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The Effects of Arginine, Lysine and Ornithine on the Transformation of Plasmid pBR322 in *E. coli* HB101

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The effects of arginine, lysine and ornithine on the transformation of plasmid pBR322 were tested against the known effects of $CaCl_2$ on transformation in *Escherichia coli* strain HB101. In the first experiment, four different sets of the strain were transformed, each using a different ion to induce transformation with 100 ng of plasmid present. The second time around, 12 different sets of the strain were transformed in groups of four, each with a different ion. The first group was transformed with 10 ng of plasmid, the second with 100 ng of plasmid, and the last with 500 ng of plasmid. The results showed that arginine and lysine could induce transformation but not as well as $CaCl_2$ and they also showed that the more plasmid present, the better the transformation frequency in the case of $CaCl_2$.

It is possible to transform *Escherichia coli* cells in the cold when treated with divalent cations. There is a procedure for this process known as the Hanahan protocol (2) and it consists of exposing *E. coli* cells and the DNA (to be taken up) to an ice-cold salt solution, such as a $CaCl_2$ solution. The entire solution is then heat-shocked and the DNA is transported into the cell (3). This procedure only has a transformation frequency that peaks around 0.01%, but it is a large increase over not treating the cells at all.

It is thought that during this treatment, the divalent cations in solution, such as Ca^{2+}, bind to the negative charges of the DNA, therefore neutralizing it, while at the same time binding to random sites on the E. coli cell surface. By holding the DNA close to the cell surface, the chance that the DNA will be taken up by the cell is greatly increased.

Based on this presumed theory, a question that is asked is: why wouldn't natural, organic, divalent cations be just as efficient if not more efficient for use in the transformation process? Candidate cations would include lysine, arginine, putresine and natural polybasic polypeptides, or artificial polypeptides such as polylysine. The above amino acids would be candidates because, while biologically significant binding of these molecules alone to DNA has not been shown, the positive charge on these amino acids is thought to be responsible for providing significant non-covalent interactions with the negative charge on DNA in the binding of DNA by DNA binding proteins such as the lac repressor (1) and several histones (4). Polylysine has long been known to condense DNA through electrostatic interactions, which is thought to bring about a hydrophobic collapse as water is excluded (website of the University of Birmingham [http://web.bham.ac.uk/can4psd4/nonviral/condense.html]). This may mediate passage of the DNA through the membrane.

If it can be shown that these molecules provide a more efficient transformation, it may not only be useful for lab purposes, but may also provide some insight as to how some of these natural molecules may be involved in helping with bacterial transformation that is known to occur in nature. For example, putresine would be expected to be available to serve this function in decaying meat, while polylysine is a common preservative used in meats. Arginine and lysine could be expected to be present in many environments supporting bacterial growth.

Since polylysine and putresine were unavailable at the time the experiment was performed, the divalent cation ornithine was chosen to replace these molecules in the experiment. Ornithine is manufactured through the body's metabolism of arginine during the production of urea. It has a structure similar to that of lysine and could be expected to be present in meats, dairy, fish and eggs (the Healthwell website [http://www.healthwell.com]).

MATERIALS AND METHODS

E. coli plasmid pBR322 was used for the first experiment and was obtained using the Promega "Magic" Minipreps Purification System kit. The concentration was then calculated using absorbance readings at A_{260}, A_{270}, and A_{280} (5). The transformation was then performed based on the following procedures (3):

Set The bacterial strain HB101 grown to OD_{600}=0.2 in 10 mL of Luria broth on ice to chill for 5 minutes. Add 1 mL of the chilled bacterial culture to four separate chilled microfuge tubes and centrifuge for five minutes and then discard the supernatant. Add 50µL of one of either 100 mM chilled arginine, lysine, ornithine or $CaCl_2$ ions to one of the four microfuge tubes with cell pellets and vortex. Place all four microfuge tubes on ice for 30 minutes. Mix each microfuge tube of cells with 100ng of the plasmid and then place on ice for another 10 minutes. Heat the cells in a 42°C water bath for 30 seconds, and then ice the cells for 2 more minutes. Add 1 mL of Luria broth to each set of cells and incubate at 37°C for

an hour to allow for recovery. After an hour, plate the dilutions 10^{-5}, 10^{-6}, and 10^{-7} on Luria broth plates, and 10^{-1}, 10^{-2}, and 10^{-3} on Luria Broth-ampicillin plates from each set of cells. Incubate the plates at 37°C for 24 hours and count the colonies

The second time the experiment was performed, the same E. coli plasmid was obtained using an alkaline lysis method. The concentration was then calculated again using absorbance readings at A_{260}, A_{270}, and A_{280} (5), and the transformation was then performed based on the procedures outlined above (3), with a minor addition. The transformations were performed using the same set of ions to make four different cultures of competent cells, but this time around, the procedure was completed three separate times using different amounts of the plasmid. First it was completed using 10 ng of plasmid, then with 100 ng of plasmid and lastly with 500 ng of plasmid.

