Abstract

In order to eliminate organophosphorus pesticide residue, we have designed an engineered bacterium, secreting OpdA enzyme to degrade the organophosphorus pesticide residue. Its expression is under temperature control and can only be activated above 32 Celsius. To avoid secondary pollution, a UV-induced suicide gene is introduced into the bacterium upon exposure to UV, the suicide procedure is induced.

Background

With increased agriculture activities around the world, it has become a common practice to use pesticides to solve pest problems. Especially in China, toxic pesticide residues on green vegetables and fruits have become a major public health problem. Therefore, our team has designed an engineered bacterium which is able to degrade organophosphorus pesticide.

Methodology

<table>
<thead>
<tr>
<th>PCR amplification for F1</th>
<th>PCR amplification for F2</th>
<th>PCR amplification for F3</th>
<th>PCR amplification for F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>single-digested with HindIII</td>
<td>single-digested with HindIII</td>
<td>single-digested with HindIII</td>
<td>single-digested with HindIII</td>
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<tr>
<td>Linked with T4 ligase</td>
<td>Linked with T4 ligase</td>
<td>Linked with T4 ligase</td>
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Plasmid pSB1C3

<table>
<thead>
<tr>
<th>PCR amplification for F 1+2</th>
<th>PCR amplification for F 3+4</th>
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<tbody>
<tr>
<td>single-digested with Spel</td>
<td>single-digested with Xbal</td>
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<tr>
<td>Linked with T4 ligase</td>
<td>Linked with T4 ligase</td>
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</tbody>
</table>

double-digested with EcoRI and PstI

double-digested with EcoRI and PstI

Ligated into pSB1C3-EcoRI-Constitutive promoter-3X4Athermometer-opdA-opdA-RecA3SOS promoter-ccdB

Results

Gel extraction results of F1 (track 1); F2 (track 2); F3 (track 3); and F4 (track 4), segments. M: DL2000Km ladder

 Verification result of two positive plasmids after double digestion. M: X-Taq DNA marker; track 1: positive plasmid 1 digested by PstI enzyme; track 2: positive plasmid 1 without digestion (negative control); track 3: positive plasmid 2 digested by PstI enzyme; track 4: positive plasmid 2 without digestion (negative control); track 5: positive plasmid 2 digested by EcoRI + PstI enzymes; track 6: positive plasmid 2 without digestion (negative control);

Conclusions & Discussion

Conclusion: We successfully constructed and submitted a plasmid that expresses temperature-controlled OpdA protein in vitro and the expression can be shut down by UV-induced suicide gene.

Discussion: Further functional tests need to be done:
- Expression dynamic curve of OpdA
- Secretion of OpdA
- Activation curve of suicide gene

Human Practices

- School Club: We founded the Life Code Club in our school. Many students with interest in biology joined us.
- T-shirt Theme Activity: Our Life Code Club organized students from our school to design and paint T-shirts to show their view of synthetic biology.
- IJEG Magazine: We and World Magazine worked together to publish a special issue about IJEG.
- Questionnaire: We did a questionnaire about Nanso wholesale market for vegetables and fruits.
- Video Promotion: We made a short video to introduce synthetic biology and our IJEG project. We put it on Tudou and YouTube.
- Sina Weibo: The weibo of our IJEG Team concentrates on synthetic biology and the IJEG competition

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