The Study on the factors affecting transformation efficiency of *E. coli* competent cells

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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 such as ionic species, strain types, plasmid concentration, ice-bath time and incubation time. The result shows that ionic species is fatal to the transformation efficiency, preparation of competent cells by monovalent ions (Li^+、Na^+、K^+) and tervalent ion (Al^{3+}) do not have capacity of transformation, preparation of competent cells by bivalent ion (Ba^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, Sr^{2+}) has capacity of transformation. On the whole, the efficiency of Ca^{2+} was found to be the optimum bivalent ion, following by Sr^{2+}>Mn^{2+}>Mg^{2+}>Ba^{2+}. On the other factors that affecting transformation efficiency, the transformation efficiency was the best when plasmid concentration was 100ng/mL, and ice-bath time should be controlled at about 30 min and incubate time selected as 60 min. Experiment of natural transformation later proves the existence of the phenomenon of natural transformation and it's also related to factors such as calcium chloride concentration, plasmid concentration and others.

Keywords: competent cell; different ion; transformation efficiency; *Escherichia coli.*

INTRODUCTION

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 ice-cold solutions of $CaCl_2$ followed by heat-shock could take up foreign DNA, this great improvement has led genetic engineering into a new era (Panja *et al*., 2006). For *E. coli* bacterial cells, transformation method was divided into two kinds: electro transformation and chemical transformation. But electro transformation 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/ug plasmid DNA (Nishimura *et al*., 1990). This low efficient competent cell is not suitable for construction of mutant and antibody, construction of gene banks.

Here, we have discuss some key but easily neglected factors (plasmid concentration, ice-bath time, incubation time) affecting transformation efficiency of *E. coli*. Each *E. coli* host has different characteristics, so we get the optimal results for best transformation efficiency.

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MATERIALS AND METHODS

Materials

Strains and plasmids

BL21 (DE3), BL21 (DE3) pLys S, DH5α, JM109 were supported by Wang Yong-gang teacher at Lanzhou University of Technology; HB101, TG1, Top10, XL1-Blue was from Beijing ding guo changsheng biotech; plasmid pUC19 was preserved in our laboratory.

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, aluminum chloride and other reagents used were of analytical reagent grade. Yeast extracts and tryptone were purchased from Sangon (Shanghai, China) Biotech Company.

METHODS

Plotting the E. coli growth curves

DH5α frozen stock (same cell density) were inoculated 1:100 into 100ml 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 were plotted according absorbance recorded at 600nm against time. All experiments were performed with three replicates.

Competent cell preparation

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

Analysis of factors influencing transformation efficiency

In this work, we have tested the transformation efficiency with different ions for different *E. coli* strains competent cells. All ion solution were chloride in this experiment, and dissolved with double distilled water, final concentration is 100 mm. Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+}, Sr^{2+}, Al^{3+} were selected as some metal ions in transformation solutions.

In addition, we investigated other factors influencing the transformation efficiency including (plasmid concentration, the ice bath time after heat shock, the recovery time), the experimental design represented in the table 1.

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 1ml LB liquid medium, add DNA to cell suspension and incubate under the condition of shaking speed 220r/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 1min 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×1000ng/μg. The experimental design represented in the table 2.

RESULTS

Analysis 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. As shown in fig. 1, we get the growth characteristics of the different strains and explore the relationship between cell density and the absorption of the culture at 600 nm reached to 0.4 (fig. 2). The results showed that the optimal incubation time for eight strains. From short to long, optimal incubation time was BL21 (DE3) (80min), TG1 (80min), DH5α (90min), HB101 (90min), XL1-Blue (100min), BL21 (DE3) pLysS (110min), JM109 (130min), Top10 (140min) respectively. In addition to, from following figs we can see the saturation value of final density for different strains was different.

