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Ability of *Escherichia coli* to Distinguish Between Self and Foreign DNA as Demonstrated by Trends in Transformation Efficiency

DUSTIN CLASSEN, NICOLE M. LEE-SON, CLIFFORD LIN, JEN M. MENZIES, AND DAWN S. YEW

Department of Microbiology and Immunology, UBC

Bacterial transformation is a widely used technique, yet its underlying mechanisms are not fully understood. This study investigated the effect of contaminating DNA on the transformation efficiency in two different strains of *Escherichia* coli (HB101 and B23) and attempted to determine whether *E. coli* is able to distinguish during transformation between its own (self) DNA and DNA originating from another strain or organism (foreign). First, p328.5 was isolated from *Escherichia coli* HB101 then transformed into *E. coli* B23 via $CaCl_2$ treatment or electroporation. Isolation of p328.5 from HB101 was relatively easy. Subsequent transformation into B23 failed with $CaCl_2$ treatment but was successful by electroporation. Secondly, we contaminated plasmid p328.5 DNA, isolated from the transformed B23 and HB101 cells, with genomic DNA originating from one of *E. coli* HB101, *E. coli* B23, or *Bacillus subtilis* WB746 and observed the effect on transformation efficiency in HB101 and B23. It was found in both strains that the presence of genomic DNA significantly affects transformation efficiency, in some cases reducing it by 50% and in other cases down to undetectable levels. It could not be concluded exactly what effect different origins of genomic DNA has on transformation, but there are indications that genomic B23 DNA has an increased ability to interfere with transformation in both HB101 and B23 strains of *E. coli.*

Transformation is a process commonly used for the incorporation and expression of cloned gene sequences in bacteria (9). However, while this technique is essential to many experimental protocols, it is relatively inefficient. There are many potential factors that can influence the efficiency of transformation including, but not limited to: the incubation conditions, the concentration of the cloned sequence to be inserted, the relative competence of the recipient cells, and the presence of contaminating DNA (1)

In a previous study (9), it was found that the transformation frequency of *Escherichia coli* HB101 with p328.5 plasmid decreased in the presence of contaminating genomic DNA of two types: foreign or self. Foreign genomic DNA can be defined as chromosomal DNA that originates from a source other than the bacterial strain that is being transformed. In the context of that experiment, foreign chromosomal DNA originated from *E. coli* B23 (wild-type K-12) and *Bacillus subtilis* WB746. Chromosomal DNA originating from the same species and strain as the bacteria being transformed is referred to as self-DNA. In fact, it was observed that the presence of foreign genomic DNA led to a greater decrease in transformation efficiency than if the contaminating DNA originated from the self *E. coli* HB101 strain (9). Transformation efficiency in both cases was lower than plasmid DNA only.

It is possible that the greater decrease in transformation frequency in the presence of foreign genomic DNA might be due to greater competition with the plasmid for binding sites on the cell compared with self-DNA, interfering to a greater extent with the integration of plasmid into the cell. Thus, we hypothesize that *E. coli* must have mechanisms that enable the cells to differentiate between foreign and self genomic DNA, which, in turn, interferes with plasmid uptake. In fact, it has been demonstrated that some gram-negative organisms, such as *Neisseria gonorrhoeae* and *Haemophilus influenzae*, possess mechanisms that enable cells to discriminate between homologous and heterologous DNA (2, 3). However, in these bacteria, these mechanisms operate to promote the preferential uptake of homologous DNA (which includes both self DNA and DNA from taxonomically related species) and to disregard DNA from disparate organisms (2, 3). It has also been shown that there are genetic loci (for example, the *hsdRMS* locus) for systems to discriminate between self and foreign DNA(5). These systems involve the recognition of specific methylation sites on DNA to determine whether the DNA is self or foreign.

In this paper, we investigated the transformation efficiency of both *E. coli* HB101 and *E. coli* B23 strains with the p328.5 plasmid in the presence of both self and foreign genomic DNA originating from *E. coli* HB101, *E. coli* B23, or *B. subtilis* WB746. This experiment sought to verify the integrity of the findings of the aforementioned study (9) in order to confirm that the existing pattern of transformation frequencies could be applied to a different strain of *E. coli* and to prove that this was not simply a trend specific to HB101. This work was performed as the important

preliminary phase of an investigation that could ultimately lead to an examination into the possible mechanisms underlying the ability of *E. coli* to differentiate between foreign and self DNA.

