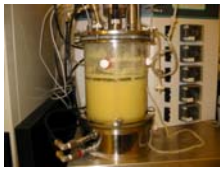
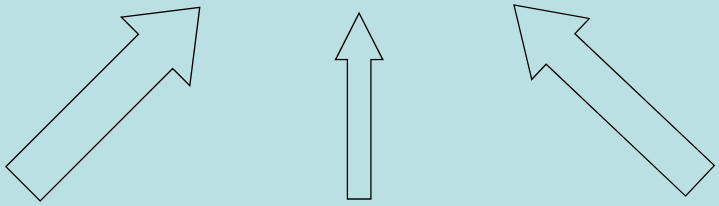
Research Overview

- Plant biotechnology/bioprocess engineering with applications to
  - Biopharmaceutical production (e.g. human α_1-antitrypsin)
    - Ting-Kuo Huang (transgenic tobacco cell suspension cultures in stirred tank bioreactors)
    - Kittipong Rattanaporn (development of improved viral amplicon expression vectors for transient agroinfiltration)
  - Biofuels applications (*in-planta* production of cellulase enzymes)
    - Ben Lindenmuth (transient production in tobacco leaves)
    - Sang-Kyu Jung (transient production in switchgrass)
  - Biopolymers (human gelatin and collagen)
    - Corey Dodge (rice cell suspension cultures in bioreactors)
    - Lucas Arzola (maize cell suspension cultures in bioreactors)



Research Goals

- Efficient production of recombinant proteins in plants and plant cell cultures



Genetic Instructions
- Amino acid sequence
- Transient vs stable production
- Inducible promoter
- Secretion signal
- Optimized genes (codon usage/introns)

Host Factors
- Choice of plant
- Stability
- Byproducts

Bioprocess Approaches
- Environmental conditions
- Operational strategies
- Scale-up

Collaborations

University:

- Abhaya Dandekar, Plant Sciences
- Bryce Falk, Plant Pathology
- Jean VanderGheynst, Bio & Ag Engr.

Industry:

- Ventria Biosciences (Applied Phytologics)
- FibroGen
- Planet Biotechnology
- Chevron
- BioRad

Ben Lindenmuth

- PhD candidate in Chemical Engineering with a Designated Emphasis in Biotechnology
- B.S. in ChE from Penn State

Transient *in planta* expression of cellulose-degrading enzymes: Plant tissues as bioreactors

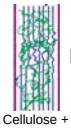
Ben Lindenmuth
CREATE-IGERT Trainee
Dept. of Chemical Engineering
& Materials Science
University of California - Davis
October 16th, 2008

Overview

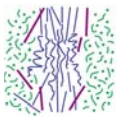
- Why produce cellulose-degrading enzymes?
- Why use harvested plant tissue as a bioreactor?
- How can plant tissue be used as a bioreactor?
- Preliminary data.

Cellulosic Biofuel Production

Cellulosic Biomass



Cellulose + Other Polymers

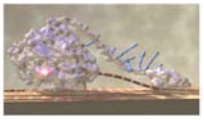


Exposed Cellulose

Disrupted Biomass

Enzyme Production

Enzymes



Sugars

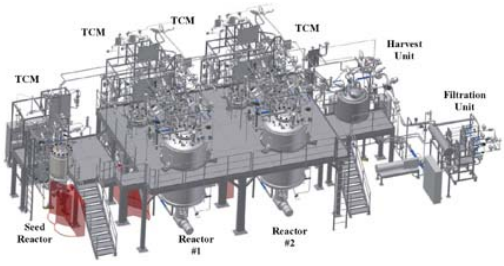
Fermentation

Distillation

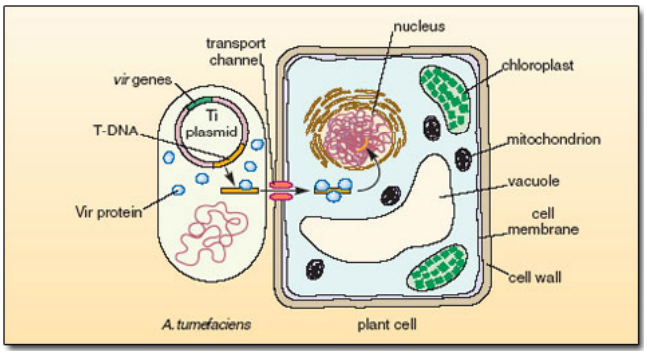
Fuel Alcohol: Ethanol, Butanol, etc.

