INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Leveraging Cryptobiosis Mechanisms To Develop Ruggedized Cells For Biological Sensing In Austere Environments

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The ability to detect diverse biological and chemical threats using engineered E. coli cells has been broadly demonstrated where E. coli represent a seemingly limitless platform for cell-based threat detection or use as a biological foundry for on-demand generation of synthetic biological sensor / reporter complexes. Despite their immense utility, E.coli cells are very fragile relative to electronic systems where the ability to ruggedize E. coli cells for improved performance as environmental sentinels and/or use in austere environments would greatly expand their utility for chemical and biological threat detection in reconnaissance applications. In the present study, we seek to utilize the innate biological mechanisms underlying the reversible arrest of life, termed “cryptobiosis”, to achieve cellular dormancy and subsequent high-fidelity reactivation to ruggedize E. coli for use in synthetic biology applications in challenging environments. Specifically, we are investigating anhydrobiosis, the most dramatic example of cryptobiosis, where cells can be reanimated post-desiccation. A common feature of anhydrobiotic organisms is the increased production of the nonreducing disaccharide, trehalose, during desiccation stress. Increased trehalose concentrations in cytoplasm stabilizing proteins and lipid membranes by replacing the primary water of hydration through the formation of amorphous glasses (vitrification). In prokaryotes, trehalose expression is facilitated by an operon containing trehalose-6-phosphate synthase (OtsA) and trehalose-6-phosphate phosphatase (OtsB). In the present study, we have constructed a plasmid to over express the OtsBA operon to investigate desiccation tolerance in wild-type (WT) and trehalose null-mutant E. coli. The trehalose operon was inserted into a pDuet plasmid chassis downstream of an inducible promoter to enable on-demand trehalose over-expression. The correct transgene insertion was confirmed by restriction enzyme digestion followed by PCR diagnostics and DNA sequencing. Host E. coli with individual gene knockouts (KOs) for OtsA and OtsB were obtained, and RT-qPCR confirmed the absence of transcriptional expression for each respective KO, whereas background expression was detectable in the WT. Transformation of the OtsBA over-expression plasmid into OtsA KO E. coli resulted in a 5 order of magnitude increase in transcriptional expression of OtsA and a 10x increase in OtsB expression relative to conspecifics with no plasmid insertion. Further, the OtsBA plasmid was inducible under desiccation stress with 5x increased expression relative to cells that were not dried down. Finally, desiccation tolerance was significantly increased in OtsA KO E. coli transformed with the OtsBA over-expression plasmid with viability differences increasing through 7, 10, and 14 days post-drying to a maximum of one order of magnitude increase by day 14. Presently, we have transformed the OtsBA over-expression plasmid into WT E. coli and are comparing OtsA and OtsB expression and extended desiccation tolerance (28 days) relative to OtsA KO and WT conspecifics with no plasmid insertion. The proof-of-concept demonstrated herein indicates that E. coli can be ruggedized by OtsBA over-expression where the viability of the cells in a desiccated state can be extended, thus providing future opportunities for development chem-biological detectors as stable environmental sentinels and/or assays for reconnaissance testing in austere environments.

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