The Journal of Microbiology, February 2006, p.126-128 Vol. 44, No. 1

NOTE

Removal of Contaminating TEM-la β-Lactamase Gene from Commercial *Taq* DNA Polymerase

Jae Seok Song[1], Jung Hun Lee[1,2], Jung-Hyun Lee[2], Byeong Chul Jeong[1], Won-Keun Lee[1] and Sang Hee Lee[1,*]

[1]*Department of Biological Sciences, Myongji University, San 38-2 Namdong, Yongin, Gyeonggido, 449-728, Republic of Korea*
[2]*Marine Biotechnology Center, Korea Ocean Research & Development Institute, Ansan P.O. Box 29, Seoul 425-600, Republic of Korea*

(Received October 21, 2005 / Accepted November 30, 2005)

This study confirms that *Taq* DNA polymerase could be contaminated with the *bla*TEM-1$_a$ gene. It also proposes two different methods that could be used to overcome DNA contamination: (i) DNase I treatment prior to PCR amplification; and (ii) the use of a highly purified *Taq* DNA polymerase which was devoid of detectable contamination.

Keywords: Decontamination, DNase I, *Taq* DNA polymerase, TEM-type β-lactamase

β-Lactamases produced by bacterium are known to protect against the lethal effect of β-lactam antibiotics (penicillins, cephalosporins, carbapenems or monobactams) on cell-wall synthesis. The production of β-lactamase is the single most prevalent mechanism responsible for the resistance to β-lactams among clinical isolates of *Pseudomonas aeruginosa* and the family *Enterobacteriaceae* (Sanders and Sanders, 1992). Extended-spectrum β-lactamases (ESBLs) are clavulanate-susceptible enzymes conferring broad resistance to penicillins, aztreonam, and cephalosporins (with the exception of cephamycins) (Livermore, 1995). ESBLs are often plasmid-mediated, and most of them are mutants of the classic TEM- and SHV-type enzymes such as TEM-1, TEM-2, and SHV-1, with one or more amino-acid substitutions around the active site (Paterson *et al.*, 2001). These changes allow the hydrolysis of extended-spectrum cephalosporins (e.g., ceftazidime and cefotaxime) and monobactams (e.g., aztreonam), which are stable to the classic TEM- and SHV-type enzymes (Bradford, 2001). The standard method for determining the specific gene for more than 130 TEM-type and more than 50 SHV-type ESBLs (http://www.lahey.org/studies/webt.asp) is the PCR (polymerase chain reaction) technique followed by nucleotide sequencing (Bradford, 2001).

The standard method was performed to detect ESBL genes from clinical isolates of *Enterobacteriaceae* in our nationwide survey (Jeong *et al.*, 2004) and our environmental metagenomic libraries (Song *et al.*, 2005). On the basis of DNA sequencing of the PCR products for TEM-type β-lactamase genes, the $bla_{\text{TEM-1a}}$ gene was frequently detected together with other TEM-type β-lactamase genes such as $bla_{\text{TEM-1b}}$ in our survey. However, this did not occur in our survey when the other β-lactamase genes such as bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{OXA}, and bla_{PER} were targeted. The $bla_{\text{TEM-1a}}$ gene was presented in pBR322 (Sutcliffe, 1978) and has been the most commonly used selective maker for expression vectors that are generally presented in multiple copies. Thus, it is likely that during *Taq* DNA polymerase purification, the DNA harboring $bla_{\text{TEM-1a}}$ gene was not completely removed. To verify this possibility and avoid cross-contamination, separated rooms for sample preparation, PCR analysis, and agarose gel electrophoresis were used. The PCR condition (30 cycles) and DNA sequencing methods were as previously described (Jeong *et al.*, 2004). Results of PCR and DNA sequencing indicated that pipette tips (Sarstedt, Germany), microcentrifuge tubes (Sarstedt, Germany), and PCR reagents were not the source of $bla_{\text{TEM-1a}}$ gene contamination. When TEM-type β-lactamase genes were targeted with a TEM primer pair (expected PCR product size: 839 bp), the contamination in the PCR reaction was strongly related to the *Taq* DNA poly-

* To whom correspondence should be addressed.
(Tel) 82-31-330-6195; (Fax) 82-31-335-8249
(E-mail) sangheelee@mju.ac.kr

merase itself (Fig. 1A). These results were confirmed from a previous report (Chiang *et al.*, 2005). Contrary to our study, this report did not show the information about the subgroup (e.g., $bla_{\text{TEM-1a}}$, $bla_{\text{TEM-1b}}$, and so on) of contaminated $bla_{\text{TEM-1}}$ gene and about any method necessary for overcoming the $bla_{\text{TEM-1a}}$ gene contamination of the *Taq* DNA polymerase. Using the negative control (double-distilled water), the *Taq* DNA polymerase from company A produced a false-positive signal, but the *Taq* DNA polymerase highly purified by company B did not (Fig. 1B). The *Taq* DNA polymerase has a high affinity for DNA, and thus a certain amount of contaminating DNA such as the $bla_{\text{TEM-1a}}$ gene may always remain protected from physical or chemical treatment (Corless *et al.*, 2000). PCR is a highly sensitive technique widely used for the rapid detection of specific DNA sequences, with numerous applications in genotyping of β-lactamase genes, clinical diagnosis, and microbial identification (Lee *et al.*, 2005). These PCR techniques can amplify a single copy of template DNA 10^6- to 10^7-fold, and thus small amounts of exogenous

