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■ RESEARCH ARTICLE

Engineering Thermal Stress Resistance in Crops Using Protein Solutions and Synthetic Biology

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ABSTRACT: Climate change poses a major threat to global food security by increasing the frequency and severity of extreme temperatures, which can significantly damage crop productivity. To address this challenge, this study explores a synthetic biology-based approach to engineer thermal stress resistance in crops using protective proteins. Specifically, we investigate the roles of heat shock protein HSP70 and trehalose biosynthesis enzymes OtsA and OtsB in enhancing cellular stability under heat and cold stress. Using *E. coli* as a model system, we constructed two plasmids: pHot, a heat-inducible system for HSP70 expression, and pCold, a cold-inducible system for OtsA/OtsB expression. These plasmids were introduced into *E. coli* BL21 STAR (DE3), and protein expression was tested under thermal stress (42°C and 20°C). Western blotting and fluorescence microscopy confirmed successful expression of the target proteins, although heat-inducible dsRED expression was not detected—likely due to inefficient T2A cleavage. Cold-inducible constructs showed strong EGFP fluorescence, supporting the role of OtsA and OtsB in cold tolerance. This work provides a foundation for developing temperature-responsive genetic tools in agricultural biotechnology and informs future strategies for building climate-resilient crops.

KEYWORDS: Biomedical Engineering, Synthetic Biology, Heat, Thermal.

■ Introduction

Global agriculture faces increasing threats due to climate change, including rising temperatures, unpredictable weather patterns, and resource scarcity. These challenges have intensified food insecurity and underscored the urgent need for adaptive agricultural strategies. Econometric studies of Taiwanese agriculture, for example, highlight significant yield sensitivities to weather variability, reinforcing the importance of resilience-focused solutions.[1] Similarly, global studies, such as those modeling rice production under fluctuating temperatures and precipitation, illustrate the economic and environmental consequences of uncontrolled stressors.[2,3]

Synthetic Biology as a Solution:

Synthetic biology offers transformative solutions by engineering protein-based systems to mitigate abiotic stress. Heat shock proteins (HSPs) and trehalose biosynthesis enzymes are critical tools for stabilizing cellular structures under extreme environmental conditions. HSP70, a molecular chaperone, prevents protein aggregation during heat stress and supports protein refolding.[4] Prior research demonstrated that HSP70 plays a role in thermal tolerance across plant species, such as in cotton[5] and carrots.[6] Similarly, trehalose biosynthesis, mediated by OtsA and OtsB, mitigates cold stress by stabilizing membranes and preserving cellular integrity. Research on cold-inducible CBF genes and their regulation of osmolyte production further underscores the trehalose pathway's importance in freezing tolerance.[7]

To enhance scalability and precision in protein expression systems, this study employs an advanced plasmid-based strategy. Heat-inducible (pHot) and cold-inducible (pCold) plasmids were designed to drive targeted protein expression under thermal stress conditions in *E. coli*. These constructs enable precise regulation of HSP70, OtsA, and OtsB synthesis, ensuring controlled and efficient protein production under relevant environmental conditions.

Recent advancements, such as split intein-mediated plasmid selection systems, have significantly improved the efficiency of synthetic biology applications in microbial expression systems.[8] Studies indicate that trehalose biosynthesis pathways not only enhance cold tolerance but also influence metabolic processes that improve plant biomass accumulation.[9] The evolutionary conservation of HSP functions in cellular stress protection is further supported by studies on Theileria annulata[10] and heat shock protein mechanisms in plants.[11] By leveraging these technologies, this study validates protein expression and stability under thermal stress conditions, providing a foundation for future applications in mitigating climate-induced stress in agricultural settings.

Research Gap and Novel Contributions:

While previous studies have investigated the roles of HSP70 and trehalose biosynthesis in stress tolerance, most have focused on their natural expression in plants rather than leveraging synthetic biology-based solutions for controlled protein expression. Additionally, existing research lacks precise regulatory mechanisms for temperature-induced expression, which limits its scalability for agricultural applications. Without efficient, inducible expression systems, the potential for real-world deployment remains constrained.