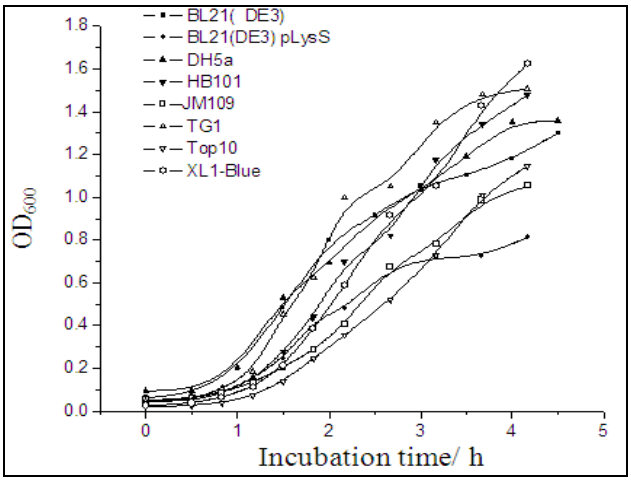

Fig. 1: The growth curves of different strains during log phase

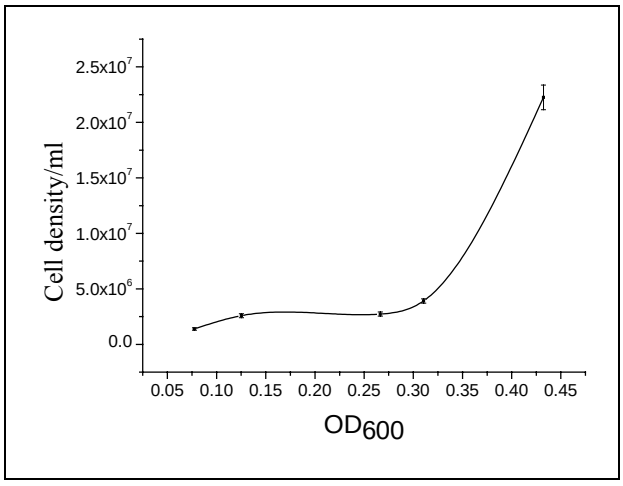

Fig. 2: The relationship between OD_{600} value and cell density

Analysis of different factors influencing transformation efficiency of E. coli

Transformation efficiency affected by different metal ions

It was found that the competent cells prepared by Li^+, K^+ were lack of the transformation ability, which has no colony found in plate after it was coated. So we can get a

conclusion that monovalent ions cannot be used for the preparation of competent cells and Na^+ in LB medium has no effect on cells preparation. Al^{3+}, which was trivalent ion, the competent cells prepared by Al^{3+} has no transformation phenomenon and exist some precipitate. After the microscopically study found that the precipitation existed on the surface of competent cells, which is aluminum hydroxide precipitation. Meanwhile, the competent cells prepared by Cu^{2+} also had no transformation phenomenon, this was due to the reason that Cu^{2+} had some toxicity in inducing cell death. Competent cells was prepared by Ba^{2+}, Ca^{2+}, Mn^{2+}, $Mg^{2+}$and Sr^{2+}, these divalent ions showed certain transformation ability in our experiment. But the transformation ability had certain differences. The transformation efficiency of the same strain for different ions was different and with different *E. coli* strains of the same ion also show some differences.

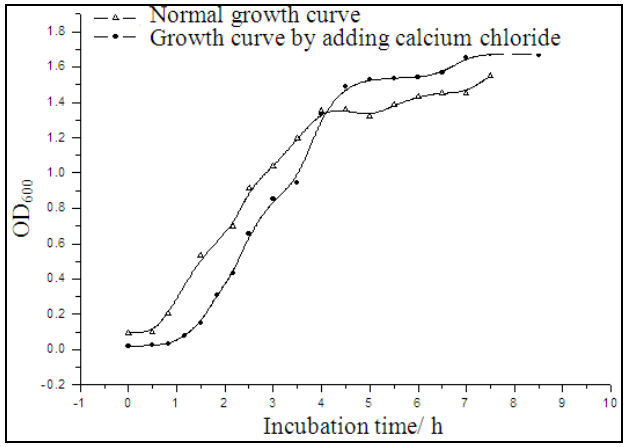

Fig. 3: The growth curve by adding calcium chloride.

