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Full Length Article

High Efficiency DNA Transformation Protocol for *Escherichia coli* using Combination of Physico-chemical Methods

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Abstract

The present study was designed to achieve high DNA transformation efficiency of *E. coli* by using the combination of chemical and physical transformation methods. The effect of low growth temperatures, osmotic agents, reducing agents, field strength, pre- and post-electroporation heat shock treatments; surfactant and different combinations of some of these factors were studied in *E. coli*. Cells grown at low temperature required longer time to reach required density and no improvement in transformation efficiency observed. Osmotic agents in growth medium changed the growth trend of bacteria and their presence in transformation mixture proved helpful in achieving up to 10^8 transformants/µg of plasmid DNA. The addition of β-mercaptoethanol in growth and transformation mixture improved transformation efficiency by 10 folds compared to control. Higher field strength of 12.5 kV cm^{-1} was found to increase the transformation efficiency. Delay in electroporation after mixing plasmid DNA and competent cells as in case of pre-electroporation heat shock and use of PEG decreased the transformation efficiency. Pre- and post-electroporation heat shock decreased cell survival as well as transformation frequency. The use of sucrose, glycine and β-mercaptoethanol (in growth and subsequent transformation media), and field strength of 12.5 kV cm^{-1} positively affected the transformation efficiency. © 2014 Friends Science Publishers

Keywords: *E. coli*; Competent cells; Plasmid; Transformation efficiency

Introduction

Transformation is the genetic alteration of a cell resulting from the uptake, incorporation and expression of exogenous DNA that is taken up through the cell envelop. The ability of bacteria to take up the exogenous DNA in their close vicinity is referred to as competence (Dreiseikelmann, 1994) which is genetically programmed physiological state permitting the efficient DNA uptake. The discovery of transferring phage DNA (Mandel and Higa, 1970) and plasmid DNA (Cohen *et al.,* 1972) into *E. coli* cells set the stage for molecular cloning, and the quest for the best artificial mean for transformation of *E. coli* began. In this regard scientists studied the effect of various chemicals and physical treatment to improve the transformation ability of cells.

Chemicals acting as osmotic stabilizers are used in various studies with the aim to enhance transformation efficiency using different bacterial species (Thompson *et al.*, 1998; Arenskotter *et al.,* 2003). Reducing agents can modify cell surface transport machinery and act as permeabilizer. Polyethylene glycol (PEG) has been shown to facilitate uptake of foreign DNA by protoplasts of Gram positive as well as Gram negative bacterial strains (Hanahan, 1983). For achieving high transformation efficiency, use of low growth temperature has been reported that may make the chemical composition or the physical characteristics of bacterial membranes more favorable for uptake of DNA (Inoue *et al.,* 1990).

Electro-transformation is the process of subjecting living cells to a rapidly changing, high-strength electric field which results in producing transient pores in their outer membranes (Szostkova and Horakova, 1998). Consequently, diffusion and exchange of intracellular and extracellular components can take place during the lifespan of the pore. Electroporation is now being widely used to transfer a variety of macromolecules, including DNA, RNA, protein, and some chemotherapeutic agents, into cells (Miller and Nickoloff, 1995). Second most frequently used physical method for DNA transformation in *E. coli* is heat shock treatment. It is an important factor for induction of free DNA uptake by *E. coli* cells and inactivates the restriction enzymes thus suppressing the digestion of penetrated exogenous DNA by these enzymes (Sambrook and Russel, 2001a, b).

No doubt some basic methods are used for bacterial DNA transformation (Sheng *et al.,* 1995; Neumann *et al.,* 1996) still transformation of ligation mixture and PCR products is a difficult task in routine molecular biology lab work. Aim of the present study was to propose a highly reliable and reproducible method for the DNA transformation in *E. coli* by using combination of chemical and physical methods of transformation.

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Materials and Methods

Bacterial Strain, Plasmid and Growth Conditions

E. coli strain DH5α was used in this study. Recombinant vector pGEMTr (Promega pGEMT® easy vector) with insert of cp2 viral protein of 4 Kb containing ampicillin resistance gene was used as transforming DNA. LB (Luria-Bertani) medium alone and supplemented with 1.5% sucrose (LB-S), 1% Glycine (LB-G), 5 mM ascorbic acid (LB-A) and 10 mM β-mercaptoethanol (LB-M) were used to culture DH5α. Super optimal broth (SOB) with 50 µg/mL ampicillin was used for selection of transformants. For making plates, medium was solidified with 1% agar.

