The Journal of Microbiology, December 2007, p. 593-596

Vol. 45, No. 6

NOTE

Development of a Simple Cell Lysis Method for Recombinant DNA Using Bacteriophage Lambda Lysis Genes

Boyun Jang, Yuna Jung, and Dongbin Lim*

Department of Bioinformatics and Life Science, Soongsil University, Seoul 156-743, Republic of Korea

(Received September 27, 2007 / Accepted November 8, 2007)

In this study, we describe the development of a simple and efficient method for cell lysis via the insertion of a bacteriophage lambda lysis gene cluster into the pET22b expression vector in the following order; the T7 promoter, a gene for a target protein intended for production, *Sam7* and *R*. This insertion of *R* and *Sam7* into pET22b exerted no detrimental effects on cellular growth or the production of a target protein. The induction of the T7 promoter did not in itself result in the autolysis of cells in culture but the harvested cells were readily broken by freezing and thawing. We compared the efficiency of the cell lysis technique by freezing and thawing to that observed with sonication, and determined that both methods completely disintegrated the cells and released proteins into the solution. With our modification of pET22b, the lysis of cells became quite simple, efficient, and reliable. This strategy may prove useful for a broad variety of applications, particularly in experiments requiring extensive cell breakage, including library screening and culture condition exploration, in addition to protein purification.

Keywords: protein purification, recombinant protein, cell lysis, autolysis, bacteriophage lambda

Proteins, regardless of their origins, are currently almost always purified from *E. coli* after the cloning of the genes into expression vectors (Studier, 2005). In these cases, it is often necessary to screen culture conditions in order to maximize the soluble expression of a particular protein. Sometimes a large library of genetic variants is generated *in vitro* and screened for proteins with improved properties in a directed genetic evolution technique (Lutz and Patrick, 2004). These processes require small-scale disruptions of cells from many different samples. Consequently, a quick and efficient method of cell lysis, which could be used to deal with large numbers of samples, would be quite useful. In order to cope with these requirements, several companies have introduced detergent-based lysis methods. Basically, these methods employ non-ionic detergents to disrupt the membrane after the cell wall has been broken by lysozyme treatment. However, these methods do have some disadvantages, including the possibility of protein denaturation, reproducibility and costs. With this in mind, we developed a simple and very efficient autolysis system by utilizing bacteriophage lambda lysis genes.

Three genes of phage Lambda, *S*, *R*, and *Rz*, form a lysis cluster located on the lambda genome (Young, 1992; Wang *et al.*, 2000; Oppenheim *et al.*, 2005). The *R* gene encodes for transglycosylase, or endolysin, which hydrolyzes glycosidic linkages within the peptidoglycan of cell walls (Imada and Tsugita, 1971; Bienkowska-Szewczyk *et al.*, 1981). The *Rz* gene is conserved among lambdoid phages, but its function remains unclear (Zhang and Young, 1999). The *S* gene product, holin, somehow renders the plasma membrane permeable, and allows the endolysin in the cytoplasm to reach its substrate outside of the plasma membrane (Smith *et al.*, 1998a). Without functional holins, such as are detected in the amber mutant of the *S* gene (*Sam7*), endolysin is accumulated in the cytoplasm and the cell is not ordinarily lysed (Smith *et al.*, 1998b). In these cases, cell lysis would be achieved by treatments with membrane-perturbing chemicals, including chloroform (Garrett *et al.*, 1981).

