Journal of Experimental Microbiology and Immunology (JEMI) Vol. 18: 87 – 90

The Temperature of the Cold Shock during Temperature Shock Transformation Has No Effect on Transformation Efficiency of *Escherichia coli*

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Temperature shock transformation (TST) uses sequential cold- and hot-step treatments (0 → 42 → 0°C) to induce the uptake of exogenous DNA by chemically competent cells. This study examined the effects of the magnitude of the temperature change, as well as the cold-step temperature on transformation efficiency. *Escherichia coli* BW25113 transformed with the pUC19 cloning vector at cold-step temperatures of 0°C, 4°C, 10°C, and 20°C demonstrated no significant difference in transformation efficiency. When the same strain was transformed using approximately a 20°C difference between the cold-step and hot-step treatments: 0 → 20 → 0°C and 20 → 42 → 20°C, no significant difference in transformation efficiency was observed between the two treatments. Our results indicated that the cold-step temperature does not significantly affect transformation efficiency within the temperature range tested, and that the difference in temperature between the cold- and hot-step treatments may be more relevant for high transformation efficiency.

Escherichia coli is commonly used for DNA transformation, but its competence is artificially induced via $CaCl_2$-treatment. Cells are then subjected to temperature shock transformation (TST), in which they are incubated at 0°C followed by a brief heat treatment at 42°C and a second incubation at 0°C (1, 2).

Protocols for TST using *E. coli* have been largely optimized empirically with the goal of maximizing transformation efficiency (3-8). For example, TST is most efficient when the hot-step temperature is 42°C (3, 5). However, no optimization of the cold-step temperature before and after the 42°C step has been formally performed, and it remains possible that using a temperature other than 0°C for these cold-steps would give higher transformation efficiency.

Furthermore, the exact mechanism for uptake of DNA during TST remains largely obscure. One model is that the heat shock (0 → 42°C) causes changes in membrane fluidity, resulting in the formation of zones of adhesion, where the outer and inner cell membranes fuse with pores in the cell wall, and through which DNA may pass (9-12). Meanwhile, other studies show that the presence of particular proteins, such as temperature-shock proteins GroEL and Histone-like Nucleoid Structuring protein (H-NS), affect transformation efficiency, suggesting they have a role in the process of TST (13-16).

If cold-shock proteins do play a role in TST of *E. coli*, then changing the temperature of the cold-step would most likely affect transformation efficiency, since the expression of cold-shock proteins may be affected (17, 18). Additionally, the extent to which transformation efficiency depends on the cold-step temperatures used in TST or the temperature difference between steps is unclear, and its investigation may open new avenues in the exploration of the mechanism of TST. In this study, the effect of the cold-step temperature and the temperature difference on transformation efficiency in TST of *E. coli* BW25113 was investigated.

MATERIALS AND METHODS

Bacterial strain and growth conditions. *E. coli* BW25113 originated from the Coli Genetic Stock Center and was supplied by the MICB 421 culture collection at the Microbiology and Immunology Department, University of British Columbia. *E. coli* BW25113 was streaked on LB agar and incubated overnight at 42°C to ensure the strain was free of the pKD46 plasmid which encodes for ampicillin resistance. Isolated colonies were used to inoculate two tubes of 20 ml LB broth and grown overnight in a 30°C shaking water bath at 125 RPM. One tube contained 100 ug/ml ampicillin to test for ampicillin sensitivity.

Isolation of plasmid. An isolated *E. coli* DH5α colony carrying the pUC19 cloning vector that was grown on an LB agar plate with 100 ug/ml ampicillin (Sigma Aldrich catalog no. A9518) was picked to inoculate 20 ml of LB broth with 100 ug/ml ampicillin. This culture was incubated at 37°C on a platform shaker at 200 RPM overnight. Using the MP Bio RapidPURE Plasmid Mini Kit, plasmid DNA was extracted from the overnight culture, as per kit procedure with the exception that each plasmid prep was eluted in 50 ul of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8). The plasmid concentration was measured using the Nanodrop 2000c Spectrophotometer to be 16 ng/ul. Plasmid was stored in one-use aliquots at -20°C.

Induction of competence by $CaCl_2$ treatment. The procedure to induce cell competence was adapted from Molecular Cloning: A Laboratory Manual (2). 35 mL of a culture at 0.360 OD_{600} was chilled on ice for 10 min and centrifuged in a Beckman Coulter JA-20 rotor at 20,000 RPM for 10 min at 4°C. The pellet was re-suspended in 21 ml of chilled $MgCl_2$-$CaCl_2$ solution (80 mM $MgCl_2$, 20 mM $CaCl_2$), centrifuged at 20,000 RPM for 10 min at 4°C , then re-suspended in 1.4 ml chilled 0.1 M CaCl2 solution and 4.67 ul of 60% v/v glycerol to make up 32 aliquots of competent cells. Each microfuge tube contained 50 ul of competent cells in 15% v/v glycerol. The microfuge tubes were placed in a pre-chilled freezer box at -80°C.

