

Comparison Between Transformation Efficiencies in Rod-shaped and Spherical-shaped *Escherichia coli* Strains

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Transformation of *Escherichia coli* with foreign DNA is dependent on DNA binding and translocation at the cell surface. *E. coli* D23 is a mutant strain that contains two additional mutations, *mreB11* (*envB*) and *sloB1*, in comparison to the parent strain *E. coli* D21. These two mutations alter the cell morphology into a sphere with decreased surface area and cause growth rate to slow down in comparison to *E. coli* D21 which maintains the growth rate and rod shape that are associated with wild type *E. coli*. This study investigated the effect of decreased cell surface area, produced by the *mreB11* and *sloB1* mutations in the *E. coli* D23, on the transformation efficiency. Both *E. coli* strains D21 and D23 were transformed with a plasmid encoding a kanamycin resistance by using a calcium chloride/heat shock method. *E. coli* D23 cells had a transformation frequency of 1.1×10^{-5} transformants/cell surviving transformation while *E. coli* D21 cells had a transformation frequency of 1.2×10^{-6} transformants/cell surviving transformation. Our results demonstrate that *E. coli* D23 cells show increased transformation efficiency compared to *E. coli* D21, with 755 and 83 transformants/ μ g DNA respectively, suggesting that one of these mutations or possibly both may contribute to this increase by means of removing the physical barrier to foreign DNA and/or upregulating competence genes to facilitate DNA entry. However, the relative contribution of each of these mutations on the enhanced transformation still needs to be determined.

Transformation is the process in which foreign DNA is taken up from the environment and incorporated into the bacterial genome (7). Competence is the ability of bacterial cells to take up exogenous DNA (7). *Escherichia coli* is not naturally competent but competence can be artificially induced through calcium chloride treatment, which increases the cell membrane permeability (7). A proposed mechanism for competence is that DNA crosses through at zones of adhesion that are rich in lipopolysaccharide (LPS) molecules and where the outer and inner cell membranes fuse with pores in the cell wall (13). LPS are negatively charged molecules and DNA, possessing a like charge, cannot enter the pores due to charge repulsion (13). Thus, divalent cations serve to stabilize these zones and facilitate formation of complexes with phosphates like those of the phosphate backbone of DNA (13). Transformation efficiency using chemical transformation procedures usually yields less than 10^7 transformants or colony forming units (cfu) per μ g DNA (14).

As transformation is linked to membrane-binding events for DNA uptake, it is likely that transformation efficiency is related to cell surface area of *E. coli*. A previous study found no visible relationship between

these two variables when increased surface area resulting from sublethal exposure to nalidixic acid did not result in a higher transformation efficiency (7). In this study, the relationship of transformation efficiency and cell surface area was re-evaluated through the use of a *mreB11* and *sloB1* double mutant, *E. coli* D23, with a smaller surface area, as nalidixic acid used in previous studies may have had detrimental effects on the viability of the cell, posing a confounding variable. The single or multiple mutations in *mreB* contribute to the circular morphology of D23 mutant, while its mutation in *sloB* causes a considerable decrease in doubling time by a factor of 2. It was hypothesized that a strain with a smaller surface area, namely *E. coli* D23, would have a lower transformation efficiency than wild type *E. coli* D21, which has a larger surface area.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 mutant strains D21 (*proA12*, *lac-28*, *tsx-81*, *trp-30*, *his-51*, *strR*, *ampCp-1*) and D23 (*proA12*, *lac-28*, *tsx-81*, *trp-30*, *his-51*, *strR*, *ampCp-1*, *sloB1*, *mreB11*) were chosen on the basis of their size and morphology, and were ordered from the Coli Genetic Stock Center.

