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Deleting the *hns* Gene for the H-NS Protein in *Escherichia coli* Reduces the Transformation Efficiency Following the Heat Shock Transformation Protocol

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One commonly used, yet little understood method for transferring DNA into cells is the temperature shock transformation method (TST) that exposes competent bacteria to successive temperatures (4°C and 42°C). This study tested the effect of the gene for the heat-stable nucleoid structuring protein (H-NS) on the efficiency of the TST method in an effort to find a way to improve the transformation efficiency of a cell. H-NS is a cold shock protein (CSP) that has demonstrated preferential binding of horizontally acquired genes. A comparison of the transformation efficiency of the parental strain and the *hns* knockout suggested that the *hns* gene is involved during TST and that the absence of the gene decreased the transformation efficiency of the cell. The absence of the *hns* gene affected the phenotype of individual colonies as well as the ability of cells to recover from cold temperature incubations.

Bacterial transformation is a powerful tool in biotechnology, allowing desired DNA sequences to be inserted into bacteria for the production of proteins. While transformation does occur naturally in certain species, the manipulation of bacterial genomes has produced strains that produce a higher number of transformants with less DNA (1). These strains have become the standard for transformation in laboratories around the world, yet the exact mechanisms they use to take up DNA remains unknown (9). These strains do contain mutations that increase the number of DNA binding proteins on the cell surface (9) permitting more DNA to enter the cell. Additionally, certain protocols have been found to further increase transformation efficiency (6).

The temperature shock method involves an incubation at low temperature (0°C) followed by a quick exposure to a high temperature (42°C) before being returned to the low temperature environment (2). When a cell is exposed to extreme temperatures like this several cascades are activated (4). These cascades produce heat shock proteins (HSP) such as chaperons and proteases, and cold shock proteins that can interact with DNA such as helicases, nucleases and ribosome associated components (10). While the proteins that are part of these cascades have been identified, their specific interactions with extracellular DNA are unknown. Of particular interest is the reason why transformation efficiency increases when the cells are subjected to the initial temperature shock step from 0°C to 42°C. Proteins that are candidates for binding the extracellular DNA would need to be in high abundance during the preliminary step and be able to survive the jump to 42°C.

A well characterized CSP is the heat-stable nucleoid structuring protein, H-NS (5) which aids in the stabilization of DNA by formation of nucleoids and also assists in transcriptional regulation activities (3). H-NS would be generated in large amounts during the 0°C incubation and would persist during the spike to 42°C. Additionally, research has suggested that H-NS preferentially binds horizontally acquired DNA sequences during regular cellular operation (7). It is reasonable to think that H-NS may also preferentially bind the extracellular DNA before it is incorporated into the genome and becomes a horizontally acquired gene.

To determine if the H-NS protein is involved in the temperature shock transformation process, the transformation efficiency of an H-NS knock-out *E. coli* strain (JW1225-2) was compared to that of the parental strain (BW25113).

MATERIALS AND METHODS

Bacterial strains and plasmids. The *hns* knockout *E. coli* strain JW1225-2 and its parental strain BW25113 were ordered from the Coli Genetic Stock center. Both strains are F- and contain mutations Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568 and hsdR514 with the JW1225-2 strain having the additional Δhns-746::kan mutation. All samples using the JW1225-2 strain are labeled with JW and all samples using the BW25113 strain are labeled with BW. The pBR322 plasmid containing an ampicillin resistance gene was obtained from the MICB 421 plasmid collection.

Culture Conditions for strain growth. Both the JW1225-2 and BW25113 strains were grown up in 10 ml Luria-Bertani (LB) media at 37°C. Selection after transformation was achieved using LB plates containing 50 ug/ml ampicillin (LB AMP) (Sigma; Catalog No. A01665G Lot No. 029K05521) with incubation at 37°C.

