

Genomic approaches to industrial and environmental applications



IEA Workshop – March 3, 2003

Topics

- The fungal genomic project
- Experiences to date
- Potentials of enzyme systems for industrial and environmental applications

Industrial sustainability

Economic



Bio-
technology

Environment

Social

Strategy

- Partnership (industrial, government, academic)
- Biocatalysts (enzymes)
- Systematic
- Target identification to application

Overall goal

- Construct enzyme library
 - pH range, temperature range, stability, substrate specificity, specific activity, K_m
- Applications
 - Pulp and paper
 - Fine chemicals
 - Remediation

Objectives

- Sequence and analyze genes of 15 fungal species
- Compare with known genes to identify targets
- Explore regulatory networks and mechanistic pathways using “gene-chip” technology
- Express and characterize specific target enzymes
- Construct web-based database for fungal gene sequences and enzymes
- Test enzymes for usefulness in industrial and environmental applications

Projected results

100,000 new fungal genes



2,000 potential targets



400 enzymes



30 application tests

Fungal species



White-rot fungi:

- *Phanerochaete chrysosporium*
- *Trametes versicolor*
- *Lentinula edodes*



Other lignin and pitch degrading fungi:

- *Gloeophyllum trabeum*
- *Ophiostoma piliferum*
- *Corpinus cinereus*

Freeze-tolerant fungi:

- *Chrysosporium pannorum*
- *Cryptococcus laurentii*

Pollutant degrading fungi:

- *Aureobasidium pullulans*
- *Amorphotheca resinae*
- *Leucosporidium scottii*
- *Cunninghamella elegans*

Thermophilic composters:

- *Thermomyces lanuginosa* (60°C)
- *Chaetomium thermophile* (50°C)

Bioinformatics

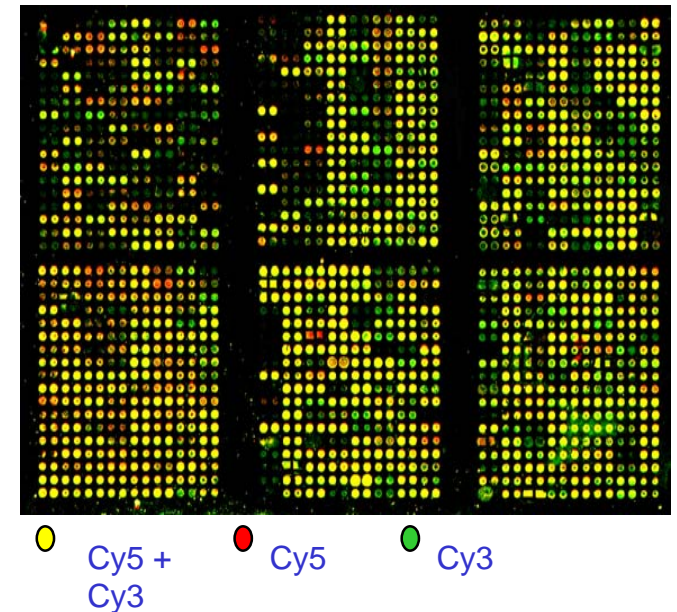
- Sequence analysis pipeline (sequence quality, assembly, sequence comparison)
- Clone-tracking (relational, transaction database)
- Target-finding toolkit

Current development

- DNA microarray database
- Annotation environment
- Enzyme database
- Phylogenetic database

DNA microarrays

- Genes (DNA) spotted on glass slides
- mRNA used to generate fluorescently labelled cDNA (control green; treated red)
- Nucleic acid hybridization
 - expressed only in control culture
 - expressed only in treated culture
 - expressed equally in control and treated cultures



Transcription profiling

- Global gene expression pattern under different environment
- Examine complexity of enzymes in degradation
- Assess substrate specificity of oxygenases
- Gene regulatory network

Fungal expression systems

- Secret proteins at high levels
- Effective modification systems – does not hyperglycosylate
- *Aspergillus niger*
- *Pichia pastoris*
- *Yarrowia lipolytica*

Aspergillus niger

- Used widely in the food processing industry
- Secretes recombinant proteins up to 25 grams/litre
- GRAS status (Generally Regarded As Safe)
- Gene expression profiles (microarray)
- Secreted protein profiles (SDS gel electrophoresis, mass spectrometry)

Progress

- ~6,000 genes analyzed



- ~150 targets identified



- 12 enzymes partially characterized

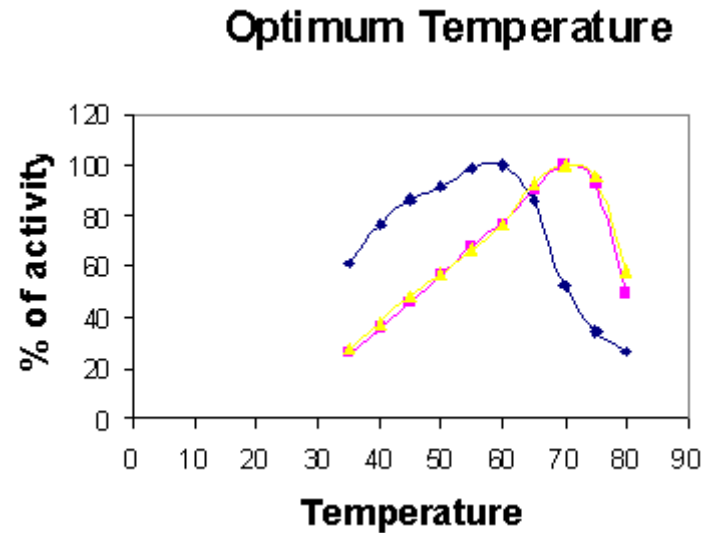


- 2 enzymes being tested for application

Target genes

	<i>P. chrysosporium</i>	<i>N. crassa</i>	<i>S. cerevisiae</i>
• Mn peroxidase	16	0	0
• Cellulase	10	18	0
• Xylanase	3	2	0
• Phytase	1	0	0
• Mannanase	2	2	0