RESULTS

In the first round of this experiment, the plasmid concentration was measured and found to be 30 μg/mL. For the transformation, 3.3 μL of DNA was used to provide the required 100 ng of DNA.

When the plates were counted, several colonies were observed on all plates. There was no perceivable difference between the plates containing Luria broth and the plates containing both Luria broth and ampicillin. In fact, some plates showed confluent lawns of colonies. Subsequently, the original strains were tested for inherent resistance to the ampicillin. Results showed that the HB 101 strain was sensitive to ampicillin. Next, the ampicillin itself was tested and was shown to be inactive when compared to a second bottle of ampicillin.

The second time the experiment was performed, plasmid concentration was measured and found to be 4.18 mg/mL. For the transformations using 500ng, 2.99 μL of plasmid was added from a 1-in-50 dilution. For the transformations using 100ng, 1.20 μL of plasmid was also added from a 1-in-50 dilution. Finally, for the transformations using 10ng, 1.20 μL of plasmid was added from a 1-in-500 dilution.

In plates containing only Luria broth, dilutions at 10^{-7} produced too few colonies, while dilutions at 10^{-5} produced too many colonies to count. Dilutions at 10^{-6} yielded colonies within the countable range (Tables 1, 2, and 3). An estimate from the average of the Arginine, Lysine, and $CaCl_2$ plates show an original bacterial concentration of 1.53×10^8 cells per mL.

A few transformants were observed in the plates containing various amino acids, but none were in the countable range (Tables 1, 2, and 3). Most contained less than 30 colonies. In fact, ornithine appeared entirely ineffective in transforming any bacteria: no colonies were counted here. However, the plates containing $CaCl_2$ showed successful transformations of approximately 310 transformants per mL with 100 ng of plasmid DNA (Table 2), and 520 transformants per mL with 500 ng of plasmid DNA (Table 1). This yielded a transformation frequency of about 1.57×10^{-4}% for 100 ng of DNA and 3.10×10^{-4}% for 500 ng of DNA.

Table 1 – Number of colonies of transformants and non-transformants using 500 ng of pBR322

	Luria Agar		Luria Agar + Ampicillin	
	10^{-7} FDP*	10^{-6} FDP*	10^{-2} FDP*	10^{-1} FDP*
Ornithine	22	149	0	0
Arginine	12	167	0	2
Lysine	18	136	0	4
$CaCl_2$	25	168	3	52

* FDP is the final plated dilution

Table 2 – Number of colonies of transformants non-transformants using 100 ng of pBR322

	Luria Agar		Luria Agar + Ampicillin	
	10^{-7} FDP*	10^{-6} FDP*	10^{-2} FDP*	10^{-1} FDP*
Ornithine	23	TNTC	0	0
Arginine	11	244	0	1
Lysine	25	220	0	0
$CaCl_2$	18	162	4	31

* FDP is the final plated dilution

Table 3 – Number of colonies of transformants non-transformants using 10 ng of pBR322

	Luria Agar		Luria Agar + Ampicillin	
	10^{-7} FDP*	10^{-6} FDP*	10^{-2} FDP*	10^{-1} FDP*
Ornithine	4	31	0	0
Arginine	10	108	0	0
Lysine	5	43	0	1
$CaCl_2$	6	127	0	7

* FDP is the final plated dilution

DISCUSSION

During our first attempt at producing transformed *E. coli,* extensive growth was observed on both ampicillin treated and untreated plates. This anomaly was likely to have one of two causes: either the *E. coli* strain used had become resistant prior to transformation, or the ampicillin used to make the plates was inactive. The *E. coli* used in this experiment was tested for susceptibility to ampicillin after the experiment and was found to be susceptible. This suggests that the ampicillin was in fact inactive, which is probable given the age of the ampicillin stock used (it was originally opened in 1982).