Induction of competency: Cells were made competent using the method outlined on p. 1.116 of Molecular Cloning a Laboratory Manual (8). All cell pelleting was done at 4100 RPM using a Sorvall SS-34 rotor. The pellet was resuspended in 4 ml ice cold 0.1 M $CaCl_2$ before 7 aliquots were made using 540 ul of sample and 160 ul of 50% glycerol. The remaining 220 ul of cells were mixed with 65 ul of 50% glycerol and stored at -80°C. This glycerol-treated stock was then frozen at -80°C for use later.

Transformation of cells: Cells were transformed using the method outlined on p.1.118 of Molecular Cloning a Laboratory Manual (8) with several exceptions and alterations. Each strain was transformed with 5 ng, 25 ng, 50 ng, 500 ng and 1 ug of plasmid. Each transformation reaction contained 50 ul of cells and 10 ul of plasmid in a 1.5 ml Eppendorf tube. Duplicates were made for each strain at each plasmid concentration. A negative control containing no plasmid DNA was also made for each strain. Heat shock was done at 42°C for 120 seconds in a non-circulating water bath but with the samples constantly being moved around the bath. Recovery was done with 450 ul of pre-warmed LB broth in 10 x 100 mm culture tubes placed in a 37°C shaker for 45 minutes at 75 RPM. Transformation mixtures were plated on both LB and LB AMP plates. LB AMP plates were inoculated. The initial cell density for each strain was calculated assuming an OD_{600} of one equals approximately 10^9 cells/ml. From this it was determined that JW1225-2 samples contained 900,000 cells/ml and BW25113 samples contained 628,000 cells/ml. Samples were diluted $\frac{1}{10000}$ through serial dilution and LB plates were inoculated with volumes giving approximately 125 cells per plate for JW1225-2 and 180 cells per plate for BW25113. Volumes less than 20 ul were made up to 20 ul using autoclaved distilled water. Cells were plated using the spread plate technique and incubated for 16 hours at 37°C.

Cell counting. Evenly spread plates containing fewer than ~50 colonies were counted as is. Plates containing more than ~50 colonies were divided into sections and one section counted, and then the number multiplied by the total number of sections.

RESULTS

Initial growth of strains. Strains were grown up in liquid medium until the BW25113 strain had an OD_{600} of 0.371 and the JW1225-2 strain had an OD_{600} of 0.363. This represents approximate cell densities of 3.7 x 10^9 cells/ml and 3.6 x 10^8 cells/ml for BW25113 and JW1225-2 strains respectively.

Phenotypic observations. During the initial growth stage of the induction of competency protocol the JW1225-2 strain took 170 minutes to reach the ~0.35 OD_{600} while the BW25113 strain took 105 minutes. When comparing individual colonies isolated on LB agar plates, JW1225-2 bacteria were approximately 50% smaller than BW25113 bacteria.

Transformation efficiencies. For both strains, the number of transformants increased with plasmid concentration (Fig. 1), however the BW25113 strain consistently had a higher number of transformants at each plasmid concentration. When corrected for the total number of viable cells in a sample, the BW25113 strain still demonstrated a higher transformation efficiency than the JW1225-2 strain (Fig. 2). The saturation point for each strain was not reached due to a lack of highly concentrated plasmid stock.

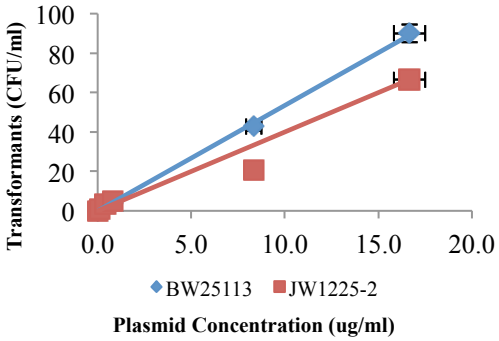

FIG 1: Effect of plasmid concentration on the efficiency of transformation Trend line shown to highlight difference between strains (typical data series shown).