In this report we describe the development of a simple and efficient method for cell lysis by the insertion of a bacteriophage lambda lysis gene cluster into the expression vector pET22b. As it was deemed desirable to break the cells and release proteins into a small volume of physiological buffer and not into the culture medium, we utilized lambda with an amber mutation on the *S* gene (*Sam7* mutant) as DNA source. The 1.85 kb fragment of lambda CI857 *Sam7* DNA from *Eco*RI to *Ssp*I (positions from 44,972 to 46,822) containing *Sam7*, *R*, and *Rz*, (in that order) was cloned into the pUC18, forming plasmid pUC18-SR. In this construct, the *Sam7* and *R* genes were under the control of the *lac* promoter. *E. coli* JM109 harboring the pUC18-SR plasmid did not burst after induction by IPTG, but was completely broken after the addition of a few drops of chloroform, as judged by an observed sharp drop in optical density (data not shown). It is known that cells which express intact *R* and *Sam7* are broken as the result of chloroform treatment, and the extent of cell lysis can be readily measured by the

* To whom correspondence should be addressed.
(Tel) 82-2-820-0452; (Fax) 82-2-824-4383
(E-mail) dblim@ssu.ac.kr

decrease in optical density (Young, 1992).

In the next experiment, the DNA fragment containing *R* and *Sam7* was transferred into the pET22b derivative pET22b-ESYP30 producing pET22b-ESYP30-SR, the structure of which is provided in Fig. 1. The ESYP30 portion of pET22b-ESYP30-SR encodes for the GroES-human dermcidin fusion protein (Schittek *et al.*, 2001; Porter *et al.*, 2003). The optical density of two cultures harboring the plasmid with or without the lysis gene (pET22b-ESYP30-SR or pET22b-ESYP30) were recorded every 30 min. As is shown in Fig. 2A, the growth of *E. coli* BL21 harboring pET22b-ESYP30 was hampered to some degree after the addition of IPTG. After full induction, the optical density reached approximately 1.4 ($O.D._{600}$=1.4), which was 70% of the value observed without induction. The optical density was only marginally affected with the addition of chloroform to the culture (Fig. 2A). This was in sharp contrast to what was observed with the cells that harbored a plasmid with lysis genes. Figure 2B showed the results from similar experiments conducted with

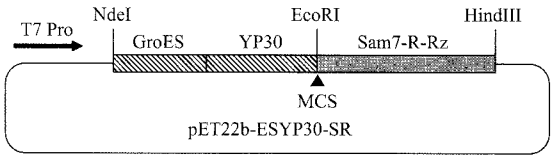

Fig. 1. Schematic drawing of plasmid pET22b-ESYP30-SR. The plasmid backbone was from pET22b. The GroES and ESYP30 indicated the genes for *E. coli* GroES and human dermcidin, which were translationally fused to generate the ESYP30 fusion protein. The *Sam7-R-Rz* region indicated the 1.85 kb fragment of lambda DNA containing *Sam7*, *R*, and *Rz*. MCS, multiple cloning site, contained restriction enzyme sites of *Eco*RI, *Bam*H, *Sst*I, and *Sal*I.

BL21 harboring the pET22b-ESYP30-SR plasmid. It is interesting to note that in cultures with or without induction, the growth rate and the final density of the cells harboring pET22b-ESYP30-SR were quite similar to those of the cell harboring pET22b-ESYP30, thereby indicating that the insertion of lysis genes under the control of the T7 promoter exerted no effects on the growth of cells even after induction. Importantly, the addition of chloroform gave rise to a sharp drop in the optical density of cells harboring pET22b-ESYP30-SR. As early as 30 min after induction, the addition of chloroform resulted in a dramatic reduction of the optical density, from 0.9 to approximately 0.2 (Fig. 2B). After 4 h of induction, the cell density reached about 1.4, but dropped to approximately 0.4 after the addition of a few drops of chloroform. Therefore, we can conclusively state that the insertion of the *R* and *Sam7* genes downstream of the target gene induced cell lysis when chloroform was added after induction.

