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In vitro comparative analysis of leaky protein expression in the BL21 strain vs. DH5α strain of *E. coli*.

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Protein expression using the bacterial expression system is routinely used not only in the research laboratories on a small scale but also in the industrial bulk productions. Usually no lethality is posed for the bacterial cells if the protein of interest (POI) is not toxic. However, certain proteins could pose lethality to the bacterial cells due to various reasons. Hence leaky expression of proteins is highly undesirable especially if the POI is toxic to the bacterial cells. In this study, we used a plasmid containing SARS CoV-2 spike protein-receptor binding domain (RBD) to test whether its expression is leaky in 2 different strains of *E. coli* viz. DH5α and BL21. Further, we evaluated any possible lethality posed by the expression of RBD in both strains. Our results suggest that RBD expression was leaky and independent of the T7 promoter that was designed in frame with RBD. Expression of RBD was not lethal to both strains of *E. coli*.

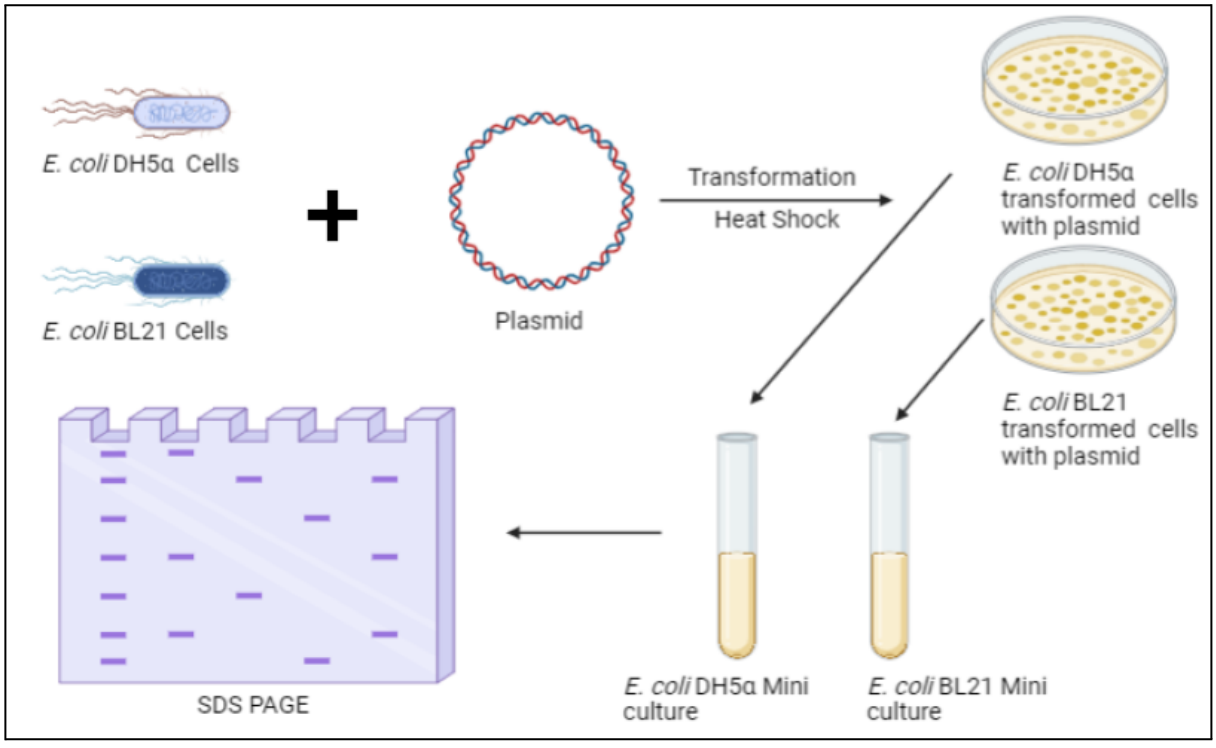

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Figure 1. Colonies of transformants, DH5α (left) and BL21 (right).

Recombinant expression and purification of proteins is in general feasible with some difficulties but the same is not true for the membrane proteins [1]. Various genetic strategies have been explored for proper expression and purification of difficult to express or insoluble proteins [2-4]. The BL21 strain of *E. coli* is prominently used because it has been genetically engineered such that it lacks proteases [5-7]. The strain "B" that lacks the lon protease "L" is the BL21 strain. Lack of proteases implies low degradation of the expressed protein of interest in the BL21 strain. On the other hand, maintenance of expression plasmids is equally important with proper strains of *E. coli* such as the DH5α that have the recA mutation and/or deletion in the genome [8, 9]. In order to ease the protein expression especially for purposes of high throughput nature, various optimizations such as codon optimization, designing disulfide bonds, co-expression of chaperones, high density bacterial cultures, etc. have been successfully tested [10-15]. Irrespective of the toxicity of the expressed protein, overexpression in combination with solubility issues may lead to the formation of inclusion bodies from which the protein of interest has to be recovered [16-19]. Usually proteins without folding problems can easily be recovered from the inclusion bodies through the denaturation process using chaotropic agents such as urea. In this study, we evaluated the leaky expression of a gene cloned into a protein expression vector using the *E. coli* DH5α and BL21 strains. Both strains were cultured with the plasmid and the whole cell extracts were used to run the SDS-PAGE for the detection of protein expression.

