Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(12):450-453

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The study on the factors affecting the natural transformation of *E. coli* DH5α

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ABSTRACT

The preparation of competent cell is the central step of bacteria transformation and has a great impact on transformation efficiency of nucleic acid. The aim of the research was to study the factors affecting the natural transformation of E.coli DH5α. The result shows that the phenomenon of natural transformation exists in the nature. and it's also related to factors such as calcium chloride concentration, plasmid concentration and others.

Keywords: competent cell; natural transformation; *Escherichia coli*

Efficient DNA transformation efficiency to competent cells is essential for successful cloning and protein expression applications. Since Griffith discovered the phenomenon of transformation, competent cells had already become a new concept. In 1973, Cohen and coworkers showed that bacteria treated with icecold solutions of $CaCl_2$ followed by heat-shock could take up foreign DNA, this great improvement has led genetic engineering into a new era[1]. For *E.coli* bacterial cells, transformation method was devided into two kinds: electrotransformation and chemical transformation. But electrotransformation method requires a very high cell density in addition to expensive cost. Chemical transformation of competent cells is achieved by $CaCl_2$ solution, which is a cost-effective choice and a simple procedure that does not require any specialized equipment. However, competent cells formed by these transformation methods were only give transformation efficiencies of about 10^6–10^7cfu/μg plasmid DNA[2]. This low efficient competent cell are not suitable for construction of mutant and antibody, construction of gene banks. Here, we have discuss some key affecting nature transformation efficiency of *E.coli*.

EXPERIMENTAL SECTION

2.1 Materials

2.1.1 Strains and plasmids

DH5α were supported by Wang Yong-gang teacher at Lanzhou University of Technology; plasmid pUC19 was preserved in our laboratory.

2.2 Apparatus and reagents

Apparatus: Ultraviolet-visible spectrophotometer (Cary 50, Varian companies in the United States); Pipette (BR704180, German Brand company); digital constant temperature water bath (HH-S, China) ; Electronic balance (AB104-N, mettler).

Reagents: Ethyl alcohol, glycerol, glucose, sodium chloride, lithium chloride, magnesium chloride, calcium chloride, copper chloride, manganese chloride, strontium chloride, aluminium chloride and other reagents used were of analytical reagent grade. Yeast extracts and tryptone were purchased from Sangon (Shanghai, China) Biotech Company.

2.2 Methods

2.2.1 Plotting the *E. coli* growth curves

DH5α frozen stock (same cell density) were inoculated 1:100 into 100 ml of LB liquid medium, under the condition of shaking speed 220r/min and 37℃. A culture of *E. coli* will be taken at 25 min intervals from the time of inoculation of the culture (0-time) through a 4-hour incubation period. Growth curves was ploted according absorbance recorded at 600 nm against time. All experiments were performed with three replicates.

2.2.2 Competent cell preparation

The Competent cell preparation protocol used for the *E. coli* strains is based on a protocol from Sambrook and Russell (2001) [3].

2.2.3 Experiment of natural transformation

The main difference between experiment of natural transformation and conventional transformation shows that: a. sodium chloride in the medium changed into calcium chloride; b. directly add DNA solution to cell suspension ; c. directly bath in ice after incubated.

Dilute transformation reaction into 1 ml LB liquid medium, add DNA to cell suspension and incubate under the condition of shaking speed 220 r/min and 37℃. After incubation, the mixture was immediately put on ice for a while. Then the cells were harvested by centrifugation at 6,000 rpm for 1 min at 4℃, plate all transformation mixture on an LB agar plate. Leave the plates for 5 minutes and place them in the 37℃ incubator for 16-20 hours, to count the number of viable cells in this plate. Transformation efficiency (transformants/μg) is calculated as follows: colonies on plate/ng of DNA plated×1000 ng/μg. The experimental design as the table 1.

Table 1 Factors influencing the natural transformation efficiency

	levels		
Mount of plasmid (μl)	5	10	15
$CaCl_2$ concentration (%)	0.5	1	1.5
Ice bath time (h)	4	5	6

2.2.4 Microscopic examination

Morphological structure was observed by light microscope between E. coli DH5α cells and treated cells, which was treated by 100mM $CaCl_2$ and transformated with plasmid pUC19.