In our second attempt at obtaining transformants, the inactive ampicillin was replaced. Also, new experimental conditions were added to the study allowing us to observe transformation efficiency given by the various reagents used. The concentrations of these reagents in our experiment were comparable to the concentrations of Ca^{2+} commonly used in established methods in the literature. However, transformation rates for Ca^{2+} were significantly lower than those found in the literature (max transformation of 3.10×10^{-4}% compared to 0.01%) (2). This may be accounted for by our deviation from established protocol, which was necessitated by practical inconvenience. Specifically, we were not able to chill the rotary of the centrifuge prior to centrifugation, which would normally be accomplished by simply refrigerating the rotor on the centrifuge prior to running the experiment. Failing to do this may have allowed the temperature of the suspension to rise above optimal levels, leading eventually to decreased transformation efficiency. Nonetheless, the efficiency of transformation using Ca^{2+} was the highest of all the compounds used, ranging from 1.57×10^{-4}% to 3.10×10^{-4}%. The highest efficiency for the Ca^{2+} condition was at 500 ng of DNA.

The use of Ornithine did not produce any transformants at any concentration of DNA. The relative significance of this is that of the four conditions used, this compound is the least effective. However, absolute conclusions based on the used of Ornithine, outside the context of comparison to the other conditions, is likely to not be valid as we did not include a negative control group in the experiment. For example, it may be possible that Ornithine somehow prevents transformation. This could be tested by repeating the experiment and adding an Ornithine + $CaCl_2$ condition and comparing the efficiency to $CaCl_2$ alone.

Arginine and Lysine gave fairly similar results, with the results from assays using both compounds giving results that are statistically suspect in that they had plate counts of less than 30 colony forming units (cfu). Again, it is difficult to draw conclusions on the effects of these compounds on transformation efficiency, other than to say that they give efficiency that is significantly less than Ca^{2+}. It is relevant to state, however, that the transformation efficiency given by these compounds may be understated in light of the fact that the Ca^{2+} condition, which may be considered a positive control, gave lower transformation efficiencies than those reported by previous studies employing the same method.

The above results, taken together, imply that naturally occurring divalent cations are not as efficient as Ca^{2+} at mediating the transport of DNA across a cell membrane, at least under the conditions used in this study. Perhaps the negative charge on the carboxyl group prevents this, or perhaps the process is sterically hindered. Alternatively, repeating the experiment with different concentrations of cations, higher plasmid concentrations, or a different heat shock procedure might increase the efficiency of transformation with the divalent cations. It should be noted that these conclusions can not be taken as absolute in the absence of a negative control. Our evidence indicates that the compounds investigated in this experiment would not be expected to facilitate transformation as well as $CaCl_2$ in a laboratory setting. However, these cations may play a role in facilitating transformation in a natural environment.

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Appendix

Sample Calculations
Bacterial Concentration:

Arginine: (167+244+108) / 3 = 173
Lysine: (43+220+136) / 3 = 133
$CaCl_2$: (127+162+168) / 3 = 152

average colonies = (173+133+152) / 3 = 153
original concentration = 153 x 10^6 = 1.53 x 10^8 cells / mL

Transformation Frequency:

$CaCl_2$:
i)100 ng of plasmid:
original concentration = 162x10^6 = 1.62x10^8 cells / mL

transformed colonies = 31
transformant concentration = 31x10^1 = 3.10x10^2 transformants / mL

transformation frequency = (3.10x10^2) / (1.62x10^8) = 1.57x10^{-6} x 100% = 1.57x10^{-4}%

ii) 500 ng of plasmid:
original concentration = 168x10^6 = 1.68x10^8 cells / mL

transformed colonies = 52
transformant concentration = 52x10^1 = 5.20x10^2 transformants / mL

transformation frequency = (5.20x10^2) / (1.68x10^8) = 3.10x10^{-6} x 100% = 3.10x10^{-4}%

Plasmid Concentration

Spectrophotometer Readings
Experiment 1:
No dilution:
A260 = 0.61
A270 = 0.476
A280 = 0.224

reading of 1.00 at A260 ≅ 50 μg/mL
reading of 0.61 ≅ 30 μg/mL in our sample
for 100 ng, add 3.3 μL of plasmid sample

Experiment 2:

diluted 1/50:

A260 = 1.67
A270 = 1.308
A280 = 0.743

reading of 1.00 at A260 ≅ 50 μg/mL

reading of 1.67 ≅ 83.5 μg/mL in a 1/50 dilution
83.5 x 50 = 4.18 mg/mL in original dilution

for 500ng, add 2.99 μL of plasmid (from a 1/50 dilution)
for 100ng, add 1.20 μL of plasmid (from a 1/50 dilution)
for 10ng, add 1.20 μL of plasmid (from a 1/500 dilution)