Competent Cell Preparation

For competent cells formation, 5 mL overnight starter culture was inoculated in 100 mL of LB, LB-S, LB-G, LB-A and LB-M media and allowed to grow at 37ºC and 25ºC with 250 rpm till growth reached mid log phase (OD_{600} 0.35-0.40). For electroporation, heat shock and PEG studies, bacterial cells were grown in LB media up to mid log phase. Competent cells were prepared according to Sambrook and Russell (2001a). Cell suspension was dispensed into 50 µL aliquots and stored at -80ºC.

Electroporation and Heat Shock Procedure

Competent cells stored at -80°C were thawed at ice and 1 µL plasmid was directly pipetted over competent cells. Competent cells were electroporated at 1.1 kV, 1.9 kV and 2.5 kV by BTX, USA model ECM 399 according to Sambrook and Russell (2001b). These cells were mixed gently by tapping followed by a heat shock treatment. After treatment, 950 µL SOC medium was added. The vials were finally incubated at 37°C for 1 h at 250 rpm in a shaking incubator. To check the effect of PEG, prior to electroporation at 2.5 kV transformation mixture was supplemented with 0%, 5.7%, 11.5% and 17% of PEG (M.W. 8000) solution to study the combination of electroporation with PEG. For pre-electroporation heat treatment, transformation mixture was incubated on ice for 2 min prior to electroporation at 2.5 kV then heat shock was given at 42°C and 46°C for 2 min. For Post-electroporation heat treatment, 42 and 46°C heat shock was given to transformation mixture after electroporation at 2.5 kV.

Reducing and Osmotic Agents

To check the effect of osmotic and reducing agents, 270 mM sucrose, 1% glycine and 0.03 M β-mercaptoethanol were used in transformation mixture to observe the change in transformation yield, before electroporation at 1.9 kV.

Calculation of Transformation Efficiency

The transformation efficiency (transformants/µg DNA) was calculated as follows:

$$\text{Transformation Efficiency} = \frac{\text{No. of Bacterial Colonies}}{\text{Plasmid DNA } (\mu g)} \times \text{Dilution Factor} \times \frac{\text{Original Transforamtion Vol.}}{\text{Plated Volume}}$$

Statistical Analysis

All the experimental data values were means of log of transformants/µg plasmid DNA from three independent experiments and results were presented as mean ± standard error. The significance of differences between the mean values was statistically evaluated by one way ANOVA and 2 Factor factorial using the MSTATC. The statistical significance was all calculated at $P < 0.05$. Least Significant Difference (LSD) test was applied where applicable.

Results

Effect of Low Temperature on the Growth of *E. coli*

Low growth temperature did not improve the transformation of *E. coli* DH5α (Table 1). There was no significant difference of 18°C, 25°C and 37°C on transformation efficiency of *E. coli* (Fig. 1).

Effect of Field Strength

In this study, effect of different field strengths was examined by electroporation at 1.1 kV, 1.8 kV or 2.5 kV potential differences in a 0.2 cm gapped cell corresponding to field strength of 5.5 kV cm^{-1}, 9.0 kV cm^{-1} and 12.5kV.cm^{-1} respectively. Pulse duration was kept constant and effect of field strength was studied (Table 2). Electric shock at 12.5 kV cm^{-1} gave highest transformation efficiency, 9.0 kV cm^{-1} medium and least efficiency was obtained with transformation at 5.5 kV.cm^{-1} (Fig. 2).

Effect of Osmoticum

The addition of 1.5% sucrose in growth medium and 270 mM sucrose in transformation mixture had pronounced effect on transformation efficiency (Fig. 3; Table 3a). Similarly in presence of glycine there was 10-100 fold increase in transformation efficiency (Fig. 3; Table 3b).

Effect of Reducing Agents

The addition of 5 mM ascorbic acid in growth medium acted as growth inhibitor and did not facilitate the transformation yield as expected (Fig. 4; Table 4a). However addition of 10 mM β-mercaptoethanol in growth medium followed by 0.03 mM in transformation medium resulted in 10^{8} transformants/µg DNA at 25°C (Fig. 4: Table 4b).

