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Designing Next Generation Platforms for Recombinant Protein Production by Genome Engineering of Escherichia coli

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Abstract : We propose a paradigm shift in our approach to design improved platforms for recombinant protein production, by addressing system level issues rather than the individual steps associated with recombinant protein synthesis like transcription, translation, etc. We demonstrate that by controlling and modulating the cellular stress response (CSR), which is responsible for feedback control of protein synthesis, we can generate hyper-producing strains. We did transcriptomic profiling of post-induction cultures, expressing different types of protein, to analyze the nature of this cellular stress response. We found significant down-regulation of substrate utilization, translation, and energy metabolism genes due to generation CSR inside the host cell. However, transcription profiling has also shown that many genes are up-regulated post induction and their role in modulating the CSR is unclear. We hypothesized that these up-regulated genes trigger signaling pathways, generating the CSR and concomitantly reduce the recombinant protein yield. To test this hypothesis, we knocked out the up-regulated genes, which did not have any downstream regulatees, and analyzed their impact on cellular health and recombinant protein expression. Two model proteins i.e., GFP and L-Asparaginase were chosen for this analysis. We observed a significant improvement in expression levels, with some knock-outs showing more than 7-fold higher expression compared to control. The 10 best single knock-outs were chosen to make 45 combinations of all possible double knock-outs. A further increase in expression was observed in some of these double knock- outs with GFP levels being highest in a double knock-out ΔyhbC + ΔelaA. However, for L-Asparaginase which is a secretory protein, the best results were obtained using a combination of ΔelaA+ΔcysW knock-outs. We then tested all the knock outs for their ability to enhance the expression of a 'difficult-to-express' protein. The Rubella virus E1 protein was chosen and tagged with sfGFP at the C-terminal using a linker peptide for easy online monitoring of expression of this fusion protein. Interestingly, the highest increase in Rubella-sGFP levels was obtained in the same double knock-out ΔelaA + ΔcysW (5.6 fold increase in expression yield compared to the control) which gave the highest expression for L-Asparaginase. However, for sfGFP alone, the ΔyhbC+ΔmarR knock-out gave the highest level of expression. These results indicate that there is a fair degree of commonality in the nature of the CSR generated by the induction of different proteins. Transcriptomic profiling of the double knock out showed that many genes associated with the translational machinery and energy biosynthesis did not get down-regulated post induction, unlike the control where these genes were significantly down-regulated. This confirmed our hypothesis of these genes playing an important role in the generation of the CSR and allowed us to design a strategy for making better expression hosts by simply knocking out key genes. This strategy is radically superior to the previous approach of individually up-regulating critical genes since it blocks the mounting of the CSR thus preventing the down-regulation of a very large number of genes responsible for sustaining the flux through the recombinant protein production pathway.

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