From the results shown in Fig. 2, we were convinced that the lambda lysis genes under the T7 promoter worked as we had desired, with no effect on cell growth. In the next experiment, we attempted to ascertain whether or not the addition of the lysis gene exerted any effect on the expression of the target gene. In an effort to address this question, *E. coli* BL21 harboring the pET22b-ESYP30 plasmid and *E. coli* BL21 containing pET22b-ESYP30-SR were cultivated, and IPTG was added when the optical density reached to approximately 0.5. The cells were then incubated for an additional 4 h. During the incubation, a portion of each culture was taken every 30 min and their proteins analyzed by SDS-PAGE. As is shown in Fig. 3, a new protein band of approximately 35 kDa, the expected size of our target GroES-YP30 fusion protein, was noted in samples from both cultures as early as 30 min after the beginning of induction. The quantity of this protein accumulated continuously up to approximately 3 h. Induction kinetics or the final amount quantity of the induced protein was quite similar in both

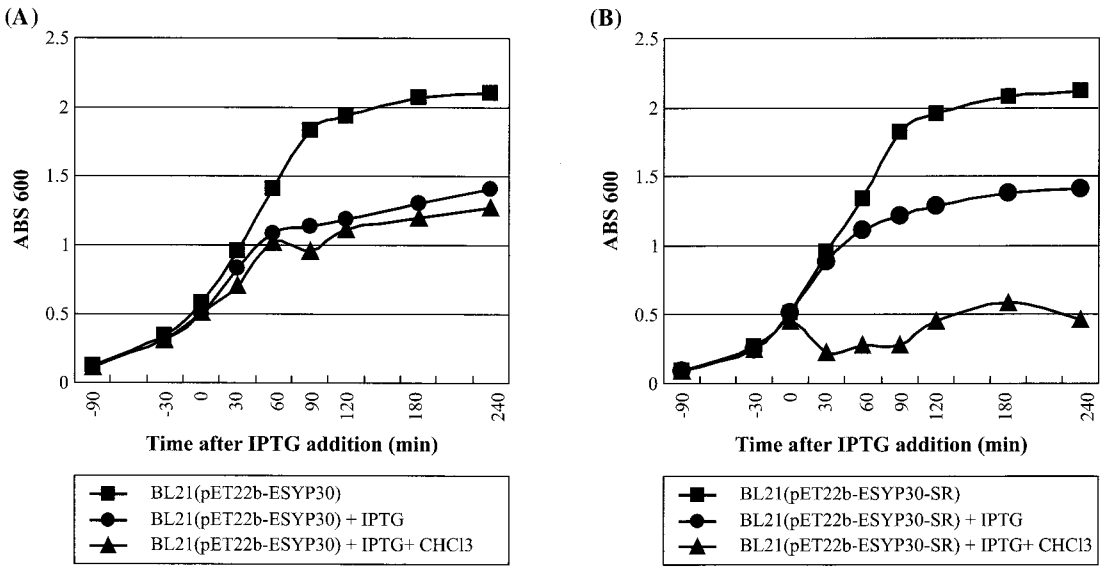

Fig. 2. Measurement of growth and lysis of cells harboring a plasmid with or without the lambda lysis region. *E. coli* BL21 harboring the pET22b-ESYP30 (A) or pET22b-ESYP30-SR (B) plasmid was cultivated in LB media at 30°C and IPTG was added to two cultures of each strain at time 0. The symbols ■, ●, and ▲ indicated the culture of no induction, induction with IPTG and induction and addition of chloroform, respectively.