This study addresses these gaps by engineering a dual-plasmid system (pHot and pCold), incorporating heat- and

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regulation of HSP70, OtsA, and OtsB expression. Unlike prior research that primarily examines trehalose biosynthesis in natural stress responses, this work harnesses synthetic biology to create a tunable, stress-responsive system. This approach builds on previous findings demonstrating trehalose's role in microbial and plant metabolic regulation, offering a more controlled and scalable platform for application in crop resilience.

Furthermore, this study bridges the gap between fundamental protein research and practical agricultural applications by using *E. coli* to validate stress-response protein expression and stability before eventual plant integration. Small heat shock proteins in *E. coli* have been shown to enhance stress resistance, reinforcing the value of microbial models for pre-screening candidate genes and regulatory systems for crop engineering. By optimizing construct design for future plant applications, this study paves the way for genome editing approaches such as Agrobacterium-mediated transformation or CRISPR/Cas9 to introduce stress resilience traits directly into crops. Research on trehalose biosynthesis genes in crops such as tomatoes and the role of HSP70 in stress and disease responses suggests further potential for genetic engineering applications in agriculture.

Methods

To evaluate the functionality and stability of heat shock proteins (HSP70) and trehalose biosynthesis enzymes (OtsA and OtsB) in mitigating thermal stress, a series of controlled experiments were conducted using *E. coli* BL21 STAR (DE3) as the expression system. The study aimed to optimize plasmid constructs, validate protein expression, and assess protein stability under controlled thermal stress conditions. The engineered plasmids (pHot for heat-inducible expression and pCold for cold-inducible expression) were designed to regulate protein synthesis in response to environmental stressors. Fluorescence microscopy and Western blot analyses were employed to confirm protein expression, localization, and cleavage efficiency. These approaches provided both quantitative and qualitative insights into the effectiveness of the constructs in driving protein production under heat and cold stress conditions.

Genomic DNA was extracted from *Pomacea canaliculata* (HSP70 source) and *Stutzerimonas stutzeri* (OtsA and OtsB sources) using a method to isolate DNA from cells. This involved breaking open the cells and using chemical reagents to separate DNA from proteins and other materials. Polymerase chain reaction (PCR) amplification of HSP70, OtsA, and OtsB genes was then performed using specifically designed primers incorporating restriction sites to facilitate plasmid integration. The amplified gene fragments were purified and inserted into the pHot and pCold expression vectors through restriction enzyme digestion and ligation, ensuring precise regulation of target protein expression. The constructs were verified via colony PCR and Sanger sequencing to confirm correct insertion and reading frame integrity.

The recombinant plasmids were transformed into *E. coli* BL21 STAR (DE3) cells, and protein expression was induced using isopropyl β-D-1-thiogalactopyranoside (IPTG) under optimized conditions. Induction parameters were set at 37°C for pHot (heat-inducible HSP70 expression) and 20°C for pCold (cold-inducible OtsA and OtsB expression), with 0.5 mM IPTG used as the optimal inducer concentration. Post-induction, bacterial cultures were harvested via centrifugation, lysed using a sonicator and buffer system, and purified using affinity chromatography. Protein purification was facilitated using His, Flag, and Myc tags to enhance specificity and improve detection efficiency in downstream assays.

To assess the functionality and stability of the expressed proteins under thermal stress, *E. coli* cultures were subjected to controlled temperature conditions. Cultures expressing HSP70 (pHot) were incubated at 42°C for 4 hours to evaluate their ability to withstand heat stress, whereas cultures expressing OtsA and OtsB (pCold) were incubated at 20°C for 16 hours to simulate cold stress conditions. Fluorescence microscopy was employed to track protein expression and localization, while Western blot analysis was conducted to evaluate protein integrity, cleavage efficiency, and expression levels under stress. Comparative fluorescence intensity measurements between treated and control groups were performed to determine protein stability and potential thermal resilience.

