World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Induction of competency and comparison of transformation efficiency in *Escherichia coli* using different salt solutions - Cations

Gunasagaran Karuna, Natesan Munuswamy*

Unit of Aquaculture and Cryobiology, Department of Zoology, University of Madras, Guindy Campus, Chennai- 600025, India

Received: 05-03-2015 / Revised: 23-03-2015 / Accepted: 28-03-2015

ABSTRACT

Escherichia coli (DH5α) was treated with different salt solutions to attain a physiological state of competence for transformation. Salts were dissolved in water to make ionic solutions containing cations of different valencies. Ionic solutions of sodium chloride (NaCl) monovalent (Na^+), magnesium sulphate ($MgSO_4$) divalent (Mg^{2+}), aluminium chloride ($AlCl_3$) trivalent (Al^{3+}) and conventionally used Calcium chloride ($CaCl_2$) divalent (Ca^{2+}) cations were used to induce competency. Competent cells prepared were stored in 50 % glycerol at – 20 °C and the transformation efficiency was compared using a plasmid vector pUC19. Efficiency of cations inducing competency with higher transformation rates was seen in the order Na^+>Ca^{2+}>Mg^{2+}>Al^{3+} for freshly prepared cells; later it was Ca^{2+}>Na^+>Mg^{2+}>Al^{3+}. Cells under storage showed transformation efficiency of 10^5-10^6 CFU/µg DNA for 5 weeks. This result concludes competency could be induced using salt solutions of different cations with difference only in their transformation efficiency. Chemical induction method being a conventional method other different salts could be used for better transformation efficiency in other organisms.

Keywords: *Escherichia coli*; Cations; Competency; Transformation; Storage

INTRODUCTION

Competency and transformation are major factors involved in molecular cloning. Cells ability to take up DNA is a process termed competency and expression of that DNA is known transformation. Some bacteria are naturally capable of taking up DNA under laboratory conditions. Many more may be able to take up DNA from their natural environment; such species carry sets of genes, specifying machinery for bringing DNA across the cell membrane [1]. This is primarily carried out to help meet nutritional needs, but can be used for acquisition of new genes that promote genetic diversity [2, 3]. The introduction of exogenous DNA into an organism requires two steps; preparation of competent cells for DNA uptake and transformation of the cells with the DNA so that the cells can acquire new genetic traits that are inheritable and stable. Biologists have transformed *E. coli* gram negative bacteria as widely used organism in molecular biology that preferentially interact and transfer double- stranded DNA [3]. Competent cells take up foreign DNA easily since they have altered cell wall, but most cells cannot uptake unless they have been exposed to certain physical or chemical treatment [4].

Induction of competency using chemicals is a conventional method where the chemical compounds are believed to react with cells membrane to make them competent. Dissolution of salt in water yields atoms or radicals as ions of various valency carrying positive or negative charge making them as anions and cations. The number of electrons that is lost, shared or gained by an atom or a radical determines its valency. In this context, salt solutions yielding cations of different valency were selected for inducing competency. The number of electrons they share could influence how strongly they can react with cells to induce competency. Sodium chloride, a monovalent cation [5]; Magnesium sulphate [6, 7], divalent cation and a trivalent cation aluminium chloride were used in the preparation of competent cells. The induction of competency and efficiency of transformation of these salts were compared with Calcium chloride [8], the most commonly used salt conventionally for the preparation of competent cells which in a solution reacts as divalent cation. Aluminium chloride is a salt and easily dissolves in water into

***Corresponding Author Address:** Prof. N. Munuswamy , D.Sc., Department of Zoology, School of Life Sciences, Life Science Building, University of Madras (Guindy Campus), Chennai - 600 025, Tamil Nadu, INDIA.; **Email:** munuswamynm@yahoo.co.in*

ions and has not used in inducing competency. Since chemical induction method is a convention method Aluminium chloride was opted for preparing solution of trivalent caiton. Although sodium and magnesium had used to prepare competent cells calcium chloride is mostly followed. Making a cell competent using chemical induction methods is considered as time consuming and laborious process but it is simpler and cheaper when compared to methods such as electroporation and commercially available competent cells that are costly and to be stored under - 80°C which most of laboratory cannot afford. Hence the study was designed with a hypothesis that the numbers of electrons as cations shared by salts in solutions could it result in inducing competency with high transformation efficiency along with an attempt to design a cost effective, simple protocol that could be stored for longer periods.