RESULTS

3.1Analysis of the *E. coli* growth curves

Cell growth period played an essential role as the factor influencing the transformation efficiency for the preparation competent cells. Furthermore, the value of OD_{600} can reflect the growth status. Generally, preparation for the competent cell require bacteria was reached to early-log phase (OD_{600} = 0.3-0.4) . Because the growth status of different strains exists some differences, so correctly examine the growth characteristics of the different strains was not only beneficial to the future experiment, but also important for improving the transformation efficiency.

Following the identity of drawing growth curves and measuring the OD_{600} values for different strains under the same conditions. we get the growth characteristics of the different strains and the relationship between cell density and the absorption of the culture at 600 nm reached to 0.4 (Fig. 1). and explore the influence of Ca^{2+} for *E. coli* DH5α by adding $CaCl_2$, as shown as Fig. 2.

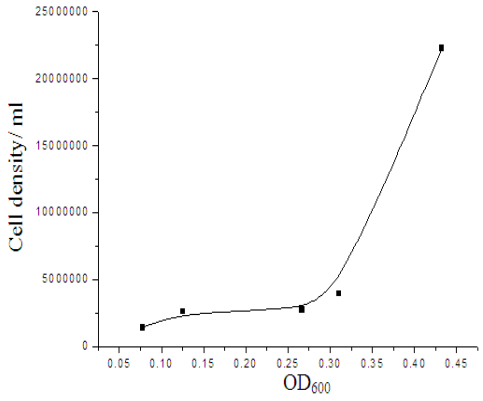

Fig. 1 The relationship between OD_{600} value and cell density

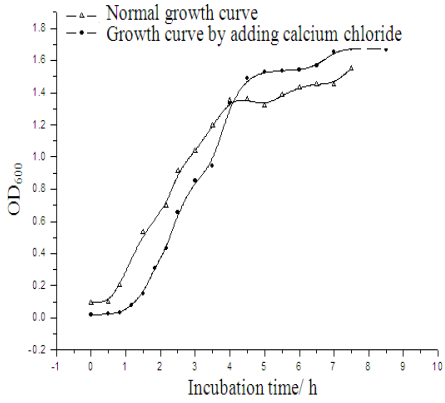

Fig. 2 The growth curve by adding $CaCl_2$

3.2 Factors affecting the natural transformation experiments

3.2.1 Effect of the concentration of $CaCl_2$ on transformation efficiency of natural transformation experiments

The concentration of calcium chloride in culture not only used for adjusting the osmotic pressure, but also as the required ions in transformation experiments. The concentration of $CaCl_2$ plays an important role in the growth of bacteria and transformation experiments. Calcium chloride concentration is too high lead to high osmotic pressure, which is unfavorable for bacteria growth [4,5]; Calcium chloride concentration is too low lead to too low osmotic pressure, which is unfavorable for transformation process. So it is particularly important to get the best concentration of calcium chloride. We can see from the Fig. 3a, which is bell-shaped distribution, maximum when calcium chloride concentration is 1.5%, so choose the concentration for the optimum concentration.

3.2.2 Effect of the mount of plasmid on transformation efficiency of natural transformation experiments

In the natural transformation experiment, when plasmid amount is less than 15 ul, with the increase of concentration of plasmid, present a tendency of increasing efficiency(Fig. 3b). So natural plasmid concentration is also a key role in changeable process and the appropriate concentration of plasmid is advantageous to the transformation.

3.2.3 Effect of the recovery treatment time on transformation efficiency of natural transformation experiments

Incubation time refers to the cultivate time after a foreign plasmid was introduced into bacteria. For general transformation experiments, the OD value requires between 0.3 to 0.5 and training time is less than 2 h [6,7,8]. The incubation time for general transformation experiment should not be too long, which easy to decrease the transformation efficiency. But for natural transformation experiments, we found that the longer the incubation time was, the higher the transformation efficiency was (Fig. 3c).

