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Transformation efficiency on *E. coli* in response to different bivalent salts

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ABSTRACT:

Bacterial transformation is the process of up taking foreign DNA molecules by bacterial cells. Bacteria, which are able to up take such foreign DNA are called "competent" cell. Foreign DNA carrying antibiotic resistance gene can be expressed in the bacteria and transforming the bacteria from antibiotic sensitive to antibiotic resistance. Such transformed bacteria can easily be isolated. The efficiency of the transformation plays a critical role in recombinant DNA technology. Several methods have been developed to improve the transformation efficiency. In our work we have studied the transformation efficiency on strains of *E. coli* and in response to different bivalents salts at different concentrations. All the experiments revealed that transformation efficiency was highest on *E. coli* DH5α strain in 50 mM $CaCl_2$ concentration. Comparing to the other *E. coli* and bivalents salts concentration, 50 mM $CaCl_2$, resulted in much faster growth of transformed bacteria. Thus, our results suggest that *E. coli* DH5α strain and $CaCl_2$ can be used as an effective agent for the transformation technique.

Keywords:
Transformation efficiency, *E. coli*, Bivalent salt.

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INTRODUCTION

Transformation is defined as the uptake and expression of foreign DNA by cells. Transformation of bacteria involves DNA binding to all cell surface followed by uptake across the wall - membrane complex into the cytoplasm. Bacterial transformation occurs naturally in many bacterial genus such as *Micrococcus, E. coli, Haemophilus* and *Bacillus* (Michod *et al*., 1988) all these organisms have proteins on their exterior surface whose function is to bind to DNA in their environment and transport it into the cell. However, it is still a rare event for most bacteria to naturally take up DNA from the environment. By subjecting bacteria to certain artificial conditions, many of them become able to take up free DNA and the cells in such state are referred to as competent**.**

In early seventies, the discoveries of transferring the phage (Mandel and Higa, 1970) and plasmid (Cohen *et al*., 1972) DNAs into *Escherichia coli* cells following a competence induction process involving treatment with calcium chloride, set the stage for molecular cloning of recombinant DNAs. The technique of DNA transformation has then become important in virtually all aspects of molecular genetics. *E. coli* has developed into a universal host organism both for molecular cloning of DNA and for a diverse set of assays involving cloned genes. In *E. coli* the competence can be developed by suspending the cells in ice-cold $CaCl_2$ and then subjecting to a brief heat shock at 42°C (Cohen *et al*., 1972; Mandel and Higa, 1970). Even after generation of competence, the technique of *E. coli* transformation is highly inefficient; the vast majority of DNA molecules added does not enter any cell, and in turn the vast majority of bacterial cell receives no DNA. With the advancement of time, the original method of competence induction by $CaCl_2$ treatment has gradually been improved to different high-transformation-efficiency protocols, which employ magnesium ions, manganese ions (Hanahan, 1983; Hanahan *et al*., 1991). Besides the chemical methods, physical treatment like electroshock with a brief pulse of high voltage electricity has been shown to be applicable to competence induction in *E. coli* and other bacteria (Neumann *et al*., 1982).

Even after all such developments, the exact mechanism of $CaCl_2$ mediated artificial transformation is still largely obscure. It is believed that the $CaCl_2$ helps in DNA adsorption of the competent cell surface and the heat-shock step facilitates for penetration of the adsorbed DNA into the cell cytosol. Earlier finding (Sarkar *et al*., 2002a) shows that during artificial transformation of *E. coli*, the naked DNA is bound to the lipopolysaccharide (LPS) receptor molecules on the competent to form surface; the divalent cation Ca^{2+} is suggested to form coordination complexes with the two negatively charged macromolecules - DNA and LPS. In this study, our primary motivation is to investigate the mechanism of DNA entry into the cell cytoplasm during artificial transformation. Here, it has been shown that heat shock step of the standard transformation procedure heavily depolarizes the membrane of $CaCl_2$-treated component cells. So, we have used different type of bivalent salts for transformation. The aim of this experimentation is to study the efficiency of transformation in respect to different bivalent salts.

