CTU Journal of Innovation and Sustainable Development
ISSN 2615-9422 | e-ISSN 2815-5602

DOI:10.22144/ctujoisd.2024.270

Repurpose the antimicrobial peptide Buforin II for plasmid transformation into *Escherichia coli*

Thanh Ngoc Nguyen[1*], and Le Minh Bui[1,2,3]

[1]*NTT Hi-tech Institute, Nguyen Tat Thanh University, Viet Nam*

[2]*Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Indonesia*

[3]*Department of Biotechnology, NTT Hi-tech Institute, Nguyen Tat Thanh University, Viet Nam*

**Corresponding author (nnthanh1080@gmail.com)*

Article info.

Received 10 Aug 2023
Revised 19 Dec 2023
Accepted 24 Dec 2023

Keywords

Antimicrobial peptide, cell penetrating peptide, Escherichia coli, plasmid, transformation

ABSTRACT

Antimicrobial peptides Buforin II, derived from histone H2A, demonstrates strong cell-penetrating activity without cell lysis and strong affinity for internal cellular nucleic acids, making it a potential candidate for macromolecule delivery into bacteria. Herein, we designed a peptide that is the fusion of Buforin II and a polycation tail $(KH)_6$ and assessed its efficiency in delivering plasmid (pGEX-RG-(TAG)5, 7,142 bp) into Escherichia coli OmniMAX. The peptide and plasmid were incubated at 25°C to form the complexes at various peptide concentrations from 5 to 50 µg/mL. After that, the complexes were incubated with the E. coli competent cells at 25°C. In comparison with the transformation efficiency and normalized transformation efficiency of conventional heat-shock method, a 1.28 and 7.83 times higher transformation efficiency, correspondingly, was achieved by using novel peptide-based delivery system at peptide concentration of 5 µg/mL. The cell viability of over 90% was displayed at peptide concentration of 5 µg/mL. This study can lead to the development of a transformation approach under mild conditions and an ideal tool for gene delivery.

1. INTRODUCTION

The application of synthetic biology and genetic engineering have appreciably contributed to the advancement of microbiome-based therapeutics (Mimee et al., 2016), biomolecular manufacturing (Pardee et al., 2016), *in vitro* and *in vivo* diagnostics (Slomovic et al., 2015), and genetic therapies (Rossi et al., 2007). Genetic delivery is among the principal steps in synthetic biology and genetic engineering processes. So far, no single available transformation technique can be applied without some limitations. Taking heat-shock transformation or chemotransformation as an example. It is one of the most common methods used for genetic transformation into bacteria, but it cannot deliver large-sized deoxyribonucleic acid (DNA) into the cells and it is limited by cell walls. Compared to the heat-shock technique, electroporation is more efficient and applicable to many more species from both Bacteria and Archaea. Despite the ability to deliver large-sized DNA and a very broad range of recipient cells, it is limited by the requirement of special equipment and the control of multiple parameters (Aune & Aachmann, 2010). The delivery technique using viral vectors applies to both bacterial and mammalian cells, but the problems are immunogenicity and cell-type specific procedure (Islam et al., 2019). Liposome-mediated is a choice for a broad range of cell types, but this method is obstructed by the cell walls. To transfer

genetic materials into some archaebacteria and circumvent the obstacle of cell walls, transformation of spheroplasts and protoplasts is a promising approach. The procedure, however, is complicated and time-consuming as well as specific for each cell type (Aune & Aachmann, 2010). Several techniques such as chitosan-mediated, biolistic and tribos methods were reported, but only a few reports have been published.

Cell-penetrating peptide (CPP) mediated transformation is a new technique that has been studied in the two recent decades. Transformation protocols using CPPs have been described for a variety of recipient cells, including bacteria, plant and animal cells (Chen et al., 2007; Lee et al., 2011; Heiat et al., 2014; Jeong et al., 2016; Islam et al., 2019). The peptides are composed of 20–50 amino acids and positively charged due to the basic residues such as lysine, arginine, and histidine (Farkhani et al., 2014; Heiat et al., 2014). These amino acids can mediate the interaction of the peptide with anionic motifs on the cell membrane, and at least eight positive charges are suggested for high-performance cellular uptake of CPPs (Farkhani et al., 2014).

