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Effects of Temperature-Induced Changes in Membrane Composition on Transformation Efficiency in *E. coli* DH5α

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One model of transformation proposed that heat shock destabilizes the cell membrane and allows for plasmid uptake. As lipid composition might also affect the stability of the cell membrane, we hypothesized that a lower growth temperature would alter membrane lipid composition so as to increase transformation efficiency after heat shock. Cells were grown at 20°C and 37°C and treated with calcium chloride to induce competency. The transformation efficiencies yielded considerable variation. We were unable to conclude an effect of growth temperature on transformation efficiency. Statistical analysis determined that increased plasmid concentration negatively impacted cell survival.

Transformation is a commonly used technique to introduce foreign plasmids into bacteria, but this process is relatively inefficient (11). In order to optimize transformation rates, it is important to understand the two competing models on the mechanism of chemically-induced transformation for bacterial cells. The first proposes that exogenous plasmid DNA becomes bound to receptor complexes and is transported through transmembrane channels, while the second maintains that heat-shocking during transformation destabilizes the membrane, allowing loops of plasmid DNA to be inserted through transient gaps (4, 9). Based on the second model, we investigated the effect of increasing membrane fluidity on the efficiency of transformation, as an increase in fluidity likely effects the ease with which plasmid DNA traverses the bilayer. At lower growth temperatures, adaptations to phospholipid and fatty acid profiles result in increased membrane fluidity (1). Here, we explored the effects of two different growth temperatures, 20°C and 37°C, on transformation efficiency in *E. coli* DH5α. Previous experiments have demonstrated increased electroporation transformation efficiencies for cells grown at lower temperatures (3); therefore, we hypothesized that cells grown at the lower temperature of 20°C will have a higher transformation efficiency as compared to cells grown at 37°C.

MATERIALS AND METHODS

Preparation of competent cells. An isolated colony of *E. coli* DH5α obtained from the University of British Columbia's MICB 421 collection was grown in SOB media at both 20 °C and 37 °C to an optical density (OD_{460}) of approximately 0.3. Aliquots of 1 ml were set aside for lipid analysis. The remainder of each culture was split into three batches for each temperature, corresponding to sets 1-6. The six batches of cells were prepared for transformation using a $CaCl_2$ protocol as previously described (12). The concentration of competent cells was determined by plate counts on LB agar. Cells were stored at -85 °C in 50 µl aliquots as described previously (12).

Plasmid isolation. pUC-19 was isolated from the *E. coli* DH5α delivery strain obtained from the University of British Columbia's MICB 421 collection using the Fermentas GENEJet Plasmid Miniprep Kit (Cat #K0503, Fermentas). No changes to the manufacturer's protocol were made. The concentration and purity of plasmid were determined by measuring A_{260} and A_{280}. DNA was stored at -20°C.

Bacterial transformation. The CFU:DNA ratio for the experiment was calculated assuming 10 µl of plasmid in the most concentrated batch of competent cells. The volume of DNA was adjusted to maintain this ratio for all six batches of competent cells. Three aliquots of each batch of competent cells were thawed on ice. The first was treated with DNA, the second was treated with water, and the third was left untreated. All were incubated on ice for 10 minutes. The cells incubated with DNA or water were subjected to a 30 second heat shock in a 42 °C water bath and recovered as described elsewhere (8). The untreated cells were not subject to heat shock but underwent recovery alongside the treated cells.

Plating of cells and calculation of transformation efficiency. Following recovery, cells were plated on LB agar and LB agar with 50 ug/ml ampicillin. Transformation efficiency was calculated using three methods as in Chan *et al*. (2): the ratio of transformants to input cells, the ratio of transformants to viable cells, and the ratio of transformants per µg of DNA.

Lipid extraction and analysis by thin layer chromatography (TLC). Lipids were extracted using chloroform/methanol mixtures as described previously (14). Extracted lipids were spotted on silica TLC plates. Plates were placed in a glass chamber with chloroform/methanol (1:1) until the solvent front reached 0.5 cm from the top of the plate. Plates were dried and visualized using bromothymol blue spray as described elsewhere (14). Deoxycorticosterone dissolved in chloroform/methanol (1:1) was used as a control.

Statistical analysis of transformation efficiencies. Correlation coefficients (r) were calculated using the Pearson product-moment correlation coefficient method.

RESULTS

Preparation of competent cells. DH5α *E. coli* cultures grown at 20°C and 37°C reached optical densities (OD_{460}) of 0.38 and 0.36 respectively. The

final concentration of each batch of competent cells ranged from 4.0 x 10^8 CFU/ml to 2.2 x 10^9 CFU/ml.

Isolation of pUC-19 plasmid. The ratio of the absorbance at 260 nm:280 nm of the isolated plasmid was 1.84. The concentration of plasmid, determined by the absorbance at 260 nm, was 24 µg DNA/ml.