MATERIALS AND METHODS

Bacterial Strains and Media. HB101 and B23 strains of *E. coli* and the WB746 strain of *B. subtilis* were used in this experiment. Cells were grown in rich media (Luria broth) (7) overnight, diluted 1/100 the next day and grown to an appropriate OD_{600}. HB101 was the only strain to contain the p328.5 plasmid, a pBR322 derivative (9). This plasmid was amplified and isolated so that a portion could be used to transform into B23 and another portion for later transformation in the presence of genomic DNA into HB101. Ampicillin was used to select for plasmid-carrying strains at a concentration of 50 ng/mL. Genomic DNA was isolated from HB101, B23, and WB746 for use as described below.

Transformation of p328.5 into B23. As mentioned previously, p328.5 was available only from HB101. However, the origin of the plasmid DNA can affect transformation frequencies (8). Since this study investigates the effect of genomic DNA on transformation frequency in two different strains of *E. coli*, it was necessary to obtain plasmid of identical origin as the host in both cases to eliminate that variable. The p328.5 plasmid was isolated from HB101 as described in the next section. Transformation of the isolated p328.5 into B23 was performed using both the Hanahan ($CaCl_2$) (6, 9) method and the electroporation method (6). Competent cells were prepared via treatment with 50 mM $CaCl_2$ and then mixed with p328.5 DNA as described previously (9). To prepare cells for electroporation, cultures were grown to an OD_{600} of 0.5 and washed with cold distilled water. The cells were transferred to a chilled electroporation cuvette, mixed with p328.5 DNA and pulsed at 2.5 kV, 25 μF (6). Transformed cells were grown on antibiotic-containing nutrient agar to select for successfully transformed colonies.

Isolation of pBR328.5 from HB101 and B23. Cultures were grown to late-log phase (OD_{600} ~0.7). At this point, 100 ng/mL chloramphenicol was added to the culture medium and grown overnight in a 37˚C shaking water bath to amplify p328.5. Plasmid isolations were carried out using a DNA Isolation MiniPrep Kit (Promega manual, Promega Co., Madison, WI.) as per manufacturer's instructions. Isolated plasmid DNA was stored at 4˚C until ready to use.

Isolation of genomic DNA. Chromosomal DNA was isolated from two strains of *E. coli*. (B23 and HB101) and also from *B. subtilis* (WB746). Cultures were grown overnight and then centrifuged @ 4,000 rpm (2,600 x g). The supernatant was discarded and the pellet processed to extract genomic DNA by phenol phase separation (A. Comeau, personal communication). Extracted DNA was stored at 4˚C until ready to use.

Plasmid Transfomation in the Presence of Genomic DNA. Plasmid p328.5 (from HB101 and B23) was transformed following the Hanahan protocol (6) into their respective plasmid-free host strains in the presence of three types of genomic DNA (B23, HB101, and WB746). The amount of plasmid DNA and genomic DNA was normalized to 5 μg and 10 μg, respectively. The final volume of the transformation reaction was also normalized to 95 μL using Tris-EDTA (10 mM Tris, 0.1 mM EDTA, pH 7.4) as diluent. The transformants were plated on selective media and transformation efficiency calculated.

RESULTS

In order to calculate the transformants/ml, transformation frequency and transformation efficiency, colony counts were only taken into consideration if they were in the range of 30-300 colonies per plate. If the colony counts were less than 30 or greater than 300, they were considered to be statistically irrelevant and were not taken into consideration for the analysis of the data. However, no colony counts fell within the 0-30 range, so the reported zero values reflect true zero.

$CaCl_2$ transformation of B23 with p328.5. The plasmid p328.5, containing the ampicillin resistance gene, was isolated from HB101. Subsequently, two individuals attempted transformation of B23 with this isolated p328.5 plasmid on two separate occasions. On both occasions the cells did not grow on the selective media (data not shown). However, confluent growth of transformants plated on non-selective media indicated that the cells were viable after the transformation process. This indicated that transformation of B23 with p328.5 by the $CaCl_2$ method was not successful.

Electroporation transformation of B23 with p328.5. As the $CaCl_2$ method of transformation did not succeed, the method of electroporation was used to transform B23 with p328.5 and to determine if B23 was capable of stably expressing p328.5. After electroporation, B23 cells were grown on nutrient agar with and without ampicillin. B23 cells plated on ampicillin containing plates grew as isolated colonies (data not shown). This indicated that electroporation was successful in transforming B23 with p328.5. B23 cells plated on LB plates grew as a confluent lawn (data not shown). This indicated that the cells were not completely killed in the electroporation process. Since the B23 cells transformed with p328.5 grew up as isolated colonies, these cells were capable of stably expressing p328.5. p328.5 was subsequently isolated from these transformed B23 cells and used in following experiments in which B23 cells were transformed with p328.5 in the presence of genomic DNA by the Hanahan method of transformation.

