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The Effects of the Ratio of Surface Area to Volume on Transformation Efficiency in *Escherichia coli* K-12

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In the process of transformation, bacteria take up DNA from the environment through their cell wall. To induce competence in the cells, the DNA for uptake must first attach to the cell surface prior to passing through the membrane. Previous studies on the effect of surface area on transformation efficiency have shown decreased transformation efficiencies in large *E.coli* cells as compared to smaller strains. The reasons remain unknown. We proposed that the surface area to volume ratio (SA:V) to be the determining parameter on transformation efficiency, with a higher ratio leading to higher transformation efficiencies. In order to assess this model, we transformed wild type *Escherichia coli* (BW25113) and a smaller mutant strain (JW2500) with different amounts of the plasmid vector pUC19.The mutant strain JW2500 with a lower SA:V ratio but a similar surface area to the parent strain displayed 70% ± 5% of the number of transformants from the wild-type strain BW25113. Such results suggest a direct relationship between SA:V ratio and the observed changes in transformation efficiency.

Transformation is the introduction of DNA into prokaryotic cells and the genetic alteration of a cell resulting from the uptake, genomic incorporation, and expression of foreign genetic material (3).The ability to transform *Escherichia coli* with plasmid DNA is crucial for a vast number of molecular biology procedures (2, 5). Cells which undergo transformation must first become competent, which can be achieved through several methods, such as calcium chloride treatment or electroporation (5).The interaction of the exogenous DNA with the cell surface prior to passing through the membrane is crucial in the transformation process (5).

A previous experiment by Hatami et al. (3) attempted to answer the question of what factors could increase the efficiency of nucleic acid transformation in *E.coli.* They tested their hypothesis that an increase of cell surface area would lead to an increase in transformation efficiency by using nalidixic acid treatment to create elongated, serpentine cells which possessed a larger surface area than the control cells (3).Their results were confounded by the observation that the nalidixic acid treatment greatly lowered cell viability. In another study conducted by Habibi et al. (4), the E. coli mutant D23 with a mutation in *mreB11* and *sloB1* and its parent strain D21 were used to test transformation efficiency. The D23 mutant displayed a smaller and spherical morphology and slower growth rate as compared to its parent strain displaying a normal rod shape. Their results showed greater transformation efficiencies for the mutant strain with a smaller cell surface area (4). Because the results were contrary to their original hypothesis, they suggested that the mutations in D23 also introduced a number of changes to the cell that may have affected transformation efficiency (4).

We hypothesized that both the surface area and volume of a cell play a role in determining its transformation efficiency. We examined the cellular geometric factors that affect transformation efficiency, specifically: surface area to volume ratio (S: V). We proposed that increases in transformation efficiency are due to higher SA: V ratios. We used a different mutant strain JW2500 (*rodZ-*) that also confers a spherical dimension to the normally rod shaped wild type cell BW25113. According to Shiomi et al. (7), the volume of the *rodZ-* round cells is about 1.5 times larger than that of rod wild-type cells, while the surface area is maintained to be roughly the same (7). Therefore, the smaller round JW2500 mutant displaying a decreased SA:V ratio was suitable for assessing the effect of SA:V ratio on transformation efficiency.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Escherichia coli* K-12 wild type strain BW25113 (::rrnB-3), *λ⁻, rph-1, Δ(rhaD-rhaB)568, hsdR514)* was compared to its mutant strain JW2500 (*Δ(araD-araB)567, ΔlacZ4787*(::rrnB-3), *λ⁻, rph-1, Δ(rhaD-rhaB)568, hsdR514)*, which should have a smaller and rounder morphology as compared to the normal rod shape. Strain were found in the Coli Genetic Stock Center (http://cgsc.biology.yale.edu/). These strains were grown in Luria-Bertani (LB) broth at 37°C at 200 rpm for 24 hours (6). LB agar was used for testing viability and growth of the two strains, while LB agar with concentration of 100 µg/ml ampicillin were used to detect successful transformants.

Plasmid Isolation. The pUC19 plasmid was isolated from the DH5alpha strain of *E.coli* using the phenol/chloroform extraction method. In order to increase our plasmid yield we treated our cultures with chloramphenicol (170ug/ml final concentration) and incubated over night before the extraction. Concentration of plasmid was measured at A_{260} and calculated to ug/ml by multiplying the reading by 50ug/ml.

