E. coli Fiber Project

Tokyo Metropolitan University

iGEM Tokyo Metropolitan
Aim of project

Use bacteria to produce cellulose cheaply and efficiently
~Introduction~

- **Cotton**
- **Glass**
- **Nylon**
- **Polyester**
- **Wool**
- **Hemp**
- **Silk**
- **Carbon nanotube**
- **Bacterial cellulose**
Bacterial cellulose

- Thin!
- Strong!
- Bacterially produced

**but**

- High cost
- Low production rate
Strong reinforcement

High elastic

Diaphragm of speaker

Very Thin

Adding resin, very strong

Bacterial cellulose

fine filter
Why Low Production...?

Need two cultures

Bacterial cellulose

Acetobacter xylinum

aerobic
anaerobic
Why Low Production...?

Acetobacter

Acetic acid

Down pH

Grow slowly

Inhibited growing by acetic acid
Transform synthetic genes into *E. coli*.

Low productivity...

Increase productivity!!
**Acetobacter xylinum**
Produces bacterial cellulose

**Escherichia coli**
Flexible as a chassis

T7 polymerase
For overexpression
Role of bacterial cellulose synthase (bcs)

- **BcsA**: binds UDP-glucose
- **BcsB**: catalyzes cellulose polymerization
- **BcsC**: forms the pore for cellulose export
- **BcsD**: exports bacterial cellulose
A. *xylinum* produces bacterial cellulose.

E. coli flexible as chassis for overexpression.

BcsA, BcsB, BcsC, BcsD form a complex on membrane.

~Approach~

T7 polymerase for overexpression of BcsA, BcsB, BcsC, BcsD.

Cellulose fiber.
E. coli produces bacterial cellulose
Why *E. coli*?

- Well characterized protein expression system
- *A. xylinum*
- Grows quickly
- Compatible with BioBricks

~Approach~
Strategy

Cluster gene

bcsA  bcsB  bcsC  bcsD

Made Biobricks

Ligate parts
~Approach~

**IPTG**

lac promoter  T7 polymerase

bcsA  bcsB  bcsC  bcsD
Quantification

~Approach~

NaOH
<table>
<thead>
<tr>
<th>Parts number</th>
<th>Domain</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K3360051</td>
<td></td>
<td>bacterial cellulose synthase A</td>
</tr>
<tr>
<td>BBa_K3360020</td>
<td></td>
<td>bacterial cellulose synthase B</td>
</tr>
<tr>
<td>BBa_K3360052</td>
<td></td>
<td>bacterial cellulose synthase C</td>
</tr>
<tr>
<td>BBa_K3360021</td>
<td></td>
<td>bacterial cellulose synthase D</td>
</tr>
<tr>
<td>BBa_K3360053</td>
<td></td>
<td>T7 polymerase expressed from lac promoter</td>
</tr>
<tr>
<td>BBa_K3360102</td>
<td></td>
<td>express bacterial cellulose synthase A from T7 promoter</td>
</tr>
<tr>
<td>BBa_K3360103</td>
<td></td>
<td>express bacterial cellulose synthase B from T7 promoter</td>
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<tr>
<td>BBa_K3360104</td>
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<td>express bacterial cellulose synthase C from T7 promoter</td>
</tr>
<tr>
<td>BBa_K3360105</td>
<td></td>
<td>express bacterial cellulose synthase D from T7 promoter</td>
</tr>
</tbody>
</table>
Result

**Completed the cloning of bcsA,B,C,D!**
We are going to continue the study

Haven't succeeded yet...
Possible productivity

Relative expression of bcs genes

Values of Acetobacter

\[
\frac{P_{\text{lac}}}{P_{\text{bcs}}} = \frac{1.83}{3.15}
\]

Values of E. coli

\[
\frac{P_{\text{T7}}}{P_{\text{lac}}} = \frac{8.00}{1.00}
\]

Over 4 times expression!!

Relative expression of bcs genes

\[
\frac{1.83}{3.15} \times \frac{8.00}{1.00} = 4.65
\]
Possible productivity

- **Number of bacteria**
  - E. coli growth
  - A. xylinum growth

- **Time**
  - E. coli product
  - A. xylinum product

- **Possible productivity**
  - E. coli growth
  - A. xylinum growth
Discussion

For more practical use

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓Amount of bacteria</td>
<td>☐Reuse waste material</td>
</tr>
<tr>
<td>✓Individual ability</td>
<td>☐Fittest condition</td>
</tr>
</tbody>
</table>
Discussion

Reuse waste for medium

waste cellulose

2008 Edinburgh

Cellulose Degradation

glucose

Bacterial cellulose

2010 Tokyo metropolitan

Bacterial Cellulose Synthesis
Discussion
Find best conditions

Component of medium

Temperatures

28°C 37°C
Future

Cellulose products will be cheaper and more available to us.

Bacterial cellulose saves trees!
iGEM JAPAN ～human practice～

- Founded “iGEM JAPAN” to make contact between Japanese iGEM teams.
- Wrote articles to popularize iGEM.
- Made “iGEM JAPAN web site” to make discussion and communication.

Human Practice... 557/1511
Have you ever heard ‘synthetic biology’?

Not famous yet

82%

15%

3%

No response

Many outreach activities!!!!

School festival, Science technology festival, Open lab, Science agora, Teaching experiment etc...
Acknowledgment

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References

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Thank you for your listening!!