Effect of PEG

Effect of different concentrations (0, 5.7, 11.5 and 17%) of

Table 1: The effect of low temperature on transformation efficiency of *E. coli*

Treatments (Temperature)	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
18°C	R1	1.35×10^8	2.00×10^7	14.80	5.10×10^6	25.50	5.10×10^7
	R2	2.50×10^7	1.80×10^7	72.00	1.70×10^6	09.40	1.70×10^7
	R3	8.30×10^7	2.20×10^7	26.00	8.90×10^5	04.00	8.90×10^6
	Average	2.43×10^8	2.00×10^7	37.60	2.56×10^6	12.96	2.56×10^7
25°C	R1	8.62×10^8	9.40×10^7	10.90	5.60×10^6	05.95	5.60×10^7
	R2	7.80×10^8	9.10×10^7	11.60	2.40×10^6	03.75	2.40×10^7
	R3	8.90×10^7	0.90×10^7	10.10	7.50×10^5	08.33	7.50×10^6
	Average	5.77×10^8	6.40×10^7	10.80	2.90×10^6	06.01	2.90×10^7
37°C	R1	1.29×10^8	2.94×10^7	22.79	5.24×10^5	01.78	5.24×10^6
	R2	1.56×10^8	4.03×10^7	25.83	4.39×10^5	01.09	4.39×10^6
	R3	2.43×10^8	9.56×10^6	03.90	5.16×10^5	05.39	5.16×10^6
	Average	1.76×10^8	7.92×10^7	17.50	4.93×10^5	02.75	4.93×10^6

Table 2: Effect of exposure to different electric field strengths for 6 mS on transformation in *E. coli* DH5α

Field Strength (kV cm^{-1})	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
12.5	R1	5.00×10^9	2.46×10^9	49.00	5.70×10^5	2.30×10^{-2}	5.70×10^6
	R2	6.22×10^8	4.18×10^8	67.00	4.10×10^5	9.00×10^{-2}	4.10×10^6
	R3	1.10×10^9	8.62×10^8	78.00	4.38×10^6	50.00×10^{-2}	4.38×10^7
	Average	2.24×10^9	1.25×10^9	64.67	1.79×10^6	20.40×10^{-2}	1.79×10^7
9.5	R1	5.00×10^9	2.97×10^9	59.40	4.70×10^5	1.50×10^{-2}	4.70×10^6
	R2	6.22×10^8	3.80×10^8	61.00	8.70×10^5	22.00×10^{-2}	8.70×10^6
	R3	1.10×10^9	6.62×10^8	60.00	1.56×10^6	20.00×10^{-2}	1.56×10^7
	Average	2.24×10^9	1.34×10^9	60.13	9.67×10^5	14.50×10^{-2}	9.67×10^6
5.5	R1	5.00×10^9	3.02×10^9	60.40	1.03×10^5	0.34×10^{-2}	1.03×10^6
	R2	6.22×10^8	6.45×10^8	100.00	1.20×10^5	1.86×10^{-2}	1.20×10^6
	R3	1.10×10^9	9.96×10^8	90.50	1.30×10^5	1.30×10^{-2}	1.30×10^6
	Average	2.24×10^9	1.55×10^9	83.60	1.18×10^5	1.17×10^{-2}	1.18×10^6

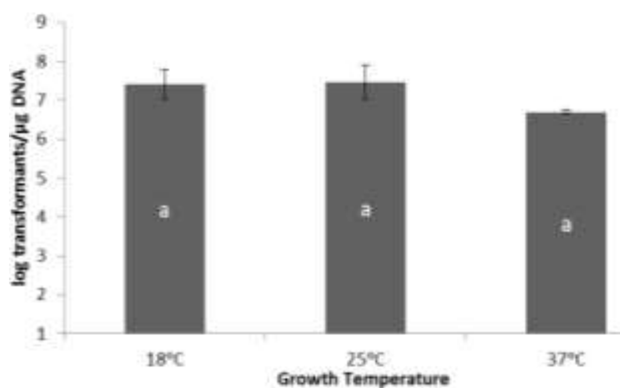

Fig. 1: Effect of different growth temperatures on transformation efficiency. Low growth temperature did not show any positive effect on transformation efficiency of *E. coli* DH5α

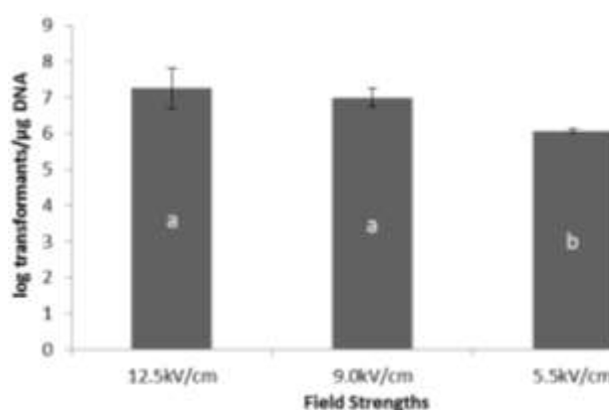

Fig. 2: Comparison of transformation efficiency of *E. coli* DH5α at different voltages. The highest efficiency was achieved at 12.5 kV cm^{-1} field strength