Preparation of competent cells. Competent cells of each wild type and mutant strains were prepared using the calcium chloride/heat shock method. Portions of overnight cultures (0.5 mL) were inoculated into 49.5 mL of pre-warmed LB broth and incubated at 37°C at 200 rpm until OD_{600} was between 0.3-0.4. Cells were then harvested at 3500 xg at 4°C for 10 minutes. Supernatant was then removed and discarded and cells were resuspended in 5.0 mL of ice cold 50mM $CaCl_2$ for 10 minutes. Cells were once again centrifuged at the same conditions as previous and resuspended in 1.0 mL of 50mM $CaCl_2$ + 15% glycerol. Aliquots of 50 µL were placed in 1.5 ml tubes and frozen at -20 °C until ready for transformation.

Cell Viability Test. Two frozen aliquots of each strain were thawed on ice. Pre-heat shock viability was performed by adding 1 mL of LB broth to the tubes and dilutions of each sample were conducted accordingly. Dilutions were plated onto LB agar plates and incubated at 37°C overnight. Post-heat shock tests were conducted similarly to pre-heat shock tests. However, prior to adding 1 mL of LB broth, the cells were heat-shocked at 45°C for 45 seconds. Comparisons between pre- and post-heat shock plates were conducted.

Transformation. Each tube was thawed on ice and 0.75 ug, 1.5ug, and 3ug of DNA (pUC19) were added. The mixtures were incubated on ice for 30 minutes and were then subjected to heat shock at 45°C for 45 seconds. Immediately after heat shock, cells were cooled on ice for 2 minutes and then diluted by the addition of 1 mL of LB broth. Each diluted tube was incubated for an hour at 37°C to allow for re-conditioning and were spread in triplicates on LB+ampicillin plates. Plates were incubated overnight at 37°C and colonies were counted from each strain 24 hours later.

RESULTS

Competent Cell Viability. Dilutions of 1/5000 of the stock competent cells were plated on LB agar. Although the wild- type BW25113 and mutant JW2500 strains have different morphology both showed similar pre-heat shock and post-heat shock viabilities. For the pre-heat shock treatments, it was calculated that on average there were 2.0 x 10^3 cfu/mL for both strains. For the post-heat shock treatments, it was calculated that on average there were 1.5 x 10^3 cfu/mL for both strains. These tests were carried out several times.

Transformation efficiency. The competent cells were transformed with 0.75µg of pUC19 plasmid in triplicates.The wild-type BW25113 strain showed an average efficiency of 78 colonies/µg of plasmid introduced while the mutant JW2500 strain showed an average efficiency of 56 colonies/µg of plasmid introduced (Fig. 1). This shows that the smaller mutant strain had only 70% ± 5% of the number of transformations of the wild-type strain. These results are consistent with our original hypothesis that the higher surface area to volume ratio strain should have a greater efficiency of transformation.

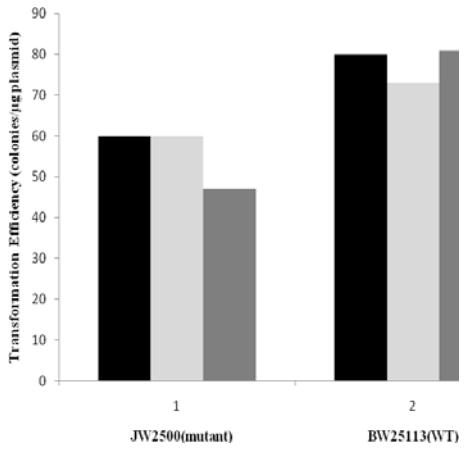

FIG. 1. Effect of SA: V ratio on transformation efficiency. Transformation efficiency of mutant (low SA: V) vs. wild type (high SA:V). The bars represent the values from separate plates in the triplicate tests using 0.75µg of pUC19 plasmid.