Buforin II is a histone H2A-derived antimicrobial peptide (AMP) that has proline hinge structure responsible for its cell-penetrating ability. It kills a broad range of microorganisms without cell lysis and has a strong affinity for internal cellular nucleic acids (Park et al., 2000). Buforin II molecules were proposed to form temporary toroidal pores, causing frequent pore disintegration and increasing the translocation rate. The Buforin II translocation was shown to withstand cargo addition, qualifying this peptide as a promising candidate for new CPP generations. We repurpose AMP Buforin II for plasmid transformation by fusing it with a polycation domain $(KH)_6$ (from now on called $B_2(KH)_6$) and assessed its effectiveness in transforming plasmid into *Escherichia coli* OmniMAX. The transformed plasmid was pGEX-RG(TAG)5 (7,142 bp) encoding red fluorescent protein (RFP) and superfolder green fluorescent protein (sfGFP) as reporter genes. The AMP-mediated transformation of pGEX-RG(TAG)5 successfully took place and brought about a higher transformation efficiency than the heat-shock technique.

2. MATERIALS AND METHOD

2.1. Materials

The One Shot™ OmniMAX™ 2 $T1^R$ *E. coli* strain (Thermo Fisher Scientific, USA) was used as the host for plasmid transformation. The plasmid pGEX-RG-(TAG)5 (7,142 bp) was used to transfer into *E. coli* OmniMAX. Dehydrated Luria-Bertani (LB) culture medium (Thermo Fisher Scientific, USA) was utilized to prepare broth and agar media for the growth, refresh of bacterial cells and the recovery of transformed cells. $CaCl_2$ (Sigma-Aldrich, USA) was used to prepare competent cells. Ampicillin (Sigma-Aldrich, USA) was utilized in transformation selection plate. Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Merck, Germany) was used to stimulate *the expression of reporter genes coding for fluorescent proteins.*

2.2. Peptide synthesis and preparation

The $B_2(KH)_6$ polycation peptide (TRSSRAGLQFPVGRVHRLLRKKHKHKHKHKHKH) was chemically synthesized based on the designed sequence. The peptide was purified and obtained with >97% High Performance Liquid Chromatography purity and confirmed with mass spectrum. The peptide was dissolved in autoclaved purified water (pH 7.0), yielding 100 µg/mL stock solution.

2.3. Transformation of pGEX-RG-(TAG)5 into *E. coli* cells

For transformation using peptide-plasmid complexes, $CaCl_2$-washed cells were prepared by the reported protocol with minor modifications (Islam et al., 2019). Briefly, harvested cells were condensed to 600-nm optical density (OD_{600}) of 3.5 and rapidly washed with ice-cold 0.1 M $CaCl_2$. After the washing step, the cells were centrifuged and resuspended in ice-cold 0.1 M $CaCl_2$ solution with 15% glycerol to obtain OD_{600} of 17.5. The cells were then stored at −80°C until use. For heat-shock transformation, competent cells were prepared using a similar protocol, but the harvested cells were incubated in $CaCl_2$ on ice for 30 minutes before centrifugation.

The complexes of $B_2(KH)_6$ and pGEX-RG-(TAG)5 were prepared by adding 10 µL of 20 ng/µL pGEX-RG-(TAG)5 to different volumes (0, 2.5, 12.5 and 25 µL) of 100 µg/mL AMP solution and adding autoclaved purified water to obtain a final volume of 50 µL. The corresponding final concentrations of peptide in 50 µL of prepared mixtures were 0, 5, 25

and 50 µg/mL. The solutions were thoroughly mixed by using a pipette then shaken for 30 min at 150 rpm at 25°C. The peptide-plasmid complex solutions were thoroughly mixed with 100 µL of $CaCl_2$-washed cells using a pipette and shaken for 30 min at 150 rpm at 25°C to allow transformation. Afterwards, 1 mL of LB broth was supplemented into each mixture. Then, the tubes containing mixtures were shaken at 150 rpm at 37°C for 1 h. Afterwards, the tubes were centrifuged at 13,000 rpm at 4°C for 1 min, and 850 µL of supernatant was removed from each tube. The recovered cells in the pellet were resuspended and plated (20 µL/plate) onto LB agar plates containing ampicillin (100µg/mL). Transformation efficiency was calculated using Equation 1:

$$TE = \frac{CFU_{Amp} \times 300}{m_p \times 20} \times 1{,}000 \quad (1)$$

where

TE is the transformation efficiency by colony forming unit (CFU);