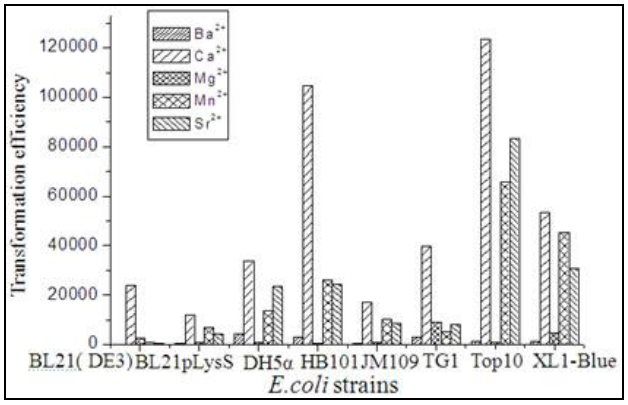

Fig. 4: The different transformation efficiency of same strain prepared by the different ions.

The different *E. coli* strains were used for testing the transformation efficiency. Fig. 4 shows transformation efficiency of the different ions for same strain. For BL21 (DE3), JM109, TG1, HB101 strains, competent cells prepared by Ca^{2+} have the highest transformation efficiency and the cells prepared by other ion have the same transformation efficiency; For BL21 (DE3) pLysS strain, competent cells prepared by Ca^{2+} have the highest transformation efficiency, which higher then competent cells prepared by Mn^{2+}; For *DH5α* strains, competent cells prepared by Ca^{2+} have the same transformation efficiency with the competent cells prepared by Sr^{2+}; For the top 10, XL1-Blue strains, competent cells prepared by Ca^{2+} have the highest transformation efficiency, which higher then competent cells prepared by Mn^{2+} and Sr^{2+}.

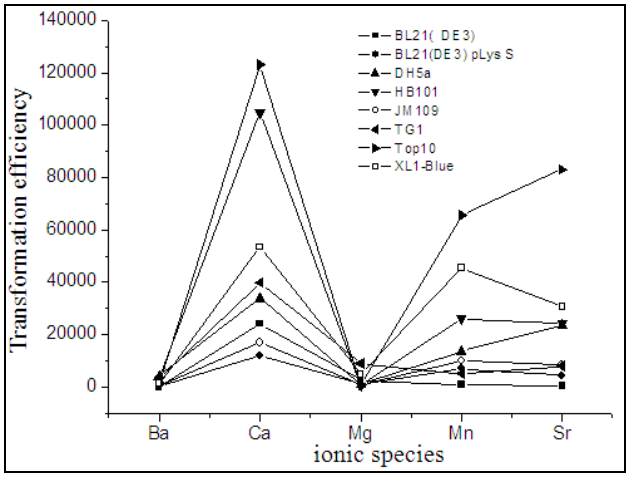

Fig. 5: The different transformation efficiency of different strains prepared by the same ion.

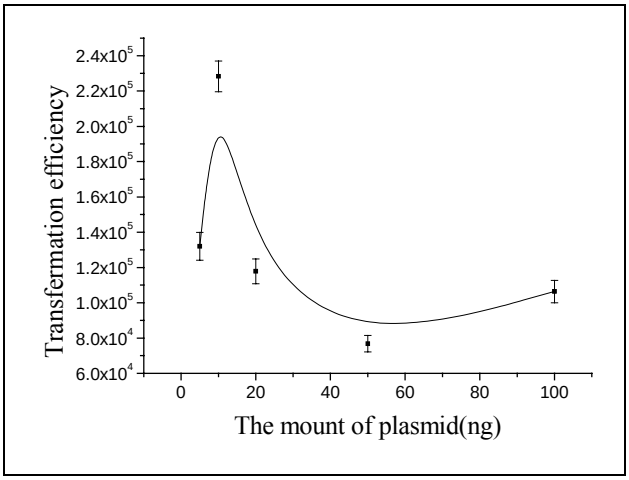

Fig. 6: Transformation efficiency of E. coli with different mount of the plasmid.

