Application of versatile Acetic Acid Bacteria to innovative bioprocesses

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Versatility of Acetic Acid Bacteria

Cellulose

New vinegars

Bioactive attractants
Feasible acetic acid fermentations of alcoholic and sugary substrates in combined operation mode
Part 1  Implementation of a combined system (static and submerged)

**A. pasteurianus UMCC 1754**

- High acetic acid production rate
- Efficient start-up and persistence
- Phenotypic stability
- No production of undesired products
- Low nutritional needs

4 DIFFERENT VINEGARS
Part 1

Selected Starter Cultures at laboratory scale

**STATIC SYSTEM**

- SSC-A to start-up submerged fermentation
- SSC-B to prototype scale
- Slow fermentation

**SUBMERGED SYSTEM**

- SSC-C, D wine
- SSC E grape must
- Fast fermentation
**Part 1**

**Cultures performance at prototypal scale**

**SCALE-UP**

Over 200L of vinegar/batch in 6 months

**BATCH B-E: Wine**

**BATCH C-D: CGM**
Combined fermentation by *A. pasteurianus* UMCC 1754 yielded viable SSCs at both laboratory and prototype scales.

Process stability in static, submerged and prototype-scale confirmed the feasibility of using SSCs in industrial vinegar fermentations.
Increased production of bacterial cellulose as starting point for scaled-up applications
Bacterial cellulose synthesis

- D-glucose with β(1-4) glycosidic bond
- Synthetized by cellulose synthase (CS)
- High purity
- High degree of crystallinity
- High W.A.R. and resistance to tensile strength
Part 2

Cellulose production

34 STRAINS SHOWED BC PRODUCTION

HIGHEST BC YIELD BY *K. xylinus* (K2G30) 23,18 g/L
Culture optimization

Strain K2G30

UMCC 2756 (K. xylinus)

FORMULATION OF AN ENHANCED BROTH

\[ \text{GET} = \text{GY} - \text{CaCO}_3 + 1.4\% \text{ ETOH} \]

+ 

S/V RATIO OPTIMIZATION

0,23 cm⁻¹

\[ \text{HIGHER BC YIELD} \]
\[ \text{LOWER BY-PRODUCTS FORMATION} \]

Ethanol is an additional energy source so it allows glucose to be used mainly for BC synthesis
CARBON SOURCES CONSUMPTION AND BC PRODUCTION

Part 2  15 days of static cultivation in vessel (S/V 0.23 cm$^{-1}$)

<table>
<thead>
<tr>
<th>Broth</th>
<th>pH</th>
<th>Glu (g/L)</th>
<th>*EtOH (g/L)</th>
<th>G.A (g/L)</th>
<th>A.A (g/L)</th>
<th>BC g/L</th>
<th>BC/cons sugar (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GY</td>
<td>Initial</td>
<td>5.40</td>
<td>50.00 ± 0.03</td>
<td>15.73 ± 0.03</td>
<td>0.00</td>
<td>22.23 ± 0.05</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>final</td>
<td>3.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GET</td>
<td>Initial</td>
<td>6.37</td>
<td>50.00 ± 0.02</td>
<td>18.00 ± 0.08</td>
<td>2.20 ± 0.08</td>
<td>11.46 ± 0.05</td>
<td>0.67 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>final</td>
<td>4.45</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

OPTIMIZED CONDITIONS

- BC production increased of 30%
- Gluconic acid was ~ 50% lower
- Final pH was higher
- g of BC produced per consumed sugar increased

*GET: Initial Ethanol: 14%
Glu: glucose
EtOH: ethanol
GA: gluconic acid
A.A: acetic acid
BC: bacterial cellulose
Structural analysis of bacterial cellulose produced by K2G30

**XRD**

- Typical peaks of BC with high degree of crystallinity
- D.o.c = > 80%
- Graphs are analogous and the peaks are typical for BC.
- Chemical bonds were not altered by the milling process
- Glass transition temperature (Tg) at about 0 °C.
- The mass loss (water removal) was 12.9%.
- W.A.R: 400%

**FT-IR**

- Hydrogen bonding (3300 cm⁻¹)
- H-O-H bending (1600 cm⁻¹) (adsorbed water)
- C-H stretching (2900 cm⁻¹)
- C-O-C stretching (1050 cm⁻¹)

**DSC**

- Absorbed water

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**Part 2**
Conclusions

- The highest yield was achieved by K2G30 by static cultivation among 34 studied strains.
- Enhanced culture conditions increased BC production of 30%.
- High-purity BC with high degree of crystallinity (80%) and WAR (400%).