MATERIALS AND METHODS

Bacterial strain: Overnight cultures of *Escherichia coli* DH5α from LB agar plates were grown in 10ml Luria Broth at 37 °C in shaker for 18h [4].

Plasmid: pUC19 was selected for its traditionally high degree of transformation efficiency and the ease of selection of transformants on ampicillin [9].

Preparation of media: *E. coli* DH5α was cultured in Luria-Bertani (LB) broth containing 10g tryptone (Himedia), 5g Yeast extract (Himedia) and 10g NaCl (SRL). pH adjusted to 7.5 with NaOH in 1L dH_2O. Solid LB-agar for plates was made by adding 15g/L agar (Himedia). The autoclaved medium was cooled to 55°C and ampicillin (AMP) at concentration of 10 µg/mL was added for LB-AMP selective medium.

Preparation of chemical solutions of different cations: Chemical induction method was followed to prepare competent cells. The chemicals viz. NaCl, $CaCl_2$, $MgSO_4$ and $AlCl_3$ were prepared as 100mM solutions using distilled water and stored at 4 °C until used.

Procedure for induction of competency: From cultured *E.coli* DH5α (containing no plasmids), 1 ml was withdrawn and added to 200ml of LB broth in 500ml conical flask, incubated at 37°C for 3-4 h in rotatory incubator. After OD of 0.4 – 0.5 was reached at 600 nm the culture was placed on ice for 20 min [9]. The culture was centrifuged at 1000g for 10 min at 4 °C in 50 ml falcon tubes the supernatant was discarded. Pellets from rest of the medium were collected in the same by repeating the centrifugation step. 20ml of 100mM chilled solutions of salt of different cation was added and mixed gently until the bacterial pellet dissolved completely in solution. Sometimes pipetting was done gently and slowly for mixing the cells into the solution of cation. The tubes were incubated on ice for 30 min and later centrifuged at 1000g for 10 min at 4 °C. Supernatant was discarded and 2ml of chilled cation was added to dissolve the treated cells and stored. A total of 4 separate 200ml cultures of *E.coli* DH5α were inoculated to treat the cells using different salt solutions and same procedure was followed.

Competent cells storage: For long term storage, 1ml of competent cells was suspended in 1ml of sterile 100% glycerol to give a final concentration of 50% glycerol and mixed. The glycerol stock was stored at -20°C for later use [10].

Procedure for bacterial transformation: Cells were transformed with pUC19 plasmid DNA. Briefly 0.5µl (1µg/µl) of plasmid DNA was added to 100 µl of competent cells, mixed and left to incubate on ice for 10 min. After incubation the cells were subjected to heat shock at 42 °C for 2 min and soon placed on ice for 10 min after which 250 µl of sterile LB broth without any antibiotic was added and incubated on shaker for 1 hour at 37°C. Once recovered 100 µl of this mixture containing plasmid and competent cells was inoculated on to LB-AMP plates for selection of transformants and transformation efficiency was calculated. The experiment was done in triplicates. For positive control, 25 µl of competent cells that have not been transformed with the plasmid were plated onto plate without antibiotic (ampicillin). For negative control, 25 µl competent cells that have not been transformed with the plasmid were plated onto plate with antibiotic.

Calculation of transformation efficiency under storage: The transformation efficiency was calculated as described by Aryadeep *et al* [6]. The efficiency of competent cells stored to transform was checked for a period of 5 weeks. The transformation efficiency per microgram (µg) of DNA was noted for each set of competent cells prepared and statistically analysed.