CFU_{Amp} is the number of colonies on Ampicillin plate;

m_p is the amount of pDNA (ng) used in transformation.

After the recovery, centrifugation and resuspension steps, the same cells were 10-fold serially diluted then plated (50 µL/plate) on LB agar plates with no antibiotics to acquire the corresponding number of surviving cells for transformation frequency calculation (Tee et al., 2017). The transformation frequency was calculated using Equation 2:

$$TF = \frac{CFU_{Amp} \times 50}{CFU_{LB} \times F_D \times 20} \quad (2)$$

where

TF is the transformation frequency;

CFU_{Amp} is the number of colonies on Ampicillin plate;

CFU_{LB} is the number of colonies on LB plate without Ampicillin;

F_D is the dilution factor.

In order to compare with the traditional heat-shock technique, the heat-shock transformation was carried out using previously reported protocol with some modifications (Chang et al., 2017). The number of *E. coli* cells in the heat-shock treatment was different from that of AMP-mediated transformation. Therefore, the transformation efficiencies of heat-shock were normalized using the ratio of cell numbers in AMP-mediated treatment and heat-shock treatment.

In order to confirm the successful transformation, the expression of reporter genes coding for fluorescent proteins was stimulated by IPTG. The colonies formed on ampicillin-LB plates were inoculated on LB agar plates containing ampicillin (100µg/mL) and IPTG (0.01 M).

2.4. Investigation of the effect of AMP concentrations on the growth of *E. coli* cells

To evaluate the effect of the peptide $B_2(KH)_6$ at various concentrations on the growth of *E. coli* cells during total 1.5 hours of transformation and recovery, the surviving cells on LB agar plates without antibiotics were counted. The viable cells in the broth includes the successfully transformed cells and competent cells. The number of colonies on LB plates obtained when calculating transformation frequency were used to calculate the viable cells using Equation 3:

$$sCFU = \frac{CFU_{LB} \times F_D \times 300}{50} \quad (3)$$

where

sCFU is the number of colonies formed from surviving cells;

CFU_{LB} is the number of colonies on LB plate without Ampicillin;

F_D is the dilution factor.

3. RESULTS AND DISCUSSION

3.1. Transformation of pGEX-RG-(TAG)5 into *E. coli* cells

The peptide B2(KH)6 (theoretical pI of 13.10 and net charge of +13 at pH 7.0; Mw of 4.027 kDa) was utilized to transform the plasmid pGEX-RG-(TAG)5 via forming the complexes of peptide-plasmid. Under 365-nm irradiation, the bacterial cells transformed with the complexes of B2(KH)6 and pGEX-RG-(TAG)5 exhibited red fluorescent color on LB agar plates containing ampicillin and 0.01 M IPTG (Figure 1). This confirmed that AMP-mediated transformation of *E. coli* OmniMAX cells with plasmid pGEX-RG-(TAG)5 was successful.

The transformation efficiency of the plasmid pGEX-RG-(TAG)5 was assessed by calculating the number

of transformants (CFUs) per microgram of pDNA (Figures 2). The transformation results were compared to the samples transformed by the traditional heat-shock method (Chang et al., 2017).

The result of the preliminary transformation experiment showed that plasmid DNA delivery mediated by B2(KH)6 was sensitive to the peptide concentration and N/P ratio. The N/P ratio is defined as the molar ratio between the cation group ($–NH_3^+$) and the anion group ($–PO_4^-$) (Chen et al., 2007). The transformation efficiencies at different peptide concentrations of 0, 5, 25 and 50 µg/mL, corresponding to N/P ratios of 0, 1.25, 6.24 and 12.47 were approximately 23,700, 73,258, 47,225, 57,883 CFU/µg, respectively.

