Investigating the effect of thermal shock factors and different cell treatments on bacterial transformation efficiency

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Abstract

Introduction: Molecular transformation of bacterial cells plays a central role in molecular DNA transfer. At present, transformation is carried out either by chemical methods or by electric shock (electroporation). This study tended to investigate the effect of thermal shock factors and different cell treatments on transformation efficiency in E.coli.
Materials and methods: In this method, E.coli cells were treated by different concentrations of peptide CM11 (0.1, 1, 2, 3 and µg/ml) and lysozyme enzyme (40 mg/ml and 80 mg/ml) based on competence by calcium chloride (100 and 200 mM). They also underwent thermal and cold shocks at different temperature intervals. Then, pUC19 plasmid (2.7 kbp) was transferred as a model and separately to the competent bacteria.
Results: The results showed that increasing the thermal shock in three temperature intervals (37, 42 and 47°C) with cold shock (0 and 5°C) led to an increase in the number of transformed colonies and it has a significant relationship with transformation ($P<0.05$). Increased CaCl2 concentration was a positive factor in the amount of transformation, but no significant relationship was found between increased lysozyme concentration and transformation ($P>0.05$). Increased CM11 peptide concentration showed a significant relationship with transformation ($P<0.05$). There was no significant relationship between three transformations ($P>0.05$), while there was a significant relationship between peptide CM11 concentration and lysozyme enzyme concentration in transformation simultaneously ($P<0.05$).
Conclusion: It can be concluded that increased thermal shock and stability of cold shock at minimum temperature (0°C), as well as increased CaCl2 concentration and increased peptide concentration to 1 µg/ml can increase the number of transformed colonies in E.coli. Finally, peptide concentration and enzyme concentration are introduced as a simple and convenient method for increasing the transformation efficiency.

Keywords: Transformation, Escherichia coli, Plasmid, Lysozyme Enzyme, Peptide CM11

Introduction

Genetic materials can be transmitted in bacteria in several ways. In all transmission mechanisms, there is a cell which sends a portion of its DNA to the receptor cell. Usually, this DNA portion of the sender cell becomes part of the receptor cell, and the rest is degraded by intracellular enzymes. In this case, the receptor cell, which contains a DNA portion of the sender cell, is called

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recombinant. Genetic material transfer is not a common phenomenon in bacteria and can only occur among a percentage of the bacterial population (1). Gene transfer is carried out in bacteria by three methods: transduction, conjugation and transformation. Plasmid transfer into competent bacterial cells by using thermal shock is a fundamental method in bacterial transformation (2). Most bacterial transformation techniques are based on observations of Mandel and Higa in 1970 (3). Success rate of transformation depends on the type of methods used. Thus, whatever the molecular transformation has more efficiency and quality, success rate of this method will increase. Transformation is a phenomenon in which the cell takes (incorporates) an alien gene released (DNA or RNA) and expresses it. The sender DNA can be isolated from cells by natural decomposition of the cell or by chemical means. In immediate transfer, recombination takes place when DNA enters the receptor cell. Bacteria receiving genes which contain certain traits from sender bacteria are called modified. This phenomenon has been seen more in bacteria and less in eukaryotes.

Therefore, when some bacteria grow in the vicinity of closely related strains, they will obtain one or more traits of the relative strain and will transfer to close strains (4). In addition to chromosome, there are also non-chromosomal elements of double stranded DNA in bacteria. These elements which can independently replicate in bacterial cytoplasm are called plasmid. Plasmids are circular; compared with chromosome size of the bacterium, they are about 0.1 to 0.5 times the chromosome length (5). E.coli and its similar bacteria transfer their genetic material through horizontal gene transfer to other similar bacteria, using mechanisms such as transformation, conjugation and transduction. For example, transfer of Shiga toxin from Shigella to E.coli O157: H7 occurred through phage (transduction) (6). E.coli has been used to transfer DNA and express many genes and produce recombinant products. For example, scientists have been able to produce insulin from E.coli using genetic engineering (7). The modified E.coli has been used for production of vaccine and bioremediation (8). Finally, this study tends to investigate the effect of thermal shock factors and different treatments on transformation efficiency of Escherichia coli.