RESULTS

The solution of salt $CaCl_2$ divalent cation showed highest transformation efficiency of $3.5x10^6$ CFU/ µg DNA after an initial lagging of $1.5\pm0.13\times10^6$ CFU/ µg DNA. $MgSO_4$ and NaCl had shown transformation efficiency between maximum of $1.9x10^6$ to least of $7.3x10^5$ CFU/ µg DNA but comparatively it was found that $MgSO_4$ induce less competency than by salts of sodium and calcium. Trivalent cation $AlCl_3$ had maximum

transformation efficiency of 6.4± 0.2 × 10^5 CFU/ µg DNA and was the least efficient cation for the preparation of competent cells (Figure 1).

Transformation efficacy of freshly prepared competent cells using NaCl and $CaCl_2$ showed high transformation efficiency. The transformation efficiency of cations in making cells competent was seen in the order Na^+>Ca^{2+}>Mg^{2+}>Al^{3+} for freshly prepared cells; later it was Ca^{2+}>Na^+>Mg^{2+}>Al^{3+}. Incubation in cations (as solutions of salts) had increased competency initially over cells that were used immediately when storage was prolonged competency reduced. Upto 20 days (3 weeks) the transformation efficiency of the competent cells were good and after which there was a decrease yet cells competent were enough to be used for transformation upto 35 days (5 weeks). Overall the competent cells of different cations stored at -20 ºC in 50 % glycerol were able to retain transformation efficiency for 5 weeks.

DISCUSSION

The success rate of a bacterial transformation is determined by several factors. For example the growth phase at which the bacterial culture is harvested [9], quality of chemical additives used, the temperature at which cells are maintained during preparation, the speed at which cells are centrifuged, and the degree of shear forces generated upon resuspension may all affect transformation efficiency [11]. It is also demonstrated that temperature and duration of competent cells stored may also affect efficiency [8]. With these facts a simple protocol was followed for making the cells competent. Cells were maintained constantly at temperature of 4 ºC which makes them rigid which can withstand the handling pressure. Centrifugation was done at 1000g for 10 min to pellet cells where greater centrifugal force within short time could do the same but it may damage the cells. With gentle tapping and slight force generated using micropipettes gentle suction and release the cells were resuspended in the solutions of cations there by not causing much damage to augment that more the number of viable cells so high could be the chance for successful transformation.

In the present study monovalent, divalent and trivalent cations induced competency. The reason for $CaCl_2$ solution to induce competency with higher transformation efficiency could be the PHB/Polyp complexes in the lipid bilayers of *E. coli* are channels related to the translocation of exogenous DNA in the organism. These PHP/Polyp channels appear to have high density of negetaive charges on their phosphate backbone and have higher affinity towards Ca^{2+} ions [12, 13]. While rest of cations used were not as good as Ca^{2+} ions but what was important is that they were also able to induce competency with good transformation efficiency.

The pH of chemical solution is an important factor in uptake of DNA [14]. It was reported decrease in pH results decrease in conductance of PHB channels to the bivalent cation such as Ca^{2+} this seems to be significant because DNA translocation *in vitro* across a lipid bilayer appears to be an electrophoretic process [15, 16]. The potential difference required for this process is generated by the activity of high conductance channels in the membrane. Salts of various cations were dissolved in double distilled water of pH 6.5 which is slightly less than the physiological pH 7.2 – 7.8 of *E. Coli* [17] and the resulting chemical solution of respective salts had no change in pH except for aluminium chloride which had acidic pH of 5.5; this reduction in pH may have resulted in lesser transformation efficiency for Al^{3+} cation when compared with other cations. This makes that the valency of cation, binding affinity of membrane channels and the pH had effect on making a cell competent. The pH of aluminium chloride solution could be adjusted at physiological range and also the pH of all the solutions could be varied to check transformation efficiency in studies made in future. The whole process of inducing competency and transformation was completed in less than 4 hrs. The competent cells were stored at -20 °C with good transformation efficiency between 10^6 to 10^5 CFU/µg DNA for 5 weeks even though transformation efficiency showed a decreasing trend the cells were usable.