PEG (M.W. 8000) solution on transformation efficiency was monitored. Transformation mixture containing 50 µL competent cells and 1 µL plasmid DNA was made in ice-cold eppendorf. Then 57% PEG solution and nanopure water were added in ratio 0 µL:22 µL, 7 µL:15 µL, 15 µL:7 µL, 22 µL:0 µL to transformation mixture for making 0, 5.7, 11.5 and 17% PEG solution respectively. After properly mixing, the contents were transferred to prechilled electroporation cells and shocked at 12.5 kV cm^{-1}. Results of One-way ANOVA showed that all four treatments were similar with reference to transformation efficiency (P>0.05) (Fig. 5; Table 5).

Pre-electroporation Heat Shock Treatment

Results of One-Way ANOVA showed that the three treatments i.e., electroporation without heat shock, pre-electroporation heat shock treatment at 42ºC and at 46ºC were significantly different (P<0.05). LSD results suggested that the transformation efficiency without heat shock was greater than either of the pre-electroporation heat shocks at

Table 3a: The effect of osmotic agents (sucrose or glycine) on transformation efficiency of *E. coli* at 37°C

Treatments	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
LB	R1	2.20×10^{8}	7.50×10^{7}	34.00	6.00×10^{5}	00.80	6.00×10^{6}
	R2	6.30×10^{8}	1.05×10^{8}	16.00	1.00×10^{6}	00.95	1.00×10^{7}
	R3	4.00×10^{8}	8.65×10^{7}	21.00	9.10×10^{5}	01.05	9.10×10^{5}
	Average	4.16×10^{8}	8.80×10^{7}	23.60	8.36×10^{5}	00.90	8.36×10^{6}
LB-S	R1	2.30×10^{8}	8.70×10^{7}	37.00	8.50×10^{5}	00.97	8.50×10^{6}
	R2	7.50×10^{9}	9.60×10^{8}	12.80	9.50×10^{6}	00.99	9.50×10^{7}
	R3	7.50×10^{8}	6.50×10^{7}	08.66	6.60×10^{6}	10.10	6.60×10^{7}
	Average	2.80×10^{9}	3.70×10^{8}	19.40	5.60×10^{6}	04.02	5.60×10^{7}
LB-G	R1	2.90×10^{7}	1.50×10^{7}	51.00	2.50×10^{6}	16.00	2.50×10^{7}
	R2	3.10×10^{9}	3.70×10^{8}	28.00	6.10×10^{7}	07.00	6.10×10^{8}
	R3	8.30×10^{8}	1.50×10^{8}	18.00	3.40×10^{7}	22.60	3.40×10^{8}
	Average	1.31×10^{9}	3.45×10^{8}	32.00	3.25×10^{7}	15.20	3.25×10^{8}

Table 3b: The effect of osmotic agents (sucrose or glycine) on transformation efficiency of *E. coli* at 25°C

Treatments	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
LB	R1	6.80×10^{8}	1.90×10^{8}	27.50	1.70×10^{6}	00.90	1.70×10^{7}
	R2	7.00×10^{8}	1.56×10^{8}	22.00	1.00×10^{6}	00.60	1.00×10^{7}
	R3	7.30×10^{8}	9.10×10^{7}	12.40	1.00×10^{6}	01.00	1.00×10^{7}
	Average	7.03×10^{8}	1.45×10^{8}	20.63	1.23×10^{6}	00.83	1.23×10^{7}
LB-S	R1	8.10×10^{8}	1.80×10^{8}	22.00	4.00×10^{7}	04.80	4.00×10^{8}
	R2	7.70×10^{8}	1.70×10^{8}	22.00	1.10×10^{7}	06.00	1.10×10^{8}
	R3	7.50×10^{8}	2.00×10^{8}	26.70	1.40×10^{7}	07.00	1.40×10^{8}
	Average	7.76×10^{8}	1.83×10^{8}	23.56	2.16×10^{7}	05.93	2.16×10^{8}
LB-G	R1	6.30×10^{8}	1.20×10^{8}	19.00	1.20×10^{7}	10.00	1.20×10^{8}
	R2	6.20×10^{8}	1.10×10^{8}	18.50	1.40×10^{7}	12.00	1.40×10^{8}
	R3	5.50×10^{8}	1.60×10^{8}	29.00	3.30×10^{7}	20.60	3.30×10^{8}
	Average	6.00×10^{8}	1.30×10^{8}	22.16	1.96×10^{7}	14.20	1.96×10^{8}

