International Journal of Biomolecules and Biomedicine (IJBB)
ISSN: 2221-1063 (Print) 2222-503X (Online)
Vol. 1, No.2, p. 27-31, 2011
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Transformation of signal sequence in *Escherichia coli* by reporter gene fusion

Ahmad Humayan Kabir[1*], FM Ali Haydar[2], M Shibli Abedin[3]

[1]*School of Biological Sciences, Flinders University, SA 5042, Australia*

[2]*Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh*

[3]*Department of Biochemistry, Sher-E-Bangla Agricultural University, Dhaka 1207, Bangladesh*

Received: 27 June 2011
Revised: 25 July 2011
Accepted: 27 July 2011

Key words: Signal sequence, *Erwinia carotovora*, gene fusion, pHSK34 plasmid.

Abstract

Transformation of signal sequence by reporter gene fusion technology was attempted. The promoter containing signal sequences was taken from *Erwinia carotovora*, a plant pathogenic soil bacterium. Plasmid DNA (Deoxyribonucleic acid) and genomic DNA were cleaved by *Bam*HI and *Sau3*A1, respectively. Result of gel electrophoresis reveals successful cutting of restriction enzyme in both plasmid and genomic DNA. Best banding was found in 0.75 U/µg DNA of *Sau3*A1 compared to other concentrations of *Sau3*A1 used. Bacterial growth was found on LB^{cmp} (Luria-Bertani) plates, since chloramphenicol resistance ability was pre-existed in this plasmid. Highest colony forming unit was observed with ligation ratio (1:5). It was also evident that the cells have been successfully transformed with the plasmid resulting notable growth of bacteria in LB^{amp} plate.

*Corresponding Author: Ahmad Humayan Kabir ✉ ahmad.kabir@flinders.edu.au

Introduction

Recombinant DNA technology is a set of techniques for manipulating DNA, including identification, cloning and transfer of genes or protein. Gene transformation is a common technique to transfer the gene of interest. Prokaryotes were used in the transformation due to some advantages. Prokaryotes grow fast, easier to manipulate. There are some drawbacks of prokaryotes in transformation including inability to splice introns and unable to take large size of DNA (Martinko and Madigam, 2006). Moreover, use of *Escherichia coli* for transformation is beneficial, since it's completely sequenced and easy to cultivate in laboratory (Albert *et al*., 2002). Genomic DNA from *E. carotovora* was used. It is a plant pathogenic bacterium containing thousand signal sequences (Sirkka *et al*., 1991; Steven *et al*., 2006). A signal sequence is a short peptide chain that directs the post-translational transport of a protein in a specific destination in the cell. The promoter and signal sequences of *E. carotovora* were incorporated into plasmid (pHSK34) with a view to transfer the antibiotic resistant protein that eventually makes the cell resistant to ampicillin. The pHSK34 is a recombinant plasmid, which contains a modified *bla* gene lacking regulatory region and signal sequence for the export of the protein (Tiina *et al*., 2002). It has no ampicillin resistance. In this study, both vector and target gene is to be cut by specific restriction enzyme. This technique has been used by other workers (Gelbart *et al*., 2005).

In vitro reporter gene fusion is a modern method to detect gene of interest (Saarilahti *et al*., 2004; Ian *et al*., 2004). It is also an important alternative for profiling gene expression and cloning (Tina et al. 2001). Enormous reporter genes have been used for studying gene expression and cloning and it is mostly depends on the quality of reporter gene. Use of the *bla* gene has been reported by several workers for gene cloning and transformation (Saarilahti, 2004; Yahua *et al*., 2004). The β-lactamase (bla) gene constitutes a heterogenous group of enzymes that confers resistance to penicillin and many of its derivatives. Expression of the *bla* gene is possible when the gene can be ligated with a DNA fragment containing regulatory sequence.

The aim of this study was to make a recombinant plasmid containing regulatory sequence and subsequent transformation in *Escherichia coli*. Transformation would reveal that the gene of interest containing signal sequence is successfully cloned and inserted to genomic DNA.

Materials and methods

The pHSK34 has been used which contains a modified *bla* gene that lacks the promoter needed for the gene regulation. These sequences have been replaced by a multiple cloning site.

Plasmid and chromosomal DNA preparation

The pHSK34 plasmid was used and purified according to the guideline of QIAGEN Spin Miniprep kit. Plasmid Preparation of chromosomal DNA from *E. carotovora*: Genomic DNA of *E. carotovora* was isolated by using DNeasy Tissue Kit.

Measurement and visualization of DNA

Measurement of both plasmid and genomic DNA concentration was done by Nanodrop during the whole experiment. Visualization of DNA molecules by size was done by for agarose gel electrophoresis was ethidium bromide. In the gel, 1kb DNA ladder was used to compare with sample DNA (Fig. 1).