Fig. 5 shows the distinguishing between the different *E. coli* strains for the same ion. For Ba^{2+}, the transformation efficiency is generally low by contrast the transformation efficiency of HB101 and TG1 strains are higher than other strains. But the low transformation efficiency is not suitable for preparation of competent cells. For Ca^{2+}, the transformation efficiency is generally high by contrast the transformation efficiency of HB101 and Top10 strains is higher than other strains, which is good for preparation of competent cells. For Mg^{2+}, the differences between different strains is obvious, the transformation efficiency

of TG1 strain is the highest, the second is the XL1-Blue, but relative value is small, so Mg^{2+} should not be used for the preparation of competent cells; For the Mn^{2+}, its transformation efficiency were completely different, the transformation efficiency of strain Top10 is the highest, the second is the XL1-Blue. Like some special strains, which were hard to transform, we can add Mn^{2+} to enhance the transformation efficiency. For Sr^{2+}, has the same consequence of Mn^{2+}, which not suitable for preparation of the competent cells.

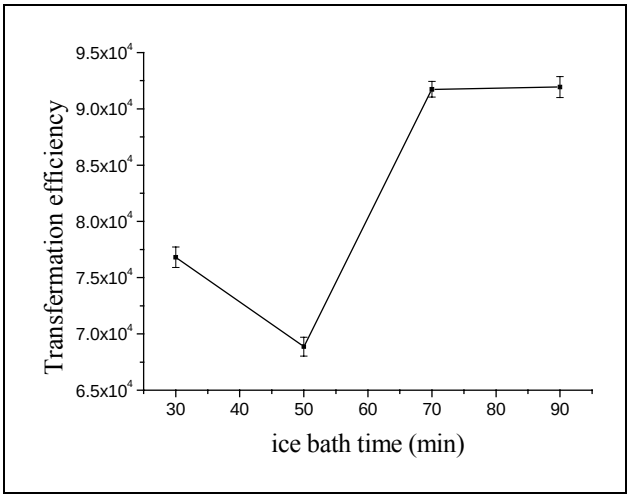

Fig. 7: Transformation efficiency of *E. coli* with different ice bath time.

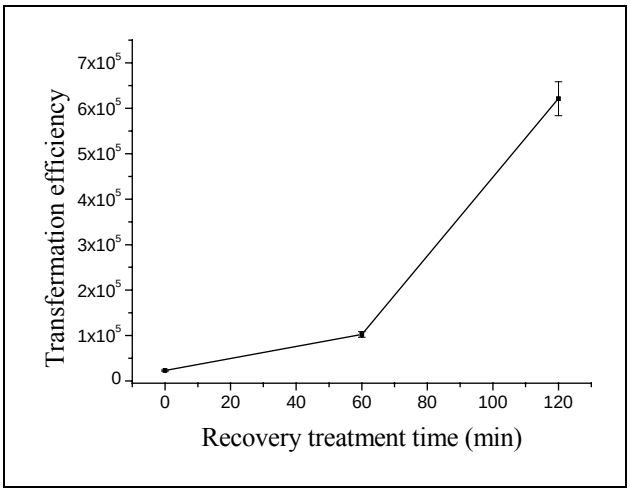

Fig. 8: Transformation efficiency of *E. coli* with different recovery treatment time.