The procedure used in this study for preparation of competent cell had only salts dissolved in water to form ionic solution of mono, di and tri valent cations and used for achieving the state of competence. No other compounds were added to aid or induce competency apart from the salts used proving cations in general irrespective of their valence have significant effect in making the cell competent. The competent cells are mixed with the plasmid DNA and given a heat shock 42 ºC for 2 min and immediately placed on ice for 20 min. Heat shock destabilises the membrane and facilitates uptake of DNA. The cold shock is thought to crystallize the membrane after heat shock as heat and cold shock are basics of chemical transformation procedures [18, 6].

The injured host cells require recovery step and are transferred to a nutrient medium to allow the injured host cells to uptake DNA and recover their physiological function and drug resistance. The

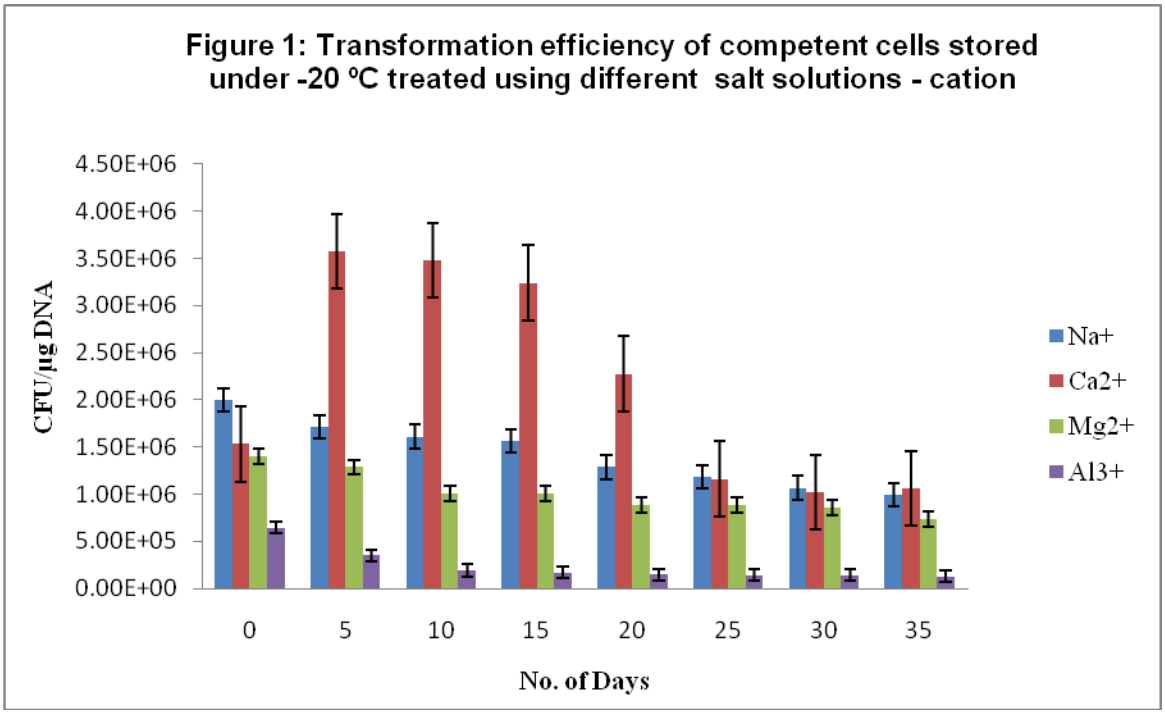
actual transport of the DNA through the cell membrane is carried out by DNA translocation machinery that has three components i.e. the DNA receptor, the permease/channel protein and the ATP binding protein classified as a DNA translocase by Transport Protein Database [14]. This process requires time and carried out during this incubation step. The host cells are plated on a selective medium to screen for transformed cells otherwise, the transformation efficiency would decrease by several times.