LB, control cells; LB-S, cells grown in presence of 40 mM sucrose and 270 mM sucrose was added to transformation mixture prior to electric pulse; LB-G, cells grown in presence of 1 % (w/v) glycine and transformation mixture also contains 1 % (w/v) glycine.

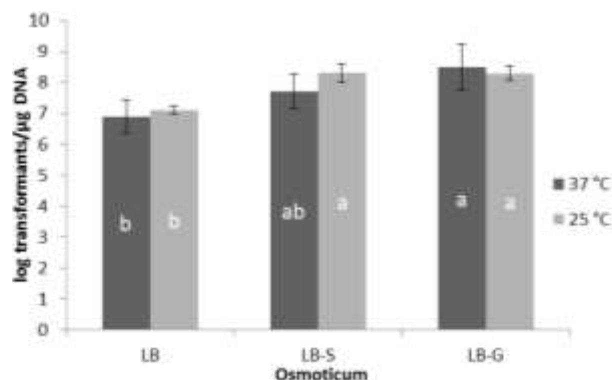

Fig. 3: Effect of sucrose and glycine as growth supplement in LB growth media and in transformation mixture on *E. coli* genetic transformation. LB-S is cells grown with sucrose (1.5%) and 270 mM sucrose in transformation mixture and LB-G is results of experiment where 1% glycine was used in both growth medium and in transformation mixture

42ºC or at 46ºC (Fig. 6). Heat shock before electroporation at either of the temperature decreased the transformation efficiency by 10 fold than control experiment (Table 6a).

Post-electroporation Heat Shock Treatment

Competent cells and plasmid DNA were shocked at 12.5 kV.cm^{-1} followed by addition of SOC medium, incubation for 6 min at 42ºC or 46ºC and then transfer to ice for 5 min before incubation for growth recovery. The three treatments were compared with One-way ANOVA and the results revealed no significant difference among all the three treatments ($P>0.05$) (Fig. 6; Table 6b).

Discussion

Results of present study for bacterial growth at low temperature were in contrast to Inoue *et al.* (1990); Han *et al*. (2003) and Wang *et al*. (2007), that there was 10-20 folds increase in transformation yield in *E. coli* when cells were allowed to grow at 25°C instead of 37°C. In this study, low growth temperature showed no improvement in transformation efficiency. Better transformation efficiency was observed when *E. coli* DH5α was transformed at high field strengths. Kinosita and Tsong (1977) reported increase in the number and radius of pores formed as a function of increased electric shock intensity, resulting in augmented transfer of vector DNA to competent cells.

The presence of sucrose in growth medium facilitates the growth process and early attainment of mid log phase, therefore exerting positive role in transformation (Arenskotter *et al.,* 2003; Wang *et al.,* 2007). In the present study, when electric pulse was applied to transformation mixture containing 270 mM sucrose, the transient pores were produced in the cell membranes with the life span of

Table 4a: The effect of reducing agent, ascorbic acid, on transformation efficiency of *E. coli* at 37°C

Treatments	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
LB	R1	4.00×10^8	1.50×10^8	37.00	1.40×10^5	00.09	1.40×10^6
	R2	1.30×10^9	3.60×10^8	27.00	1.80×10^5	00.05	1.80×10^6
	R3	6.00×10^8	3.00×10^8	50.00	2.00×10^5	00.06	2.00×10^6
	Average	7.60×10^8	2.70×10^8	38.00	1.73×10^5	00.20	1.73×10^6
LB-A	R1	2.60×10^8	1.00×10^8	38.00	1.60×10^5	00.16	1.60×10^6
	R2	7.60×10^8	1.00×10^8	13.00	1.50×10^4	00.01	1.50×10^5
	R3	6.30×10^8	9.90×10^7	25.30	1.70×10^6	01.00	1.70×10^7
	Average	1.83×10^8	9.96×10^7	25.40	6.25×10^5	00.40	6.25×10^6

Table 4b: The effect of reducing agent, ascorbic acid, on transformation efficiency of *E. coli* at 25°C