Above all, we can clearly see the different *E. coli* strains has the selectivity of different ions for preparation of the competent cells and different ions has some difference for same strain, the transformation efficiency of competent cells prepared by same ion were different. As for different strains, we should use proper methods of competent cells preparation. But overall, the highest transformation efficiency of competent cells is still the Ca^{2+}, the other in the order: Sr^{2+} > Mn^{2+} > Mg^{2+} > Ba^{2+}. Rational combination of different ion to the preparation of competent cells should be able to receive the good effect, which can further improve the transformation efficiency laid a foundation for the laboratory preparation of competent cells. In our experiment, we observed the competent cells prepared by these ions of second price has certain dispersion phenomenon on the centrifugal pipe after centrifuged and different strains has some differences, which only associated with the valence state of ion itself. What does make its appearance remains to be further discussed? Other factors influencing transformation efficiency of different *E. coli* strains.

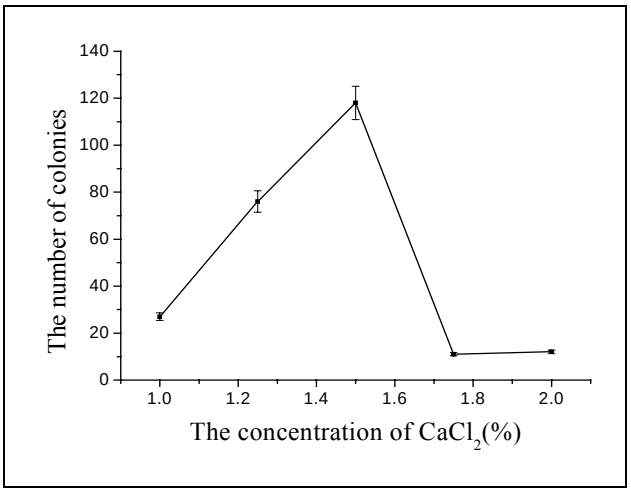

Fig. 9: The number of *E. coli* colonies with the concentration of CaCl2 for natural transformation experiments.

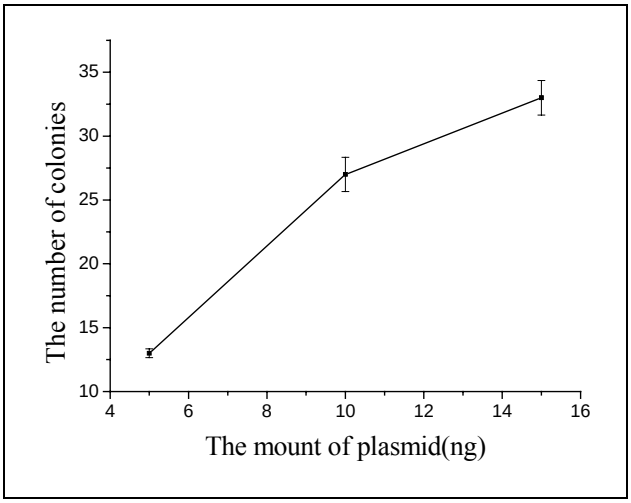

Fig. 10: The number of *E. coli* colonies with the mount of plasmid for natural transformation experiments.

Effect of the amount of DNA on transformation efficiency of competent cells

Since the amount of DNA needed for transformation is impractical, so we carried out the experiment to find the

optimal concentration of plasmid for each bacteria. From the fig. 6 we can see that when the plasmid amount added to 5ng, the transformation efficiency reached the highest. With the increase of the plasmid concentration, transformation efficiency decreased. The bigger plasmid concentration resulting in a toxic effect on the cells and have a significant negative effect on the transformation efficiency. In addition, since the plasmid is extracted by us, so the purity becomes one of the important limits.