Media preparation and growth conditions. Four different types of media were prepared to be used in multiple parts of this experiment. Luria-Bertani (LB) broth was used for growth of *E. coli* wild type

containing pACYC177 vector (12). Modified LB broth was prepared to compensate for the mutations present in *E. coli* strains D21 and D23: L-Tryptophan (Sigma Chemical Company, T-0129) added at 50 µg/ml, 0.2% Bacto Casamino Acids (Difco Laboratories) and 0.2% glucose (Gibco® Laboratories) (6). Modified LB agar plates used to assess the efficiency and frequency of transformation were supplemented analogously to LB broth without the addition of glucose (6). A portion of the modified LB agar plates were supplemented with Kanamycin at final concentration of 50 mg/L to create selective plates required for transformation (12). The cultures in LB broth and modified LB broth were grown at 37°C in a shaker (New Brunswick Scientific Excella E24) set to 200 RPM and colonies on modified LB agar plates were grown at 30°C.

Morphology assessment of bacterial strains. Turbidity measurements were taken for both *E. coli* D21 and D23 strains following their overnight growth. Both cultures were diluted into fresh modified LB broth, with *E. coli* D21 being diluted 1/10 and *E. coli* D23 being diluted 1/5, allowing the cultures to grow for 3 more hours at 37°C. Two wet mount slides were prepared, one containing 10 µl *E. coli* D21 strain, and one containing 10 µl *E. coli* D23 strain. Phase microscopy at 400X total magnification was used to visualize the strains and photos were taken with a Canon SD1100 digital camera.

Plasmid isolation. The plasmid pACYC177, conferring kanamycin resistance was used to select for successful transformants. Following the overnight growth of *E. coli* cells with plasmid, cells were isolated using the Alkaline Lysis Miniprep protocol (12). The concentration of plasmid was measured at A_{260} . The plasmid was diluted to a concentration of 600 ng/µl and 12 ng/µl.

Preparation of competent cells. The competent cells were prepared according to the calcium chloride/heat shock method (8). Six ml of modified LB broth were inoculated with overnight cultures of *E. coli* D21 and D23 strains for final dilutions of 1/100 and 1/50, respectively. The turbidity measurements were taken following 4 hours of growth of new cultures, a time determined by preliminary testing, until final turbidity reached 0.2 OD₆₀₀. Each culture was vortexed and split in half, with the rest of the experiment proceeding in duplicate. The cells were then harvested by centrifugation at 14,000 RPM for 5 minutes and, subsequently, decanting the supernatant. The pellet of each culture was resuspended in 50 µl of 100 mM CaCl₂ and incubated in an ice bath for 30 minutes.

Viability tests. As a pre-heat shock test, 50 µl of competent cells from each culture were mixed with 0.95 ml modified LB broth. The cells were then plated on non-selective modified LB agar plates to ensure that the cells were capable of growing on the agar plates. As a post-heat shock test, 50 µl of competent cells from each culture were heat shocked at 42°C for 30 seconds and, following the mixing with 0.95 ml LB broth, were plated on non-selective modified LB agar plates to ensure that cells were still viable after heat shock. To accommodate for the mutation in *sloB*, the plates containing *E. coli* D21 were incubated for 1 day, while the plates growing *E. coli* D23 strain were incubated for 2 days before the colony count was performed.

Transformation. The two strains of competent cells were each mixed with 0, 25 or 1200 ng/µl of pACYC177 vector. The mixture was then heat shocked at 42°C for 30 seconds and, subsequently, placed on ice to chill. The cells were then mixed with 0.95 ml of modified LB broth and incubated at 37°C for 1 hour. The cultures were then plated on selective modified LB agar plates and, to accommodate for the mutation in *sloB*, the plates containing *E. coli* D21 were incubated for 1 day, while the plates containing *E. coli* D23 strain were incubated for 2 days before the colony counts was performed.

RESULTS

The cellular morphology and size of each strain type. Microscope images (Fig. 1) reveal the distinct morphologies of *E. coli* D21 and D23 strains, with the

D21 strain displaying a typical rod shape and having a larger surface area than the spheres of the D23 strain.



FIG. 1. Comparison of cell morphology between *E. coli* (A) D21 and (B) D23 as observed by phase microscopy.