Materials and methods

The E.coli strain containing DH5α was used. The plasmid DNA used was the plasmid pUC19 (2.7 kbp) containing ampicillin resistance gene. Agarose gel (given the considered DNA fragment size) was made at a concentration of 1%. The sample was poured in the sink; then, the electrophoresis tank was connected to DC voltage of 5-10 V/Cm in the presence of a TBE buffer. Electrophoresis was applied until the gel nearly turned into bromophenol blue. Nucleic acid molecules were studied in the presence of ethidium bromide and its luminescence against UV light by a transluminator (UV) device. Plasma DNA is different from other nucleic acid molecules based on size and circular shape. Plasma DNA concentration was calculated by measuring invisible light absorbance of the solution containing DNA at a wavelength of 260 nm and by Bio Photometer, Eppendorf. Finally, 50 µl inoculum liquid from E.coli tubes stored in glycerol at -70°C was added to loosely capped tubes containing 3 ml LB medium and 100 µg/ml ampicillin (LB+Amp); its light absorbance rate was determined at 0.5 at a wavelength of 600 nm. The obtained cultures were the same overnight cultures.

Treatments used for evaluating transformation included:

a) Treatment by $CaCl_2$

Calcium chloride treatment was carried out according to Cohen et al. For this purpose, E.coli cells were mixed with calcium chloride buffer (100 mM) and suspended at 0°C for one hour. For transformation,

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DNA was added to competent cell suspension (1 µl 1 plasmid pUC19 containing 100 ng DNA), incubated for one hour and cultured at 37°C on a LB agar + amp plate (100 mg/ml) (9).

b) Treatment by CM11 peptide

Initially, 1 mg/ml CM11 peptide solution synthesized in a phosphate saline buffer, pH = 7.2, was made. In order to evaluate the effect of CM11 peptide on enhancing the ability of E.coli to receive external DNA, bacterial cells became competent based on standard CaCl2 method and treated by peptide. In this method, E.coli was first cultured. Then, 50 µl medium containing E.coli cultured previously (overnight) was incubated to 5 separate Falcon tubes containing 5 ml LB medium, and incubated at 37°C, 150 rpm, to absorb ambient light to 0.5. Then, cells were centrifuged for 10 min at 2000 g and they were collected. The cell precipitates were suspended in 5 separate tubes with 4 ml of cold 100 mM CaCl2 (0°C) and kept in ice (0°C) for 1 h. Then, the tubes were centrifuged for 5 min at 2000 g and the obtained cell precipitate was suspended in cold 100 mM CaCl2. Then, different concentrations of CM11 peptide (0.1, 1, 2, 3 and 5 µg/ml) were added to the tubes.

c) Treatment by lysozyme

In order to investigate the effect of lysozyme on increased ability of *E.coli* to receive external DNA, bacterial cells became competent based on standard $CaCl_2$ method and treated by lysozyme. In this method, *E.coli* was first cultured. Then, 50 µl medium containing *E.coli* cultured previously (overnight) was incubated to 5 separate Falcon tubes containing 5 ml LB medium, and incubated at 37°C, 150 rpm, to absorb ambient light to 0.5. Then, cells were centrifuged for 10 min at 2000 g and they were collected. The cell precipitates were suspended in 5 separate tubes with 4 ml of cold 100 mM $CaCl_2$ (0°C) and kept in ice (0°C) for 1 h. Then, the tubes were centrifuged for 5 min at 2000 g and the obtained cell precipitate was suspended in cold 100 mM $CaCl_2$ (10).

Finally, transformation was calculated based on Tu et al by following equations (11).

Statistical analysis

Finally, all experiments were repeated twice to examine repeatability and statistical study. Results were expressed as mean values ± standard deviation. The data obtained were analyzed by the use of ANOVA, using the SPSS software version 21 statistical package. Multiple comparisons were done using the least significant difference test, t-test or Chi-square to compare differences between results. Results were considered significant at 95 % confidence level ($P < 0.05$).