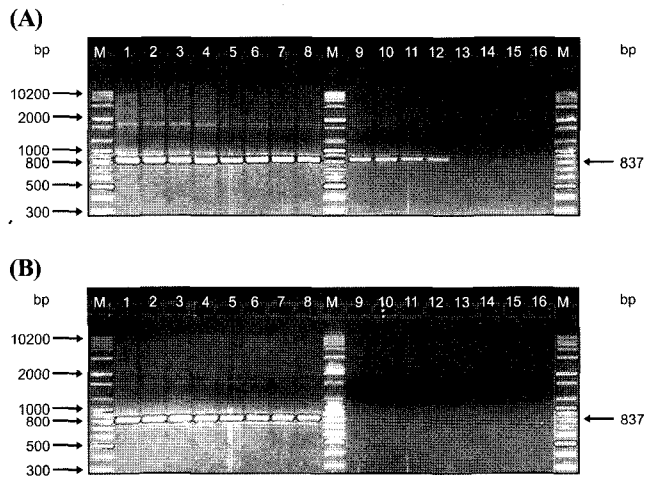

Fig. 1. PCR amplification (30 cycles) using the primer pair derived from TEM-1 β-lactamase gene and commercial *Taq* DNA polymerase from company A (A) and company B (B). PCR products were run on a 1% agarose gel with 0.5x Tris-acetate-EDTA buffer. Lane M, 100-bp plus DNA size marker (sizes in base pairs are indicated on the left edge of the gel); lane 1, *Taq* DNA polymerase with genomic (template) DNA from a clinical isolate producing TEM-1b and without DNase I treatment; lanes 2-8, *Taq* DNA polymerase with the template DNA after treatment of DNase I the amount of which is 0.05, 0.075, 0.1, 0.25, 0.5, 0.75 or 1.0 unit, respectively; lane 9, *Taq* DNA polymerase with double-distilled water instead of the template DNA and without DNase I treatment; lanes 10-16, *Taq* DNA polymerase with double-distilled water instead of the template DNA after treatment of DNase I the amount of which is 0.05, 0.075, 0.1, 0.25, 0.5, 0.75 or 1.0 unit, respectively. The arrow on the right edge of the gel indicates the amplified $bla_{\text{TEM-1b}}$ (lanes 1-8) and $bla_{\text{TEM-1a}}$ (lanes 9-12) fragments (837 bp).

DNA is a limitation to PCR approaches (Corless *et al.*, 2000; Newsome *et al.*, 2004). The identification of exogenous $bla_{\text{TEM-1a}}$ gene contamination in *Taq* DNA polymerase must be important to the investigators, especially those who work on TEM-type ESBLs. The tainted *Taq* DNA polymerase can produce false-positive results that can be extremely confusing and misleading. It can also be very time-consuming for investigators to follow up on the false-positive products. Thus, some strategy is necessary for overcoming the $bla_{\text{TEM-1a}}$ gene contamination of *Taq* DNA polymerase.

Since the most effective method for the elimination of exogenous DNA was a DNase I treatment (Corless *et al.*, 2000; Tondeur *et al.*, 2004), RQ1 RNase-Free DNase (Promega, USA) was added to the PCR reaction mixture without the primer set. For each 13 μl of PCR mixture, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, or 1.0 unit of the enzyme was added together with 2 μl of its specific buffer. The mixture solution was incubated for 30 min at 37°C, then for 10 min at 65°C after the addition of 2 μl of the RQ1 DNase Stop Solution (Promega, USA) to inactivate the DNase I. After the addition of 2 μl primer set and 1 μl template DNA (or double-distilled water) into the mixture, a PCR was performed. The best results for overcoming the $bla_{\text{TEM-1a}}$ gene contamination were obtained with 0.25 unit of the DNase I for 13 μl of PCR mixture (Fig. 1A). This amount of DNase I was sufficient to eliminate any trace of contaminated $bla_{\text{TEM-1a}}$ genes from the PCR mixture without altering the efficiency of the PCR reaction (Fig. 1A). These results were highly reproducible in several experiments.