Quantitative fluorescence data were collected using a fluorescence microscope, allowing for the assessment of protein expression patterns and localization within *E. coli* cells. Fluorescence intensity measurements were normalized against non-induced control samples to provide a comparative analysis. Western blot analysis was used to confirm molecular weight accuracy and assess relative protein abundance across different stress conditions. Densitometry software was used to quantify Western blot bands, and statistical analyses including t-tests and ANOVA were performed to determine significant differences in protein expression levels. All experiments were conducted in triplicate to ensure reproducibility and minimize variability.

To control for confounding variables, a series of negative and positive controls was implemented. Negative controls consisted of *E. coli* cultures transformed with empty plasmids or non-induced cultures without IPTG to establish baseline fluorescence and protein expression. Positive controls included cultures expressing previously validated stress-tolerant proteins to compare expression efficiency. Experimental conditions, such as temperature variations (20°C vs. 42°C), IPTG concentration (0.1–1.0 mM), and induction duration, were carefully standardized to isolate the effects of the target proteins and minimize external variability.

The following equipment was used to ensure precision and reliability in data collection: a thermal cycler for PCR amplification, a NanoDrop spectrophotometer for nucleic acid and protein quantification, a fluorescence microscope for protein localization and expression analysis, and an SDS-PAGE and Western blot apparatus for evaluating molecular weight accuracy and protein integrity. Additionally, a shaking incubator was used to maintain bacterial cultures under controlled temperature and induction conditions, while a centrifuge and sonicator facilitated cell lysis and protein extraction.

By integrating synthetic biology-based approaches with rigorous experimental design and quantitative analysis, this study provides a systematic evaluation of protein-based thermal

stress mitigation strategies. The use of *E. coli* as a model system allows for scalable, cost-effective validation of genetic constructs, paving the way for future applications in crop engineering and environmental stress resilience.

Results and Discussion

Plasmid Transformation and Validation:

The transformation of the pHot and pCold plasmids into *E. coli* BL21 STAR (DE3) cells were successfully achieved, enabling the expression of heat-inducible HSP70 and cold-inducible OtsA and OtsB, respectively. The plasmid map for pHot (Figure 1) illustrates the inclusion of the ibpA promoter, which enables heat-inducible expression of HSP70, along with a HisTag for protein purification. Similarly, the pCold plasmid (Figure 2) features the cspA promoter for cold-inducible expression of OtsA and OtsB, with Flag and Myc tags for downstream protein analysis and tracking.

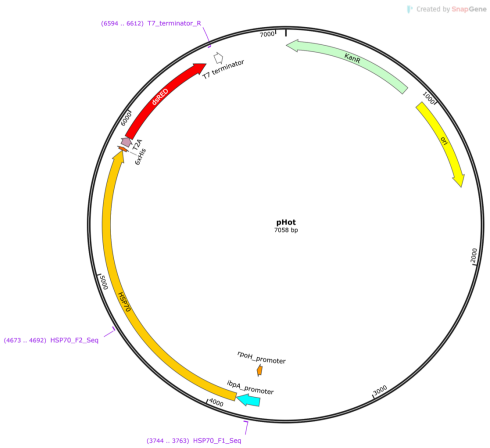

Figure 1: Plasmid map of pHot created using SnapGene Viewer. Illustrates the ibpA promoter-driven heat-inducible expression of HSP70, along with a HisTag for purification. The construct enables targeted expression under heat stress conditions.

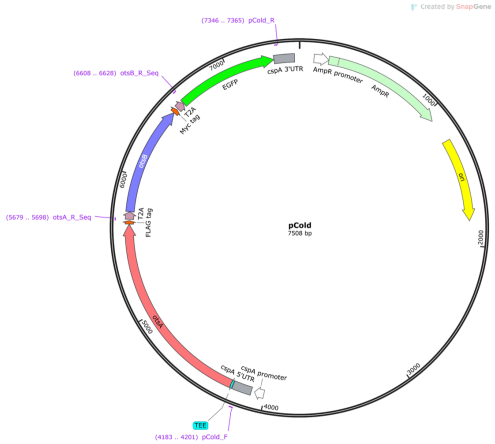

Figure 2: Plasmid map of pCold created using SnapGene Viewer. Shows the cspA promoter regulating cold-inducible expression of OtsA and OtsB, tagged with Flag and Myc, respectively. This construct facilitates protein expression under cold stress.