Results

This project evaluated the effect of thermal shock factors and different cellular treatments on bacterial transformation efficiency. In culture and processing of bacterial suspension, common conditions were used to have equal, comparable number of bacterial cells which participated in different competence and transformation operations. After transformation process, some colonies grown on LB + Amp medium were randomly selected and the plasmid was extracted; then, electrophoresis was processed on 1% agarose gel. The experiment showed that increased thermal shock from 37 to 47°C in a constant cold shock (0°C) was associated with increased transformation and there was a significant relationship between thermal shock at the above temperatures and the cold shock (0°C) and transformation rate ($P<0.05$). Chi-square was used to analyse data. A significant relationship was found between increased thermal shock at constant cold shock 5°C and transformation ($P<0.05$); however, this increased transformation was reduced compared to cold shock 0°C.By comparing transformation rate in

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increased thermal shock and cold shock 0°C and 5°C (Table 1), it can be concluded that increased thermal shock with minimum cold shock (0°C) increased transformation efficiency in *E.coli* (P<0.05).

Table 1. Number of colonies transformed at thermal shock temperatures 37, 42, 47°C with cold shock 0 and 5°C.

Thermal shock	Cold shock 0°C		Cold shock 5°C	
	Number of colonies	Transformation rate	Number of colonies	Transformation rate
37C°	137	1.37×10^3	74	0.74×10^3
42C°	154	1.54×10^3	91	0.91×10^3
47C°	178	1.78×10^3	107	1.07×10^3

The results showed that increased DNA bond to outer surface of the bacterial wall as a result of increased $CaCl_2$ concentration was a positive factor in transformation and increased $CaCl_2$ concentration from 100 to 200 mM was associated with increased number of transformed colonies.

Chi-square test showed a significant relationship between CM11 peptide concentration and transformation (p<0.05). The experiments showed that increased concentration of peptide from 0.1 to 1 µg/ml increased transformation rate, while increased concentration to >1 µg/ml reduced the number of colonies transformed. Moreover, Chi-square test showed that although increased concentration of lysozyme from 40 to 80 µg/ml slightly increased number of colonies undergoing transformation, there was no significant relationship between enzyme concentration and transformation; that is, the treatment acts independently (Table 2).

Table 2. Number of colonies transformed in treatment by CaCl2, Peptide CM11 and Lysozyme enzyme.

	Concentration	Number of colonies	Transformation rate
$CaCl_2$ (mM)	100	51	5.1×10^2
	200	87	8.7×10^2
Peptide CM11 (µg/ml)	0.1	291	2.91×10^3
	1	342	3.42×10^3
	2	245	2.45×10^3
	3	191	1.91×10^3
	5	57	5.7×10^2
Lysozyme (µg/ml)	40	53	5.3×10^2
	80	58	5.8×10^2

Comparison of treatments by thermal shock, enzyme and peptide (cold shock 0°C): ANOVA was used to analyse data. No significant relationship was found between three treatments at cold shock 0°C in transformation. This means that all three treatments separately increased transformation and they were not significantly different. Each treatment could replace the other (P>0.05). Pairwise comparison of treatments at cold shock 0°C showed a significant relationship only between peptide and enzyme concentrations; that is, simultaneous use of these two treatments increased the transformation (P<0.05) (Table 3). *Comparison of treatments by thermal shock, enzyme and peptide (cold shock 5):* ANOVA was used to analyse data. A significant relationship was found between three treatments at cold shock 5°C in transformation. Pairwise comparison of treatments at cold shock 5°C showed a significant relationship between peptide

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concentration and enzyme concentration; that is, peptide concentration and enzyme concentration could be used simultaneous (P<0.05) and other treatments act independently.

Table 3. Pairwise comparison of transformation methods (Treatment protocol) at cold shock 0 and 5 ℃.

	Cold shock 5℃		P value	Cold shock 0℃		P value
LSD*	Thermal shock	Enzyme concentration	0.062	Thermal shock	Enzyme concentration	0.295
		Peptide concentration	0.657		Peptide concentration	0.227
	Peptide concentration	Enzyme concentration	0.049	Peptide concentration	Enzyme concentration	0.045

* Least significant difference

Discussion

One of the processes widely used in genetic and biotechnological laboratories is transformation process in which DNA is transferred to the target cell, and high transformation efficiency is dependent on the amount of DNA transferred to the host cell. In this sense, the more DNA transfer to the target cell with a higher probability, the better results will be achieved (5). In most biotechnology research laboratories, it is very common to use bacterial cells as an adjunctive DNA recipient, because growth and proliferation are fast in bacteria and work with them is very simple and cheap compared to other cells.