Treatments	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
LB	R1	1.40×10^9	3.80×10^8	27.00	9.60×10^6	02.50	9.60×10^7
	R2	3.90×10^8	1.20×10^8	30.00	2.80×10^6	02.30	2.80×10^7
	R3	6.50×10^8	1.50×10^8	23.00	5.40×10^5	00.36	5.40×10^6
	Average	8.13×10^8	2.36×10^8	26.60	4.30×10^6	01.72	4.30×10^7
LB-A	R1	4.10×10^8	5.30×10^7	12.00	2.90×10^5	00.55	2.90×10^6
	R2	1.70×10^8	1.60×10^7	09.40	9.40×10^4	06.20	9.40×10^5
	R3	5.60×10^8	4.90×10^7	08.70	1.70×10^6	03.40	1.70×10^7
	Average	3.80×10^8	3.93×10^7	10.30	6.90×10^5	03.38	6.90×10^6

LB, control cells; LB-A, cells grown in presence of 5 mM L-ascorbic acid in LB medium.

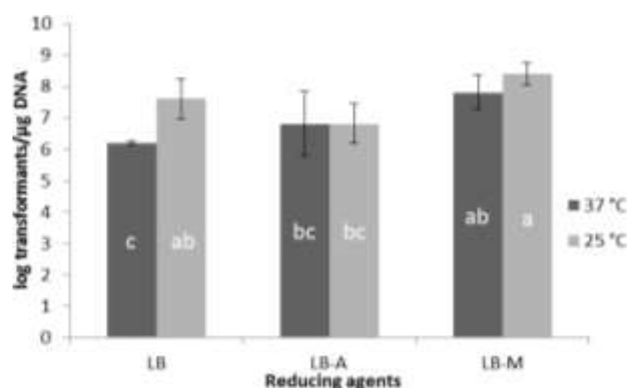

Fig. 4: Effect of ascorbic acid and β-mercaptoethanol supplemented medium on transformation efficiency in *E. coli*. LB represents control cells; LB-A are cells grown with ascorbic acid (5 mM) and LB-M is indicating results of experiment performed in presence of β-mercaptoethanol in growth medium as well as in transformation mixture

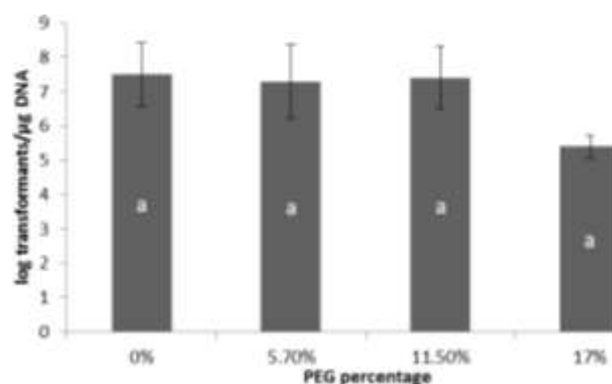

Fig. 5: Comparison of *E. coli* transformation efficiencies with different PEG cocentrations. The control with 0% PEG gives better transformation yield compared to average transformation efficiencies obtained with 5.7, 11.5 and 17%

30 min. The sucrose being higher in concentration in extra cellular medium than inside the cell cause mass flow of sucrose through pores from outside to inside of the cell resulting in concomitant DNA delivery into the cells (Enyard, 1992).

Glycine acts as inhibitor of bacterial growth but it has positive impact on transformation yield because its presence in growth medium interferes in membrane biosynthesis and replaces L- and D-alanine found in the peptide units of peptidoglycan rendering membrane more permeable (Kaderbhai *et al.,* 1997).

Presence of ascorbic acid in growth medium was supposed to reduce membrane proteins and weaken the membrane that could have resulted in breaking permeability barrier for macromolecule, nevertheless our results indicated that ascorbic acid act only as a growth inhibitor not rather than a transformation facilitator. The addition of β-mercaptoethanol (10 mM) in growth medium only, had neither any effect on transformation efficiency nor on growth pattern. However, its presence in growth medium (10 mM) as well as in transformation mixture (0.03 M) resulted in 10 fold increase in transformation efficiency (Fig. 4). It has been reported by Puyet *et al.* (1990) that *S. pneumonia* harbor a membrane-bound nuclease required for transformation. It was therefore, hypothesized that presence of β-mercaptoethanol in transformation mixture prior to addition of DNA may prevent inactivation of similar surface exposed nucleases possessed by *E. coli* required for facilitating the process of DNA uptake.