DISCUSSION

Habibi et al. (4) had previously attempted to determine whether the effect of an increased surface would be increased transformation efficiency. They based their hypothesis on the knowledge that exogenous DNA requires an interaction with the outer membrane of cells in order for a successful transformation event to occur. Intuitively, a large surface area would lead to increased interactions and thus increase uptake of exogenous DNA (transformation efficiency). They, in fact, observed the opposite. Thus observation by Habibi et al. (4) could have arisen from inappropriate selection of a mutant strain. As they suggested, the D23 mutant contains two additional mutations, *mreB11* and *sloB1*, as compared to the parent strain D21. The MreB complex interacts with the penicillin-binding proteins required for synthesis of the peptidoglycan layer. The absence of any complex component results in abnormal cell shapes, such as round or Y-shaped due to their inability to properly synthesize the peptidoglycan layer and divide correctly. Thus such defects lead to the eventual death of these cells (7). Since MreB plays a major role in ensuring cell integrity, any mutations such as *mreB1* that affect its function could change the normal cellular response to environmental stress and might affect transformability. The original sphere-like mutant D23 exhibited a hypersensitivity to penicillins due to the *mreB* mutation (7). Such hypersensitivy would be consistent with an increased permeability arising from the inhibition of peptydoglycan synthesis as suggested

by Habibi et al. (4). To avoid the problem and increased permeability confounding the results, our approach was based on the comparison of two completely different strains, mutant JW2500 (*rodZ*) and its parent strain BW25113. The mutant strain used here is only believed to have one mutation in the *rodZ* locus (7). These strains have a constant surface area but the volume was about 1.5 times larger in JW2500 than in wild-type BW25113 cells (7). Even though the volume of the JW2500 mutant is larger, this strain displayed a shorer morphology than BW25113 cells under the microscope.

We ultimately found the mutant JW2500 with a lower SA:V ratio to have a lower transformation efficiency than its parent strain BW25113. The base for the effect of SA: V on transformation efficiency is still unclear; however, the answer could be found by comparing this phenomenon with other cellular methods of control. Previous studies have shown that transcriptional repressor proteins play a key regulatory role in plasmid replication initiation. The concentration of such regulatory proteins is greatly reduced under normal conditions, if this concentration decreases greatly due to enlargement of the cell, plasmid replication inhibition would decrease, thus leading to an increase in copy number (1). Such dosage-dependent manner of control is likely to be functioning in various bacterial mechanisms such as transformability control. We propose the decreased transformation efficiency in the mutant strain to be due to dilution of cytoplasmic signals for plasmid stability and proper partitioning. Not only is plasmid uptake important but also the ability for the plasmid to equally partition when the cell divides (1). With a larger volume, any molecules present in the JW2500 cytoplasm will be diluted in comparison to the wild-type strain displaying smaller volumes. Dilution of stability and partitioning signals would thus lead to loss of the plasmid vector by either degradation or unequal distribution of the plasmid upon cell division and lead to lower transformability.

In conclusion, a lower SA:V ratio leads to decreased transformability displaying a direct relationship between SA:V and transformation efficiency. This model, however, will not be complete until it is repeated with bacteria of varying SA:V ratios with no constant parameters. Only then, will the effect of varying the SA:V ratio on transformation efficiency be uncovered. If only surface area or volume is changed, one could attribute the change to the changing parameter only and not the ratio between surface area and volume.

FUTURE DIRECTIONS

This experiment should be attempted with strains of varying surface areas and volumes (accurately measured). If one of the two variables are kept constant and the other changed (SA or V), despite a consequential change in SA:V ratio, only the effect of the changing variable on transformation efficiency will be studied. Thus, both parameters of surface area and volume need to be adjusted to determine the true effects of SA:V ratios on transformation efficiency.

It would be worthwhile to investigate the phenomenon of transformation further by studying the localization, as well as the amount of plasmid DNA that enters the cell during the transformation process. To this end, it might be plausible to use fluorophore-labeled plasmids in the initial transformation step of this experiment. Then using fluorescence microscopy, one could visualize the localization of the plasmid between the two groups of cells (with differing SA:V ratios), as well as measure fluoresce intensity as a measure of total plasmid DNA taken up. Comparisons between the two groups would hopefully provide insight into the mechanism behind DNA uptake in different cell types. Colocalization studies with potential stability or partitioning factors could be performed in order to define potential interactions between these factors and the plasmid and how these differ in larger and smaller cells.

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