Cellulase Enzyme Production

- Protein production on an unprecedented scale
  - Base case: replace 1% of U.S. gasoline consumption with cellulosic ethanol.
  - Current estimate: requires 31,000 tons of enzymes per year.
- State of the art process: fungal cell fermentation
  - 76 x 100,000L bioreactors running constantly
  - Capital cost: $800 million
  - Alternative process:
    - Use bioreactor cultures to produce genes, not proteins
    - Transfer genes to plant tissue to produce enzymes



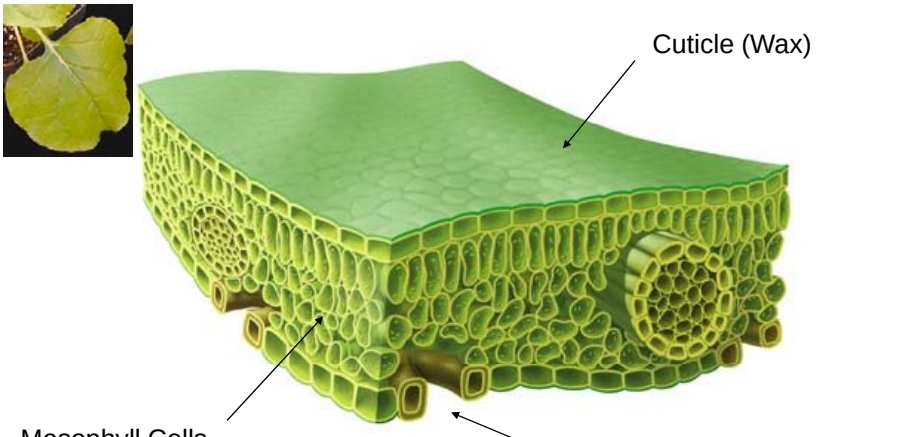
Transient *In Planta* Expression of Enzymes



- Agrobacteria known as “nature’s genetic engineers” - can transfer DNA to plant cells.
- Plant cell transiently transcribes the transferred genes.
- For these experiments: T-DNA replaced with an expression cassette.
  - Viral promoter (CaMV 35S)
  - Endoglucanase (thermostable enzyme from *A. cellulolyticus*)

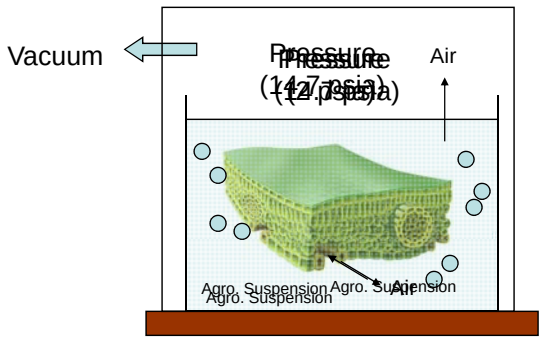
http://openlearn.open.ac.uk/file.php/2808/S250_1_003i.jpg

Leaf Tissue



http://www.science-art.com/gallery/24/24_120200693546.jpg

Vacuum Infiltration of Leaf Tissue



Fungal Cell Culture vs. Agroinfiltration

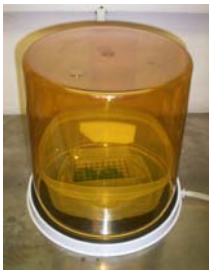
- Advantages of using plant tissue
  - Plants grown outside using energy from sunlight.
  - Fungal cultures grown in bioreactors require a carbon source for energy.
- Advantages of using Agrobacteria
  - Bacteria grow faster than fungal cells
  - Less bioreactor volume is needed
- Capital cost comparison
  - Base case: enough enzyme to displace 1% of domestic gasoline consumption
  - Fungal cell culture bioreactors: $800 million
  - Agrobacteria bioreactors: $58 million