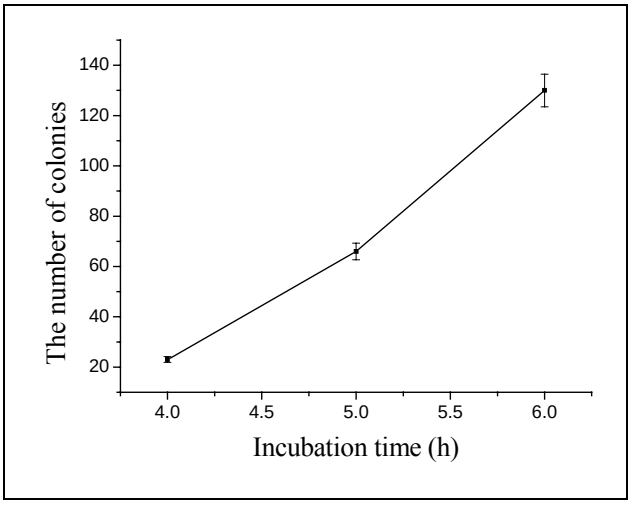

Fig. 11: The number of *E. coli* colonies with incubation time for natural transformation experiments.

Effect of ice bath time on transformation efficiency of competent cells

The process of competent cells preparation must be carried out at low temperature, but the length of the ice bath time also becomes an important factor influencing transformation efficiency of competent cells. In these experiments, the ice bath time refers to the period after joining plasmid. As show in fig. 7, with the prolongation of ice bath time, the transformation efficiency was increased. But in order to save time, the ice bath time is for 30 minutes.

Table 1: Factors influencing the transformation efficiency

Factor	Levels		
Mount of plasmid (ug)	5	50	100
Ice bath time (min)	30	50	70
Recovery time (min)	0	60	120

Effect of recovery treatment time on transformation efficiency of competent cells

After transformation, we should have a recovery treatment to make the cells recovery from the osmotic stress damage, cell division or internal restriction, thus increase the number of selected single colony. In addition, the recovery treatment is beneficial to stable expression of exogenous genes, so do not add any antibiotic in recovery medium avoid affecting transformation efficiency of competent cells. As show in fig. 8, the recovery treatment time has enhancing effect on transformation efficiency. When prolonged the recovery treatment time one hour, the transformation efficiency was increased seven times. The longer recovery treatment time will be beneficial to bacterium for growth and reproduction (Hanahan *et al*., 1991). But this process does not require too long time, which can lead to a false positive consequence. Factors affecting the natural transformation experiments.

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 (Castuma *et al*., 1995); 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. 9, which is bell-shaped distribution, maximum when calcium chloride concentration is 1.5%, so choose the concentration for the optimum concentration.

Table 2: Factors influencing the natural transformation efficiency

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

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

In the natural transformation experiment, when plasmid amount is less than 15ul, with the increase of concentration of plasmid, present a tendency of increasing efficiency (fig. 10). So natural plasmid concentration is also a key role in changeable process and the appropriate concentration of plasmid is advantageous to the transformation.

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 (Dagert and Ehrlich 1979; Inoue *et al*., 1990; Umemoto *et al*., 1996). 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 as shown as fig. 11.

DISSUSION

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 (ionic species, 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 cannot only provide the experimental basis to the discovery of conversion phenomenon, but also laid the foundation to simplify the transformation process (Pope and Kent 1996; Castuma *et al*., 1995; Zhiming *et al*., 2005). 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:

(1) Competent cells prepared by different ions from the same strain have different transformation efficiency. Competent cells prepared by monovalent ions and trivalent ions lose the transformation ability. Preparations of divalent ions cells have a certain ability, which has the highest conversion efficiency of Ca^{2+}, other in the order: Sr^{2+} **>** Mn^{2+} **>** Mg^{2+} **>** Ba^{2+}.

(2) The different strains prepared by the same ion also have different transformation efficiency, which suggesting that different strains have the selectivity of different ions for preparation the competent cells.

(3) Other factors influencing the transformation efficiency, we can draw conclusion that transformation efficiency is highest when plasmid concentration reached 100 ng/mL; Ice bath time is 30 min can meet the demand of transformation; The recovery treatment time is too long will greatly improve transformation efficiency, but prone to form false positives, we can properly raise the incubation time which can not form false positives.

(4) 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.

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