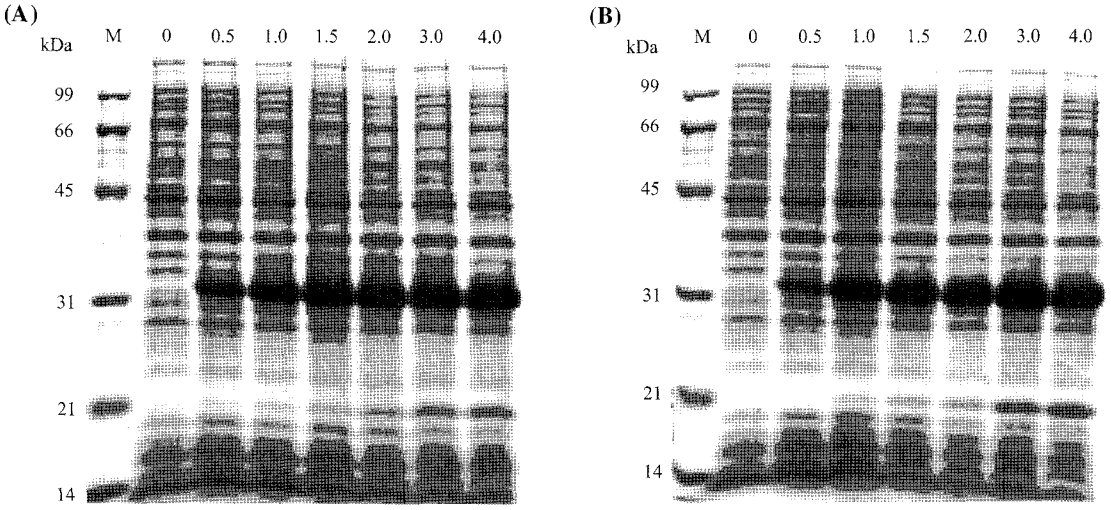

Fig. 3. Comparison of protein production in the cells containing pET22b derivative with or without lysis genes. *E. coli* BL21 harboring the pET22b-ESYP30 (A) or pET22b-ESYP30-SR (B) plasmid was cultivated in LB media at 30°C and IPTG was added at an $O.D._{600}$ of approximately 0.5. Samples were taken after the addition of IPTG at the time indicated in hours at the top of each gel. Samples with approximately equivalent amounts of cells were loaded into each lane.

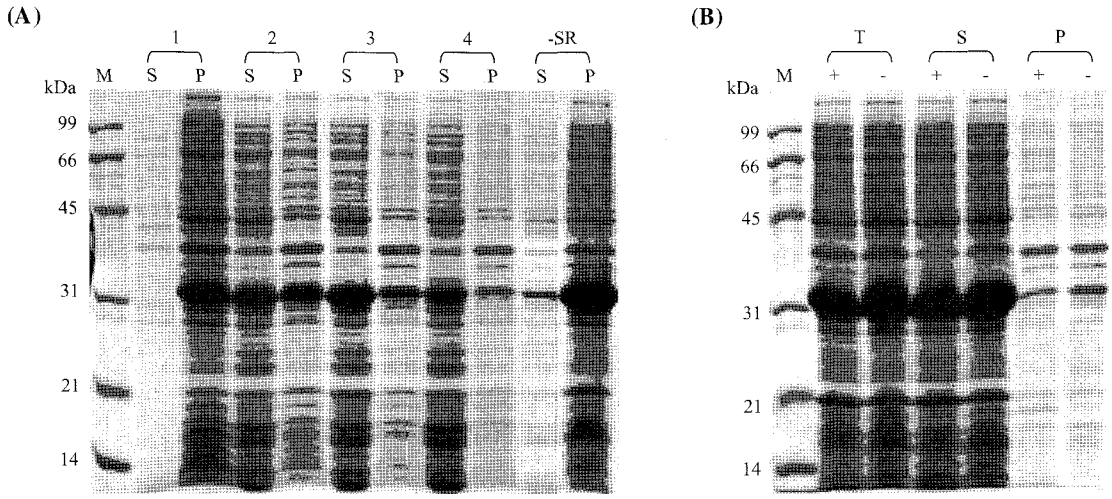

Fig. 4. Comparison of lysis by freezing and thawing to sonication. (A) Samples from *E. coli* BL21 (pET22b-ESYP30-SR) were taken after the addition of IPTG at the time indicated in hours at the top of gel and subjected to freezing and thawing. Supernatants were separated from pellets and analyzed via SDS-PAGE. The lanes indicated with '-SR' were samples from *E. coli* BL21 (pET22b-ESYP30) after 4 h of induction. S and P, supernatant and pellet after centrifugation. (B) Comparison of lysis by freezing and thawing to sonication. The cells were subjected to freezing and thawing (*E. coli* BL21 harboring pET22b-ESYP30-SR) or sonication (*E. coli* BL21 harboring pET22b-ESYP30) and centrifuged. T, S, and P indicate total, supernatant, and pellet, respectively. Samples with (+) were from *E. coli* BL21 (pET22b-ESYP30-SR) and samples with (-) were from *E. coli* BL21 (pET22b-ESYP30).

