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Integrating Cell-Free Protein Synthesis to develop a Low-Cost Vaccine Manufacturing Platform

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Introduction

Cold-chain logistical solutions are used to transport vaccines to remote locations and comprise roughly 50% of the cost of a vaccine dose [1]. A manufacturing platform can be used to produce vaccines at their point-of-use and save costs for healthcare systems in the developing world.

This project proposes a platform that is based on cell-free protein synthesis, whereby the protein synthesis machinery of E. coli bacteria is extracted (lysate) and manipulated to optimize the production of desired proteins [2].

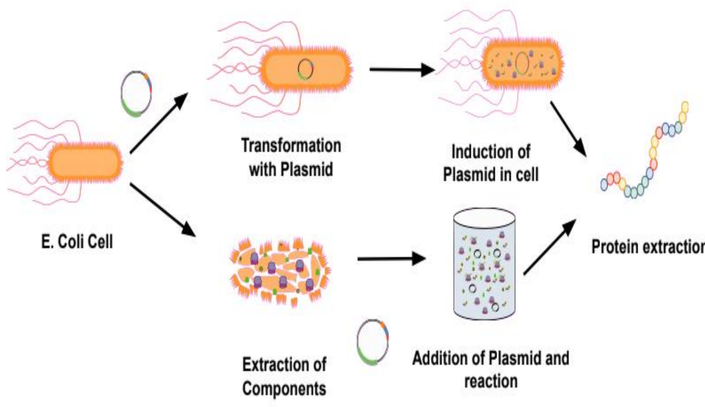

Figure 1: The figure depicts the process of in-vivo (top) and in-vitro (bottom) protein production from E. Coli bacteria. The extracted solution of components from the cell is called **lysate**. This figure was created by Athanasios Kritharis.

Methodology

- Cell-free reaction mixture is combined with lysate and DNA template (plasmids)
- Protein synthesis over 24 hours at 30 °C
- Mixture is diluted, centrifuged and purified in an Ni-NTA column
- Proteins are dialyzed overnight, for renaturation
- SDS-PAGE and Bradford Assay to confirm desired protein was produced and measure its yield

Results

Reaction Optimization

Figure 2 shows an example of reaction optimization, whereby the effect of one element of the reaction mixture was observed over time.

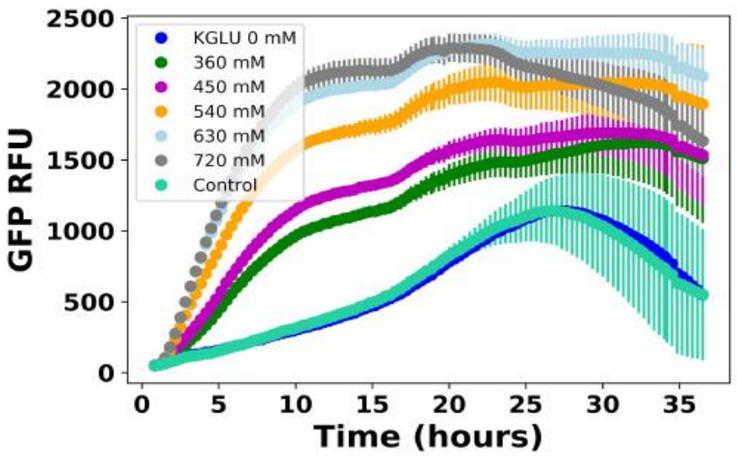

Figure 2: This graph shows the effect of Potassium Glutamate (KGLU) concentration on the production of biomarker protein, GFP, over time. It shows that GFP production peaks at 540 mmol of KGLU. GFP is used as a cheap test protein, to first simulate production of the desired Fimbraie (FIM) protein.