Table 1. Number of transformants per ml of cells.

Transformation Condition			Selective or Non-selective plating media	Number of transformants/ml of cells
Recipient Strain	Amount of plasmid (μg)[+]	Source of genomic DNA		
HB101	0	None	Selective	0
HB101	0	HB101	Selective	0
HB101	0	B23	Selective	0
HB101	0	WB746	Selective	0
HB101	5	None	Non-selective	*3.3 x 10^7
HB101	5	None	Selective	1810
HB101	5	HB101	Selective	0
HB101	5	B23	Selective	0
HB101	5	WB746	Selective	420
B23	0	None	Selective	0
B23	0	HB101	Selective	0
B23	0	B23	Selective	0
B23	0	WB746	Selective	0
B23	5	None	Non-Selective	*3.2 x 10^8
B23	5	None	Selective	620
B23	5	HB101	Selective	305
B23	5	B23	Selective	160
B23	5	WB746	Selective	305

* Total number of viable cells per ml of cells.
[+] HB101 was treated with p328.5 plasmid isolated from HB101, B23 was treated with p328.5 plasmid isolated from B23

Table 2. Transformation frequency and transformation efficiency of HB101 and B23 transformed with p328.5 by the $CaCl_2$ method of transformation in the presence or absence of genomic DNA.

Transformation Condition			Selective or non-selective plating media	Transformation frequency (10^{-6} transformants/total viable cells)	Transformation efficiency (transformants/5 μg of p328.5)
Recipient Strain	Amount of plasmid (μg)	Source of genomic DNA			
HB101	5	None	Selective	56.0	362
HB101	5	HB101	Selective	0	0
HB101	5	B23	Selective	0	0
HB101	5	WB746	Selective	13.0	84
B23	5	None	Selective	2.0	124
B23	5	HB101	Selective	1.9	61
B23	5	B23	Selective	0.5	32
B23	5	WB746	Selective	1.0	61

$CaCl_2$ transformation of HB101 with p328.5 in the presence of genomic DNA. The techniques used in the $CaCl_2$ transformation of HB101 or B23 with p328.5 were modified such that they would favour a successful transformation outcome. Most notably, it was important to ensure that all the necessary materials and equipment were cooled prior to $CaCl_2$ transformation and the plasmid used to transform B23 came from B23 cells stably expressing p328.5.

HB101 controls did not contain the plasmid but underwent the same $CaCl_2$ treatment as HB101 transformed with the plasmid. As indicated in Table 1, the HB101 controls did not grow on ampicillin-containing plates. This was to

be expected as these cells should not carry the p328.5 plasmid and are therefore ampicillin-sensitive. The controls also showed that genomic DNA from B23, HB101 and WB746 did not confer ampicillin resistance.

Five ug of p328.5 was used to transform HB101 in the presence or absence of 10 µg of genomic DNA in a total reaction volume of 95 µl. HB101 transformed with p328.5 in the absence of genomic DNA and subsequently plated on nutrient agar plates was present at a total viable cell concentration of 3.3×10^7 cells/ml. This figure was used to determine the transformation frequency of HB101 with p328.5. HB101 transformed with p328.5 in the absence of genomic DNA and plated on ampicillin-containing plates occurred at a concentration of 1810 transformants/ml of cells. Therefore, the transformation frequency of HB101 with p328.5 was 5.6×10^{-5} transformants/ml of viable cells as indicated in Table 2. However, in the presence of genomic DNA, the transformation frequency of HB101 with p328.5 decreases significantly. In the presence of HB101 and B23 genomic DNA, it appears as if HB101 transformation with p328.5 did not occur at all as indicated by the lack of transformants/ml and frequency of transformation indicated in table 1 and table 2. However, in the presence of WB746 genomic DNA, transformation of HB101 with p328.5 occurred but at a diminished frequency of 1.3×10^{-5}, 23% of the transformation frequency of HB101 with p328.5 in the absence of genomic DNA. The transformation efficiency decreased as the transformation frequency decreased as indicated in table 2. This was to be expected as the same amount of HB101 cells were transformed with identical amounts of plasmid and genomic DNA.

$CaCl_2$ transformation of B23 with p328.5 in the presence of genomic DNA. The p328.5 plasmid used to transform B23 was isolated from B23 cells carrying p328.5 prepared from electroporation of B23 as described earlier. The strain of B23 that was to be transformed did not contain p328.5. Table 1 shows that B23 controls did not grow on ampicillin-containing plates which indicated that the B23 strain was sensitive to ampicillin and that B23, HB101 and WB746 genomic DNA did not confer ampicillin resistance.