Transformation at different cold-step temperatures. For each replicate, a microfuge tube containing 50 ul of frozen competent *E. coli* BW25113 cell mix was moved from the -80°C freezer and placed on ice to thaw for 10 min. 5 ul of pUC19 was then added into each tube and mixed by flicking gently. For the cold-step, tubes were placed into water baths of varied temperature (0°C, 4°C, 10°C, 20°C) for 20 min prior to heat

shock. To cause the heat shock, tubes were placed into a 42°C water bath for 30 sec without agitation. The cells were then incubated in the water baths of the original cold-step temperatures for 2 min. Next, 950 ul of LB broth was added to each reaction volume and incubated in a 37°C shaking water bath at 150 RPM for 1 h. LB agar plates with and without 100 ug/ml ampicillin were spread in duplicate at final plated dilutions up to 10^{-3} and x 10^{-7} respectively and incubated at 37°C for 16-20 h. The TST procedure was performed in duplicate for every cold-step temperature, with the exception of 10°C, which was performed in triplicate.

Transformation across a 20°C temperature change. The method was performed as above in duplicate with the TST temperatures at 0 → 20 → 0°C.

Calculation of transformation efficiency. Transformation efficiency was calculated by dividing the number of transformants by the number of viable cells at each experimental condition. The number of viable cells and the number of transformants for each experimental condition was based on the colony counts on LB plates and LB plates supplemented with 100 ug/ml ampicillin, respectively.

RESULTS

Transformation at different cold-step temperatures. We found that the transformation efficiency (TE) was approximately 1.6-fold higher with cold-step temperatures of 4°C and 10°C, compared to the standard procedure performed at 0°C (Fig. 1), however this difference was not statistically significant. Furthermore, the TE at 20°C was observed to be 70% of the TE at 4°C and 10°C, however there was large variation between experiments. Additionally, the TE also was 1.2-fold higher at a cold-step temperature of 20°C compared to 0°C.

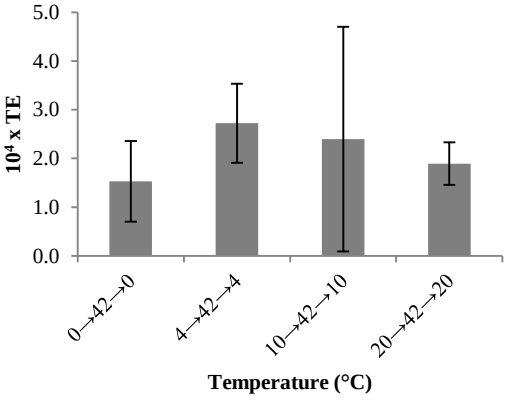

FIG 1 Comparison of transformation efficiency (TE) of *E. coli* BW25113 acclimatized at different initial cold-step temperatures before shocking at 42°C. Error bars represent the standard deviation between 2 samples. TE was measured in transformants per viable cell.

Transformation across a 20°C temperature change. No significant difference in TE was observed when *E. coli* BW25113 was subjected to a 20 → 42 → 20°C compared to a 0 → 20 → 0°C TST protocol (Fig. 2). The standard deviation for TE measured for the 20 → 42 → 20°C protocol was approximately 9- fold higher than that of the 0 → 20 → 0°C protocol.

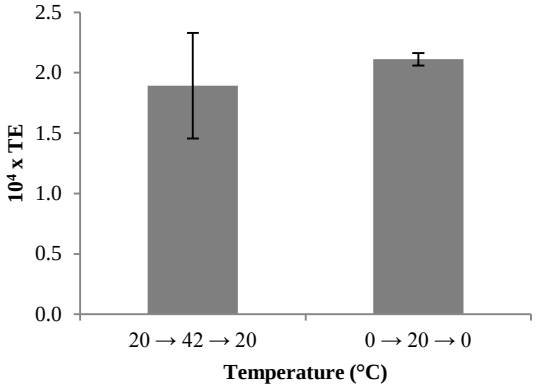

FIG 2 Comparison of transformation efficiency (TE) of *E. coli* BW25113 with approximately 20°C difference between cold- and hot-steps. Error bars represent the standard deviation between 2 samples. TE was measured in transformants per viable cell.