Use of PEG in combination with electroporation is not

Table 5: Transformation efficiencies in different concentrations of polyethylene glycol

Treatments	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
0%	R1	1.40×10^9	5.73×10^8	40.90	6.80×10^6	119.00×10^{-2}	6.85×10^7
	R2	1.90×10^9	4.96×10^8	26.10	3.06×10^6	60.00×10^{-2}	3.06×10^7
	R3	2.90×10^9	1.95×10^8	6.70	1.20×10^5	06.15×10^{-2}	1.20×10^6
	Average	2.07×10^9	4.21×10^8	24.57	3.34×10^6	62.00×10^{-2}	3.34×10^7
5.7%	R1	1.40×10^9	5.64×10^8	40.20	5.14×10^6	90.00×10^{-2}	5.14×10^7
	R2	1.90×10^9	4.66×10^8	24.52	4.70×10^5	10.00×10^{-2}	4.70×10^6
	R3	2.90×10^9	2.25×10^8	7.76	4.00×10^4	01.70×10^{-2}	4.00×10^5
	Average	2.07×10^9	4.18×10^8	24.16	1.88×10^6	33.90×10^{-2}	1.88×10^7
11.5%	R1	1.40×10^9	5.39×10^8	38.50	8.38×10^6	155.00×10^{-2}	8.38×10^7
	R2	1.90×10^9	4.31×10^8	22.68	6.40×10^5	14.80×10^{-2}	6.40×10^6
	R3	2.90×10^9	2.49×10^8	8.58	1.40×10^5	00.56×10^{-2}	1.40×10^6
	Average	2.07×10^9	4.06×10^8	23.25	3.05×10^4	58.00×10^{-2}	3.05×10^7
17%	R1	1.40×10^9	3.75×10^8	26.78	4.00×10^4	1.00×10^{-2}	4.00×10^5
	R2	1.90×10^9	3.81×10^8	20.00	1.00×10^4	0.20×10^{-2}	1.00×10^5
	R3	2.90×10^9	1.97×10^8	6.79	3.33×10^4	1.69×10^{-2}	3.33×10^5
	Average	2.07×10^9	3.18×10^8	17.86	2.78×10^4	0.96×10^{-2}	2.78×10^5

Table 6a: Transformation efficiency obtained with pre-electroporation heat shocks at different temperatures

Treatments	Replications	No. of cells/mL	No. of cells survived/mL	% survival	No. of cells transformed/mL	% Transformants	Transformation efficiency
Control	R1	1.20×10^{10}	4.87×10^9	40.58	2.50×10^5	5.10×10^{-3}	2.50×10^6
	R2	3.33×10^9	1.24×10^9	37.23	1.53×10^5	12.00×10^{-3}	1.53×10^6
	R3	1.50×10^9	3.58×10^8	23.86	2.20×10^5	60.00×10^{-3}	2.20×10^6
	Average	5.61×10^9	2.16×10^9	33.89	2.08×10^5	25.70×10^{-3}	2.08×10^6
42ºC	R1	1.20×10^{10}	4.01×10^9	33.42	3.00×10^4	0.70×10^{-3}	3.00×10^5
	R2	3.33×10^9	1.43×10^9	42.90	1.67×10^4	1.20×10^{-3}	1.67×10^5
	R3	1.50×10^9	7.60×10^7	5.06	1.00×10^4	13.00×10^{-3}	1.00×10^5
	Average	5.61×10^9	1.84×10^9	27.13	1.89×10^4	5.90×10^{-3}	1.89×10^5
46ºC	R1	1.20×10^{10}	2.09×10^9	17.41	4.00×10^4	1.90×10^{-3}	4.00×10^5
	R2	3.33×10^9	7.85×10^8	23.57	2.00×10^4	2.60×10^{-3}	2.00×10^5
	R3	1.50×10^9	5.90×10^7	3.90	1.00×10^4	13.00×10^{-3}	1.00×10^5
	Average	5.61×10^9	9.78×10^8	14.96	2.33×10^4	5.80×10^{-3}	2.33×10^5