Fimbraie Expression

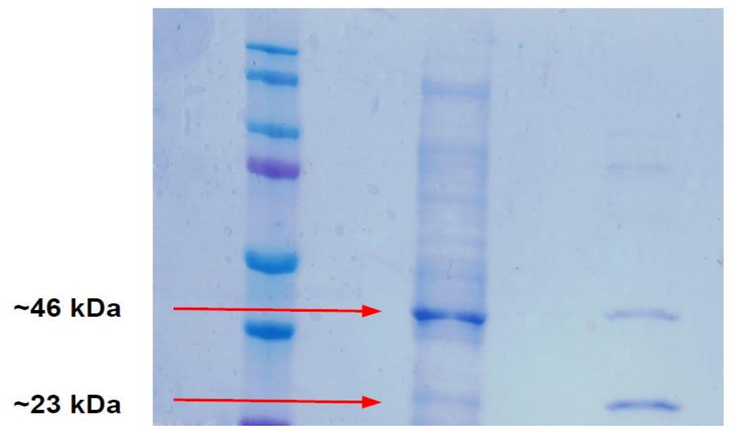

Figure 3: This figure shows the in-vivo expression of desired proteins, FIM-2 (left) and FIM-3 (right). These proteins were produced in-vivo to first determine whether a hypothesized recombinant plasmid could produce the desired protein. The figure is the result of the SDS-PAGE Protein Isolation technique.

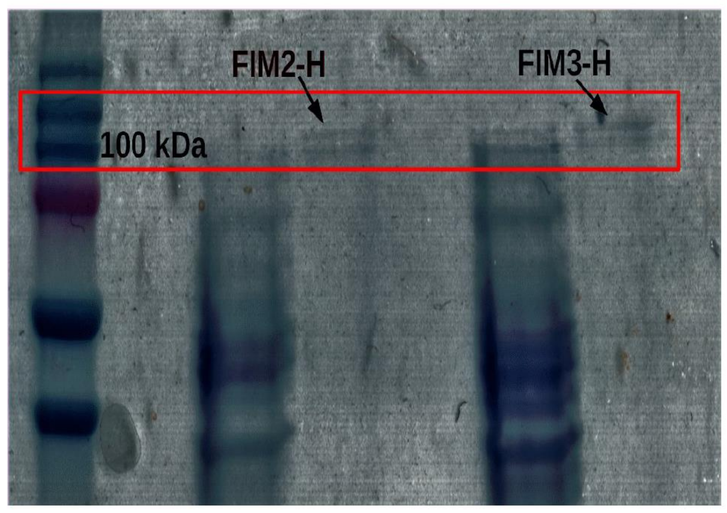

Figure 4: This figure shows the in-vitro expression (cell-free protein synthesis) of desired proteins ,FIM-2-H and FIM-3-H . SDS-PAGE isolation is run for the crude reaction mixture (left side) and the Ni-NTA purified reaction mixture (right side) for each protein. The first vertical plot on the outmost left side of the Figure represents the protein ladder that was used to identify the proteins' size.

Conclusion

- An optimized low-cost formulation for in-vitro protein synthesis has been developed.
- The proteins of interest, FIM-2 and FIM-3. have been expressed, using cell-free protein synthesis.

Implications

The first objective of the project, cell-free protein synthesis of FIM proteins, has been achieved. Subsequent experimentation is underway to develop a disposable bioreactor for decentralized vaccine production.

This would be actualized upon completion of the following objectives:

- Optimizing productivity of antigen synthesis
- Demonstrating cell-free protein synthesis in a specially designed bag that will be directly integrated into the final design
- Developing methodology for chromatographic purification of the antigen, including a pumping system
- Demonstrating fluid flow between interconnected chambers of the bioreactor using a simple roll-up mechanism

Reference / Bibliography

[1] Lydon P, Zipursky S, Tevi-Benissan C, Djingarey MH, Gbedonou P, Youssouf BO, et al. Economic benefits of keeping vaccines at ambient temperature during mass vaccination: the case of meningitis A vaccine in Chad [Internet]. Bulletin of the World Health Organization. World Health Organization; 2014 [cited 2020Feb16]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3949534/

[2] Lee K-H, Kim D-M. Recent advances in development of cell-free protein synthesis systems for fast and efficient production of recombinant proteins [Internet]. FEMS microbiology letters. U.S. National Library of Medicine; 2018 [cited 2020Feb16]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30084930

Acknowledgement

We would like to thank Athanasios Kritharis, for his great mentorship as well as Dr. Vikramaditya G. Yadav for his expert advice and guidance throughout this interesting project. We would also like to extend our thanks to Dr Melih Tamer and his team at Sanofi Pasteur.

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