For the transformation of 200 ng of plasmid pGEX-RG-(TAG)5 into *E. coli* OmniMAX cells, the peptide concentration and N/P ratio significantly influenced the transformation efficiency ($P < 0.001$). The complex prepared at peptide concentration of 5 µg/mL (N/P of 1.25) exhibited the highest transformation efficiency of 73,258 CFU/µg, which was approximately 1.28 and 7.83 times of the transformation efficiency and normalized transformation efficiency, respectively, obtained using the traditional heat-shock method. In comparison with the control (peptide concentration of 0 µg/mL), the complex prepared at peptide concentration of 5 µg/mL resulted in 3.09 fold higher transformation efficiency.

Similar protocols for preparing peptide-plasmid complex and transformation were reported by Islam et al. (2019) for the delivery of large plasmid DNA (205 kb). The optimal N/P ratio was 0.1 for the transformation of 205 kb plasmid into *E. coli* DH5α cells using peptide which is the fusion of CPP BP100 and polycation (KH)9 (Islam et al., 2019). In this study, the optimal N/P ratio for plasmid delivery was 1.25 at the peptide concentration of 5 µg/mL in complex formation mixture. With the N/P ratio of 1.25, the molar concentration of positive charge ($–NH_3^+$) was larger than that of negative charge ($–PO_4^-$). This suggests that the remaining positive charges of B2(KH)6 molecules after complex formation possibly interacted with the negatively charged groups in the cell membrane via one of the models described before (Huan et al., 2020). The positive charged-peptide might also aid in transformation activity by reducing electrostatic repulsion between the plasmid and cell membrane.

In the present study, more positive charges were presented in the treatments at higher peptide concentrations in the complex formation mixtures. Increasing of peptide concentration above 5 µg/mL led to the decline in transformed bacteria number. Transformation efficiency of the treatment at 50 µg/mL concentration was higher than that at 25 µg/mL concentration although the higher peptide concentration reduced the survival ability of bacterial cells. After complex formation, more remaining positive charges in the treatment of 50 µg/mL peptide might be the main factor enabling the peptide-plasmid complexes to effectively interact with cell membranes, which increased the rate of pore formation and caused a slight increase in transformation efficiency. Further studies (e.g. simulation studies) are necessary to investigate the transformation mechanism enabled by the complex of $B_2(KH)_6$ and plasmid DNA. Further optimization (e.g. peptide concentration, plasmid amount, N/P ratio, complex formation and transformation temperature, complex formation and transformation time) could potentially increase the transformation efficiency for OmniMAX cells and other bacteria strains.

For bacteria, the most popularly used genetic transformation technique is heat-shock transformation (also known as chemical transformation). The existence of several drawbacks of this method, however, makes it not an optimal method for many transformation processes, especially for large sized DNA or thick cell-wall microorganisms (Aune & Aachmann, 2010). In recent years, several studies have been carried out on peptide-mediation transformation with the purposes of improving the conventional DNA transformation method or inventing facile method (Chen et al., 2007; Lee et al., 2011; Heiat et al., 2014; Jeong et al., 2016; Islam et al., 2019).

In previous study of Heiat et al. (2014), the transformation efficiency of heat-shock method was improved by preparing *E. coli* BL21 (DE3) competent cells using CaCl2 and CM11 peptide which is composed of Cecropin (1–7 residues) and Mellitin (5–8 residues). In the presence of CM11 peptide, the transformation efficiencies for 100 ng of pUC19, pGEX4T-1 and pET-28a(+) plasmids were 4, 4.4 and 4.7 fold higher than control, respectively. The two methods for preparing *E. coli* competent cells required CM11 peptide at various concentrations from 0.5 to 6 µg/mL in the broth medium used for cell culture (Heiat et al., 2014). This will require a considerable amount of peptide

for each experiment; hence, economic efficiency is not achieved.