Table 6b: Transformation efficiencies with post-electroporation heat treatment

Treatments	Replications	No. of Cells / mL	No. of Cells Survived /mL	% Survival	No. of Cells Transformed /mL	% Transformants	Transformation Efficiency
Control	R1	4.67×10^9	1.17×10^9	25.05	1.00×10^5	8.50×10^{-3}	1.00×10^6
	R2	6.00×10^9	1.67×10^9	27.80	1.90×10^5	11.00×10^{-3}	1.90×10^6
	R3	5.00×10^9	1.43×10^9	28.60	1.50×10^5	10.00×10^{-3}	1.50×10^6
	Average	5.22×10^9	1.42×10^9	27.15	1.47×10^5	9.80×10^{-3}	1.47×10^6
42°C	R1	4.67×10^9	1.39×10^9	29.76	1.60×10^5	11.50×10^{-3}	1.60×10^6
	R2	6.00×10^9	1.91×10^9	31.80	1.00×10^4	8.30×10^{-3}	1.00×10^5
	R3	5.00×10^9	1.76×10^9	35.00	1.10×10^5	2.20×10^{-3}	1.10×10^6
	Average	5.22×10^9	1.69×10^9	32.19	9.33×10^4	7.30×10^{-3}	9.33×10^5
46°C	R1	4.67×10^9	1.30×10^9	27.84	6.00×10^4	4.60×10^{-3}	6.00×10^5
	R2	6.00×10^9	9.40×10^8	15.67	1.00×10^4	1.00×10^{-3}	1.00×10^5
	R3	5.00×10^9	8.00×10^8	16.00	1.00×10^4	1.25×10^{-3}	1.00×10^5
	Average	5.22×10^9	1.01×10^9	19.83	2.67×10^4	2.28×10^{-3}	2.67×10^5

previously reported; hence this combination was also tested, although with no superior outcome in terms of transformation efficiency. Tu *et al.* (2005) and Hanahan *et al.* (1991) observed that addition of PEG to the plasmid DNA and competent cells increased transformation efficiency but heat shock was not necessary for increasing number of transformants. PEG functions to condense and consequently increase the chances of DNA entry to competent cells (Himeno *et al.* 1984). PEG facilitates DNA entry into the chemically prepared competent cells (Hanahan *et al*., 1991; Tu *et al*., 2005) but if other physical treatment as heat shock was used in combination with PEG, then efficiency was either same or even declined.

When both heat and electric shocks were administered in this study, it may have resulted in complete extinction of membrane potential and decrease in recovery which may be a consequence of difficulties to the cells owing to exposure to two different, albeit consecutive shocks. Resultantly the percentage of cells transformed may have dropped thus offsetting any increase in ultimate transformation efficiency.

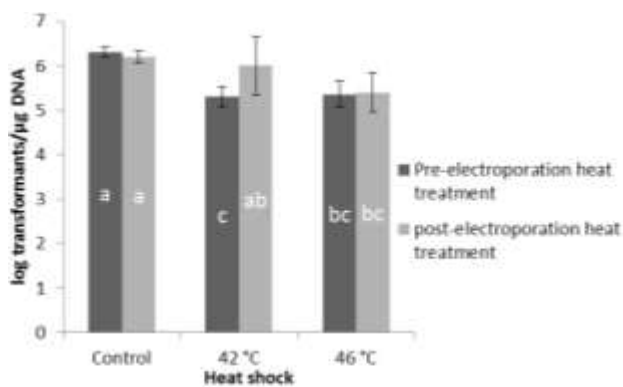

Fig. 6: Effect of pre- and post-electroporation heat shock on transformation efficiency. Control (transformation with electroporation) gave better efficiency than electroporation combined with heat shock at any of above mentioned temperatures

In order to improve the transformation efficiency, an effort was made to administer heat shock after electroporation expecting DNA molecules to move into the cells through transient pores. The results are shown in Fig. 6, which are in contrast to those of Arenskotter *et al.* (2003) in *Gordonia polyisoprenivorans,* where they observed enhancement in transformation efficiency with post-electroporation heat shock treatment at 46ºC for 6 min. This difference in results might be due to differences in cell wall composition and structure between *G. polyisoprenivorans* and *E. coli*.

In conclusion, the highest transformation efficiency was obtained when sucrose was added in growth medium as well as in transformation mixture. Use of glycine in growth medium and transformation mixture also give high transformation efficiency. Thus, osmoticum on the whole have positive effect on transformation yield. Higher transformation efficiency was also obtained with β-mercaptoethanol. Among the three field strengths tested during this study, 12.5 kV cm^{-1} proved to be the best field strength. Use of low temperature, PEG, ascorbic acid and pre and post electroporation treatments did not show any positive effect on transformation efficiency.

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