Competent cell viability. The viability of *E. coli* D21 and D23 mutant strains were determined pre and post heat shock. The difference between *E. coli* D21 and D23 before heat shock was found to be over two-fold, $2.0 \times 10^8 \pm 1.4 \times 10^8$ cfu/ml compared to $9.0 \times 10^7 \pm 3.0 \times 10^7$ cfu/ml, respectively, based on the Poisson method. Similarly, the difference between *E. coli* D21 and D23 after the heat shock was found to be just under two-fold, $2.0 \times 10^8 \pm 1.4 \times 10^8$ cfu/ml and $1.2 \times 10^8 \pm 3.0 \times 10^7$ cfu/ml, respectively, based on the Poisson method. However, the numbers are imprecise, as a result of low colony counts (<30 colonies).

Transformation efficiency. The cells transformed with 25 ng of plasmid produced no viable cells. Consequently, the calculations were based on the cells transformed with 1.2 µg of plasmid. There was a nine fold difference between transformed cells of *E. coli* D21 and D23 mutant strains, resulting in 100 ± 20 and 906 ± 30 transformants/ml, respectively, with both completely enduring the treatment, as evidenced by 100% survival. The error calculation was based on combining the plated volumes and treating the resultant volume as if the colonies were grown on one plate.

With respect to transformation efficiency and frequency, the difference between the strains of *E. coli* D21 and D23 was determined to be nine-fold for both calculations (Table 1).

DISCUSSION

Contrary to our hypothesis, it was found that *E. coli* D23 had a nine-fold higher transformation efficiency and frequency compared to *E. coli* D21. It is worth

Table 1. The effect of the strain type of *E. coli* K-12 on the effectiveness of the transformation performed using calcium chloride/heat shock method.

Strain	Transformation efficiency (transformants/ μ g DNA)	Transformation frequency (transformants/ 10^6 cells surviving transformation)
D21	83 \pm 8	1.2 \pm 0.1
D23	755 \pm 100	11.0 \pm 1.4

noting that the transformation frequency was calculated based on plate counts that contained less than 30 colonies, potentially reducing the accuracy of these results. Additionally, the transformation efficiency in both strains was significantly lower than that of typical *E. coli* strains, a magnitude less than 10^7 as reported by chemical transformation procedures (15). These results may be explained by the fact that the strains used were compromised of a number of additional mutations,

potentially decreasing the strains' transformation potential. Since the total difference in relative transformation efficiencies between the two strains tested (658.5 transformants/ μ g DNA) is much greater than the absolute uncertainty (40.8 transformants/ μ g DNA), however, it is reasonable to conclude that the difference between the strains is statistically significant. Similarly, the transformation frequency is also significant, because it displays the analogous difference between the uncertainty and the discrepancy between the results. Furthermore, this difference is further amplified by the observation that *E. coli* D21 had a concentration twice as high as that of *E. coli* D23, which has a slower growth rate because of its mutation in *sloB*.

There are a number of explanations that can address the differences between the results. With respect to the *mreB11* mutation present in *E. coli* D23, in addition to conferring a spherical shape to the cell (Fig. 1), it also induces a number of changes that may pertain to transformation efficiency. Previous papers have reported that the mutation in *mreB* results in a decreased number of pores, despite maintaining LPS levels comparable to those of wild type (11). This would cause one to expect a decrease in the strain's ability to take up DNA, as DNA is hypothesized to be taken up in zones of adhesion where inner and outer membrane fuse with pores in the cell wall and are rich in LPS (13). Nevertheless, these mutants display an increased transformation efficiency. One possible explanation is based on the observation that these mutants also display increased sensitivities to certain β -lactamases (11). We propose that there is an alternate means of entry for foreign molecules into the cell, which may make up for both the increased permeability

of β -lactam antibiotics as well as DNA. It is known that MreB plays a role in the structural integrity of the cell (10). Therefore, mutations in this gene may simply remove this physical barrier such that foreign elements including DNA, proteins and some chemicals are more accessible to the cell. Furthermore, other factors including LPS, murein sacculus and other components may contribute to the barrier function (5).