The presence of DNA contamination surrounding *Taq* DNA polymerase was previously reported in several PCR studies (Böttger, 1990; Rand and Houck, 1990; Schmidt *et al.*, 1991; Corless *et al.*, 2000; Hughes *et al.*, 2000; Newsome *et al.*, 2004). Most of the contamination reported was exogenous bacterial DNA. In all of these previous reports, PCR amplification was performed with universal primers for the highly-conserved 16S rDNA gene, whereas in the present study, amplification was done with primers targeting the TEM-type β-lactamase gene. Here we have revealed that standard preparations of *Taq* DNA polymerase are contaminated with $bla_{\text{TEM-1a}}$ gene and proposed two methods to efficiently detect ESBL genes from clinical isolates by a standard PCR, using either decontamination with low concentrations of DNase I prior to PCR amplification or a highly purified *Taq* DNA polymerase without exogenous DNA. These methods that overcome the $bla_{\text{TEM-1a}}$ gene contamination of *Taq* DNA polymerase were highly reproducible. They could be useful in microbial studies where the presence of contaminating exogenous

DNA is a limitation to PCR approaches.

Acknowledgement

This work has been supported by a research grant from BioGreen 21 Program (20050301034479), Rural Development Administration, Republic of Korea, by the Driving Force Project for the Next Generation of Gyeonggi Provincial Government, and by in-house program (PE87200) of KORDI.

References

Böttger, E.C. 1990. Frequent contamination of *Taq* DNA polymerase with DNA. *Clin. Chem.* 36, 1258-1259.

Bradford, P.A. 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev.* 14, 933-951.

Chiang, C.-S., C.-P. Liu, L.-C. Weng, N.-Y. Wang, and G.J. Liaw. 2005. Presence of β-lactamase gene TEM-1 DNA sequence in commercial *Taq* DNA polymerase. *J. Clin. Microbiol.* 43, 530-531.

Corless, C.E., M. Guiver, R. Borrow, V. Edwards-Jones, E.B. Kaczmarski, and A. J. Fox. 2000. Contamination and sensitivity issues with a real-time universal 16S rRNA PCR. *J. Clin. Microbiol.* 38, 1747-1752.

Hughes, M.S., L.A. Beck, and R.A. Skuce. 1994. Identification and elimination of DNA sequences in *Taq* DNA polymerase. *J. Clin. Microbiol.* 32, 2007-2008.

Jeong, S.H., I.K. Bae, J.H. Lee, S.G. Sohn, G.H. Kang, G.J. Jeon, Y.H. Kim, B.C. Jeong, and S.H. Lee. 2004. Molecular characterization of extended-spectrum β-lactamases produced by clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* from a Korean national survey. *J. Clin. Microbiol.* 42, 2902-2906.

Lee, S.M., S.Y. Yoo, H.-S. Kim, K.-W. Kim, Y.-J. Yoon, S.-H. Lim, H.-Y. Shin, and J.-K. Kook. 2005. Prevalence of putative periodontopathogens in subgingival dental plaques from gingivitis lesions in Korean orthodontic patients. *J. Microbiol.* 43, 260-265.

Livermore, D.M. 1995. β-lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev.* 8, 557-584.

Newsome, T., B.-J. Li, N. Zou, and S.-C. Lo. 2004. Presence of bacterial phage-like DNA sequences in commercial *Taq* DNA polymerase reagents. *J. Clin. Microbiol.* 42, 2264-2267.

Paterson, D.L., W.-C. Ko, A. Von Gottberg, J.M. Casellas, L. Mulazimoglu, K.P. Klugman, R.A. Bonomo, L.B. Rice, J.G. McCormack, and V.L. Yu. 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: Implications for the clinical microbiology laboratory. *J. Clin. Microbiol.* 39,. 2206-2212.

Rand, K.H. and H. Houck. 1990. *Taq* polymerase contains bacterial DNA of unknown origin. *Mol. Cell. Probes* 4, 445-450.

Sanders, C.C. and W.E. Jr Sanders. 1992. β-lactamase in Gram-negative bacteria: global trends and clinical impact. *Clin. Infect. Dis.* 15, 824-839.

Schmidt, T.M., B. Pace, and N.R. Pace. 1991. Detection of DNA contamination in *Taq* polymerase. *BioTechniques* 11, 176-177.

Song, J.S., J.H. Jeon, J.H. Lee, S.H. Jeong, B.C. Jeong, S.-J. Kim, J.-H. Lee, and S.H. Lee. 2005. Molecular characterization of TEM-type β-lactamases identified in cold-seep sediments of Edison seamount (south of Lihir Island, Papua New Guinea). *J. Microbiol.* 43, 172-178.

Sutcliffe, J.G. 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. *Proc. Natl. Acad. Sci. USA* 75, 3737-3741.

Tondeur, S., O. Agbulut, M.-L. Menot, J. Larghero, D. Paulin, P. Menasche, J.-L. Samuel, C. Chomienne, and B. Cassinat. 2004. Overcoming bacterial DNA contamination in real-time PCR and RT-PCR reactions for LacZ detection in cell therapy monitoring. *Mol. Cell. Probes* 18, 437-441.