MATERIALS AND METHODS

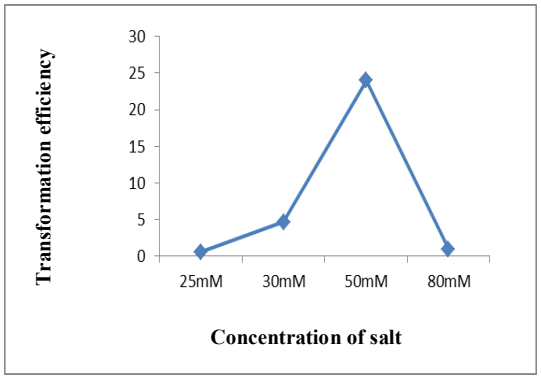
Bacterial Strain – *E. Coli* DH5α.

Plasmid – pUC-19

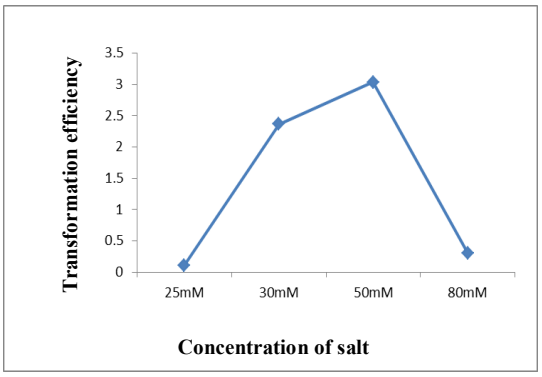
Media for bacterial growth

LB medium

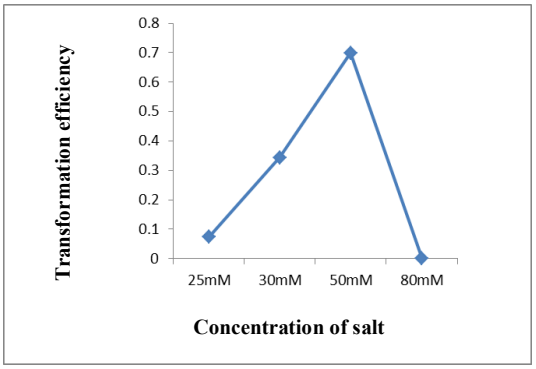
Bacto-tryptone-10 gm/L, Bacto-yeast-5 gm/L, Nacl-10 gm/L, pH was adjusted to 7.0-7.4 with NaOH (10 N) and was autoclaved to sterilize. The autoclaved medium was cooled to 55°C and ampicillin was added (final concentration 50 mg/ml). For LB-agar plates, 1.7% Bactoagar (17 gm/L) was added prior to autoclaving.



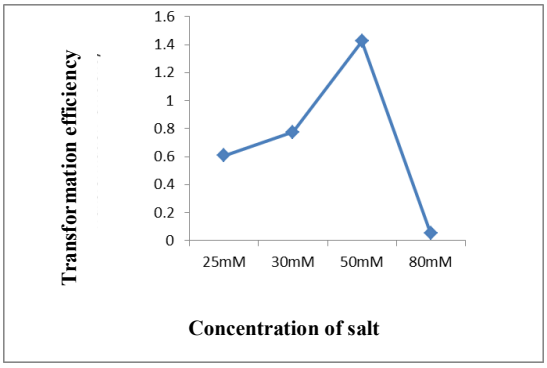
a. Calcium chloride ($CaCl_2$)



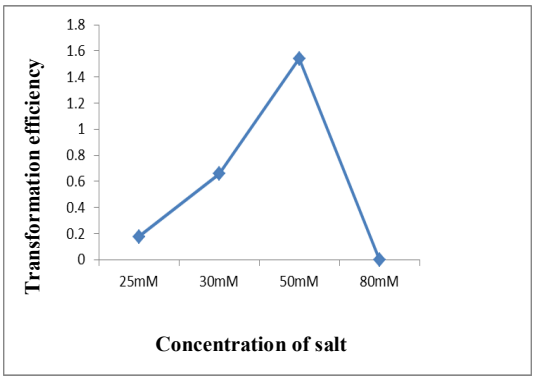
b. Magnesium sulphate ($MgS0_4$)