Five µg of p328.5 was used to transform B23 in the presence or absence of 10 µg of genomic DNA in a total reaction volume of 95 µl. B23 transformed with p328.5 in the absence of genomic DNA and plated on LB plates was present at a total viable cell concentration of 3.2×10^8 cells/ml. Therefore, the transformation frequency of B23 with p328.5 was 2.0×10^{-6} transformants/ml of viable cells as indicated in Table 2. However, in the presence of genomic DNA, the transformation frequency of B23 with p328.5 decreased significantly. In the presence of genomic HB101, B23 and WB746, the transformation frequency decreased to 9.7×10^{-7}, 5.1×10^{-7} and 9.7×10^{-7} transformants/ml of viable cells respectively. As indicated in table 2, the transformation frequency of B23 with p328.5 in the presence of genomic B23 DNA was approximately half that in comparison to the transformation frequency in the presence of genomic HB101 and WB746 DNA. The transformation efficiency decreased as the transformation frequency decreased as indicated in Table 2. This was to be expected as the same amount of B23 cells were transformed with identical amounts of plasmid and genomic DNA.

DISCUSSION

In previous experiments, foreign genomic DNA contaminants were found to hinder the plasmid transformation of *E. coli* HB101 cells (9). In this experiment, we attempted to investigate the effect of different genomic DNA contaminants on the transformation efficiency of the wild-type *E. coli* strain, B23, and to determine if similar transformation trends existed among different *E. coli* strains.

$CaCl_2$ transformation p328.5 into B23 failed twice in two separate experiments, possibly due to experimental error such as the lack of chilled instruments to be used in the process of making competent cells. Furthermore, in preparing competent cells it is important to remove residual medium that can interfere with the cells' ability to take up DNA. First, because such small volumes are involved, even a small amount of leftover media can significantly lower the Ca^{2+} concentration, an important ion in this process. Second, it is possible that cell starvation plays an important role during the competence procedure. Residual media contains nutrients which can interfere with the starvation process of the cells.

However, the fact that the process failed on two independent occasions suggests that factors other then experimental error may be involved. Firstly, B23 is an endonuclease positive *E. coli* strain, which increases the likelihood that internalized plasmids would be degraded, thus reducing transformation efficiency. Secondly, the plasmid is not optimized for stability or for replication in B23 and so is less likely to escape degradation. HB101 is mutated for *hsdR* and *hsdM*. The gene product of *hsdR* is an EcoK type I endonuclease which cleaves DNA containing the EcoK cleavage site unless specific adenine residues are methylated by the EcoK methylase, encoded by the *hsdM* gene (5). Plasmids replicating inside HB101 are thus unmethylated due to a lack of EcoK methylase, but safe from restriction due to a lack of EcoK endonuclease. However, B23 is wild-type for both these gene products. Hence, it is conceivable that unmethylated plasmid that enters during $CaCl_2$ transformation is degraded by

the endonuclease before it has a chance to become established. On the other hand, electroporation might allow much more DNA to enter into the cell, allowing plasmid DNA the chance to be methylated, and so protected from degradation, before it is attacked by the EcoK endonuclease. Thus electroporation seemed to be a suitable technique for overcoming this restriction barrier to transformation.

Hence, electroporation was able to stably transform p328.5 into B23. The $CaCl_2$ transformation with this plasmid isolated from B23 was successful, although transformation frequency with B23 was still about 28 times lower than transformation of HB101 with the same type of plasmid isolated from HB101 (Table 2). This difference is not unexpected considering the presence of endonucleases in B23. In fact, the transformation frequency of B23 was lowest in the presence of B23 genomic DNA, which opposes our hypothesis that foreign DNA would interfere to a greater extent than "self" DNA. The similarity in the transformation frequency of B23 contaminated with genomic DNA originating from either WB746 or HB101 suggests that B23 is unable to differentiate between different sources of foreign DNA.

