Biochemical Features Influencing Mushroom-Lignocellulosic Substrate Compatibility

John Buswell

Institute of Edible Fungi,
Shanghai Academy of Agricultural Sciences,
Shanghai, China
Large amounts of lignocellulosic wastes are generated annually through:

- agricultural and forestry practices
- pulp and paper industries
- timber industries
- agroindustries
- food processing industries
Represents a valuable resource if appropriate technology, i.e.

MUSHROOM CULTIVATION

is applied
MUSHROOM GROWTH

SUBSTRATES

Cereal straws
Bagasse
Wood pulp
Cotton wastes
Coffee grounds
Water hyacinth
Sawdust
Corn cobs
Oil palm waste
Coconut husks
Banana leaves
Tree bark
Lentinula edodes

Volvariella volvacea

Pleurotus ostreatus
PREFERENCES

*Lentinula edodes* grows and fruits well on sawdust-based substrates.

*Volvariella volvacea* grows and fruits poorly on woody substrates – higher yields obtained on cotton waste ‘composts’.

*Pleurotus ostreatus* grows and fruits well on a wide variety of lignocellulosic residues.
LIGNOCELLULOSE

Major components:

CELLULOSE

HEMICELLULOSE
  Xylans
  Mannans

LIGNIN
Lignocellulose in plant cell walls is a complex composite of polysaccharides and lignin.
### Cellulose, hemicellulose and lignin content of some representative mushroom cultivation substrates

<table>
<thead>
<tr>
<th>Residue</th>
<th>Lignin (%)</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat straw</td>
<td>18.0</td>
<td>30.5</td>
<td>28.4</td>
</tr>
<tr>
<td>Barley straw</td>
<td>11.0</td>
<td>48.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Rice straw</td>
<td>12.5</td>
<td>32.1</td>
<td>24.0</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>24.0</td>
<td>49.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Cotton waste</td>
<td>8.1</td>
<td>73.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Bagasse</td>
<td>18.9</td>
<td>33.4</td>
<td>30.0</td>
</tr>
<tr>
<td>Peanut hulls</td>
<td>23.0</td>
<td>42.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Groundcorn cobs</td>
<td>7.0</td>
<td>28.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Soy bean hulls</td>
<td>2.0</td>
<td>48.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Softwood sawdust</td>
<td>22.0-31.3</td>
<td>39.5-45.0</td>
<td>17.2-30.8</td>
</tr>
</tbody>
</table>
ENZYMES INVOLVED IN THE BIODEGRADATION OF AGRO-WASTES

• CELLULASES – hydrolases

• HEMICELLULASES – hydrolases, esterases

• LIGNINASES – peroxoxidases, oxidases
CELLULOSE

- **Polysaccharide** consisting of a linear chain of several hundred to over ten thousand $\beta(1\rightarrow4)$ linked D-glucose units; basic repeating unit is CELLOBIOSE

- Hydroxyl groups on the glucose units of one chain form hydrogen bonds with oxygen atoms on the same or on an adjacent chain, holding the chains firmly together side-by-side to form *microfibrils*, which contain both crystalline (ordered) and amorphous (less-ordered) regions
Repeating unit is **CELLOBIOSE**
ENZYMES OF THE “CELLULASE” COMPLEX

Hydrolytic enzymes

1. ENDOGLUCANASE
   - attacks amorphous regions in the cellulose chain

2. CELLOBIOHYDROLASE
   - releases cellobiose units and cello-oligomers

3. β-GLUCOSIDASE (CELLOBIASE)
   - hydrolyses cellobiose and cello-oligomers to glucose
Postulated mode of action of cellulolytic enzymes

Cellulose

Crystalline Regions

Endoglucanase (EG)

Fibrils

Amorphous Regions

Cellobiohydrolase (CBH)

β-glucosidase (BGL)

Glucose
Ultrastructural Analysis

Target enzyme
Primary antibody
Secondary antibody
Fluorescent marker
HEMICELLULOSE

Two major types

- XYLANS: backbone consists of $\beta$-1,4-linked XYLOSE residues
  These usually have various side-chain substituents (e.g. arabinose, acetyl, methyl glucuronic acid, feruloyl, $p$-coumaryl) attached by different linkages