Additionally, a mutation present in the *sloB* gene of *E. coli* D23 may similarly result in increased transformation efficiency. While only limited research has been done on *sloB* in *E. coli*, this gene has been well researched in the *Streptococcus* genus. It is, nevertheless, possible to assume that the functions of *sloB* in both genera are similar because the mutants appear to possess the same phenotype. In *Streptococcus mutans*, for example, *sloB* encodes a component of the ATP-binding cassette (ABC) transport system, namely an integral membrane protein (9). In bacteria, ABC transporters are important virulence factors, because, amongst other functions, they play roles in nutrient uptake (2). Being limited in nutrient uptake capabilities as a result of a mutation in *sloB* in *E. coli* D23 essentially creates a nutrient limiting environment for the bacteria despite being grown in rich media, which has been shown to increase transformation efficiency in bacteria by upregulating competence genes, such as those present in the *crp* operon (1).

With respect to the research which has been done on *sloB* in *E. coli* concerning its position in the genome, it has been shown that the gene is located in the *cai* operon of *E. coli* K-12. The genes, *caiE* and *caiF*, and the approximate location of *sloB*, appear to be implicated in amine catabolism (4). Hence, as a result of not being able to utilize amines as a nutrient source, *E. coli* D23 appears to be in nutrient-limiting conditions, which, as shown, may contribute to its transformation efficiency.

While the exact process of chemically induced transformation is still unknown, this study has attempted to evaluate the mutations in *sloB* and *mreB* in the context of transformation. Our discussion shows that smaller surface area does not correlate to lower transformation efficiency. Potentially, one of these two mutations or both of them might contribute to higher transformation efficiencies by means of removing the physical barrier and/or upregulating competence genes in *E. coli*. However, it is yet to be empirically determined the relative contributions of each of these genes to transformation since the effects of each mutation were not studied in this experiment.

Additionally, although *E. coli* D23 showed an increase in the transformation efficiency in comparison to *E. coli* D21, its transformation efficiency is still significantly lower than that of wild type *E. coli* K12. Therefore, it follows that *E. coli* D23 should not be

used to conduct transformations as part of other experiments since it has a number of mutations, which decreases its transformation ability. Alternatively, we can use *E. coli* K12 strain with only *mreB11* and *sloB1* mutations in order to see the effects of the nature of mutations on transformation efficiency clearly.

FUTURE EXPERIMENTS

Due to complications imposed by several additional mutations in both *E. coli* D21 and D23, it would be worthwhile to construct a strain defective in only the *sloB* gene to evaluate its absolute contribution to transformation efficiency as a strain defective in only the *mreB* gene is not viable long enough for the transformation process. The transformation efficiency of the cell with the mutation in *sloB* mutant can be compared to both the wild type and a constructed double mutant (with respect to *sloB* and *mreB*), and if the *sloB* mutant has a comparable transformation efficiency with the double mutant, then it can be concluded that *sloB* is the major contributor to the increased transformation efficiency witnessed in the above experiment, whereas if the *sloB* mutant shows no significant difference in transformation efficiency compared to the wildtype while the double mutant does, it would be possible to conclude that the *mreB* gene plays a larger role in increasing transformation efficiency. Alternatively, to examine the effects of surface area on the transformation efficiency, it is possible to utilize temperature-sensitive mutants, such as *rodA*(Ts) and *pbpA*(Ts) mutants that grow as rods at 30°C and as spheres at 42°C (3). Both mutants are able to grow and divide at almost normal growth rates, therefore, they provide an ideal test system (3). The mutants can be grown to log phase at their respective temperatures and then electroporation can be performed instead of the Hannahan protocol, because the former does not involve leaving the cells on ice for 30 minutes to facilitate the binding of the DNA, a step during which the spherical cells might revert to rod-shaped cells. By comparing the transformation efficiencies of these mutants in their coccal form to their respective rod-shaped form, it is possible to deduce whether surface area is an important factor in transformation. Furthermore, the coccal cells are about four to six times greater in volume than the rod-shaped cells with an average diameter close to the length of the rod-shaped cells (3). Consequently a higher transformation efficiency in coccal cells than rod-shaped cells will indicate that a larger cell surface area contributes to a higher transformation efficiency and vice versa.