Cleavage of plasmid DNA

Plasmid and genomic DNA was digested by *Bam*HI and *Sau*3A1, respectively. The recombinant plasmid carries a gene that is resistant to chloramphenicol. Cleavage of plasmid DNA was undertaken by the restriction enzyme *Bam*HI (20 U/μg of DNA). On the other hand, partial digestion of genomic DNA was done by using *Sau*3AI at three different concentrations (0.25, 0.75 and 2.0 U/μg of DNA).

Ligation of plasmid and genomic DNA

Two different tubes were used for ligation reaction. In the first tube, 50ng of pHSK34 plasmid and 150 ng of genomic DNA (1:3) were taken. In the second tube, 250 ng of genomic DNA was taken in addition to 50 ng pHSK34 plasmid DNA (1:5). These tubes were incubated overnight at 16°C in a ligation block. After ligation, the cloned gene was then cultured in the competent cells of *E. coli*. The competent *E. coli* cells with cloned gene have been grown in Luria-Bertani media (LB media) containing chloramphenicol, ampicillin and no antibiotics. The aim of using LB^{blank} was to check the viability of competent cells after incubation.

Transformation and plating

After purification, concentration of plasmid and genomic DNA were 31.2 ng/µl and 110 ng/µl, respectively. The sample containing highest amount of DNA was further used for cleaving and transformation. The recombinant DNA was cultured with competent cells of *E. coli* for transformation and plating was done in LB media containing antibiotics. Two different amounts (300 µl and 150 µl) of ampicillin were added in the medium.

DNA purification

Both plasmid and genomic DNA purification was done in Qiagen purification tube. In these tubes, 50 µl and 100 µl EB buffer was added to elute the plasmid and genomic DNA, respectively.

Analysis of transformed cells

After the incubation of transformed cells, analysis was done by observing the growth of bacteria on different plates.

Results and discussion

Gel electrophoresis of digested and undigested DNA showed evidence of cutting by the restriction enzyme. The images of gel electrophoresis revealed that the specific restriction enzyme successfully cut both the plasmid and genomic DNA (Fig. 1 and 2). It is observed that the restriction enzyme cut in many places of DNA. It was also evident that 0.75 U/µl of *Sau*3AI found to be best for cleaving genomic DNA of *E. carotovora* (Fig. 2). Sau3AI is a large type II restriction enzyme with sequence homology to the mismatch repair protein and that's why it has been widely used (Peter *et al*., 2001; Gregory *et al*., 2006).

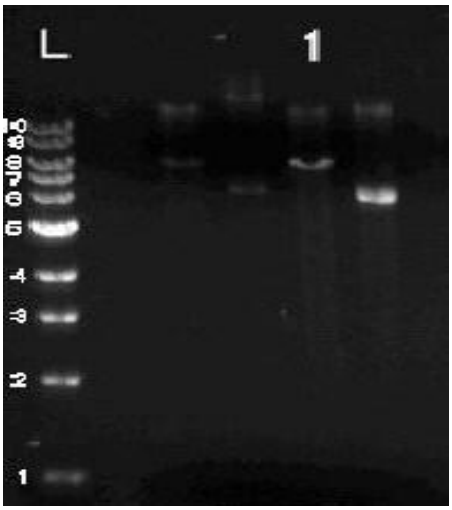

Fig. 1. Gel electrophoresis of pHSK34 plasmid DNA. Lane 1 shows the undigested plasmid DNA while lane L shows the ladder DNA.

It was found that bacterial growth on control (LB^{blank}) was enormous, which indicates that competent cells were live (Table 1). The modified promoter-less *bla* gene cannot be expressed by itself, therefore mutant bacterial cells carrying this plasmid are not resistant to ampicillin. Expression of the promoter-less *bla* gene is possible only if the gene is fused (ligated) behind a DNA fragment containing regulatory sequences. Growth of bacteria is not supposed to see if any wrong sequence or promoter would have been inserted and transformed. It was also found that 1:3 ligation ratio was better than that 1:5.

The growth of bacteria in LB^{amp} plates revealed that cloned genes with the promoter were transformed well in *E. coli* competent cells. Highest 88 colony forming

unit (CFU) was found in LB^{amp} media containing 300µl ampicillin. Cloning of the β-lactamase gene and its expression was noticedn in ceftazidime-susceptible and-resistant strains (Pannika and Vanaporn, 2002). Cloning and transformation of signal sequence was also reported to be successful in *E. coli* by other researchers (Hideakmi *et al*., 1993). Use of LB media contained antibiotics is popular for screening clone genes (Shinichi *et al*., 2004).