To confirm successful plasmid uptake, colony PCR was performed, followed by agarose gel electrophoresis. The expected fragment sizes of 2869 bp for pHot and 1516 bp for pCold were observed, confirming successful transformation. Additionally, the co-transformation of both plasmids was verified, as evidenced by the stability of bacterial colonies under selection pressure, indicating the retention of both constructs.

Colony growth patterns further validated transformation success. *E. coli* colonies harboring pHot formed distinct colonies on LB agar plates (Figure 3), demonstrating effective transformation. Similarly, transformation with pCold resulted in well-defined bacterial colonies, which were further validated using colony PCR (Figure 4). The stability of these constructs in transformed bacteria supports their potential for regulated protein expression under induced thermal stress conditions.

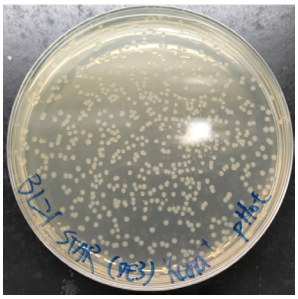

Figure 3: Colony growth on LB agar plates after transformation with pHot plasmid. Demonstrates successful transformation of *E. coli* with pHot, validated by colony PCR. The distinct colony formation indicates plasmid retention and expression capability.

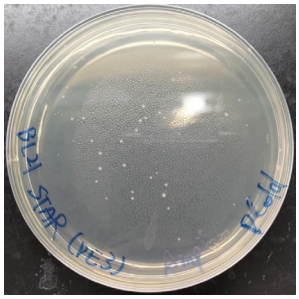

Figure 4: Colony growth on LB agar plates after transformation with pCold plasmid. Confirms successful pCold transformation in *E. coli*, verified through colony PCR. The stable colonies suggest the construct remains functional under selective conditions.

Protein Expression and Analysis:

Western blot analysis confirmed the successful expression of HSP70, OtsA, and OtsB, each tagged with His, Flag, and Myc, respectively, to facilitate detection and purification. The presence of distinct bands at the expected molecular weights validated the effectiveness of the plasmid constructs in driving protein synthesis under their respective heat- and cold-inducible conditions. Specifically, the pHot construct induced HSP70 expression at ~72 kDa (Figure 5), while pCold successfully expressed OtsA-FlagTag (~57 kDa, Figure 6) and OtsB-MycTag (~31 kDa, Figure 7) under cold-inducible conditions. These results demonstrate the specificity and efficiency of the designed plasmid systems.

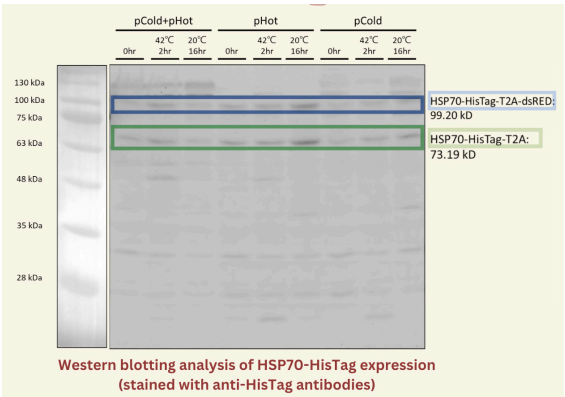

Figure 5: Western blot analysis of HSP70-HisTag protein expression in *E. coli* transformed with the pHot plasmid. HisTag was fused to the HSP70 protein to enable affinity purification and facilitate detection via anti-HisTag antibodies. Bands at ~72 kDa confirm successful expression under heat-

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inducible conditions. These results validate the pHot plasmid's ability to drive heat-inducible protein expression and demonstrate HisTag's reliability for protein identification and purification.

