Intelligent Microbial Heat Regulating Engine
Background and Issues

NAME: COCO
SEX: UNKNOWWN
DATE OF BIRTH: 2013.08.08
FAVORITE FOOD: LB
Background and Issues
Background and Issues

**Note:** Including organic acid, amino acid, starch sugar, yeast, etc. Not including antibiotic, vitamin, Alcohol, etc.
Background and Issues

50 °C

37°C

Cooling system

So comfortable

Log, or exponential growth, phase
Stationary phase
Death, or logarithmic decline, phase

E. coli growth curve
Background and Issues

1 °C

¥ 1,000,000,000

345,000 kW·h

111,373,056 tons

CO₂

CO₂

¥ 1,000,000,000

CO₂
Background and Issues
Ideas

Extending their living temperature

Making them live in the optimizing density
Ideas

Thermotolerant Mechanism

- Heat shock protein
- Cell membrane
- Transcription factor
- Some special enzyme
- Global regulator
Ideas

Controlling the cell density to reduce metabolic heat

\[ Q_{\text{Fermentation}} = Q_{\text{Biology}} + Q_{\text{Mechanism}} - Q_{\text{Evaporation}} - Q_{\text{Sensible heat}} - Q_{\text{Radiation}} \]
### Regulation of cell density

#### T-A systems in *Escherichia coli*

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Antitoxin</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CcdB</td>
<td>CCdA</td>
<td>Found on the F plasmid of <em>Escherichia coli</em></td>
</tr>
<tr>
<td>HicB</td>
<td>HicA</td>
<td>Found in archaea and bacteria, it is not clear which component is toxin and which is antitoxin.</td>
</tr>
<tr>
<td>Kid</td>
<td>Kis</td>
<td>Stabilises the R1 plasmid and the CcdB/A system.</td>
</tr>
<tr>
<td>MazF</td>
<td>MazE</td>
<td>A system induced by the SOS response to DNA damage in <em>E. coli</em>.</td>
</tr>
<tr>
<td>ParE</td>
<td>ParD</td>
<td>Found in multiple copies in <em>Caulobacter crescentus</em>.</td>
</tr>
</tbody>
</table>

*Well-studied and low toxicity*
Design and Modeling

- Customized Thermotolerance System
  - Heat Shock Protein
  - RNA Thermometer

- Intelligent Quorum Regulating System
  - Device I (Quorum-sensing)
  - Device II (Oscillating Circuit)
  - Device III (Programmed Cell Death)
# Heat Shock Protein

**Thermoanaerobacter tengcongensis MB4**

<table>
<thead>
<tr>
<th>Gene</th>
<th>classification</th>
<th>Property</th>
<th>Gene</th>
<th>classification</th>
<th>Property</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>GroEL</td>
<td>HSP60</td>
<td></td>
<td>FliA</td>
<td></td>
<td>σ factor</td>
<td>Heat-induced Spore</td>
</tr>
<tr>
<td>GroES</td>
<td>HSP10</td>
<td>Assist and correct unfolded and non-native</td>
<td>RpoD</td>
<td></td>
<td>σ factor</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>protein</td>
<td>RpoE1</td>
<td>σ factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DnaK</td>
<td>HSP70</td>
<td></td>
<td>RpoE2</td>
<td>σ factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DnaJ</td>
<td>HSP40</td>
<td></td>
<td>RpoE3</td>
<td>σ factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GrpE</td>
<td>HSP</td>
<td></td>
<td>RpoE4</td>
<td>σ factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ThiF</td>
<td>ubiquitin</td>
<td></td>
<td>RpoE5</td>
<td>σ factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTE</td>
<td>ubiquitin</td>
<td>Mark Decomposed protein</td>
<td>RpoE6</td>
<td>σ factor</td>
<td></td>
<td>Be activated under specific</td>
</tr>
<tr>
<td>LytS</td>
<td>HSP90</td>
<td>protease</td>
<td>RpoE7</td>
<td>σ factor</td>
<td></td>
<td>conditions</td>
</tr>
<tr>
<td>LbpA</td>
<td>HSP20</td>
<td>Protein digest</td>
<td></td>
<td></td>
<td></td>
<td>Cell envelope</td>
</tr>
</tbody>
</table>

(pdslab.biochem.iisc.ernet.in/hspir/chaperone.php)
The GroES and IbpA confer E. coli much better heat-resistance at 40°C.
The GroEL and ThiF confer E. coli much better heat-resistance at 43°C.
The DnaK and IbpA confer E. coli much better heat-resistance at 46°C.
We found that overexpressing a protein may affect the growth of the host to a certain degree.
Different strength promoters control different expression level of different heat shock proteins (HSPs) to achieve the goal of Hierarchy Heat-resistant.
Design and Modeling

- Customized Thermo-tolerance System
  - Heat Shock Protein
  - RNA Thermometer

- Intelligent Quorum Regulating System
  - Device I (Quorum-sensing)
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  - Device III (Programmed Cell Death)
Design and Modeling

Intelligent Quorum Regulating System

Device I (Quorum-sensing)
Device I (Quorum-sensing)
Model

- N species react through M reaction channels.
- $X_i(t)$ is the number of molecules of species i, in the system at time t.