Liu et al. (2018) reported a way to improve the competence of *E. coli* DH5α by supplementing LFcin-B peptide in the transformation buffer used in the competent cell preparation step. In this approach, the peptide can increase the permeability of cell membrane at the sub-lethal concentration (0.35 mg/L). In their study, high concentrations of Ca^{2+} and Mn^{2+} may be important to inhibit the antibacterial properties of LFcin-B without losing the effect on increasing the permeability of the DH5α cell membrane (Liu et al., 2018).

Several positively charged nanomaterials can be used as plasmid delivery agents by forming complexes. Aminoclays (3-aminopropyl functionalized metal phyllosilicates) containing Mg^{2+} or Ca^{2+} in the backbone can form aminoclay-DNA complexes via the bonding between their protonated amine groups and the phosphate groups of plasmid DNA. Choi et al. (2013) reported that magnesium-aminoclay led to transformation efficiencies of ~6 × 103 and ~2 × 105 CFU/μg in *Streptococcus mutans* and *E. coli* XL1-Blue, respectively, whereas calcium-aminoclay created higher transformation efficiency in *S. mutans* than that in *E. coli* XL1-Blue (Choi et al., 2013). In the investigation on the transformation of plasmid pUC19 into non-competent *E. coli* JM109, the OD_{600} of *E. coli* JM109 and spreading time were studied. The OD_{600} of 1.0 and spread time of 90 s were favorable for the transformation process (Mendes et al., 2021a). In the study of Mendes et al. (2021b), 2-pyrrolidinone-functionalized needle-shaped magnetic nanoparticles were used to form the complexes with plasmid pUC19 and the translocation of nanoparticle-pUC19 complexes across the cell envelope of *E. coli* JM109 were promoted by frictional stimulation, leading to a maximum transformation efficiency of 3.1 × 102 CFU/μg plasmid. In their study, magnetic stimulation was not able to induce the transformation of *E. coli* JM109, which highlights the disadvantages of magnetic stimulation for magnetic nanoparticle-assisted transformation in microbes and the necessity of the delivery agent with a high aspect ratio for successful transformation of prokaryotes (Mendes et al., 2021b)

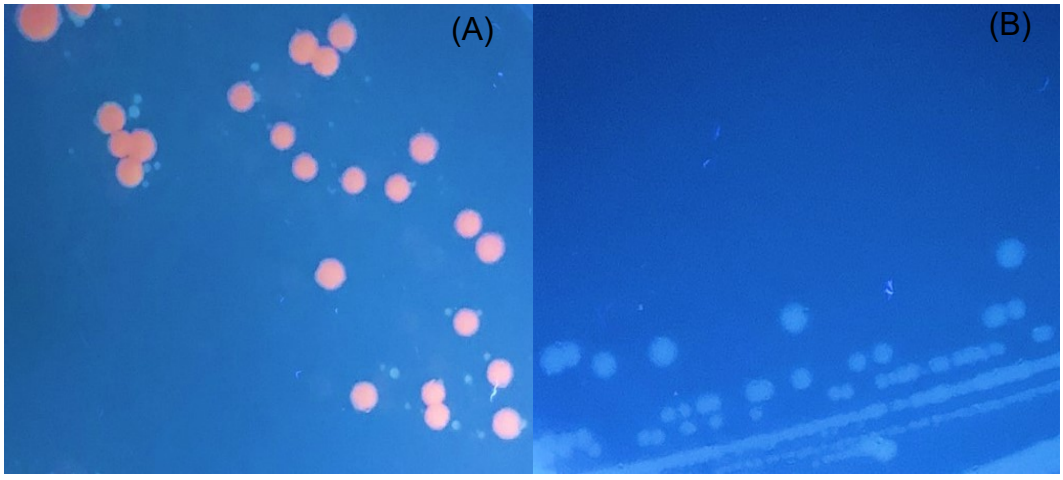

Figure 1. Colonies with expression of red fluorescent protein in *E. coli* OmniMAX cells under 365-nm irradiation

(A) E. coli OmniMAX transformants harboring pGEX-RG-(TAG)5 encoding red fluorescent protein. (B) Normal E. coli OmniMAX cells as a negative control.