Various expressive systems have been used to produce recombinant proteins (12). Many advantages have been demonstrated for *E.coli* expressive system and it remains a valuable microorganism for production of recombinant proteins at high levels. Because of well-known genetic and physiological characteristics of this bacterium, its proliferation in short time, its ease of manipulation, its proven fermentation knowledge and ultimately high capacity for accumulation of recombinant proteins (more than 20% of total protein content of the cell), *E.coli* is one of the most suitable hosts for production of recombinant protein (13).

Nejad Moghadam et al. (2009) used a new solution called competent transformation solution (CTS) cells. The results of this study showed an increase in transformation rate in the presence of DMSO and PIPES, while thermal shock was discarded; moreover, its transformation rate was about 10 times higher than usual treatments (10^7 cells transformed per μg plasmid DNA) on average. In this study, a complete CTS solution was stored for 6 months in a refrigerator (4°C) away from light radiation and used to repeat the experiments; no significant difference was observed in the number of transformed bacteria compared to before. This can be a reason for stability of this solution at 4°C and time (14).

In the experiment, combination of two methods based on enzyme and peptide concentration increased transformation (P=0.04). However, all three methods worked independently in either 0°C or 5°C cold shock (p>0,05). Snyder et al. used the carpet method. At threshold concentration, CM11 peptide attached to membrane and caused initial instability in its structure; by increasing the peptide concentration and reaching a threshold, it created a hole or cavity in the membrane. It is noteworthy that primary bond and subsequent ability to form a cavity by peptide are independent but continuous processes which are dependent on peptide concentration (15).

In the present study, the results showed that peptide concentration from 0.1 as threshold and increase to 1 μg/ml increased transformation but increased concentration to >1 μg/ml reduced the transformation process. Thus, this process depends on peptide concentration

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(P<0.05). Tan et al. examined a DNA transfer into the bacteria based on the Yoshida method. The results showed that even when high amount of pET15b plasmid concentration was used, transmission rate was low. Therefore, $CaCl_2$ transfer method was used on the same plasmid to improve the transfer rate. Using carbon microtubes, transfer rate was more than 15000, and this method was referred to as the third widespread method in addition to conventional chemical methods and electrical shock methods in vitro (16).

Heler et al. (2011) used TFB instead of $CaCl_2$ solution. The number of transformed cells has been reported using conventional $CaCl_2$ method at 37°C (17). Previous studies have shown that addition of DMSO greatly increased transformation rate.

In the present study, bacterial competence by using chemical increase in $CaCl_2$ concentration from 100 to 200 mM with removal of thermal shock doubled the transformation rate, and according to Tan et al. (2012) it is as a competent factor for transformation. Moreover, increased thermal shock in the temperature range of 37, 42 and 47°C in 45 s and constant cold shock (0° and 5°C) in 10 min increased the number of colonies undergoing transformation, contrary to Chang et al., who found no significant difference when using thermal shock (P<0.05).

Hara et al. studied the transformation of Bacillus subtilis protoplast by DNA plasmid influenced by low concentration of lysozyme. The plasmid used in this study was pUB110. The results showed that optimal transformation of protoplast was obtained at concentration of 2.5 μg/ml lysozyme (18).

The results of the above experiments showed that although increased concentration from 40 to 80 μg/ml slightly increased the number of colonies undergoing transformation, there is no significant relationship between enzyme concentration and transformation. The difference between current findings and Hara et al. is due to low enzyme concentration, while minimum concentration used in this study is.

Conclusion

By the above findings, it can be concluded that increased thermal shock and stability of cold shock at minimum temperature (0°C), as well as increased $CaCl_2$ concentration and increased peptide concentration to 1 μg/ml increased the number of transformed colonies in *Escherichia coli*. The three methods also work similarly and increase the transformation. In pairwise comparison of methods, peptide and enzyme concentrations simultaneously increased the transformation. Our results suggest that treatment of the cells by each combined method play a crucial role in increasing the membrane permeability and is more than heat shock. At the same time, it should be noted that in the lab protocol cells were undergone a heat shock (0 →25 → 37 ℃) process which can be optimised for transferring DNA by treating agents.

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Conflict of Interest

No conflict of interest exists.

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