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

Saad Chaudhry, Kenechukwu Ekwemalor , Shobhit Keswani & Odutayo Odunuga

Dept. of Chemistry & Biochemistry, Stephen F. Austin State University, Nacogdoches, TX

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 genotype. 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_{600nm} of 1.6 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 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

Name of Construct	Size of Protein (kD)	Promoter	Expression Level	Antibiotic Selectivity
p202	42	T7	High	Kanamycin
pEX202	42	Trc	Moderate	Ampicillin
pPROEXCeHsp70-1	76	Trc	Moderate	Ampicillin
pEX202CeHsp70-1NBD	80	Trc	Moderate	Ampicillin
p202CeHsp70-1NBD	80	T7	High	Kanamycin
pEX202 CeHsp70-1 no tag	108	Trc	Low	Ampicillin

SDS-PAGE analysis of protein expression levels

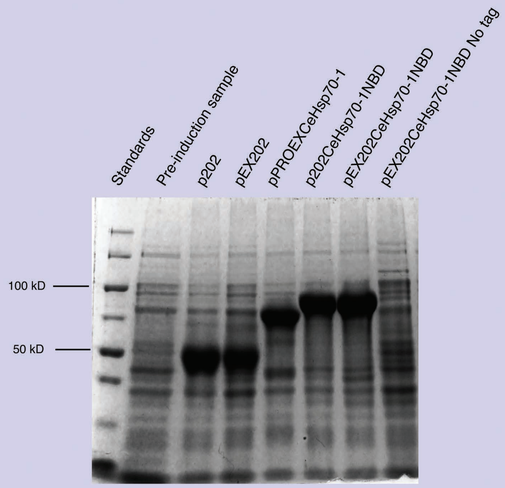

Fig. 1: 1 mL aliquot of each cell culture taken 3 hours post induction at 37 ºC was centrifuged and the cell density was normalized by resuspension of the pellets in buffer.16 μL of the whole cell lysate was analyzed on a 10% gel.