cultures, thereby indicating that the addition of the lysis gene had no effect on the expression of our target gene. Based on the information displayed in Fig. 2B, we knew that as early as 30 min after the beginning of induction, the endolysin sufficient for the cell lysis was generated. However, a band corresponding to the calculated molecular weight of endolysin (17.8 kDa) could not be identified, even after full induction.

As it is known that the cells containing lambda with *R* and *Sam7* can be readily broken by freezing and thawing (Young, 1992), we attempted to determine whether or not the induced cells were lysed by freezing and thawing. *E. coli* BL21 (pET22b-ESYP30-SR) was cultivated and 20 ml of culture was taken every hour after induction. The cells were then harvested by centrifugation. Following, they were frozen at -20°C for 1 h, and thawed at room temperature. The cells were resuspended in phosphate-buffered saline and centrifuged in order to separate the soluble fraction from the pellet. Both fractions were analyzed via SDS-PAGE. Figure 4A shows that, although a large quantity of ESYP30 was generated after 1 h of induction, a very small quantity of proteins were released into the supernatant, indicating

that only a small portion of cells was broken by freezing and thawing. After 2 h of induction, however, we observed more proteins in the supernatant than in the pellet, thereby indicating that more than half of the cells were now broken. It appeared that 4 h of induction was required for complete lysis by freezing and thawing (Fig. 4A). This result was in sharp contrast to the earlier observation that cells were completely broken as early as 30 min of induction by the addition of chloroform to the culture (Fig. 3). Even after four hours of induction, the cells harboring pET22b-ESYP30 gave rise to only approximately 10% lysis after freezing and thawing (Fig. 4, lane -SR), indicating the positive role of endolysin in cell lysis.

Finally, we compared the efficiency of cell breakage by our freezing and thawing to that achieved by the traditional sonication method. Two cultures harboring either pET22b-ESYP30-SR or pET22b-ESYP30 were grown and induced fully for 4 h. The harvested cells were treated with either freezing and thawing (cells with pET22b-ESYP30-SR) or sonication (cells with pET22b-ESYP30). The insoluble fractions were separated via centrifugation and the supernatant and pellet were analyzed by SDS-PAGE. As shown in Fig. 4B, both methods were equally effective with regard to cell disruption, releasing the majority of proteins into the solution. Importantly, almost all ESYP30 proteins were found in the supernatants of both samples. Therefore, it was clear that simply freezing and thawing caused the complete disintegration of cell structure, thus releasing proteins into the solution.

Several attempts have been made previously to develop an autolysis system based on phage lysis genes (Jain and Mekalanos, 2000; Xu *et al.*, 2006; Li *et al.*, 2007). In these cases, lambda lysis cluster was inserted into an extra plasmid, and an extra antibiotic and an inducer was added for the maintenance of the plasmid and for the induction of lysis system. Since wild type lysis genes were used, cells were automatically broken shortly after induction (Xu *et al.*, 2006; Li *et al.*, 2007). In this study, we have described the development of a simple and very efficient lysis system by the insertion of a lambda lysis gene cluster into the popular expression vector pET22b. We used mutant (*Sam7*) rather than wild type *S* gene. With this construction, the induction of the T7 promoter did not in itself result in the autolysis of cells, rendering a target protein fully accumulating in cells (Fig. 3). Simple freezing and thawing of cells harvested after induction caused complete disintegration, and released target protein into the solution. We conclude that cell lysis by freezing and thawing is as efficient as that by sonication.

