Efficient Lysine production from sustainable biomass feedstock using a modified industrial bacterium

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DLG supply: Vagn Hundebøll, Hans Aae

Biovalue meeting, ARLA Foods, Viby, 2017
Amino acids for balancing animal feed

<table>
<thead>
<tr>
<th></th>
<th>First</th>
<th>Second</th>
<th>Third</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growing Pig</td>
<td>Lysine</td>
<td>Threonine</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Broiler</td>
<td>Methionine</td>
<td>Lysine</td>
<td>Threonine</td>
</tr>
</tbody>
</table>
Lysine production

• Lysine is produced from sugar by fermentation, using engineered or classically derived bacterial strains
• Annual world production: ~2 million tons
Production process at Vitalys

Raw materials: C6 sugars

Fermentor: C. glutamicum DSMZ 20300

Spray drying

Vitalys production closed in 2016 – no lysine production in DK
Can we find a more sustainable feedstock for lysine production?
Wheat straw hydrolysates - challenges

190°C for 10 minutes (Risø)

Enzymatic hydrolysis (Cellic® CTec3) & concentration (Novozymes)

Analysis (DTU Chemical Engineering)

<table>
<thead>
<tr>
<th></th>
<th>Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>121.9</td>
</tr>
<tr>
<td>Xylose</td>
<td>84.1</td>
</tr>
<tr>
<td>Arabinose</td>
<td>2.5</td>
</tr>
</tbody>
</table>

- *C. glutamicum* cannot metabolize xylose
- Microbial inhibitors are present in the hydrolysate
Goal of project 4: a bacterium for efficient lysine production from wheat straw hydrolysates

Strategies:

Model strain:
- Produce lysine
- Metabolize xylose
- Inhibitor tolerant

Transfer of traits from model to industrial strain
1. Metabolize xylose
2. Inhibitor tolerance

Industrial strain:
- High lysine yield
- High titer

Transfer of traits from industrial to model strain
1. High lysine yield
2. High titer
Construction of a model strain producing lysine

Three genetic modifications are required for lysine production on the basis of wildtype strain ATCC 13032:

1. **Remove the feedback inhibition of aspartate kinase (lysC)**

2. **Down-regulate the branch pathway of homoserine dehydrogenase (hom)**

3. **Up-regulate the production of precursors for lysine (pyc)**

Wildtype $\rightarrow$ Lysine producing strain

Yield $= 0.08$ (mmol lysine/mol glucose)

Further optimization has been performed and published or patented in project 4 with respect to C6 fermentation.

Ohnishi, J. 2002. Applied Microbiology and Biotechnology
Model strain - Lysine production from xylose

1. A plasmid containing xylA and xylB from *L. lactis*, introduced into our lysine-producer *C. glutamicum*

2. Adaptive evolution

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**Growth rate on 1% xylose**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Growth rate (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZW90</td>
<td>0.1</td>
</tr>
<tr>
<td>ZW91</td>
<td>0.1</td>
</tr>
<tr>
<td>ZW92</td>
<td>0.3</td>
</tr>
<tr>
<td>ZW94</td>
<td>0.5</td>
</tr>
<tr>
<td>Control (glucose)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Lysine yield on 1% xylose**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lysine yield (C·mmol/C·mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZW90</td>
<td>80</td>
</tr>
<tr>
<td>ZW91</td>
<td>80</td>
</tr>
<tr>
<td>ZW92</td>
<td>80</td>
</tr>
<tr>
<td>ZW94</td>
<td>80</td>
</tr>
<tr>
<td>Control (glucose)</td>
<td>80</td>
</tr>
</tbody>
</table>
Adaptation to feedstock inhibition

- Adaptive evolution in feedstock based medium or MM with furfural

- Adapted for growth in 40% wheat hydrolysate and 5 g/l furfural

![Graph showing inhibition concentration and growth of WT, Mutant α, Mutant β, and Mutant γ](image)

<table>
<thead>
<tr>
<th>Wheat hydrolysate</th>
<th>80%</th>
<th>40%</th>
<th>20%</th>
<th>Furfural g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.3</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

![Image showing test tubes with different concentrations of wheat hydrolysate and furfural](image)
Goal of project 4: a bacterium for **efficient** lysine production from wheat straw hydrolysates

**Strategies:**

**Model strain:**
- Produce lysine
- Metabolize xylose
- Inhibitor tolerant

**Industrial strain:**
- High lysine yield
- High titer

**Transfer of traits from model to industrial strain**
1. Metabolize xylose
2. Inhibitor tolerance

**Transfer of traits from industrial to model strain**
1. High lysine yield
2. High titer
Genome sequencing and subsequent comparison revealed more than 1000 mutations in the industrial strain compared to the model strain.

Transfer traits from industrial to model strain

Model strain: lysine from xylose
Inhibitor tolerant

Industrial strain: High lysine yield
High titer

1. Ability to metabolize xylose
2. Inhibitor tolerance
Transfer of traits from the model to the industrial strain: xylose consumption

Preliminary characterization on xylose:

1. ZW111 produced more lysine than the model strain

2. Final biomass of ZW111 was lower
High yield of lysine from xylose in lab-scale fermentation

Stage 1. Growth phase (CGXII with 2% glucose and 0.2% YE), 24 hrs, OD ~ 15-18. Volume: 50 ml/500ml shake flask. Stage 2. Collect the cell pellet and resuspended in production medium (CGXII with 2% glucose or xylose respectively). Volume: 20 ml/250ml shake flask.

Lysine yield:

On glucose: ~ 200 mmol/mol
On xylose: ~ 450 mmol/mol
(*1.2 to convert to c-moles)
Future plans

1. Characterize adaptive mutants of industrial strain on xylose
2. Test glucose/xylose co-feeding strategy
3. Test/improve the inhibitor tolerance of the industrial strain
4. Test real lignocellulose hydrolysate
5. Reach high titer, repeated fed-batch fermentation
Should we produce amino acids in Denmark?

• Sustainability
  – Exploit waste and side streams as cheap? and sustainable feedstocks

• Organic amino acids, new market
  – Possibility for balancing amino acids content in animal feed in a fast growing organic market
Thank you!