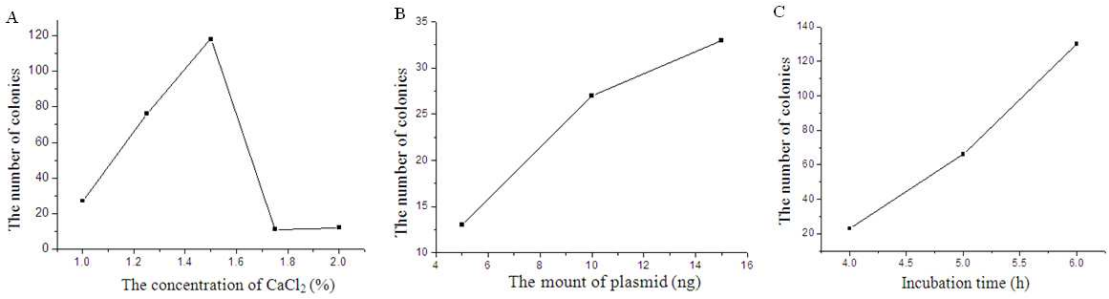

Fig. 3 The effect on number of *E. coli* colonies with the different conditions
A: the concentration of $CaCl_2$ B: the mount of plasmid C: Incubation time

3.3 Observation by light microscope

Morphological structure was observed by light microscope between *E. coli* DH5α cells and treated cells, which was treated by 100mM $CaCl_2$ and transformated with plasmid pUC19. The results were shown as Fig. 3. Normal non-spore *E. coli* cells are with rod-shaped and rounded ends (Fig.3a), The competent cells are bigger than normal non-spore *E. coli* cells as shown as Fig. 3b, but decrease in the number. The cells which were transformed with plasmid pUC19, are bigger and decrease in the number compared with the control (Fig. 3c).

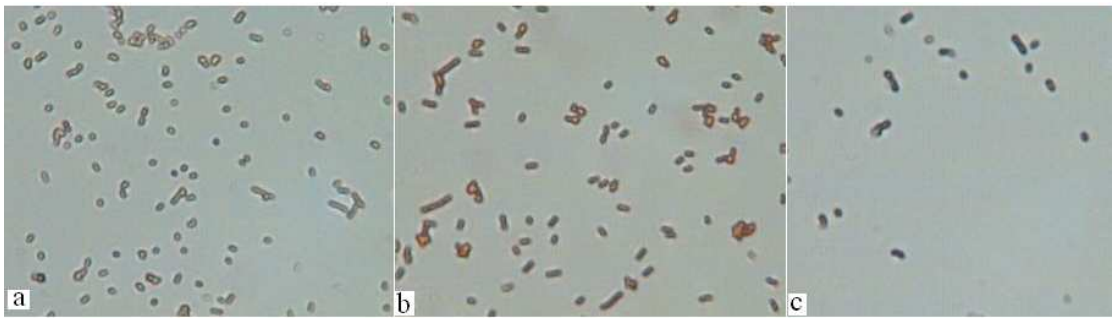

Fig. 3 Microscope morphological of the cells
a: E. coli DH5α cells b: Competent cells c: Competent cells transformated by pUC19

DISCUSSION

This research mainly used the traditional chemical methods to obtain a better understanding about a series of factors affecting transformation efficiency and analyzed every factor by the natural transformation experiments. Experiments explored the factors (plasmid concentration, ice bath time, the recovery treatment time) effecting transformation efficiency. This result provides further insight on the improvement of transformation efficiency. Natural transformation experiment proves that transformation occurs under the natural conditions and influenced by some factors. This research can not only provide the experimental basis to the discovery of conversion phenomenon, but also laid the foundation to simplify the transformation process [9,10,11,12]. Our studies have showed that the ice bath time plays an important role in the later transformation experiment. No matter how long the ice bath time is, it is not advisable to avoid to bath competent cells in ice a period of time, only a few colonies was found in agar plate. But after the ice bath time, transformation resulted in a 7-fold increase in efficiency. Therefore, it may be get the following valuable conclusions:

Natural transformation experiment found that: a. ice bath time is necessary for transformation. b. bivalent ions for transformation are necessary. c. natural transformation process can occur under natural condition and affected by many factors.

Acknowledgements
This research was supported by Agricultural Scientific research projects: Forage feed resources development and utilization of technology research and demonstration (201203042), Chinese National Natural Science Foundation (No.31060041, 51366009), and Natural Science Foundation of Gansu Province (No. 1212RJYA008) and Teaching Research Project of LUT (No. JY2012045).

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