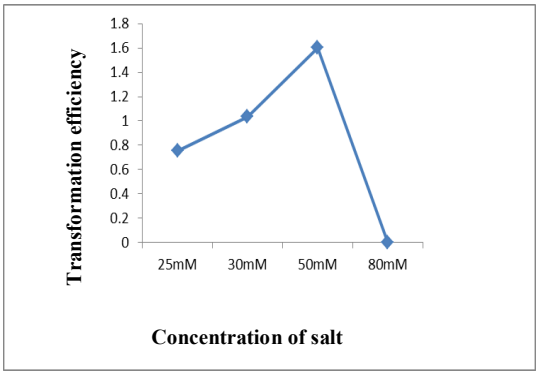
c. Magnesium Chloride ($MgCl_2$)



d. Ammonium-Ferrous sulphate (NH_4FeS0_4)



e. Sodium Chloride (NaCl)



f. Cobalts Chloride ($CoCl_2$)

Figure 1 In all the transformation process (a, b, c, d, e, f) 50 mM concentration is the highest for with above bivalents salt.

Salts

$CaCl_2$, $CoCl_2$, $MgCl_2$, NaCl, NH_4FeSO_4, $MgSO_4$ was used in 25 mM, 30 mM, 50 mM and 80 mM concentration. All salts were added as solids, always kept and used in cold.

Methods

Preparation of *E. coli* DH5a competent cell

The following simple procedure is a variation of that of (Cohen *et al.,* 1972) and is frequently used to prepare batches of competent bacteria that yield, $5x10^6$ to $2x10^7$ transformed Colonies per microgram of super coiled plasmid DNA. The standardization of procedure is established in our research laboratory.

- Pick a single Colony (2-3 mm in diameter) from a plate freshly grown for 16-20 h at 37°C and transfer into 100 ml of Lysogeny Broth (LB) broth in a 1-liter flask. Incubate the culture for 3 h at 37°C with Vigorous shaking (300 cycles/h) minute in a

rotary shaker). To monitor the growth of the culture, determine the OD_{600} nm every 30 minutes. OD_{600} nm = 0.25-0.30 is better for *E. coli* DH5α transformation.

- Aseptically transfer 10ml of the culture to sterile, ice-cold 15 ml polypropylene tubes. Store the tubes on ice for 15 minutes to inhibit the metabolic activity of the bacteria.
- Recover the cells by centrifugation at 4000 rpm for 5 mins at 4°C in a Sarvall GS3 rotor.
- Decant the media from the cell pellets. Stand the tubes in an inverted position for 1 minute to allow the last traces of media to drain away.
- Resuspend each pellet in 5 ml of ice-cold salt solution of different concentrations respectively and store it on ice for 30 minutes.
- Recover the cells by centrifugation at 2500 rpm for 10 mins at 4°C in a Sarvall GS3 rotor.
- Decant the fluid from the cell pellets and stand the tubes in an inverted position for 1 minute to allow the last trace of fluid to drain away.
- Resuspend the pellet in 1 ml of the salt solution of different concentrations respectively.
- Put the cells back on ice until they are used for transformation (minimum two hours)

Bacterial transformation

According to (Zhiming *et al.,* 2005) and we modify the Transformation process in our laboratory.

Plasmid transformation and antibiotic selection

Calcium chloride treatment of bacterial cells produces competent cells that will take up DNA following a heat shock step. DNA molecules, i.e. plasmid, which are introduced by this method, will then be replicated in the bacterial host cells. To aid the bacterial cells' recovery, the cells are incubated briefly with non-selective growth medium following the heat shock treatment. However, due to the low percentage of bacterial cells that have been transformed with the

Table 1 shows that transformation rate is high in 50 mM concentration of each bivalent salts.