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

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

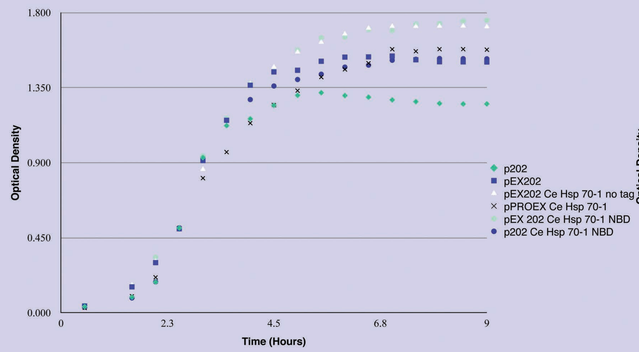

Fig. 4B: Growth curve of induced cells at 25 ˚C

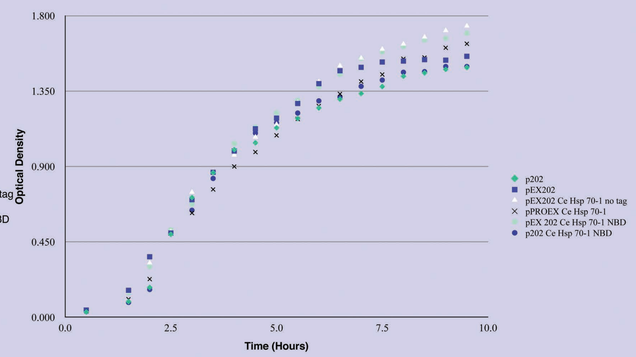

Fig. 4C: Growth curve of induced cells at 18 °C

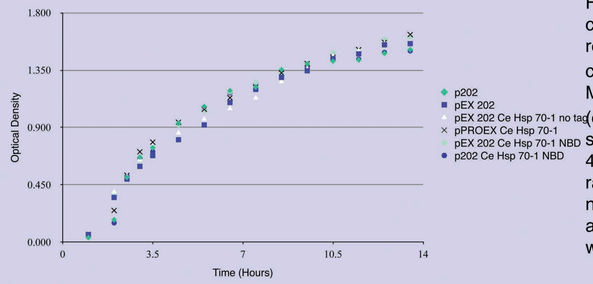

Fig. 4: Overnight bacterial cell cultures were used to inoculate fresh broth containing appropriate antibiotics. Cultures were grown at 37 ºC until they reached OD_{600nm} of approximately 0.5. Densities were normalized before cultures were induced with IPTG and incubated at desired temperatures. Monitoring of cell growth was continued by measuring OD_{600nm} every 30 minutes (every 1 hour at 18 ºC) for the entire duration of incubation. Expression at 37 ºC 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 ºC, the rates of growth of the expression cultures did not show any significant difference, Fig 4B, while at 18 ºC, all the cultures grew at the same rates, Fig 4C. Experiments were performed in triplicates and data were averaged.

Antibiotic conc. vs bacterial culture growth rates

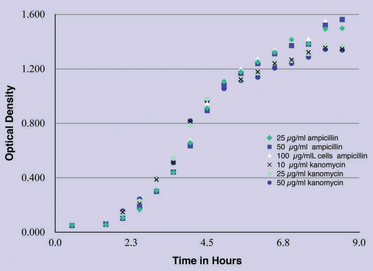

Fig. 2: Normalized bacterial broth cultures harboring the plasmids, pEX202CeHsp70-1NBD and p202CeHsp70-1NBD, were grown in various concentrations of their respective antibiotics at 37 ºC. Cell growth was monitored by measuring OD_{600nm} every 30 min, before and after induction with IPTG. Generally, no significant difference in growth rate was observed. The difference observed in growth rate after 8 hours may be due to depletion of nutrient in the cultures. The experiments were performed in duplicates and data were averaged.

Growth of pre-induced bacterial broth cultures

Fig. 3A: Genes from different organisms cloned into p202 vector

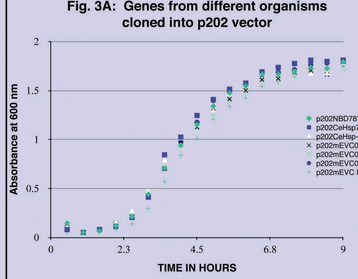

Fig. 3B: Genes from different organisms cloned into pEX202 vector

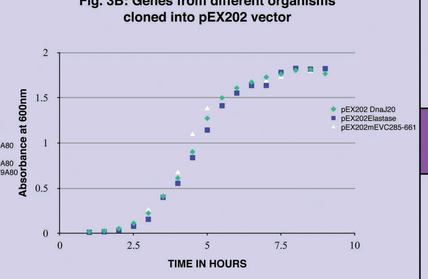

Fig. 3: Overnight cultures were all normalized to an OD of 0.600 and fresh LB broth media, containing the appropriate antibiotic was then inoculated with these overnight cultures. All cultures were incubated at 37° C and cell growth was monitored by measuring optical density (OD) at 600 nm at 30 min intervals. All bacterial cultures reached the stationary phase between OD of 1.6 and 2.0 regardless of the type of plasmid construct used to transform the bacterial cells. It appears that cultures of bacterial cells transformed with pEX202 constructs take longer to reach log phase (arbitrary OD of 0.4) when compared to cells transformed with p202 constructs. 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

- Donovan, RS, Robinson, CW & Glick, BR., Optimizing inducer and culture conditions for expression of foreign proteins under the control of the *lac* promoter, J. Ind. Microbiol. 16 (1996) 145-154.
- Baneyx, F., Recombinant protein expression in *Escherichia coli,* Curr. Opin. Biotechnol. 10: (1999) 411-421.
- Donovan, RS, Robinson, CW & Glick, BR., Optimizing the expression of a monoclonal antibody fragment under the transcriptional control of *Escherichia coli lac* promoter, Can. J. Microbiol. 46 (2000) 532-541.

Acknowledgements

This work was funded by SFA University Research miniGrants and the Robert A. Welch Foundation Research Grant (# AN-0008) awarded to the Dept. of Chemistry and Biochemistry of SFA University.