In this plasmid delivery system, the peptide-plasmid complex formation and transformation happened at room temperature with no special equipment. As a result, this is an advantage of the system and $B_2(KH)_6$ may become a powerful tool for simple gene delivery platform under mild conditions.

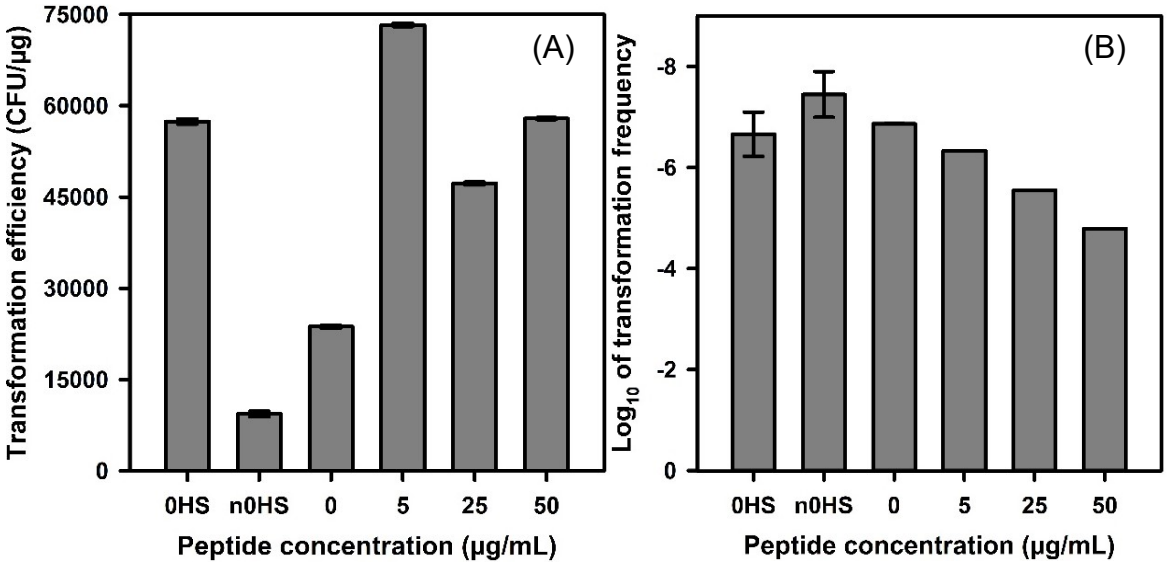

Figure 2. AMP-mediated transformation of pGEX-RG-(TAG)5 into *E. coli* OmniMAX cells

(A) Normalized transformation efficiency from heat-shock (n0HS) and transformation efficiency from heat-shock (0HS) and AMP-mediated transformation at various peptide concentrations. (B) Normalized log10 of transformation frequency from heat-shock (n0HS) and log10 of transformation frequency from heat-shock (0HS) and AMP-mediated transformation at various peptide concentrations. Error bars show the 3-fold standard error. Each data point is the average of the three replicates (n = 3). In each replicate, the CFUs were counted three times to obtain the average value for each treatment.

3.2. Effect of AMP concentrations on the growth of *E. coli* cells

The growth of *E. coli* cells during 1.5 hours of transformation and recovery stages was determined by counting the CFUs on LB agar plates. The inverse relationship between number of surviving cells and peptide concentration was observed, but only the AMP concentration of 50 µg/mL caused a significant decrease in the number of surviving cells (Table 1). Ca^{2+} ions play an important role in transformation by neutralizing the negative charges present in cell membranes and DNA molecules and decreasing the electrostatic repulsion between bacterial outer membranes and DNA molecules (Lim et al., 2015). The bacterial growth, however, can be inhibited by elevated osmolality caused by $CaCl_2$ salt addition (Alahakoon et al., 2014). coexistence of AMP and $CaCl_2$ caused slower growth of *E. coli* cells, presented by the decline in number of transformants on LB agar plates. The peptide concentration of 5 µg/mL led to over 90% cell viability, so this concentration was cytocompatible and suitable for plasmid transformation.