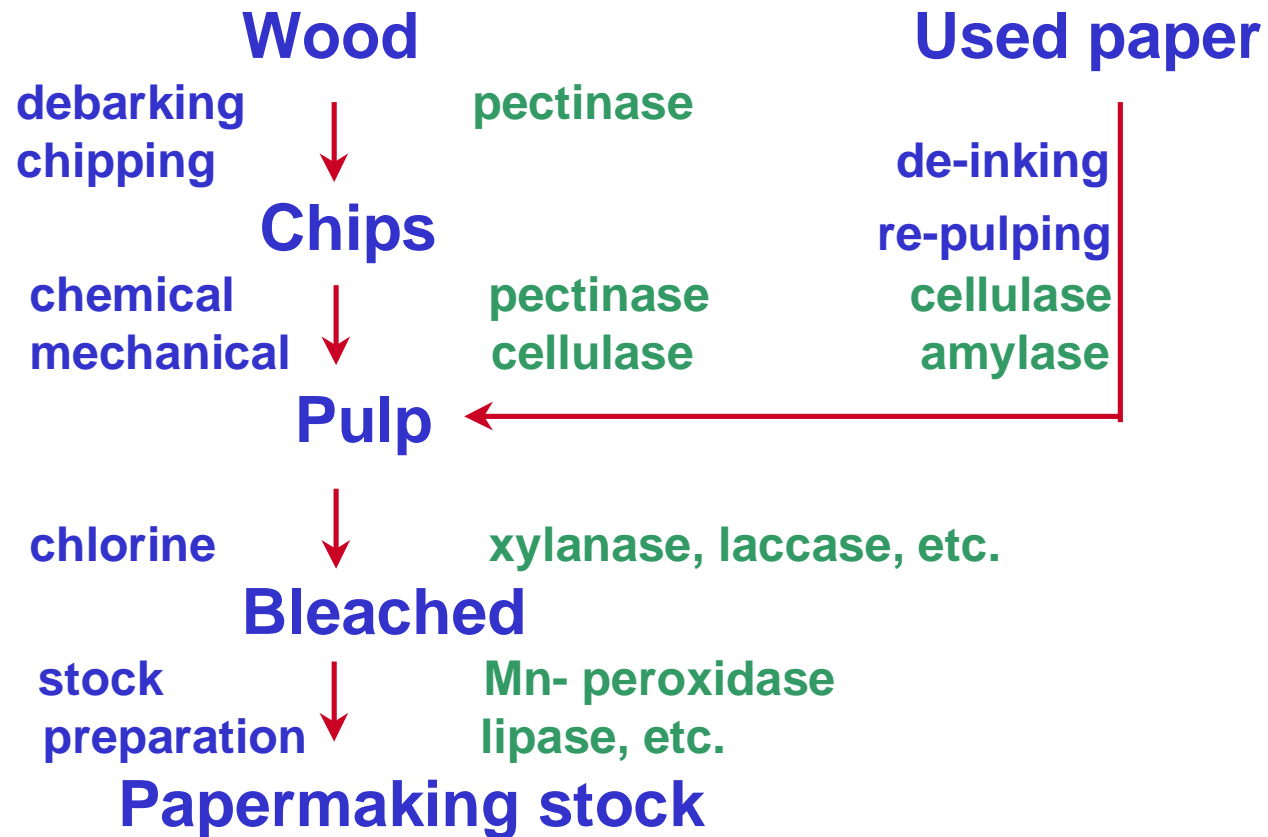
Temperature Optimum



- Tested *Phanerochaete* enzymes function at relatively high temperature

Potentials of fungal enzymes in industrial and environmental applications

Enzymes in papermaking



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- Biopolymers – lignin, cellulose
 - Synthetic polymers – nylon, polyacrylamide
 - Dyes – azo-dyes, cresol red
 - Munitions – TNT, nitroglycerin
 - Pesticides – DDT, lindane
 - Chlorinated aromatic compounds - PCB
 - Others – azide, cyanide

Extracted from M.D. Cameron, S. Timofeevski & S.D. Aust (2000)

Appl. Microbiol. Biotechnol. 54: 751-758.

Involves extracellular enzymes

Non-specific reactions

Oxidation, reduction and mineralization under aerobic conditions

Free-radical intermediates; no epoxides and other toxic intermediates detected

Lignin peroxidase

Mn-dependent peroxidase

Laccase (copper-containing
phenol oxidase)

Cellobiose dehydrogenase

Membrane methyl
transferase

Softwood lignin

Synthesis of fine chemicals

Over 20 monooxygenases identified from
P. chrysosporium

Perform chemically difficult reactions under
mild conditions

Know to display stereo-specific
hydroxylation of pharmaceuticals

Rapid method of identifying target enzymes

Powerful tool in unravelling complex
degrading systems

New approach to explore the substrate
specificity of monooxygenases

Logistics – multiple high-throughput
technology platforms

Multiple disciplines from different academic
cultures

Forging fruitful partnerships with end-users

Collaborators



Molecular biology

Reg Storms Adrian Tsang

Enzyme biochemistry

Justin Powlowski Luc Varin

Computer science

Greg Butler Clement Lam
Ahmed Seffah

Gene evolution

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Fine chemicals

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