ACKNOWLEDGEMENTS

We would like to thank Dr. William Ramey and Kristen Schurek for their time, advice, and guidance throughout this experiment. Gratitude is also extended to the media room staff. Furthermore, we would like to thank the Department of Microbiology and Immunology, UBC for funding this project.

REFERENCES

1. **Cameron, A. D., and R. J. Redfield.** 2006. Non-canonical CRP sites control competence regulons in *Escherichia coli* and many other gamma-proteobacteria. *Nucleic Acids Res.* **34**:6001-6014.
2. **Davidson, A. L., and J. Chen.** 2004. ATP-binding cassette transporters in bacteria. *Annu. Rev. Biochem.* **73**:241-268.
3. **Donachie, W. D., and K. J. Begg.** 1989. Cell length, nucleoid separation, and cell division of rod-shaped and spherical cells of *Escherichia coli*. *J. Bacteriol.* **171**:4633-4639.
4. **Eichler, K., A. Buchet, R. Lemke, H. P. Kleber, and M. A. Mandrand-Berthelot.** 1996. Identification and characterization of the *caiF* gene encoding a potential transcriptional activator of carnitine metabolism in *Escherichia coli*. *J. Bacteriol.* **178**:1248-1257.
5. **Gustafsson, P., K. Nordström, and S. Normark.** 1973. Outer penetration barrier of *Escherichia coli* K-12: Kinetics of the uptake of gentian violet by wild type and envelope mutants. *J. Bacteriol.* **116**:893-900.
6. **Haggstrom, B. W., and S. Normark.** 1975. Genetic and physiological analysis of an *envB* spherelike mutant of *Escherichia coli* K-12 and characterization of its transductants. *J. Bacteriol.* **123**:75-82.
7. **Hatami, N., G. Naderkhani, N. Shah, and B. Young.** 2004. The effects of pretreatment of competent cells with nalidixic acid on efficiency of chemically-induced transformation in *Escherichia coli* B23. *J. Exp. Microbiol. Immunol.* **12**:84-87 [Online].
8. **Hinze, E.** 2007. Microbiology 323 microbiological techniques II laboratory manual. University of British Columbia.
9. **Kitten, T., C. L. Munro, S. M. Michalek, and F. L. Macrina.** 2000. Genetic characterization of a *Streptococcus mutans* Lral family operon and role in virulence. *Infect. Immun.* **68**:4441-4451.
10. **Kruse, T., J. Bork-Jensen, and K. Gerdes.** 2005. The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. *Mol. Microbiol.* **55**:78-89.
11. **Oppezzo, O. J., B. Avanzati, and D. N. Anton.** 1991. Increased susceptibility to beta-lactam antibiotics and decreased porin content caused by *envB* mutations of *Salmonella typhimurium*. *Antimicrob. Agents Chemother.* **35**:1203-1207.
12. **Sambrook, J., T. Maniatis, and E. F. Fritsch.** 1982. *Molecular Cloning, A Laboratory Manual.* Cold Spring Harbor Laboratory, United States of America.
13. **Sarkar, S., S. Chaudhuri, and T. Basu.** 2002. Mechanism of artificial transformation of *E. coli* with plasmid DNA –Clues from the influence of ethanol. *Curr. Sci.* **83**:1376-1380.
14. **Zeng, W., Y. Deng, Z. Yang, W. Yuan, W. Huang, C. Zhu, Y. Bai, Y. Li, Z. Peng, Y. Wang, Y. Zhu, M. Liu, and X. Wu.** 2006. High transformation efficiency of *Escherichia coli* with plasmids by adding amino modified silica-nanoparticles. *Biotechnol.* **5**:341-343 [Online].