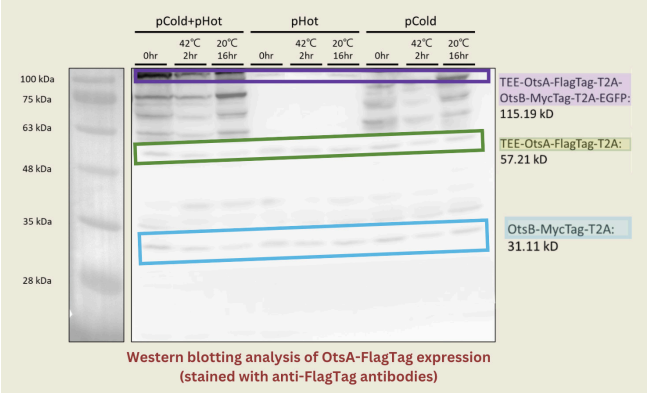

Figure 6: Western blot analysis of OtsA-FlagTag protein expression in *E. coli* transformed with the pCold plasmid. FlagTag was fused to the OtsA protein for detection using anti-FlagTag antibodies. A protein band observed at ~57 kDa confirms successful OtsA-FlagTag synthesis under cold-inducible conditions, validating the pCold plasmid's function. The precise banding pattern suggests efficient transcription and translation, with the FlagTag facilitating post-translational identification.

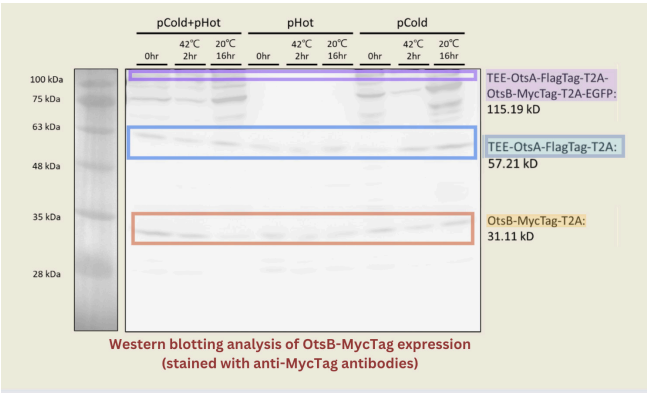

Figure 7: Western blot analysis of OtsB-MycTag protein expression in *E. coli* transformed with the pCold plasmid. The MycTag was fused to OtsB for characterization via anti-MycTag antibodies. The band at ~31 kDa confirms proper OtsB-MycTag expression under cold-inducible conditions. The observed molecular weight confirms the pCold plasmid's effectiveness in expressing OtsB, a critical enzyme in the trehalose biosynthesis pathway for cold stress adaptation.

To further confirm plasmid integrity, agarose gel electrophoresis was performed following PCR amplification of transformed *E. coli* colonies. The presence of the expected 2869 bp band for pHot (Figure 8) and 1516 bp band for pCold (Figure 9) validated the successful transformation and integration of the target genes. Minor background bands were observed in some samples, potentially indicating nonspecific amplification or residual genomic DNA contamination. However, the primary bands aligned with the expected fragment sizes, reinforcing the reliability of the cloning strategy.

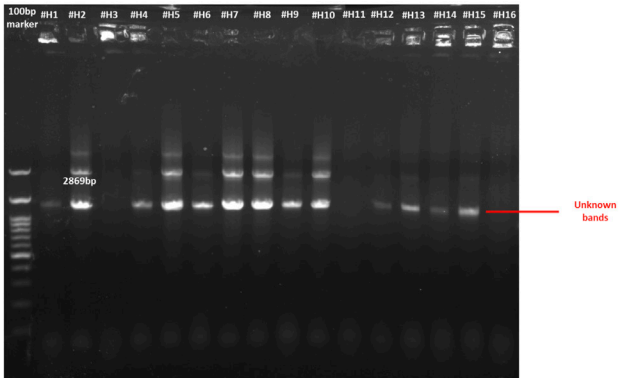

Figure 8: Agarose gel electrophoresis of PCR products for plasmid pHot validation. The expected band at 2869 bp confirms successful plasmid transformation. The presence of additional unexpected bands may be attributed to nonspecific amplification or experimental variability. Further optimization may be required to improve band specificity.