Selected strain was able to produce high amount of BC suitable for biomedical applications and food processing.
Screening and selection of acetic acid bacteria for fructans production
Some AAB are levan producers $\beta(2\rightarrow6)$
- Prebiotics, anti-cancer and anti-viral effect
- Thickening and gelling agents
- Biodegradable plastics, glues, cosmetics, textile coatings, detergents

Selection of strains to develop prebiotic beverages

Some AAB are high fructan (Levan) producers
### Identification of the screening conditions

#### Standard Conditions
- Sucrose concentration: 70 g/L
- Shaking speed: 140 rpm

#### Optimized Conditions
- Sucrose concentration: 250 g/L
- Shaking speed: 200 rpm

#### Screening of Fructan Production

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sucrose concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250 g/L</td>
</tr>
<tr>
<td>NBRC 101099&lt;sup&gt;T&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>UMCC1754</td>
<td>+</td>
</tr>
<tr>
<td>UMCC 1789</td>
<td>+</td>
</tr>
<tr>
<td>DSM 3509&lt;sup&gt;T&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>DSM 2343</td>
<td>+</td>
</tr>
<tr>
<td>DSM 2004&lt;sup&gt;T&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>UMCC 2756</td>
<td>+</td>
</tr>
</tbody>
</table>

#### Osmotolerance Screening
- Most AAB strains can grow at 300 g/L of sucrose
- *A. pasteurianus* strains prefer lower concentration

**250 g/L Maximum Sucrose Concentration**
Part 3

Fructan production optimization

**STANDARD CONDITIONS**

- **Neoasaia chiangmaiensis** showed the highest fructan production in both conditions

**OPTIMIZED CONDITIONS**

- **Neoasaia chiangmaiensis**
- **K. xylinus**

- **Acetobacter** and **K. xylinus strains** produced about 5 g/L in both conditions
**CELLULOSE SCREENING AND EXTRACTION**

<table>
<thead>
<tr>
<th>Strain</th>
<th>QUALITATIVE BC PRODUCTION TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBRC 101099&lt;sup&gt;T&lt;/sup&gt;</td>
<td>HS-G -</td>
</tr>
<tr>
<td>DSM 2343</td>
<td>HS-S -</td>
</tr>
<tr>
<td>DSM 2004&lt;sup&gt;T&lt;/sup&gt;</td>
<td>HS-G +</td>
</tr>
<tr>
<td>DSM 3509&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>UMCC 2756</td>
<td>HS-G +</td>
</tr>
</tbody>
</table>

**FRUCTANS EXTRACTION PROTOCOL**
- Centrifugation
- + 2 Volume of cold ETOH
- Precipitation
- Centrifugation
- Levan-pellets collected
- Levan purification

**FRUCTAN CHARACTERIZATION**
- Preliminary results showed a FT-IR spectra of pure levan
- Fructans extracted by ethanol precipitation
Conclusions

Neoasaia chiangmaiensis NBRC101099 showed the highest fructan production in both standard and optimized culture conditions.

Strains suitable for industrial production

Komagataeibacter strains showed a considerable production of both fructans and cellulose.

K. xylinus strain UMCC 2756 is suitable to develop functional foods and beverages.
General conclusions of the Ph.D. project

• High versatility of Acetic Acid Bacteria opens wide perspectives in both food and non food industry

• Possibility to develop new products

• Research on small scale is essential to improve the processes on large scale

Performing a further engineering of the investigated processes is the most suggested development, both in research and industrial scale
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