Bacterial Contaminants of Fuel Ethanol Production

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Bioproducts and Biocatalysis Research
Bacterial Contaminants of Fuel Ethanol Production

- National Center for Agricultural Utilization Research
- Bioethanol Research Program
- Bacterial Contamination in fuel ethanol plants
- Implications for distiller’s grains
NCAUR Research Units:

- **Fermentation Biotechnology Research** (FBT)
- **Bioproducts and Biocatalysis Research** (BBC)
- Crop Bioprotection Research
- Cereal Products and Food Science Research
- Food and Industrial Oil Research
- Microbial Genomics and Bioprocessing Research
- Mycotoxin Research
- New Crops and Processing Research
- Plant Polymer Research
Useful Products from Agricultural Materials

Starch
- Enzymatic Saccharification
- Microbial, Chemical

Sugars

Microbial Conversions
- Fumaric acid
- Heat
- Maleic anhydride

- Ethanol
- Dehydration
- Ethylene
- Butadiene

Butylene glycol

Chemical

Plastics, Fibers, Rubber, Resins

Citric acid
Lactic acid
Gluconic acid
Acetic acid
Acetone
Butanol
Antibiotics
Carotenoids
Enzymes
Polysaccharides
Vitamins
Amino acids
Single-cell protein
Biological control agents

Agri-Residues
- Ligno cellulose
- Cellulose
- Hemicellulose
Bioproducts and Biocatalysis Research Unit

Mission: To develop new microorganisms and biocatalysts that can be employed in the bioconversion of renewable agricultural materials into high value products.
BBC Research

- Valuable Chemicals
- Polysaccharides & Novel Carbohydrates
- Fuel Alcohol
Energy Policy Act of 2005
Renewable Fuels Standard

- Double the current production of renewable fuels by 2012

- Establishes an RFS that starts at 4 billion gallons in 2006 and increases to 7.5 billion gallons in 2012.

- By 2013, a minimum of 250 million gallons/year of cellulosic derived ethanol

- Promotes research on alternative feedstocks and biomass to ethanol
Current Paradigm

- Wheat
- Sugarcane
- Corn
**Dry Grind**

- Corn → Mill
- Enzyme → Liquefy
- Enzyme → Saccharify & Ferment (SSF)
- Yeast → CO₂ → Distill
- Distill → Dehydrate
- Dehydrate → Ethanol
- Distiller's Dry Grains

**Wet Mill**

- Corn → Steep
- Stein → Oil
- Corn → Degerm/Defiber
- Gluten Separation → Corn Gluten Meal
- Enzyme → Liquefy
- Enzyme → Saccharify
- Yeast → Ferment
- Ferment → CO₂ → Distill
- Distill → Dehydrate
- Dehydrate → Ethanol
- Corn Gluten Feed
Current and Proposed Locations of Ethanol Production

Source: www.ethanolrfa.org
Potential of corn to replace oil for U.S. market

(RFA & NCGA, 2006)
Potential cellulosic substrates for ethanol production:

- Cottonwoods
- Paper
- Switchgrass
- Wood chips
- Cornstover
- Bagasse
- Corn Fiber
So........

Why not just ferment these materials to alcohol like we do with corn starch???
Constraints to Bioconversion of Fibrous Biomass into Ethanol

- More severe pretreatment required (physical, chemical, thermal)
- Lower sugar concentrations, pretreatment often limits final ethanol concentration
- Microbial inhibitors generated from side-reactions
- Hydrolyzing enzymes less efficient and more sensitive to end product inhibition
- Multiple sugars present in fermentation broth: glucose, xylose, arabinose, galactose…
Composition of Corn Fiber

- Hemicellulose significant
- Mixed sugars
- Need new enzymes and organisms to deal with this
  - *Saccharomyces* doesn’t ferment pentoses
    - So… discover or develop new bugs
Microbial Catalysts to Produce Fuel Ethanol and Value Added Products

♦ Novel microorganisms (extremophiles)
  – Thermophilic, acidophilic bacilli

♦ Recombinant Saccharomyces
  – Inserting xylose-utilization pathway

♦ Metabolic engineering of lactic acid bacteria
  – Converting to ethanologen
Bacterial contaminants

- Gram-positive lactic acid bacteria
  - pH, temperature, ethanol tolerant
  - Faster growth than yeast
- Compete for fermentable sugars and nutrients
- Produce organic acids
  - Inhibitory to yeast
- Reduce yields 2 - 4%
Fuel Ethanol fermentations are not performed under pure culture conditions.
Survey of bacterial contaminants in fuel ethanol plants

♦ Sampled ethanol plants multiple times over 1 year; various points along production line

♦ Sources: One Wet-mill (No antibiotics)
  Two Dry-grinds (+ Antibiotics)

♦ Isolate bacterial strains on selective media and identify by Biolog and API
### Survey of bacterial contaminants in fuel ethanol plants

<table>
<thead>
<tr>
<th>Genus</th>
<th>% of total&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Wet mill</th>
<th>Dry Grind #1</th>
<th>Dry-Grind #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium</td>
<td>0 – 20</td>
<td>1 – 2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Clostridium</td>
<td>0 – 9</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td><strong>Lactobacillus</strong></td>
<td><strong>44 – 60</strong></td>
<td>37 – 39</td>
<td>69 – 87</td>
<td></td>
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<tr>
<td>Lactococcus</td>
<td>0 – 4</td>
<td>0 – 6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>0 – 6</td>
<td>1 – 8</td>
<td>0 – 8</td>
<td></td>
</tr>
<tr>
<td>Pediococcus</td>
<td>0 – 6</td>
<td>19 – 24</td>
<td>0 – 4</td>
<td></td>
</tr>
<tr>
<td>Weisella</td>
<td>0 - 2</td>
<td>18 – 24</td>
<td>0 – 6</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values represent a range over multiple samplings.

Skinner and Leathers, 2004
Microbial control measures

♦ Cleaning and sanitation

♦ Antibiotics
  – Virginiamycin, Penicillin

Any application of antibiotics is a selection experiment for resistant microorganisms!
Antimicrobial susceptibility of *Lactobacillus* contaminants

**Summary of results**

♦ Dry-grind isolates less susceptible to antibiotics (PEN, VIR)

♦ Streptogramin acetyltransferase gene (*vatE*) found only in dry-grind isolates
  – Virginiamycin presumable selection pressure

♦ Potential for acquired resistance to virginiamycin

♦ Most isolates susceptible at recommended dosage of 0.25 to 2.0 ppm

♦ Multi-drug resistance possible
  – *Lactobacillus* $\text{MIC}_{\text{PEN}} = 8$ ppm and $\text{MIC}_{\text{VIR}} > 16$ ppm
  – *Enterococcus* $\text{MIC}_{\text{PEN}} = 8$ ppm and $\text{MIC}_{\text{VIR}} 8$ ppm
Implications for Distiller’s Grains?

- Drug residues
  - PEN labile during fermentation
  - VIR activity reduced to 2.6% after distillation

- Alternative bacterial control measures (new drugs, hop acids)?
  - Safety issues for beef product?

- Microbial load (wet distillers grains)
  - Resistant organisms?

- Reservoir of antibiotic resistance?
  - Potential for transfer to pathogens?
Cellulosic ethanol co-products

- Composition/nutrition of co-product?
- Recombinant organisms?
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