DISCUSSION

From the initial growth data, it appeared that the JW1225-2 strain had a harder time recovering from cold temperatures than the BW25113 strain. This difference can be attributed to the lack of the H-NS protein which is known to stabilize DNA and assist in cell recovery from cold temperature exposure. The lack of *hns* gene was likely also responsible for the altered phenotype of individual colonies. Alternatively this could be the result of the processes that the JW1225-2 cells underwent to create the *hns* gene knockout.

Without reaching the saturation plateau for each strain, it remains possible that the difference in transformation efficiency was due to availability of plasmid as opposed to the H-NS protein. When the initial number of viable cells in each sample is taken into account, the difference between the strains persisted (Fig. 2). The relationship was not as linear as the direct comparison of plasmid concentration and number of transformants, indicating that each strain reacts differently when exposed to similar plasmid concentrations.

Interestingly, in both comparisons the difference in transformation efficiency between the two strains was much more exaggerated at higher plasmid concentrations. This suggests that the *hns* gene was of minimal importance when there was only a small amount of plasmid present. If the H-NS protein was

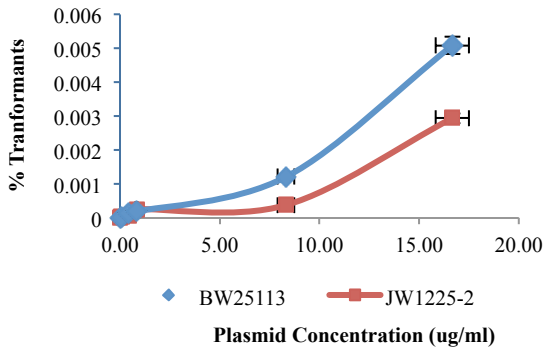

FIG 2: Efficiency of transformation at various plasmid concentrations (typical data series shown).

responsible for directly binding onto extracellular DNA during the temperature shock transformation process, it would be reasonable to think that the parental strain would have considerably more transformants than the knockout strain at lower plasmid concentrations where the likelihood of DNA 'falling' into the cell by chance is much lower. However, an extensive number of replicates would be needed to provide enough data to overcome the masking effect of the low numbers of transformants for both strains at these low concentrations.

The use of the empty pBR322 plasmid provided a stable, comparable basis for evaluating transformation efficiency between the strains. However, the plasmid may not contain the region of DNA that the H-NS protein recognizes when associating with horizontally acquired genes and therefore any difference in transformation efficiency between the two strains could not be attributed to the H-NS protein. This would suggest that the observed difference in transformation efficiency is more likely attributed to the ability of the BW25113 strain to recover from the heat shock protocol more effectively than the JW1225-2 strain.

CONCLUSION

The difference in size and initial growth time between the two strains provides evidence that the *hns* gene is involved in regular cellular functions. Given the transformation efficiencies of the two strains at various plasmid concentrations, the BW25113 strain generated more transformants than the JW1225-2 strain at similar plasmid concentrations and initial numbers of viable cells. This suggests that the *hns* gene was involved at least partly with the temperature shock transformation process without being an essential component.

FUTURE EXPERIMENTS

In the future, when looking at the concentration of plasmid and its affect on transformation efficiency, it would be advisable to use several very high concentrations to achieve saturation. That way any difference in transformation efficiency at saturation between the two strains can be attributed to the missing gene and not the availability of plasmid.

To determine if the difference in transformation efficiency was due to each strains capacity for recovery from the heat shock protocol, a control experiment could be run where a known number of cells for each strain were submitted to the heat shock protocol and the recovery of the cells monitored on non-selective media. Additionally, it may prove interesting to use plasmids with different gene inserts and DNA sequences to identify if the function of the H-NS protein during the temperature shock transformation process is dependent on the sequence of the DNA being transferred. This could be further improved if a common sequence was identified in horizontally acquired genes which H-NS is shown to preferentially bind.

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