Materials & Methods:

Transformation of E. coli cell with plasmid: Both strains of *E. coli*, DH5α and BL21 were transformed with the plasmid carrying the gene of interest. This plasmid has ampicillin selection with β-lactamase gene along with mCherry tag followed by the gene of interest. Heat-shock method of transformation was used. Fresh vials of both strains each containing 100 µl of competent cells were taken to which 2 µl of plasmid per vial was added and incubated on ice for 30 min. The vials were then kept at 42 ℃ for 30 sec and were immediately placed on ice. One ml of presterilized SOC medium was added to each vial and both vials were incubated at 37 ℃ for 1 hr. Freshly prepared LB agar plates using autoclaved LB agar medium were used for plating the transformants. Post incubation, the transformed cells were plated on LB agar plates containing 30 mcg ampicillin. Plates were incubated at 37 ℃ overnight.

Overnight bacterial minicultures: Freshly prepared LB broth was taken in two culture tubes 5 ml each. One tube was inoculated with the DH5α colonies and the other was inoculated with the BL21 colonies. Both tubes were incubated at 37 ℃ overnight. Fresh LB broth (5 ml per tube) was added to the overnight cultures and the cultures were further incubated for 6 hours before harvesting them.

Cell lysis and sample preparation: Cells were harvested by centrifugation at 5000 rpm for 20 min. Supernatants were discarded safely into a beaker containing bleach or 70% alcohol. The pellets were then resuspended in the residual supernatant. Equal volumes of lysis solution were added to both tubes and the contents were boiled at 95 ℃ for 10 min. After cooling down the samples, they were centrifuged at 14,000 rpm for 15 min. Supernatants were carefully transferred to new tubes to load and run the SDS-PAGE.

SDS-PAGE: The 12% separating gel was first prepared in a centrifuge tube containing 6 ml of 30% acrylamide-bisacrylamide + 6 ml of 2.5X Tris-SDS buffer (pH 8.8) + 10% ammonium persulfate + TEMED + deionized water. The separating gel was poured in between the plates and isopropanol on the top to prevent uneven edges on the top. Once the separating gel is solidified, the 5% stacking gel is prepared in a centrifuge tube containing 1.3 ml of 30% acrylamide-bisacrylamide + 1.6 ml of 5X Tris-SDS buffer (pH 6.8) + 10% ammonium persulfate + TEMED + deionized water. The isopropanol was removed and the stacking gel was poured and the comb was placed on the top. Samples were loaded (15 µl per well) and the gel was run at 120 V. Gel was stained/destained to visualize protein bands.

Results and Discussion:

Multiple colonies were obtained for both DH5α and BL21 transformants: The LB agar plates containing colonies of transformants are shown in Figure 1. Both DH5α and BL21 transformants yielded multiple colonies on the ampicillin plate confirming the presence of the plasmid (transformants). The number of colonies in the case of BL21 plate are visibly countable while the other plate showed a lawn of bacterial colonies suggesting that the transformation was very efficient in both cases.

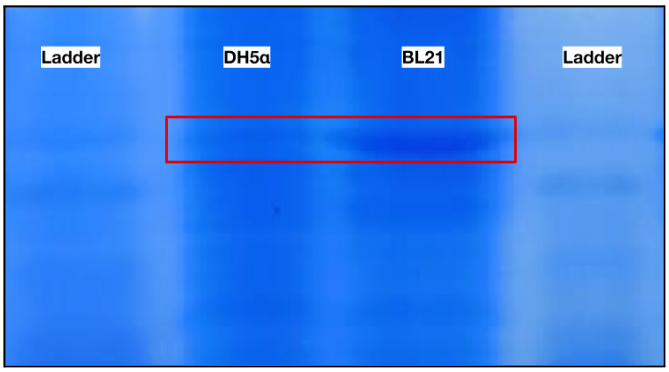

Figure 2. SDS-PAGE analysis of leaky expression.

Leaky expression was seen in both strains on SDS-PAGE: The whole cell extracts of the bacterial cells were analyzed by using SDS-PAGE as shown in Figure 2. Both strains showed the protein expression that is highlighted by the red box in Figure 2. The BL21 strain showed a significantly visible thick band while the other strain gave a faint band that aligned to the same molecular weight. This suggests that both strains were capable of expressing the protein of interest (POI) without inducing the promoter with IPTG. The POI tested here is a non-toxic protein hence, it did not pose any lethality to the bacterial cells. However, if the POI were toxic, then the final yield of POI would have been significantly low due to several reasons such as inefficient growth of the cultures, degradation of the excess POI that was produced, etc. Both bands were quantified using ImageJ software [20]. We found that the pixel-based intensity of the BL21 band is more than 10-fold higher when compared to the DH5α band in the SDS-PAGE. The current study proves that certain plasmids, even though have a promoter that can be externally controlled, there might still be some leaky expression of the POI. However, if the POI is toxic to the cells then the overall yield of the POI will be low thus affecting the final yield of the recombinant protein.

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