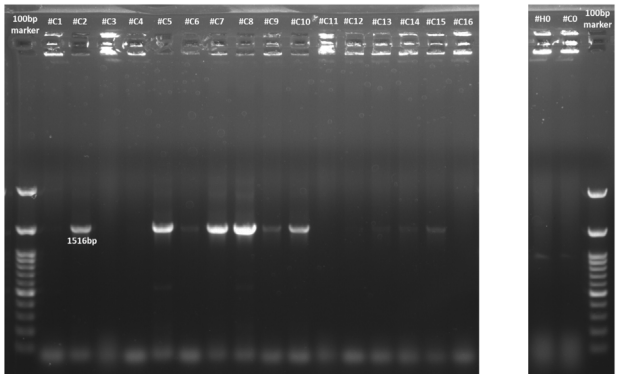

Figure 9: Agarose gel electrophoresis of PCR products for plasmid pCold validation. The presence of a distinct band at 1516 bp confirms successful plasmid transformation. Minor background bands may result from nonspecific amplification or residual genomic DNA, but the primary band aligns with the expected fragment size, supporting successful construct verification.

Fluorescence microscopy analysis provided additional insights into the protein expression profiles. EGFP fluorescence confirmed successful cold-inducible expression of OtsA and OtsB, indicating active translation and localization of trehalose biosynthesis enzymes under cold stress (Figure 10). However, dsRED fluorescence, which was intended to confirm the heat-induced expression of HSP70, was not detected. This absence suggests inefficiencies in T2A cleavage or structural challenges affecting dsRED folding and stability, necessitating further optimization.

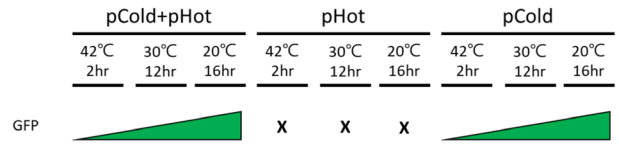

Figure 10: Fluorescence microscopy of EGFP. This shows a successful expression of EGFP under cold-inducible conditions but the absence of detectable dsRED fluorescence. There is an observed lack of prominent dsRED signal, which suggests inefficiencies in T2A cleavage or dsRED folding, requiring further construct optimization.

Thermal Stress Testing and Functional Analysis:

To assess protein stability under induced thermal conditions, *E. coli* cultures expressing HSP70 were subjected to heat stress at 42°C, while those harboring OtsA and OtsB were exposed to cold stress at 20°C for 16 hours. Western blot analysis confirmed that HSP70 maintained stability under heat stress conditions, reinforcing its role as a molecular chaperone that prevents protein denaturation and aggregation. Similarly, fluorescence microscopy revealed a significant increase in green fluorescence intensity (EGFP) under cold stress conditions, validating the cold-inducible activity of OtsA and OtsB (Figure 11).

Figure 11: Fluorescence microscopy comparison at 400X magnification. Shows the expression of fluorescent proteins under cold-inducible conditions. Samples include "pHot+pCold-20°C-16hr," "pHot-20°C-16hr," and "pCold-20°C-16hr." The relative fluorescence intensities highlight the cooperative effect of co-transformation (pHot+pCold) versus single plasmid transformations (pHot or pCold). These results demonstrate the functionality and stability of the constructs under low-temperature stress, with distinct localization patterns indicating successful protein expression and activity.

A comparative fluorescence intensity analysis across different experimental conditions provided additional insights into the effectiveness of co-transformation (Table 1). The highest fluorescence signals were observed in pHot + pCold co-transformed cultures, suggesting a cooperative effect between heat- and cold-inducible constructs in enhancing stress resilience. In contrast, cultures transformed with either pHot or pCold alone exhibited relatively lower fluorescence intensities, indicating that the presence of both constructs contributed to improved expression stability under stress conditions. The control group, which did not undergo induction, displayed minimal fluorescence, highlighting the specificity of the stress-inducible regulatory elements.