Table 1. CFUs of surviving cells at different peptide concentrations

Peptide concentration (µg/mL)	CFUs ± SE
0	$3.4923 \times 10^{10} \pm 1.1260 \times 10^{8a}$
5	$3.1703 \times 10^{10} \pm 2.9627 \times 10^{7ab}$
25	$3.3337 \times 10^{9} \pm 3.1798 \times 10^{6ab}$
50	$7.1700 \times 10^{8} \pm 5.7735 \times 10^{5b}$

[a, b]: Means with different letters in the same row are significantly different (P≤0.05).

4. CONCLUSION

In our study, the peptide $B_2(KH)_6$ successfully formed the complexes with the plasmid pGEX-RG-(TAG)5 at room temperature. At the peptide concentration of 5 µg/mL, the $B_2(KH)_6$-pGEX-RG-(TAG)5 complex showed higher transformation efficiency of approximately 1.28- and 7.83-fold than the transformation efficiency and normalized transformation efficiency of heat-shock method. In addition, high cell viability was achieved in the presence of *5* µg/mL peptide. These results can be used as fundamental data for further studies on this peptide and similar peptide types, contributing to the development of a simple approach for transformation under mild conditions and an ideal

tool for gene delivery in synthetic biology and genetic engineering platforms.

REFERENCES

Alahakoon, A. U., Jayasena, D. D., Jung, S., Kim, H. J., Kim, S. H., & Jo, C. (2014). Antimicrobial effect of calcium chloride alone and combined with lactic acid injected into chicken breast meat. *Korean Journal for Food Science of Animal Resources*, *34*(2), 221-229. https://doi.org/10.5851/kosfa.2014.34.2.221

Aune, T. E. V., & Aachmann, F. L. (2010). Methodologies to increase the transformation efficiencies and the range of bacteria that can be transformed. *Applied Microbiology and Biotechnology*, *85*(5), 1301-1313. https://doi.org/10.1007/s00253-009-2349-1

Chang, A. Y., Chau, V., Landas, J. A., & Pang, Y. (2017). Preparation of calcium competent *Escherichia coli* and heat-shock transformation. *Journal of Experimental Microbiology and Immunology Methods*, *1*(22-25).

Chen, C. -P., Chou, J. -C., Liu, B. R., Chang, M., & Lee, H. -J. (2007). Transfection and expression of plasmid DNA in plant cells by an arginine-rich intracellular delivery peptide without protoplast preparation. *Federation of European Biochemical Societies Letters*, *581*(9), 1891-1897. https://doi.org/10.1016/j.febslet.2007.03.076

Choi, H. -A., Lee, Y. -C., Lee, J. -Y., Shin, H. -J., Han, H. -K., & Kim, G. -J. (2013). A simple bacterial transformation method using magnesium- and calcium-aminoclays. *Journal of Microbiological Methods*, *95*(2), 97-101. https://doi.org/10.1016/j.mimet.2013.07.018

Farkhani, S. M., Valizadeh, A., Karami, H., Mohammadi, S., Sohrabi, N., & Badrzadeh, F. (2014). Cell penetrating peptides: efficient vectors for delivery of nanoparticles, nanocarriers, therapeutic and diagnostic molecules. *Peptides*, *57*, 78-94. https://doi.org/10.1016/j.peptides.2014.04.015

Heiat, M., Aghamollaei, H., Moosazadeh Moghaddam, M., & Kooshki, H. (2014). Using CM11 peptide as a cell permeable agent for the improvement of conventional plasmid transformation methods in *Escherichia coli* and *Bacillus subtilis*. *Minerva Biotecnologica*, *26*, 149-157.