Salt	Concentration (mM)	Average Transformation Efficiency (cfu/mg)
a. Calcium chloride ($CaCl_2$)	25	0.590
	30	4.675
	50	23.98
	80	0.980
b. Magnesium sulphate($MgSo_4$)	25	0.110
	30	2.367
	50	3.037
	80	0.310
c. Magnesium Chloride($MgCl_2$)	25	0.073
	30	0.344
	50	0.699
	80	0.000
d. Ammonium-Ferrous-sulphate (NH_4FeS0_4)	25	0.607
	30	0.774
	50	1.424
	80	0.052
e. Sodium Chloride (NaCl)	25	0.178
	30	0.658
	50	1.543
	80	0.000
f .Cobalts Chloride ($CoCl_2$)	25	0.756
	30	1.035
	50	1.601
	80	0.000

plasmid and the potential for the plasmid not to propagate itself in all daughter cells, it is necessary to select for bacterial cells that contain the plasmid. This is commonly performed using antibiotic selection.

E. coli strain such as DH5α is sensitive to common antibiotics such as ampicillin. Plasmids used for the cloning and manipulating of DNA have been engineered therefore to harbor genes for antibiotic resistance too. Only bacteria that posses the plasmid DNA will have the ability to metabolize ampicillin and form colonies. Bacterial cells containing plasmid DNA has been selected following this technique.

Protocol of Bacterial transformation

- 100 µl of competent cells is taken in a round bottom test tube and place it on ice.
- Add DNA (<5 µl) to the cells and mix
- The tube put back on ice for 30 min.
- Prepare a water bath at 42°C.
- Heat shock the cells and hold the tube at 42°C for 90 seconds.
- Put the tube back on ice for 2 min.
- Add 1 ml L.B medium in each tube.
- Incubate the cells at 37°C for 1 h (200 cycles/min) with gentle shaking.
- Plate different amount of transformation culture on antibiotic- containing LB agar plate.
- Incubate plates overnight at 37°C.

RESULTS AND DISCUSSION

Using cells in early log phase of growth is an important factor for preparation of competent cells. We have chosen the optimum optical density at 600 nm (OD_{600}) for the preparation of competent cells using strain DH5α where it was 0.25-0.30. We have studied the effects of different bivalent salts on the transformation efficiency of DH5α using the growth medium LB as the most suitable one.

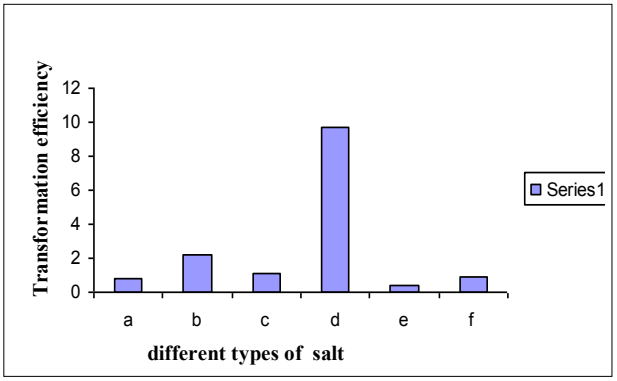


Figure 2 shows that the transformation efficiency is highest in using $CaCl_2$ (d) as a bivalent salt according to above table.

Calculation of transformation efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1 mg of plasmid DNA, and each measured by performing a control transformation reaction using a known quantity of DNA, then calculating the number of cfu formed per mg of DNA.

Equation for transformation efficiency

Transformant cfu = No. of bacteria colonies × dilution ratio × original transformation volume / plated volume.

Transformation efficiency = Transformant cfu/plasmid DNA added (mg)

The plasmid DNA was added 1.4 mg,

The following table shows the average transformation efficiency to that of the salts used.

Transformation efficiency is very important in molecular cloning experiments, and can be affected by many factors. Takahashi have reported a simple method of plasmid transformation of *E. coli* by rapid freezing

Salt	Average of Transformation Efficiency
a. NaCl	0.7932
b. $MgSo_4$	2.2136
c. $CoCl_2$	1.1306
d. $CaCl_2$	9.7483
e. $MgCl_2$	0.4172
f. NH_4FeSO_4	0.9350