Algorithm

- Determine probability that, starting at time $t$, reaction $\mu$, $R_\mu$, will be the next reaction to occur in the interval $[t+\tau, t+\tau+d\tau]$
- Execute reaction $\mu$ and propagate time.
Stochastic simulation of protein degradation

Design and Modeling

![Graph showing stochastic simulation of protein degradation.](image.png)
Stochastic simulation of production and degradation

Stationary Distribution

Number of Molecules
Design and Modeling

Intelligent Quorum Regulating System

--- Device II (Oscillating Circuit)

Device I

TetR → TetR

CI → CI

tetR → CI

cl → lacI

R0011 B0034 C0040 B0010 B0012 R0040 B0034 C0051 B0010 B0012 R0051 B0034 C0012 B0010 B0012

LacI
Device II (Oscillating circuit)
Device I

Device II

Intelligent Quorum Regulating System

----Device III (Program Cell Death)

Actually I prefer MazE....
Device III (Programmed Cell Death)
Design and Modeling

Intelligent Quorum Regulating System

Device I

Device II

Device III

Result: Device III PCD

Graph showing OD600 over time for different conditions.
Intelligent Quorum Regulating System

Design and Modeling

Device I

Device II

Device III

Density of E. coli

Time
## Summary

<table>
<thead>
<tr>
<th>Tube</th>
<th>Part</th>
<th>Plasmid Backbone</th>
<th>Resistance Status</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BBa_K1117001</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
</tr>
<tr>
<td>2</td>
<td>BBa_K1117002</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
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<tr>
<td>3</td>
<td>BBa_K1117003</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
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<tr>
<td>4</td>
<td>BBa_K1117004</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
</tr>
<tr>
<td>5</td>
<td>BBa_K1117005</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
</tr>
<tr>
<td>6</td>
<td>BBa_K1117006</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
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<tr>
<td>7</td>
<td>BBa_K1117007</td>
<td>pSB1C3</td>
<td>C</td>
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<td>8</td>
<td>BBa_K1117008</td>
<td>pSB1C3</td>
<td>C</td>
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<td>9</td>
<td>BBa_K1117009</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
</tr>
<tr>
<td>10</td>
<td>BBa_K1117010</td>
<td>pSB1C3</td>
<td>C</td>
<td>Accepted Sending Plasmid Backbone</td>
</tr>
<tr>
<td>11</td>
<td>BBa_K1117011</td>
<td>pSB1K3</td>
<td>K</td>
<td>Requires Exception(U) Sending Plasmid Backbone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>we have no time to transfer the part to pSB1C3, but we want to sending this part to iGEM.</td>
</tr>
<tr>
<td>12</td>
<td>BBa_K1117012</td>
<td>pSB1K3</td>
<td>K</td>
<td>Requires Exception(U) Sending Plasmid Backbone</td>
</tr>
</tbody>
</table>
Summary

Advantages

Reducing energy consumption

Improving production efficiency

I’m Here for one green earth!
With the help of HSP, the enzyme can catalyze efficiently during normal cell growth at a higher temperature.
Future Plan

Intelligent variable frequency oscillator
Human Practice

- Primary School
- High School
- University
- Enterprise

Kindergarten
Future Plan

Genetic Engineering & Transformation

Flask Small-scale Pilot Production

Fermentation Small-scale test

Mass product

Pilot Large-scale test
Cooperation

TJU & BIT-China

GC Parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume</td>
<td>1 μL</td>
</tr>
<tr>
<td>Inlet heater temperature</td>
<td>250°C</td>
</tr>
<tr>
<td>Inlet pressure</td>
<td>7.07 psi</td>
</tr>
<tr>
<td>Total inlet flow</td>
<td>0.5 mL/min</td>
</tr>
<tr>
<td>Septum purge flow mode</td>
<td>Standard</td>
</tr>
<tr>
<td>Inlet Mode</td>
<td>Splitless</td>
</tr>
<tr>
<td>Column</td>
<td>Agilent 19091S-433: 325°C: 30 m x 250 μm x 0.25μm</td>
</tr>
<tr>
<td>Column flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Column Pressure</td>
<td>7.07 psi</td>
</tr>
<tr>
<td>Average velocity</td>
<td>36.262 cm/sec</td>
</tr>
<tr>
<td>Holdup Time</td>
<td>1.3789</td>
</tr>
<tr>
<td>Initial oven temperature</td>
<td>40°C for 1 min</td>
</tr>
<tr>
<td>Ramp 1</td>
<td>Heat 15°C/min until 100°C</td>
</tr>
<tr>
<td>Ramp 2</td>
<td>Heat 25°C/min until 320°C, hold for two min</td>
</tr>
</tbody>
</table>
Acknowledgment

Instructors

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Yue Wang
Shanqin Liang
LiChao Zhu
Bangjie Liao
Jiexin Zhang
Yinan Luan
Tianhui Zhang
Han Yue
Thanks for your attention!