Huan, Y., Kong, Q., Mou, H., & Yi, H. (2020). Antimicrobial peptides: Classification, design, application and research progress in multiple fields. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.582779

Islam, M. M., Odahara, M., Yoshizumi, T., Oikawa, K., Kimura, M., Su'etsugu, M., & Numata, K. (2019). Cell-penetrating peptide-mediated transformation of large plasmid DNA into *Escherichia coli*. *ACS Synthetic Biology*, *8*(5), 1215-1218. https://doi.org/10.1021/acssynbio.9b00055

Jeong, C., Yoo, J., Lee, D., & Kim, Y.-C. (2016). A branched TAT cell-penetrating peptide as a novel delivery carrier for the efficient gene transfection. *Biomaterials Research*, *20*(1), 28. https://doi.org/10.1186/s40824-016-0076-0

Lee, C. Y., Li, J. F., Liou, J. S., Charng, Y. C., Huang, Y. W., & Lee, H. J. (2011). A gene delivery system for human cells mediated by both a cell-penetrating peptide and a piggyBac transposase. *Biomaterials*, *32*(26), 6264-6276. https://doi.org/10.1016/j.biomaterials.2011.05.012

Lim, G., Lum, D., Ng, B., & Sam, C. (2015). Differential transformation efficiencies observed for pUC19 and pBR322 in *E. coli* may be related to calcium chloride concentration. *Journal of Experimental Microbiology and Immunology*, *20*, 1-6.

Liu, J., Chang, W., Pan, L., Liu, X., Su, L., Zhang, W., Li, Q., & Zheng, Y. (2018). An improved method of preparing high efficiency transformation *Escherichia coli* with both plasmids and larger DNA fragments. *Indian Journal of Microbiology*, *58*(4), 448-456. https://doi.org/10.1007/s12088-018-0743-z

Mendes, G. P., Kluskens, L. D., Lanceros-Méndez, S., & Mota, M. (2021a). Magnesium aminoclays as plasmid delivery agents for non-competent *Escherichia coli* JM109 transformation. *Applied Clay Science*, *204*, 106010. https://doi.org/10.1016/j.clay.2021.106010

Mendes, G. P., Kluskens, L. D., Mota, M., Lanceros-Méndez, S., & Hatton, T. A. (2021b). Spherical and needle shaped magnetic nanoparticles for friction and magnetic stimulated transformation of microorganisms. *Nano-Structures & Nano-Objects*, *26*, 100732. https://doi.org/10.1016/j.nanoso.2021.100732

Mimee, M., Citorik, R. J., & Lu, T. K. (2016). Microbiome therapeutics - Advances and challenges. *Advanced Drug Delivery Reviews*, *105*(Pt A), 44-54. https://doi.org/10.1016/j.addr.2016.04.032

Pardee, K., Slomovic, S., Nguyen, P. Q., Lee, J. W., Donghia, N., Burrill, D., Ferrante, T., McSorley, F. R., Furuta, Y., Vernet, A., Lewandowski, M., Boddy, C. N., Joshi, N. S., & Collins, J. J. (2016). Portable, on-demand biomolecular manufacturing. *Cell*, *167*(1), 248-259.e212. https://doi.org/10.1016/j.cell.2016.09.013

Park, C. B., Yi, K. S., Matsuzaki, K., Kim, M. S., & Kim, S. C. (2000). Structure-activity analysis of Buforin II, a histone H2A-derived antimicrobial

ACKNOWLEDGMENT

This research is funded by Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam.

peptide: The proline hinge is responsible for the cell-penetrating ability of Buforin II. *Proceedings of the National Academy of Sciences*, *97*(15), 8245-8250. https://doi.org/10.1073/pnas.150518097

Rossi, J. J., June, C. H., & Kohn, D. B. (2007). Genetic therapies against HIV. *Nature Biotechnology, 25*(12), 1444-1454. https://doi.org/10.1038/nbt1367

Slomovic, S., Pardee, K., & Collins, J. J. (2015). Synthetic biology devices for *in vitro* and *in vivo* diagnostics. *Proceedings of the National Academy of Sciences, 112*(47), 14429-14435. https://doi.org/10.1073/pnas.1508521112

Tee, K. L., Grinham, J., Othusitse, A. M., González-Villanueva, M., Johnson, A. O., & Wong, T. S. (2017). An efficient transformation method for the bioplastic-producing “Knallgas” bacterium *Ralstonia eutropha* H16. *Biotechnology Journal*, *12*(11), 1700081. https://doi.org/10.1002/biot.201700081