Applications of genetic transformation of Aspergillus oryzae with bleomycin resistant selection

Satoshi Suzuki1, Sawaki Tada1, Mari Fukuoka1, Yoshiki Tsukakoshi1, Mayumi Matsushita1, Yutaka Kashiwagi1, Masanori Sugiyama2, Ken-Ichi Kusumoto1

1Natl. Food Res. Inst., Japan; 2Hiroshima Univ.

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Abstract
Introduction: Since Aspergillus oryzae produces large quantity of enzymes, it is significant to establish a host-vector system to use this microorganism as a host for heterologous protein expression. In various filamentous fungi, the genes, which confer resistance to hygromycin B, aureobasidin, and G418, have been used as genetic markers for the gene manipulation. However, A. oryzae is resistant to these antibiotics. We have recently developed a transformation system for A. oryzae RIB40 by using bleomycin-resistance gene as a selectable marker. In the present study, we generated ligD knock out strain, by using bleomycin resistance selection. And we generated exogenous or endogenous enzyme over-expressor by transformation with expression vector carrying bleomycin resistant gene.

Methods: Synergistic activity of several agents with bleomycin was evaluated by Cross-paper-strip assay. A strip of filter paper (0.7*8 cm) containing bleomycin and each one of the filter papers containing agents were placed crosswise in the test agar plates. The paper-strip assay. A strip of filter paper (0.7 * 8 cm) containing bleomycin and each one of

Results: The disruption of the ligD locus in genomic DNA of transformants was confirmed by colony PCR and the Southern blot analysis. Activity of each enzyme was detected from cell free extract or culture filtrate of the each transformant selected by bleomycin resistance.

Table 1: Natural resistance of A. oryzae to antibiotics

<table>
<thead>
<tr>
<th>Antibiotics (200µg/ml)</th>
<th>Growth of A. oryzae</th>
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<tbody>
<tr>
<td>bleomycin</td>
<td>-</td>
</tr>
<tr>
<td>hygromycin B (1mg/ml)</td>
<td>+++</td>
</tr>
<tr>
<td>G418 (1mg/ml)</td>
<td>+++</td>
</tr>
<tr>
<td>blasticidin S (1mg/ml)</td>
<td>++</td>
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</tbody>
</table>

• Bleomycin

Fig. 1: Effect of the chemosensitizers

Triton X-100, Tween 20, Tween 80, Saponin
X: 100 µg bleomycin, Y: 10 µl detergent

Verapamil, Diltiazem, Chlorpromazine, Trifluoperazin
X: 100 µg bleomycin, Y: 20 mg pump inhibitor

• Bleomycin family is metalloglycopeptide antibiotics produced as mixtures of derivatives with different side-chains by Streptomyces verticillus.
• Bleomycin is used in cancer chemotherapy.
• Bleomycin cleavages nucleic acid of both eukaryotes and prokaryotes.

To overcome the natural resistance, we should...
• Decrease the target molecule
• Enhance the drug uptake
• Inhibit the export of the drug from cell
• Inhibit the detoxification of the drug...etc.

Fig. 2: The ligD disruption construct

ligD 5′ framing
2 kb
sCer
blmB
H2Bpro
0.5 kb
ligD 3′ framing
BmR

The bleomycin acetyltransferase expression cassette that is consisting with A. oryzae histone H2B promoter followed by blmB (bleomycin acetyltransferase gene from Streptomyces) and A. nidulans sC terminus was named as BmR.

Fig. 3: Disruption of the ligD gene of A. oryzae

Fig. 4: The β-glucuronidase over-expressing strain

RIB40, host strain; GUS, β-glucuronidase over-expressing strain

Fig. 5: The polygalacturonase over-expressing strain

W.B., wheat bran adjusted 60% water-content; P.P., potato pulp adjusted 60% water-content with 1% urea and Ammonium phosphate RIB40, host strain; PGB3, polygalacturonase over-expressing strain

Summary: In the present study, we successfully generated a gene disruptant and over-expresser of two kinds of enzymes by using bleomycin resistance transformant selection system of A. oryzae.

satosuz@affrc.go.jp