Transformation of DH5α *E.coli*. The CFU:DNA ratio used was 1.1x10^8 CFU to 240 ng DNA. The volume of DNA used to achieve this CFU:DNA ratio for each set of competent cells is listed in Table 1. The average survival rate of competent cells after exposure to heat shock at 42°C in the presence of DNA was 12 % (Table 1). When this process was repeated in the presence of water instead of DNA, the average survival rate was 38 % (Table 1). There was a negative correlation between DNA volume and cell survival (Figure 1). The Pearson product-moment correlation coefficient (r) was calculated to be -0.677 (p=0.10).

TABLE 1. Comparison of heat shock survival rates by six sets of competent *E. coli* DH5α.

Growth Temperature (°C)	Set	Survival of heat-shock with H_2O (%)	Survival of heat-shock with pUC19 (%)	Volume of H_2O or DNA (µl)
20	1	38	1	5
	2	56	22	3
	3	55	6	10
37	4	24	9	4
	5	30	29	2
	6	25	7	6

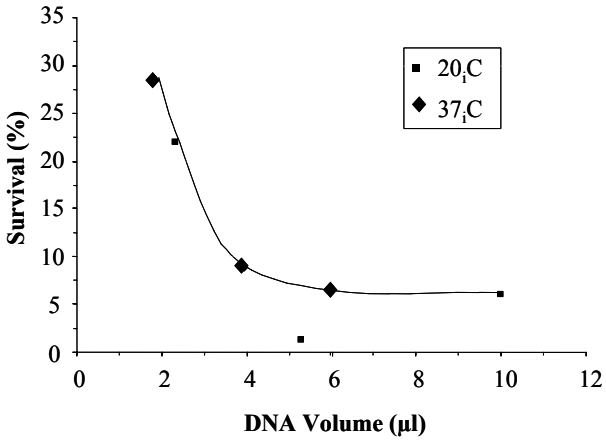

FIG. 1. Effects of added volume of pUC19 plasmid on *E. coli* DH5α cell survival following a 30 s heat shock at 42°C.

Transformation efficiencies exhibited 100-fold differences when derived using all three calculation methods (Table 2). This variation is unexpected. Figure 2 depicts the trend between the percentage of DH5α cells surviving heat shock in the presence of pUC19 and the measured transformation efficiency per viable cell. The correlation coefficient (r) was calculated to be -0.534 (p=0.13).

Thin layer chromatography of isolated lipids. Visualization of the TLC plates revealed smears that were inconclusive (data not shown).

DISCUSSION

Here we investigated the effect of growth temperature on transformation efficiency in *E. coli* DH5α. Cells were grown at 20°C and 37°C, and the two cultures were split, and three sets of competent cells were prepared from each culture using salt solutions. Each of the six sets of competent cells was assessed independently for transformation efficiency.

Transformation efficiencies, calculated using three methods, are presented in Table 2. It is important to address the unexpected difference between the transformants per viable cell and the transformants per input cell (Table 2). As input cells represent those present before heat shock, they should be greater in number than the viable cells because some cells die during heat shock. Considering this, the ratio of transformants per input cell is unexpectedly low. This can be explained by the observation that after the one-hour recovery, the input cells were mistakenly plated on LB-amp instead of LB. Instead, transformants per input cell was calculated using the number of competent cells present before the experiment began. It is likely that the input cells were underestimated as they should have been enumerated after one hour of recovery. A greater number of input cells would have decreased the number of transformants per input cell, yielding an expected ratio when compared to the transformants per viable cell.

TABLE 2. Effects of growth temperature on the transformation efficiency of competent *E. coli* DH5α

Growth Temp. (°C)	Set	Calculated Transformation Efficiency		
		10^{-7} Transformants per input cell	10^{-7} Transformants per viable cell	10^3 Tranformants/ µg plasmid DNA
20	1	180	140	34
	2	390	18	7
	3	83	2	2
37	4	790	90	140
	5	73	3	13
	6	11	2	2

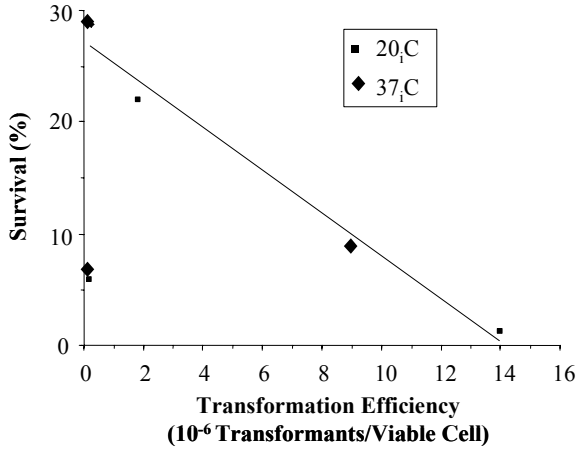

FIG. 2. Relationship between the survival of DH5α cells after a 30 s heat shock at 42°C and the transformation efficiency.