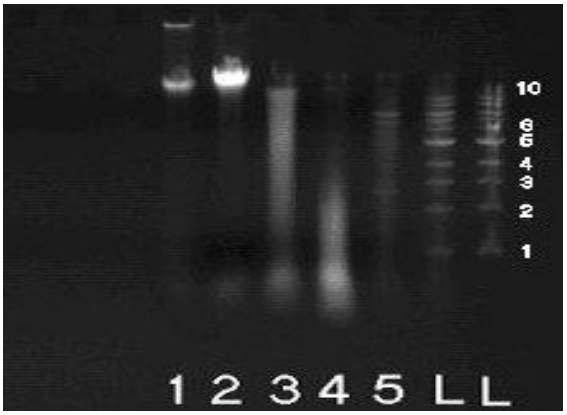

Fig. 2. Gel electrophoresis of digested and undigested genomic DNA. Lane 1 shows the undigested DNA. Lane 2, 3 and 4 shows the digestion of genomic DNA with *Sau*3AI having the concentration of 0.25 U/µg DNA, 0.75 U/µg DNA and 2.0 U/µg DNA, respectively. Lane 5 shows the digested plasmid DNA and lane L shows the ladder DNA in size in KB.

Table 1. Result of transformed bacteria (colony forming unit) in LB plates.

Ligation ratio	LB^{amp} (300µl)	LB^{amp} (150µl)	LB^{cmp} (50µl)	LB media
1:5	plate 1 – 76 plate 2 – 88	plate 1 – 58 plate 2 – 56	26 CFU	Uncountable
1:3	plate 1 – 40 plate 2 – 52	plate 1 – 16 plate 2 – 34	19 CFU	Uncountable

It is to be concluded that gene of interest was successfully transformed in *E. coli* via in vivo gene fusion and β-lactamase gene proved to be ideal reporter system for monitoring gene expression in live *E. coli* cells. These findings could be used for further purposes in molecular biology and proteomics study.

References

Albert B, Johnson A, Lewis J, Raff M, Roberts K, P Walter. 2002. Molecular Biology of the Cell (4th Ed.). New York, Garland Science.

Gelbart WM, Griffith, AJF, Lewontin RC, MIller JH, Suzuki D. 2005. An Introduction to Genetic Analysis. (8th Ed.). New York, W.H. Freeman and Company.

Hideakmi A, Tsutoshmi O, Azuhitofu J, Nichki I, Toshiomyio S. 1993. Cloning, sequence analysis and expression in Escherichia coli of a gene encoding an alginate lyase from *Pseudomonas* sp. OS-ALG-9. Journal of General Microbiology **139,** 987-993.

Ian A, Jonathan S, Anna G, Josie H, David LC, Michael AB, Marc V. 2004. Feasibility of Genome-Scale Construction of Promoter::Reporter Gene Fusions for Expression in Caenorhabditis elegans Using a MultiSite Gateway Recombination System. Genome Research **14(10b),** 2070–2075.

Gregory JG, Rachel M, Douglas ES. 2006. Tension-dependent DNA cleavage by restriction endonucleases: Two-site enzymes are "switched off" at low force. PNAS **103(31),** 11555-11560.

Martinko JM, Madigam MT. 2006. Brock Biology of Microorganisms. (11th Ed.). USA, Pearson Education.

Pannika N, Vanaporn W. 2002. Cloning of the class D β lactamase gene from *Burkholderia pseudomallei* and studies on its expression in ceftazidime-susceptible and-resistant strains. Journal of Antimicrobial Chemotherapy **50,** 445–455.

Peter F, Rudi L, Gerhild L, Alfred P. 2001. Sau3AI, a Monomeric Type II Restriction

Endonuclease That Dimerizes on the DNA and Thereby Induces DNA Loops. The Journal of Biological Chemistry **276,** 23581-23588.

Saarilahti HT, Pirhonen M, Karlsson MM, Flego D, Palva DE. 2004. Expression of pehA-bla gene fusions in *Erwinia carotovora* subsp. carotovora and isolation of regulatory mutants affecting polygalacturonase production. Molecular and General Genetics **234 (1),** 81-88.

Shinichi N, Shinichi U, Masamitsu T, Akira H, Masami N, Katsumi A. 2004. Cloning of various promoters for foreign gene expression in *Erwinia an*anas. Journal of General Plant Pathology **70(1),** 69-73.

Sirkka RM, Kyostio CL, George HL. 1991. *Erwinia carotovora* subsp. carotovora Extracellular Protease: Characterization and Nucleotide Sequence of the Gene. Journal of Bacteriology **173(20),** 6537-6546.

Steven DB, George PCS. 2006. Exploitation of a b lactamase reporter gene fusion in the carbapenem antibiotic production operon to study adaptive evolution in *Erwinia carotovora*. Microbiology **152,** 1089–1097.

Tina KVD, Yan W, Michael KH, Maureen D, Mary JGR, Antoni R, Lucia BR, Robert AL. 2001. A genomic approach to gene fusion technology. PNAS **98(5),** 2555–2560.

Tiina P, Richard P, Ruusu R, Martin R, Hannu TS. 2002. A putative three-dimensional targeting motif of polygalacturonase (PehA), a protein secreted through the type II (GSP) pathway in *Erwinia carotovora*. Molecular Microbiology **43(3),** 585–596.

Yahua Chen, Fred CT, Theresa MK. 2004. Beta-lactamase Gene Expression in a Penicillin-Resistant Bacillus anthracis Strain. Antimicrobial Agents and Chemotherapy **48(12),** 4873–4877.