Agroinfiltration Process

N. benthamiana Leaves

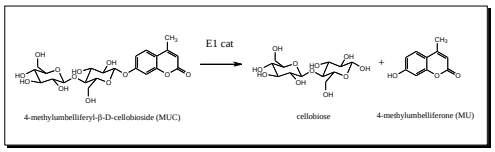
+

Agrobacteria Suspension



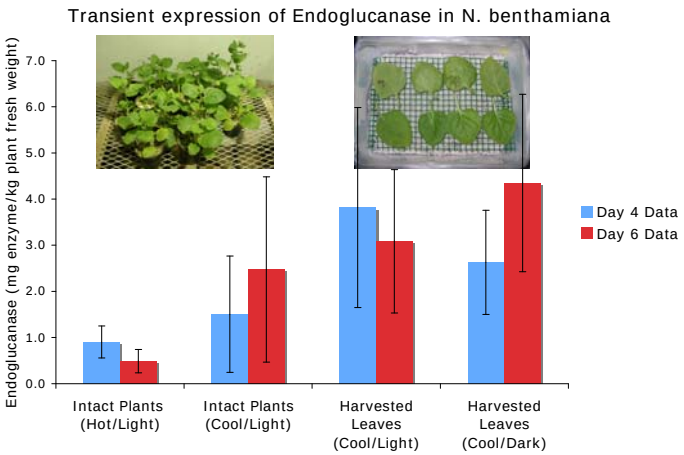
Vacuum Infiltration

Incubate Leaves



Analyze Enzyme Yield

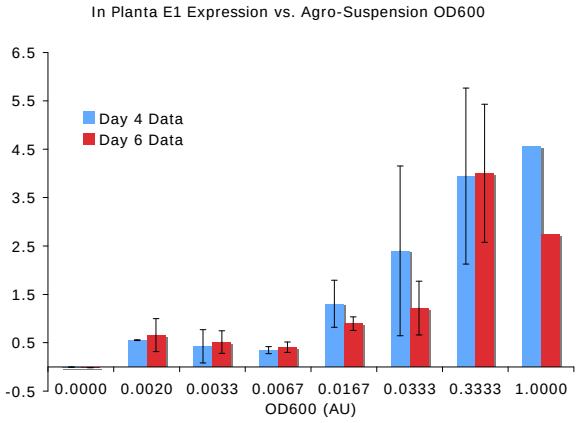
Enzyme Production in Harvested Leaves



- Harvested leaves have the same enzyme yield as intact plants.
- Leaves do not need light to produce high amounts of enzyme.

Concentration of Agrobacteria Suspension

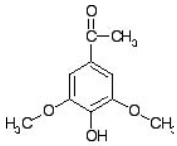
Agrobacteria Suspension



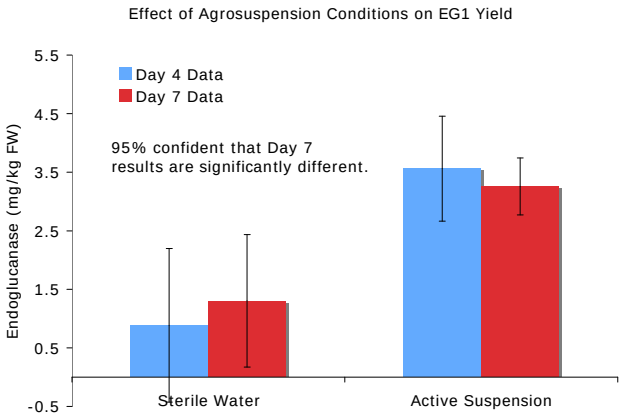
- OD = 0.33 or 1.00, same enzyme yield.
- 10X OD increase (0.03 to 0.33), only 2X enzyme yield increase.

Additional Chemicals Needed for Activation?

Agrobacteria Suspension



Acetosyringone



- Agrobacteria activated with low pH & phenolic compounds produce 2X more enzyme.
- Is the additional yield worth the cost of buffers and phenolic compounds?

Conclusions & Future Work

- Cellulose-degrading enzymes can be produced in harvested plant tissue.
  - Similar yield as in intact plants.
  - Both show low yield, future work includes using Agrobacteria with a viral replicase system to increase yield.
- Concentration of Agrobacteria infiltrated into leaves can be optimized.
  - Experiment will be repeated with Agrobacteria/viral replicase system.
- Agrobacteria do not have to be chemically activated prior to infiltration.
  - Will factor into cost analysis when higher yields are achieved.

Acknowledgments

Advisor/CREATE-IGERT Trainer:

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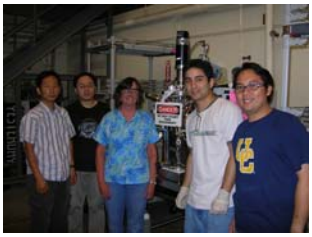
& other students in the McDonald Lab



Collaborators:

Prof. Abhaya Dandekar - UCD Plant Biology
Sandra Uratsu, Ph.D. - UCD Plant Biology
Prof. Bryce Falk - UCD Plant Pathology
Minsook Hwang, Ph.D. - UCD Plant Pathology

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Sources for images:

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