The study thus documents that cations of different valence could induce competency in *E. coli*. The difference found in result was, the higher transformation efficiency of Ca^{2+} compared to the other cations used but the most important was basically all the salt solutions of associated cations were able to induce competency. In nature, the pH and cations of various salts present may influence the microorganisms to be in constant state of competence to uptake DNA which would result in acquisition of new genes leading in development of new strains. The transformation efficiency achieved was high and cells retained competency for 5 weeks stored at -20°C. When compared to other protocols reported so far this is highly cost effective and simple method that could be used in day to day routine.

ACKNOWLEDGEMENTS

This work was supported by the financial assistance from University Grants Commission-Basic Science Research (BSR) grant (to G.K), New Delhi, India.

Figure 1: Transformation efficiency of competent cells stored under -20 ºC treated using differen salt solutions - cation



REFERENCES

[1] Chen I, Dubnau D. DNA uptake during bacterial transformation. Nat Rev Microbiol 2004; 2(3): 240-49.
[2] Dubnau D. DNA uptake in bacteria. Annu Rev Microbiol 1999; 53(1): 217-44.
[3] Addison CJ et al. Polyhydroxybutyrate-enhanced transformation of log-phase *Escherichia coli*. Biotechniques 2004; 37: 376-82.
[4] Hanahan D. Studies on Transformation of *Escherichia coli* with plasmids. J Mol Biol 1983; 166(4): 557-80.
[5] Ito H et al. Transformation of intact yeast cells treated with alkali cations. J Bacteriol 1983; 153(1): 163-68.
[6] Roychoudhury A et al. Analysis of comparative efficiencies of different transformation methods of *E. coli* using two common plasmid vectors. Indian J Biochem Biophys 2006; 46: 345-400.
[7] Tang X et al. The optimization of preparations of competent cells for transformation of *E. coli*. Nucleic Acids Res 1994; 22(14): 2857-58.
[8] Dagert M, Ehrlich SD. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene 1979; 6(1): 23-28.

[9] Yanisch-Perron C et al. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mpl8 and pUC19 vectors. Gene 1985; 33(1): 103-19.
[10] Ryu J, Hartin R. Quick transformation in *Salmonella thyphirium.* Biotechniques 1990; 8(1): 43-44.
[11] Tu Z et al. An improved system for competent cell preparation and high efficiency plasmid transformation using different *Escherichia coli* strains. Electro J Biotechnol 2005; 8(1): 113-20.
[12] Reush RN et al. Poly-3-Hydroxybutyrate/Polyphosphate complexes from voltage-activated Ca^{2+} channels in the plasma membranes of *Escherichia coli*. Biophys J 1995; 69(3): 754-66.
[13] Reusch RN et al. Posttranslational modification of E. coli histone-like protein H-NS and bovine histones by short-chain poly-(R)-3-hydroxybutyrate (cPHB). FEBS Lett 2002; 527(1-3): 319-22.
[14] Das S, Reusch RN. pH regulates cation selectivity of Poly-(R)-3-hydroxybutyrate/Polyphosphate channels from *E. coli* in planar lipid bilayers. Biochemistry 2001; 40(7): 2075-79.
[15] Chan JOANNA et al. Influences of growth temperature and preparation of competent cells on efficiency of chemically-induced transformation in *Escherichia coli* DH5α. J Exp Microbiol Immunol 2006; 9: 92-96.
[16] Szabo I et al. DNA translocation across planar bilayers containing *Bacillus Subtilis* ion channels. J Biol Chem 1997; 272(40): 25275-82.
[17] Martinez KA et al. Cytoplasmic pH response to acid stress in individual cells of *Escherichia coli* and *Bacillus subtilis* observed by fluorescence ratio imaging microscopy. Appl Environ Microbiol 2012; 78(10): 3706-14.
[18] Mandel M, Higa A. Calcium dependent bacteriophage DNA infection. J Mol Biol 1970; 53(1): 159-62.