High level protein expression slows down rate of growth of bacterial broth cultures

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Introduction

Escherichia coli is one of the most widely used systems for large-scale protein production. This is due to its ability to grow rapidly at high density in simple broth cultures, availability of a large array of cloned plasmid vectors and well-characterized genetics. Several factors affect the level of expression of foreign genes in bacteria. One of such factors is toxicity of the gene being expressed or its protein product or both. This toxicity is generally attributed to the nature of the gene product and is assessed in terms of its effect on growth rate of the bacterial culture. Using a matrix of plasmid constructs, we investigated the effect of protein expression on the growth of broth cultures of E. coli. Cell growth was monitored by measuring optical density (OD) of broth culture at 600 nm every 30 minutes before and after induction of protein production by addition of IPTG. Interestingly, all bacterial cultures reached the stationary phase between OD_{max} of 1.8 and 2.0 regardless of the type of plasmid construct and the type of antibiotic selection. Similarly, optical density was monitored after induction to determine effect of protein expression on cell growth. Our data show that bacterial cultures harboring plasmid constructs that express high level protein production have slower growth rate than cultures producing low amount of protein. The effect was reversed when expression was carried out at lower temperatures. We propose that sustained high-rate protein production imposes increased metabolic stress on cells but can be reversed by slowing down expression rate at low temperatures.

Description of plasmid constructs

<table>
<thead>
<tr>
<th>Name of Construct</th>
<th>Size of Protein (kDa)</th>
<th>Promoter</th>
<th>Expression Level</th>
<th>Antibiotic Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBAD24</td>
<td>42</td>
<td>T7</td>
<td>High</td>
<td>Kanamycin</td>
</tr>
<tr>
<td>pEX202</td>
<td>42</td>
<td>Tc</td>
<td>Moderate</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>pBADDECEhsp70-1</td>
<td>78</td>
<td>Tc</td>
<td>Moderate</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>pEX202DECEhsp70-1NBD</td>
<td>80</td>
<td>Tc</td>
<td>Moderate</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>pBADDECEhsp70-1NBD</td>
<td>80</td>
<td>T7</td>
<td>High</td>
<td>Kanamycin</td>
</tr>
</tbody>
</table>

SDS-PAGE analysis of protein expression levels

Fig. 1: 1 mL aliquots of each cell culture were taken 3 hours post induction at 37 °C were centrifuged and the cell density was normalized by resuspension of the pellets in buffer. 16 μL of the whole cell lysate was analyzed on a 10% gel.

Antibiotic conc. vs bacterial culture growth rates

Growth of pre-induced bacterial broth cultures

Fig. 2: Normalized bacterial broth cultures monitoring the plasmids, pBADDECEhsp70-1 and pBADDECEhsp70-1NBD, were grown in various concentrations of their respective antibiotic at 37 °C. Cell growth was monitored by measuring OD_{max} at 600 nm. It was observed that the optical density reached a maximum of 2.0 regardless of the type of plasmid construct and the type of antibiotic selection. Experiments were performed in duplicates and data were averaged.

Conclusions

- Choice of plasmid vector does not affect the growth of cultures containing transformed bacterial cells.
- Concentration and choice of antibiotic was found to not have any significant effect on rate of growth of transformed bacterial cultures.
- Induced bacterial cultures expressing proteins at high levels have slow growth rate compared to low protein producing cultures.
- High level protein expression has no significant effect on bacterial culture growth rate at low temperature.
- Growing bacterial cultures at low temperature may reduce portion toxicity during recombinant protein expression.

References


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