The transformation of HB101 was performed with the intention of confirming the results previously reported. However, although the transformation of HB101 with p328.5 was successful, the transformation frequency was 50 times lower than previous experiments (Table 2). A possible explanation for this decrease is that the volume of the transformation reaction was almost twice the volume previously used and this would have decreased the opportunity for interaction between the plasmid and the cells as the distance between the two increased. HB101 transformed in the presence of contaminating genomic DNA from HB101 itself and from the wildtype B23 resulted in no observed transformants (Table 1). Controls indicated that the cells were viable after the transformation reaction, which suggest the lack of transformants was due to reasons other than cell viability. Plate spreading techniques were also not likely to be the cause of the lack of transformants since the transformation reaction was plated in triplicate, and it is unlikely that all three platings would have been performed in error. In this case, other experimental errors such as inadequate heat shock treatment are possible since the heat shock treatment was done in groups where the tubes were in close proximity to each other. It is possible that the two tubes containing the transformation reactions for *E.coli* strain HB101 with genomic contaminants HB101 and B23 respectively were situated in the middle of these groups, thus receiving inadequate heat treatment. Nonetheless, if experimental error was not the cause of the lack of transformants, the 2:1 ratio of contaminating genomic DNA to plasmid used instead of 1:1 as in experiments previously reported could have provided large enough quantities of the contaminating DNA to occupy most of the relevant HB101 receptors needed for DNA uptake. Considering the already low transformation efficiency as indicated in the previous study, this additional hindrance may have been sufficient to lower the transformation frequency to undetectable levels (Table 1). However, it is interesting to note that the contaminating WB746 genomic DNA did not cause as great a decrease in transformation efficiency as contaminating HB101 or B23 genomic DNA. The observation that genomic DNA contamination from HB101 affects transformation to a greater extent than WB746 contradicts the observation previously reported in which foreign chromosomal DNA was responsible for a greater decrease in transformation frequency than self-DNA(9). This suggests that the DNA from WB746 associates with receptors different from those used by the genomic DNA derived from HB101 or B23. There is also the possibility that genomic DNA from WB746 may not be associating with any of the receptors found on HB101. In this case, the lowering of transformation frequency could have resulted from physical interference of plasmid interaction with the appropriate receptors due to the presence of the WB746 DNA randomly attached to the cell surface.

It was previously hypothesized that the difference in the degree of hindrance by the various genomic DNA contaminants was a result of the identities of these contaminants (9), where strain HB101 was able to recognize its own DNA and so was less affected by its presence. Through this experiment, it was demonstrated that this is not likely the trend in wildtype strain B23 and that the trend previously seen in HB101 was not reproducible under our experimental conditions. Transformation of HB101 was most affected by Gram (-) HB101 and Gram (-) B23 genomic DNA and least by the genomic DNA of Gram (+) WB746, suggesting that the type of bacteria, perhaps by its Gram stain status, is a factor. However, transformation of B23 with Gram (-) B23 genomic DNA and Gram (+) WB746 genomic DNA gave similar transformation frequencies (Table 2), thus dispelling the trend. Since the size of the *E. coli* genome (4397 kbp) is roughly the same as that of *B. subtilis* (4170 kbp) (http://www.dur.ac.uk/~dbl0www/Bioinformatics/dogs.htm), effects due to size can be ruled out. The one similarity is that genomic B23 DNA causes the most interference with transformation in both B23 and HB101 cells. This was also the case in the HB101 transformation of a previous experiment (9), suggesting that genomic B23 DNA may possess certain characteristics that are absent or are in lower quantities in HB101 and WB746 DNA. This certain characteristic could be causing hindrance of transformation by allowing the genomic DNA to bind and occupy receptors on the cell that would otherwise be required by the plasmid for uptake into the cell. There is also the possibility that the genomic B23 DNA is able to bind p328.5, thus preventing its uptake into the cell.

Many other factors may have affected transformation, including the degree of plasmid purity, and experimental error. It should be noted that the experiments were done only once and that it should be repeated for confirmation of results. Furthermore, most of the statistics discussed were the result of colony counts from a single plate, since most plates were not in the range which would have allowed for statistical accuracy.

The question as to why bacterial cells react differently to genetic material derived from different sources was hypothesized to be dependent on the ability of the cell to recognize its own DNA. Although through our experiments, we were unable to confirm or deny this possible trend, other studies have looked at the means by which certain bacterial species were able to identify their own DNA. One such mechanism is through the use of specific nucleotide sequences which enables the cell to recognize DNA from its own species. This has been suggested to be the case in both *Neisseria gonococci* and *Haemophilus spp,* although it is believed that the sequences recognized are different between the two species (4). In fact, a study by Dougherty et al suggests the possibility of two different DNA receptors which are each responsible for either homologous and heterologous DNA (2). To determine if there is a particular mechanism used by the different strains of *E. coli* and to verify a possible trend, further experiments will have to be performed.

The transformation frequencies of *E. coli* B23 and *E. coli* HB101 with p328.5 decrease in the presence of genomic DNA. However, the observation that foreign genomic DNA decreases transformation frequency in comparison to the transformation frequency in the presence of "self" DNA could not be re-confirmed. It can be concluded that decrease in transformation frequency in the presence of genomic DNA is not an isolated trend of *E. coli* HB101 and that the source of the contaminant genomic DNA plays a likely role in the determination of the transformation frequency.

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