₂)</th>
<th>EU28 2018 vs 1990 (CO₂)</th>
<th>EU28 2015 vs 1990 (GHG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power Industry</td>
<td>+ 82%</td>
<td>- 30%</td>
<td>- 24%</td>
</tr>
<tr>
<td>Other Industrial combustion</td>
<td>+ 60%</td>
<td>- 40%</td>
<td>- 39%</td>
</tr>
<tr>
<td>Buildings</td>
<td>+ 6%</td>
<td>- 24%</td>
<td>- 26%</td>
</tr>
<tr>
<td>Transport</td>
<td>+ 77%</td>
<td>+ 21%</td>
<td>+ 16%</td>
</tr>
<tr>
<td>Other sectors</td>
<td>+ 110%</td>
<td>- 20%</td>
<td>- 25%</td>
</tr>
</tbody>
</table>

How fungi may help us?

- CO₂ level in the atmosphere has increased >40% since anno 1750
- Global CO₂ emissions from fossil fuels combustion and processes: 40 Gtn/year (37.9 Gtn in 2018)
- Annual increase: 1.9 %
- China, US, India, E28, Japan: 2/3 of total global fossil CO₂ emissions
- To mitigate accumulation of CO₂
  - -> replace fossil fuels with renewable biomasses and energy sources
  - -> process recycled organic wastes by microbes and fungi
  - -> fungal biotechnology and bioconversions for
  - -> renewable biofuels, novel bio-based and biodegradable materials for industry and consumers
Solution 1: Fungal genomics

- New CAZYmes and genes from white rot fungi for lignocellulose conversion and valorization of biomasses, wastes and lignins
Phlebia radiata 79 comparative genomics

- 7 phlebioid fungal species
- comparative genomics on CAZy genes
- phlebioid white-rot decay specific set of CAZy genes against wood lignocelluloses
- genomic clustering of AA9 and GH10 genes

Mäkinen, Kuuskeri et al. 2019 BMC Genomics 20: 430

Clustered localization of CAZy genes
- co-regulation?
- transcriptome RNA-Seq meta-analysis, Mattila HK et al. 2020 Biotechnol Biofuels
Solution 1: Fungal genomics

Towards understanding wood decay fungal metabolism and regulation to allow systems biology approaches for bioconversions

- Clustering of 14 transcriptomes of *Phlebia radiata* on
- Different substrates and lignocelluloses
- Under aerobic and anaerobic (fermentative) atmospheres
- Mattila H, et al. (2020) Biotechnology for Biofuels
- Poster A2-27
Solution 2: Fungal ecology & physiology

-> to understand fungal interactions

- Wood decay Basidiomycota species
- Polyporales
- Hymenochaetales
- Natural and specific co-cultures
- Enzyme activities
- VOCs & gases
- Dissolved degradation products
- Interactomes
2017

RESEARCH ARTICLE

Interactions affect hyphal growth and enzyme profiles in combinations of coniferous wood-decaying fungi of Agaricomycetes

Tuulia Mali, Jaana Kuuskeri, Firoz Shah, Taina Kristina Lundell

Microbiology and Biotechnology, Department of Food and Environmental Sciences, Viikki Campus, University of Helsinki, Helsinki, Finland

2019

RESEARCH ARTICLE

Decomposition of spruce wood and release of volatile organic compounds depend on decay type, fungal interactions and enzyme production patterns

Tuulia Mali1, Mari Mäki2,3, Heidi Hellén4, Jussi Heinonsalo1,3,4, Jaana Bäck2,3 and Taina Lundell1,*1
Solution 2: Fungal ecology & physiology

- **Interactions of wood-decay Agaricomycetes, enzyme activities and decomposition events**
- Tuulia Mali PhD project

- 3 species of *Basidiomycota Agaricomycetes*
- isolated in Finland on decaying wood
- colonize Norway spruce (*Picea abies*) dead wood

- BR fungus *Fp* supreme colonizer
- WR fungus *Pr* forms dense yellow mycelial front
- against the second WR fungus *Tr*

*Mali T, et al. 2019 FEMS Microbiology Ecology 95:*
*Mali T, Mäki M, Hellén H, Bäck J, Lundell T, 2020*
Signature VOCs released by wood decay fungi from spruce wood

Solution 3: Fungal bioconversions

- Biofuels by wood decay fungi: low cost, single-step bioprocessing and fermentation to ethanol

- Sustainability: lignocellulose instead of food plant biomasses
- Circular economy: waste as raw material
- CO₂ mitigation: wood fibers and products are biotransformed, not burned
- 2nd generation biofuels, metabolites, natural products


Ethanol, organic acids and sugars by fungi from lignocelluloses and wastes
Ethanol production by Phlebia species on lignocellulose wastes

Hans Mattila PhD project, poster A2-27

Table 2: Yield of ethanol production in white rot fungal single-organism and combined organisms using fermentations on various lignocellulosic substrates, extrapolated to 100 g of the solid substrate.

<table>
<thead>
<tr>
<th>Ethanol/substrate (g/100 g)</th>
<th>Single organism</th>
<th>White rot fungus</th>
<th>Duration (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core board</td>
<td>Phlebia radiata 29</td>
<td>9</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td>Core board</td>
<td>Phlebia sp. MG-60</td>
<td>9</td>
<td>Karmi et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>Core board</td>
<td>P. chrysosporium</td>
<td>19</td>
<td>Rak et al. (2009)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Combined organisms</th>
<th>Substrate</th>
<th>Duration (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn stover</td>
<td>P. chrysosporium with yeast</td>
<td>7 (5)</td>
<td>Suresh et al. (2008)</td>
</tr>
<tr>
<td>Corn stover</td>
<td>P. chrysosporium</td>
<td>9</td>
<td>Vincent et al. (2014)</td>
</tr>
</tbody>
</table>

*Unbleached hardwood kraft pulp.

**Sodium hydroxide pre-treatment.

Metabolic pathways for degradation of wood polysaccharides are regulated by atmosphere in a white rot fungus.

poster A2-27 Feb-18th
Solution 3: Fungal bioconversions

- Natural products from waste lignocelluloses by wood decay fungi
- Eero Kiviniemi, poster C1-43 Feb 20th
- Released compounds and metabolites
- Medicinal and bioactive responses tested

Cultivation of bracket fungi in semisolid substrate

RNA extraction

mRNA sequencing Illumina

Extraction of extracellular culture media

Metabolome fractioning & analysis, Ethyl acetate extraction, UHPLC, MS

Secondary metabolism associated genes identification

Bioactivity screening

Iron reduction, hydrogen peroxide generation, Antimicrobial & cancer suppressing activity, Anti-inflammatory activity, etc.
Biotechnology applications on wood-decaying fungi: conclusions

Growth on wood and waste lignocelluloses producing

- Enzymes, CAZymes, decompose and convert wood carbohydrate polymers
- Sugars from wood polysaccharides
- Secondary metabolites, bioactive compounds
- Ethanol, other alcohols, organic acids
- Fruiting bodies and mycelium for food
Thank you for your attention! Kiitos! Grazie!

More information on us:
Fungal Co-life, Omics and Ecophysiology Research Group