- GLUCOMANNANS: backbone consists of $\beta$-1,4-linked GLUCOSE and MANNOSE residues
  These also have various side-chain substituents (e.g. galactose, acetyl), attached by different glycosidic linkages
AcMeGlcA

endoxylananase
β-xylosidase
α-glucuronidase

arabinofuranosidase
acetylersterase
feruloyl (p-coumaryl) esterase

Fer (p-Coum)
Ara

MeGlcA

-Ar-Ara-Fer (p-Coum)-

-Ac-Ara-XYL-XYL-XYL-XYL-XYL-XYL-XYL-

-Ac-Ara-XYL-XYL-XYL-XYL-XYL-XYL-

-Ac-Ara-XYL-XYL-

-Ac-Ara-XYL-

-Ac-Ara-
LIGNIN

- aromatic, amorphous, and heterogenic polymer present in all layers of woody cell walls.

- comprises a three-dimensional network of phenylpropanoid precursors (i.e., coniferyl alcohol, sinapyl alcohol, and $p$-coumaryl alcohol), which are polymerized to guaiacyl-, syringyl-, and hydroxyphenyl-type lignin subunits.

- subunits are joined together with a variety of bond types, mainly carbon-carbon and ether bonds, the $\beta$-aryl-ether ($\beta$-O-4) bonds being the most abundant.
coniferyl alcohol

$p$-coumaryl alcohol

sinapyl alcohol
THREE MAJOR TYPES OF LIGNIN

1. GUAIACYL (softwood)
   - composed mainly of coniferyl alcohol units
   + small amounts of \(p\)-coumaryl and sinapyl units

2. GUAIACYL-SYRINGYL (hardwood)
   - composed of approximately equal amounts
   of coniferyl and sinapyl residues +
   minor amounts of \(p\)-coumaryl units

3. GUAIACYL-SYRINGYL-\(p\)-COUMARYL (grass lignin)
   - approximately equal amounts of all 3 cinnamyl alcohols;
   also contains ester-linked \(p\)-coumaric and ferulic acid residues
Representative section of the lignin macromolecule
14C-Ring-labelled dehydropolymerisate (DHP) of coniferyl alcohol
LIGNIN-DEGRADING ENZYMES

• LIGNIN PEROXIDASES (LiPs)
  - oxidize both phenolic and non-phenolic residues in presence of hydrogen peroxide

• MANGANESE-DEPENDENT PEROXIDASES (MnPs)
  - oxidize both phenolic and non-phenolic residues through the generation of Mn$^{3+}$
VERSATILE PEROXIDASES (VPs)
- hybrids of LiPs and MnPs

LACCASES:
- oxidize phenolic residues
- oxidize non-phenolic residues in the presence of a ‘primary’ substrate
- produce Mn$^{3+}$ chelates in the presence of Mn$^{2+}$ and a phenolic substrate
Distribution of lignin-modifying enzymes among selected mushrooms based on biochemical and genomic data

<table>
<thead>
<tr>
<th>Mushroom</th>
<th>LiPs</th>
<th>MnPs</th>
<th>VPs</th>
<th>Laccases</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pleurotus ostreatus</em></td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td><em>Lentinula edodes</em></td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Volvariella volvacea</em></td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>
ROLE OF LACCASE IN MUSHROOM DEVELOPMENT

• LIGNIN MODIFICATION

• DETOXIFICATION OF PHENOLIC COMPOUNDS

• FRUIT BODY MORPHOGENESIS
EFFECT OF AROMATIC COMPOUNDS ON LACCASE PRODUCTION BY VOLVARIELLA VOLVACEA
Miniaturised solid-state growth system
Extracellular laccase production during the developmental cycle of *V. volvacea*

Development stages:
- 0-16 days, substrate colonisation;
- 17 days, pinheads;
- 22 days, button stage;
- 23 days, egg stage;
- 24 days, elongation stage; 25 days, mature fruit body.
Summary

Mushroom-substrate compatibility is influenced by the capacity of the mushroom species to synthesize lignocellulolytic enzymes, which contribute to:

• nutrient availability – cellulases, hemicellulases

• nutrient accessibility – Lips, MnPs, VPs, laccases

• non-toxic growth environments – laccases

• fruit body morphogenesis
Thank you