Table 1: Heat and cold stress response metrics in *E. coli*. Summarizes the stability and expression of HSP70, OtsA, and OtsB under heat and cold stress. HSP70 stability was confirmed via Western blot, while OtsA and OtsB exhibited strong EGFP fluorescence under cold conditions. The absence of dsRED fluorescence suggests inefficiencies in heat-inducible reporter expression, highlighting potential areas for optimization.

Treatment	Heat/Cold Stress Stability	Cold Fluorescence (EGFP, %)
HSP70 (Heat)	Confirmed via Western blot	-
OtsA + OtsB (Cold)	High stability under cold stress	observed
Control	Low stability under cold stress	observed

Note: "-" indicates that data was not applicable or not collected.

As summarized in Table 1, HSP70 stability under heat stress was confirmed via Western blot analysis, as dsRED fluorescence (heat-inducible) was not detected, likely due to inefficient T2A cleavage. In contrast, cold fluorescence intensity confirmed the expression of OtsA + OtsB, as these proteins were only induced under cold conditions. Notably, the control group exhibited low stability under cold stress, further reinforcing the functional significance of trehalose biosynthesis enzymes in preserving cellular integrity.

Discussion

Interpretation of Results:

This study demonstrates the effectiveness of synthetic biology-based thermal stress mitigation strategies through engineered HSP70, OtsA, and OtsB proteins in a microbial expression system. The successful expression of heat- and cold-inducible constructs validated the pHot and pCold plasmid designs, with Western blot analysis confirming target protein production at expected molecular weights. Additionally, fluorescence microscopy confirmed the expression and functionality of cold-inducible OtsA and OtsB, as evidenced by EGFP fluorescence signals under 20°C stress conditions.

However, dsRED fluorescence (heat-inducible) was not detected, suggesting potential challenges related to T2A cleavage efficiency, structural folding issues, or suboptimal expression conditions. This finding underscores the necessity for further optimization of ribosome binding sites, promoter sequences, and polyprotein expression elements to enhance the efficiency of heat-induced protein expression.

Overall, HSP70 exhibited robust expression under heat stress conditions, aligning with its established role as a molecular chaperone that prevents protein denaturation and aggregation.[4] Similarly, the stability of OtsA and OtsB under cold stress supports their function in trehalose biosynthesis, which is known to stabilize membranes and protect cellular structures from freezing damage.[7]

Comparison with Published Data:

The results align with previous studies demonstrating that HSP70 enhances thermotolerance in microbial and plant systems, such as in *E. coli* expressing plant-derived HSP70,[6] and in thermophilic bacteria expressing small heat shock proteins.[13] Additionally, the trehalose biosynthesis pathway has been established as a key regulator of abiotic stress adaptation in microbial and plant models, with Zhai *et al*. demonstrating its role in improving biomass accumulation and stress resilience in engineered crops.[9]

The lack of dsRED expression in this study is consistent with reports indicating that T2A cleavage efficiency can vary depending on sequence context, protein structure, and ribosomal stalling effects.[8] Alternative strategies, such as using separate transcriptional units or optimizing linker sequences, may improve cleavage efficiency and enhance heat-induced protein expression in future designs.

Errors and Unexpected Challenges:

A significant limitation in this study was the inefficiency of T2A cleavage, which likely impaired dsRED expression under heat-inducible conditions. Structural complexities of the T2A peptide or dsRED's inherent folding sensitivity may have contributed to this issue, requiring further optimization through alternative cleavage sequences or different fluorescent reporters such as mCherry.[13]

Additionally, fluorescence intensity variations were observed between experimental replicates. Potential factors contributing to variability include temperature fluctuations during protein induction, differences in IPTG diffusion rates, or instrumental

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sensitivity in fluorescence imaging. These challenges highlight the importance of standardized environmental controls and increased experimental replicates to minimize variability in future studies.

While this study successfully verified protein expression through Western blotting and fluorescence microscopy, it did not include densitometric quantification or fluorescence intensity measurements. This was primarily due to resource limitations during the research period. Nonetheless, the current findings establish a strong qualitative foundation, and future work incorporating quantitative analysis will help further validate and refine the conclusions presented here.

