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 cloning plasmid vectors and well-characterized genotypes. 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 ODmax of 1.8 and 2.0 regardless of the type of plasmid constructs and the type of antibiotic selection. Similarly, optical density was monitored after induction to determine the effect of protein expression on cell growth. Our data show that bacterial cultures harboring plasmid constructs that drive high level protein expression 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 (KD)</th>
<th>Promoter</th>
<th>Expression Level</th>
<th>Antibiotic Selection</th>
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<tbody>
<tr>
<td>pBR282</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>
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<td>Ampicillin</td>
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<tr>
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<td>Moderate</td>
<td>Ampicillin</td>
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</tbody>
</table>

**Growth of broth cultures of IPTG-induced bacterial cells at different temperatures**

**Fig. 4A:** Growth curve of induced cells at 37°C

**Fig. 4B:** Growth curve of induced cells at 25°C

**Fig. 4C:** Growth curve of induced cultures at 18°C

**Fig. 4:** Overnight bacterial cell cultures were used to inoculate fresh broth containing appropriate antibiotics. Cultures were grown at 37°C until they reached ODmax, of approximately 0.5. Densities were normalized before cultures were induced with IPTG and incubated at desired temperatures. Monitoring of cell growth was continued by measuring ODmax every 30 minutes (every 1 hour at 18°C for the entire duration of incubation. Expression at 37°C cDNA showed significant difference in growth rate starting 2 hours after induction, Fig 4A. Cells harboring highly expressing plasmid constructs showed lower growth rates and vice versa. At 25°C, the rates of growth of the expression cultures did not show any significant difference. Fig 4B, while at 18°C, all the cultures grew at the same rates, Fig 4C. Experiments were performed in triplicates 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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