A few aspects are worth noting for practical purposes. We observed no difference in the growth of cells harboring a plasmid with or without the lysis gene cloned downstream of the target protein gene (Fig. 2). This was true under a variety of culture conditions, including growth temperature (30°C or 20°C) or induction point ($O.D._{600}$=0.5 or 1.0). The cells were not lysed in itself by the four hour-induction at 30°C or by overnight induction at 20°C. However, overnight induction at 30°C occasionally resulted in cell lysis.

This work was supported by the Soongsil University Research Fund.

References

Bienkowska-Szewczyk, K., B. Lipinska, and A. Taylor. 1981. The *R* gene product of bacteriophage lambda is the murein transglycosylase. *Mol. Gen. Genet.* 184, 111-114.

Garrett, J., R. Fusselman, J. Hise, L. Chiou, D. Smith-Grillo, J. Schulz, and R. Young. 1981. Cell lysis by induction of cloned lambda lysis genes. *Mol. Gen. Genet.* 182, 326-331.

Imada, M. and A. Tsugita. 1971. Amino acid sequence of lambda phage endolysin. *Nature New Biol.* 233, 230-231.

Jain, V. and J.J. Mekalanos. 2000. Use of lambda phage S and *R* gene products in an inducible lysis system for *Vibrio cholerae* and *Salmonella enterica* Serovar Typhimurium based DNA vaccine delivery systems. *Infect. Imm.* 68, 986-989.

Li, S., L. Xu, H. Hua, C. Ren, and Z. Lin. 2007. A set of UV-inducible autolytic vectors for high throughput screening. *J. Biotechnol.* 127. 647-652.

Lutz, S. and W.M. Patrick. 2004. Novel methods for directed evolution of enzymes: quality, not quantity. *Curr. Opin. Biotechnol.* 15, 291-297.

Oppenheim, A.B., O. Kobiler, J. Stavans, D.L. Court, and S. Adhya. 2005. Switches in bacteriophage lambda development. *Annu. Rev. Genet.* 39, 409-429.

Porter, D., S. Weremowicz, K. Chin, P. Seth, A. Keshaviah, J. Lahti-Domenici, Y.K. Bae, C.L. Monitto, A. Merlos-Suarez, J. Chan, C.M. Hulette, A. Richardson, C.C. Morton, J. Marks, M. Duyao, R. Hruban, E. Gabrielson, R. Gelman, and K. Polyak. 2003. A neural survival factor is a candidate oncogene in breast cancer. *Proc. Natl. Acad. Sci. USA* 100. 10931-1096.

Schittek, B., R. Hipfel, B. Sauer, J. Bauer, H. Kalbacher, S. Stevanovic, M. Schirle, K. Schroeder, N. Blin, F. Meier, G. Rassner, and C. Garbe. 2001. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat. Immunol.* 2, 1133-1137.

Smith, D.L., C.Y. Chang, and R. Young. 1998a. The lambda holin accumulates beyond the lethal triggering concentration under hyperexpression conditions. *Gene Expr.* 7, 39-52.

Smith, D.L., D.K. Struck, J.M. Scholtz, and R. Young. 1998b. Purification and biochemical characterization of the lambda holin. *J. Bacteriol.* 180, 2531-2540.

Studier, FW. 2005. Protein production by auto-induction in high density shaking cultures. *Protein Expr. Purif.* 41, 207-234.

Wang, I.N., D.L. Smith, and R. Young. 2000. Holins: The protein clocks of bacteriophage infections. *Annu. Rev. Microbiol.* 54, 799-825.

Xu, L., S. Li, C. Ren, Z. Cai, and Z. Lin. 2006. Heat-inducible autolytic vector for high-throughput screening. *Biotechniques* 41, 319-323.

Young, R. 1992. Bacteriophage lysis: Mechanism and regulation. *Microbiol. Rev.* 56, 430-481.

Zhang, N. and R. Young. 1999. Complementation and characterization of the nested *Rz* and *Rz1* reading frames in the genome of bacteriophage lambda. *Mol. Gen. Genet.* 262, 659-667.