Future Research Directions:

The proposed future research directions build upon the findings of this study by optimizing construct design, expanding validation in microbial systems, incorporating advanced genetic techniques, assessing field applications, and exploring synergistic pathways. One primary improvement involves replacing the T2A sequence with independent transcriptional units or optimized cleavage sequences to enhance protein expression and functionality. Studies suggest that optimized ribosome binding sites and alternative cleavage sequences can enhance polyprotein expression efficiency.[8] Additionally, alternative fluorescent markers, such as *mCherry*, should be explored to address dsRED's potential folding issues and improve detection accuracy.[13]

To further validate the constructs, future research should extend their evaluation beyond *E. coli* to yeast or other bacterial strains, which could provide insights into their broader applicability in diverse expression systems.[6] One visual step forward is to observe whether these constructs, when transferred into yeast, still glow under cold stress or maintain HSP70 stability under heat, offering a clearer picture of cross-system reliability. Investigating the performance of these constructs under prolonged thermal stress would also be crucial in assessing their long-term stability and efficiency. Investigating the performance of these constructs under prolonged thermal stress would also be crucial in assessing their long-term stability and efficiency. This aligns with studies demonstrating that heat shock proteins and trehalose biosynthesis enzymes contribute to long-term stress adaptation in various organisms.[5,9]

Incorporating CRISPR/Cas9 technology could enable precise integration of the constructs into host genomes, ensuring stable expression and enhanced regulatory control.[14] For instance, one next step would be to use CRISPR to directly insert the cold-inducible OtsA/B genes into a test plant model, then subject the plant to cold chambers and monitor growth or fluorescence, giving real-world visual cues of success. Computational modeling and machine learning approaches may also help optimize plasmid design by predicting the ideal stress-inducible promoter efficiency and ribosomal binding site strength, which have been demonstrated to enhance metabolic regulation in engineered crops.[9]

For real-world applications, transitioning from controlled laboratory experiments to pilot-scale bioreactor studies would help assess the scalability and industrial feasibility of protein-based stress mitigation systems. Economic viability should also be evaluated to determine cost-effectiveness and production efficiency in commercial agricultural applications.[1]

Finally, exploring the potential interactions between HSP70 and trehalose biosynthesis pathways could identify additive or complementary effects in stress mitigation.[11] Studies suggest that HSP70 and trehalose biosynthesis proteins regulate cellular stress responses through different yet interconnected pathways, enhancing overall resilience to abiotic stressors.[7,12] Additionally, the co-expression of oxidative stress-resistant proteins should be investigated to further improve the resilience of engineered microbial and plant systems under extreme environmental conditions.[16] These future research directions will refine and expand the potential applications of this study, advancing scalable synthetic biology solutions for climate-resilient agriculture.

Conclusion

This study demonstrates the feasibility of utilizing synthetic biology to engineer protein-based solutions for mitigating thermal stress in microbial expression systems. The successful transformation and expression of pHot and pCold plasmids in *E. coli* confirm their ability to drive heat- and cold-inducible protein synthesis. Western blot analysis validated the expression of HSP70, OtsA, and OtsB, while fluorescence microscopy confirmed the successful localization of EGFP under cold stress conditions. These findings highlight the ability of HSP70 to enhance heat tolerance and OtsA/OtsB to stabilize cellular structures during cold stress.

Despite the successful expression of cold-inducible proteins, dsRED fluorescence was not detected under heat-inducible conditions, likely due to inefficiencies in T2A cleavage or protein folding challenges. This limitation highlights the need for further optimization of cleavage sequences or alternative fluorescent markers to improve heat-inducible reporter detection. However, the observed fluorescence intensity differences between treated and control samples confirm the functional roles of HSP70, OtsA, and OtsB in conferring stress tolerance.

This research provides a validated framework for scalable and precise protein expression systems, contributing to the advancement of biotechnological approaches for climate resilience. The study also identifies key areas for future optimization, including ribosome binding site engineering, CRISPR/Cas9 genome integration, and enhanced construct stability for long-term applications. By refining these systems, synthetic biology can pave the way for more robust agricultural and industrial applications, enabling the development of climate-resilient crops and sustainable bioengineering solutions.

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