(Takahashi *et al*., 1992). The most important being that the bacterial cells must in their early logarithmic growth period, Ryu and other authors have pointed out the importance of the early log phase for transformation (Ryu and Hartin, 1990). Bacteria that are able to take up DNA are called "competent" and competency can be induced by treatment with calcium chloride in the early log phase of growth. Bacteria that are able to take up DNA are called “competent” and competency can be induced by treatment with bivalent chloride and sulphate salts in the early log phase of growth. The bacterial cell membrane is permeable to chloride and sulphate ions, but is non permeable to Calcium ions. As the Chloride ions enter the cells, water molecules accompany the charged particle. This influx of water causes, the cells to swell and is necessary for the uptake of DNA, The exact mechanism of this uptake is unknown. Our experiments have shown that *E. Coli* DH5α, the optimum OD_{600} is 0.25-0.30, is optimum for bacterial transformation efficiency. For competent cells preparation it would be routinely cultured to OD_{600} = 0.25-0.03.

Salt has an impact on transformation efficiency, in response to bivalent salts like $CaCl_2$, $CoCl_2$, $MgCl_2$, NaCl, NH_4FeSO_4, $MgSo_4$, the transformation efficiency was found to be highest in $CaCl_2$ as depicted by figure-1.

According to (Brian and Heller, 1996) shows that $CaCl_2$ 75 mM concentration is optimum in TB solution. Another important factor is the concentration of divalent salt like $CaCl_2$, $CoCl_2$, $MgCl_2$, NaCl, NH_4FeSO_4, $MgSO_4$, although 25-80 mM salt concentration was used but 50 mM was shown to be optimum for all the cases as depicted by figure-2.

CONCLUSION

The present study shows that bacterial transformation process is associated with the bivalent salt and also associated with bacterial optimum growth phase and also salt concentration. We observed that *Escherichia coli* DH5α strain bacterial transformation processes were optimum on OD_{600} = 0.25-0.03 and the transformation efficiency was found to be highest in $CaCl_2$ at 50 mM concentration. $CaCl_2$ a bivalent salt can be used as an effective agent for the transformation technique.

REFERENCES

Brian P and Heller Mk. 1996. High efficiency 5 min transformation of *Escherichia coli*. Nucleic Acids Research 24(3):536-537.

Hanahan D, Jessee J, Bloom FR. 1991. Plasmid transformation of Escherichia coli and other bacteria. Methods in Enzymology. 204:63-113.

Hanahan D. 1983. Studies on transformation of *E.coli* with plasmids. Journal of Molecular Biology. 166:557-580.

Mandel M and Higa A. 1970. Calcium dependent bacteriophage DNA infection. Journal of Molecular Biology. 53(1):159-162.

Michod RM, Wojciechowski M, Hoelzer M. 1988. DNA repair and the evolution of transformation in the bacterium *Bacillus subtilis*. Genetics (1):118:31-39.

Neumann E, Schaefer-Ridder M, Wang W, Hofschneider PH. 1982. Gene transfer in mouse lyoma cells by electroporation in high electric fields. European Molecular Biology Organization. Journal 1(7):841-845.

Ryu J and Hartin RJ. 1990. Quick transformation in *Salmonella typhimurium LT2*. Biotechniques 8(1):43-44.

Stanley N. Cohen, Annie C. Y. Chang, and Leslie Hsu. 1972. Nonchromosomal Antibiotic Resistance in Bacteria: Genetic Transformation of Escherichia coli by R-Factor DNA. Proceedings of the National Academy of Sciences of the United States of America. 69(8):2110-2114

Sarkar S, Choudhuri S, Basu T. 2002a. Ethanol-induced enhancement of the transformation of *Escherichia.coli* by plasmid DNA. Ind. Indian Journal of Biotechnology.1 (2):209-211.

Takahashi R, Valeika SR, Glass KW. 1992. A simple method of plasmid transformation of *E.coli* by rapid freezing. Biotechniques.13(5):711-715.

Zhiming Tu, Guangyuan He, Kexiu X. Li., Mingjie J. Chen, Junli Chang, Ling Chen, Qing Yao, Dongping P. Liu. 2005. An improved system for competent cell preparation and high efficiency plasmid transformation using different *Escherichia coli* strains. Electronic Journal of Biotechnology 8(1):114-120.