The variation in the transformants per viable cell and transformants per µg of DNA should also be addressed. Previous published results suggest that a 10-fold variation in transformation efficiency can be expected between replicates (6), so the 100-fold variation observed here is unlikely to be resolved with further replicates. Due to extreme variation in these results, it is impossible to conclude that there are significant differences in the transformation efficiency of cells grown at temperatures of 20 and 37 °C. Therefore, it is important now to consider potential explanations for the observed variation in transformation efficiency.

One possible explanation for the observed variation is the effect of the DNA solution on cell viability. There was variation in the volume of DNA added in order to maintain the same CFU:DNA ratio, and it was found that increased DNA volume was correlated to a decreased cell survival rate (Figure 1). The correlation coefficient of -0.677 suggests a statistically significant, moderate negative correlation. Since no such pattern of reduced cell viability was observed in the cells treated with water rather than DNA (Table 1), it is unlikely that experimental procedure influenced viability.

It is possible that a component in the DNA solution reduced cell viability following heat shock. Although the ingredients of elution buffers are proprietary, elution buffers are typically neutral, low salt solutions and unlikely to cause cell death. Cell death was more likely caused by a contaminant or the DNA itself. As an A_{260}:A_{280} ratio between 1.8 and 2.0 is generally indicative of high plasmid purity (5), our measured value of 1.806 suggests that our isolated pUC19 plasmid was pure. The impact of DNA on cell viability likely played a role in influencing the resulting transformation efficiencies (Table 2), as shown by the calculated correlation coefficient (r value) of -0.534.

A second possible explanation for the variation in transformation efficiency considers the CFU:DNA ratio. As the amount of DNA increases, the transformation efficiency increases in a linear fashion until saturating conditions are reached (13). In this experiment we added 240 ng DNA for $1.1x10^8$ cells, which was calculated by adding 10 µl of plasmid DNA to the most concentrated batch of competent cells. In our least concentrated batch of cells we added 43 ng of DNA. Above 1 ng, the relationship between DNA and transformation efficiency is not linear (7). Therefore the ratios calculated as transformants per µg of DNA are not reliable measures of transformation efficiency.

Amounts of DNA greater than 200 ng may be saturating (7), and this was true for replicate number 3. If the plasmid is in excess, we can expect 3-5% of viable cells to be transformed (7). However, the transformation efficiency of 2 transformants per 10^7 viable cells was quite low (Table 2). The CFU:DNA ratio is not sufficient to explain the variation observed in the transformants per viable cell ratios (Table 2).

A third explanation for the variation in transformation efficiency is the arrangement of the tubes during heat shock. Tubes were arranged in two rows of six. The two replicates that were flanked on either side by other tubes (sets 3 and 4 in Table 2) had reduced heat shock exposure. However, the transformation efficiencies for these replicates (Table 2) do not indicate such a pattern. Replicate 3 exhibits below-average transformation efficiency and replicate 4 exhibits above-average transformation efficiency. The removal of these replicates from the data set does not yield a pattern among the remaining replicates.

Although it is not possible to conclude from these results that growth temperature affected transformation efficiency, it is important to acknowledge the effect of growth temperature on lipid composition as this was the basis of our hypothesis. Thin layer chromatography was unable to provide insight into lipid composition. Lipid extraction using chloroform/methanol would have extracted only neutral lipids, so it would have excluded some membrane phospholipids. It is expected that as growth temperature increases, there will be an increase in saturated fatty acids along with a decrease in unsaturated fatty acids (10). The most abundant saturated fatty acid, palmitic acid, increases continuously with temperature increase. It has been previously shown that palmitic acid increases from 25.4% at 20□C to 31.7% at 35□C. Therefore we would have expected a noticeable difference in lipid composition, but a more appropriate extraction protocol is needed to successfully extract membrane methyl esters. Diethyl ether would successfully extract these lipids from a cell hydrolysate, but this chemical was not immediately available in this laboratory. Without a measurement of lipid composition, it is not possible to conclude that growth temperature affected

transformation efficiency because of its effects on lipid composition. It is apparent, however, that the volume of DNA affected cell viability and transformation efficiency.

FUTURE EXPERIMENTS

Continuation of this work should examine the basis of our initial hypothesis that alternate growth temperatures cause adaptations to phospholipid and fatty acid profiles. Due to a poor lipid extraction protocol, we were not able to demonstrate differences in lipid profiles between cultures grown at 20° C and 37° C. We suggest future groups isolate lipids from bacteria grown at different temperatures using a diethyl ether extraction protocol for the extraction of membrane methyl esters. Multiple methods for measuring lipid composition could be performed such as TLC, gas chromatography, and mass spectrometry.

This work uncovered a significant affect of DNA on cell survival. It would be useful to investigate the effects of DNA concentration on transformation efficiency and cell survival. One could isolate the plasmid DNA using different methods to investigate the effects of plasmid preparation on transformation. Saturating conditions and the effects of different plasmids could also be investigated. Although we attributed the decrease in cell survival to DNA concentration, the contents of the elution buffer remain unknown. It is possible that the elution buffer affects cell survival. One could investigate the effects of different in-house and commercial elution buffers on cell survival. Alternatively, one could directly assess the composition of different buffers or test for the presence of potentially toxic compounds.

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