DISCUSSION

We hypothesized that an increase in cold-step temperature would decrease TE, because it would cause less significant heat- and cold-shocks. A TST without any shocks (0 → 0 → 0°C) produces very few transformants (11). However, this hypothesis was not supported by our data. Transformation was more efficient when the initial cold-step temperature was in the range of 4°C to 10°C instead of at 0°C. This might be because the temperature difference using 4 → 42 → 4°C and 10 → 42 → 10°C was less extreme when compared to 0 → 42 → 0°C. Larger temperature shifts result in higher cell death (17, 18), so the differences in transformation efficiency may be due to differences in cell viability. It is also possible it was too cold at 0°C for bacteria to make protein and thus generate a proper cold-shock response. Meanwhile, TST using a cold-step temperature of 20°C may not involve enough of a temperature difference to induce transformation as efficiently as the cold-step temperatures of 4°C or 10°C.

The lack of a significant difference between TST using 0 → 20 → 0°C compared to 20 → 42 → 20°C suggested that the magnitude of the temperature difference between cold- and hot-steps has a role in determining transformation efficiency. This is significant because the expression of cold-shock proteins in bacteria is dependent upon the magnitude of the temperature downshift (17). Our result suggests that cold shock proteins may have a role in the transformation process because of this correlation.

In contrast to the widely accepted procedure in TST of incubating the cells at 0°C (6, 19) before and after the heat-shock, our findings showed that the cold-step incubation temperature of the cells did not significantly affect TE, and that higher temperatures up to 20°C were able to produce comparably efficient transformations. Many labs can save resources if a smaller temperature

change in TST can be used to confidently transform competent *E. coli*. For example, if a change from 10°C to 30°C is enough for comparably good TE, labs may save resources by not requiring ice for transformation, which may be important in research locations around the world where these resources are scarce. Furthermore, it appeared that a temperature change of approximately 20°C between the cold- and hot-steps of TST was sufficient for efficient transformation, regardless of whether the cold- and hot- temperatures were 0°C or 20°C and 20°C or 42°C respectively. This suggested that the difference of the temperature change between the cold-step and heat-shock treatments is a larger contributory factor to TST efficiency than changes in absolute temperatures.

The definition of 'cold-shock' in scientific literature is an ambient, rapid decrease in temperature (17). Cold-shock has been described as a temperature downshift from 42°C to ice baths (~0°C) (3), and also from 37°C to 15°C (20). Our results lend further support to the little-explored idea that the range of temperature change is more critical than the absolute temperatures for successful DNA transformation in *E. coli*, and that transformation of *E. coli* sometimes occurs in the natural environment (21) because extreme temperature jumps may not be necessary for transformation.

Although we were unable to validate these proposed explanations due to the large standard deviations in our data, our results showed that comparable TE can be achieved using different cold-step temperatures, which may affect how we view possible models of DNA transformation. Overall, our results suggested that the absolute temperature of the cold-step does not affect transformation efficiency. Instead, the difference in temperatures used in the temperature shock transformation is of significance for efficient transformation in *E. coli*.

FUTURE DIRECTIONS

The experiment should be repeated to generate more replicates and conclusively determine which if any cold-step temperature of TST results in the highest TE. The expression of cold-shock proteins using varying magnitudes of temperature differences in TST can be measured to determine if it positively correlates with TE, which would imply the involvement of cold-shock proteins in the mechanism of TST. Furthermore, the possibility that the lower TE using 0 → 42 → 0°C compared to 4 → 42 → 4°C and 10 → 42 → 10°C is due to the 0°C being too cold for protein expression can be tested by comparing the expression levels of known cold-shock proteins between these TST schemes.

Alternatively, the proposed mechanism of transformation due to decreased membrane fluidity and pore formation in reaction to temperature shock can also be explored. A possible follow-up experiment would be to add a fluorescent marker that binds to membrane proteins to observe changes in membrane fluidity, and correlating the fluidity changes at different temperature ranges to transformation efficiency.

It would also be of interest to observe whether a 42°C difference, such as 10 → 52 → 10°C, yields efficiencies similar to the standard 0 → 42 → 0°C. Furthermore, the range of the TST can be changed to determine the minimum temperature jump necessary.

ACKNOWLEDGEMENTS

This study was funded by the Department of Microbiology and Immunology at the University of British Columbia. We would like to extend our gratitude to Dr. William Ramey and Dr. David Oliver for their guidance. We would also like to acknowledge the Wesbrook media room staff Nick Cheng and Lando Robillo for access to supplies and equipment.

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