INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Assessing The Relationship Between Functionality And Proteome Of Cell-free Protein Systems From Yersinia Pestis

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Cellular lysates capable of transcription and translation have become valuable tools for prototyping and evaluating genetic circuits, screening engineered functional parts, and producing biological components. Despite the increasing use of lysates, analysis of the composition of proteins in these lysates as well as their contribution to overall productivity has yet to be fully explored. The ability to characterize a lysate as productive vs non-productive before a major investment in time and resources would be a useful capability for future biotechnological pursuits. Here we report the overall productivity, as determined by expression of sfGFP (superfolder Green Fluorescent Protein), of several lysates derived from attenuated strains of Yersinia Pestis grown at 21°C, 26°C, and 37°C. Lysates from all temperatures were generally capable of in vitro transcription and translation but certain strains and temperatures were shown to produce higher amounts of desired protein. To explore this relationship, we conducted a full proteomic analysis of several strains of Yersinia pestis grown at varying temperatures to identify correlative relationships of the amount of protein produced with the presence of specific proteins within the lysates. We also assessed the functionality of expressed protein to determine if certain lysates produced more functional proteins than others. Finally, we compared our Y. pestis lysates to standard systems made from E. coli from both commercial sources and those made in-house following accepted protocols to determine if Y. pestis lysates offer any advantage over traditional systems. Together these data demonstrate that the Y. pestis lysate productivity is dependent on strain as well as growth temperature which effect the proteomic profile of the organism. The ability to generate these functional cell-free lysates from organisms other than E. coli provides an additional tool to deliver solutions to the warfighter via several avenues including the discovery of enhanced functionalities for biotechnological applications or by mitigating the risks associated with pathogen and emerging threat assessments.

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