Rapid detection of the gene of *Legionella pneumophila* using the fluorescence polarization with the asymmetric PCR

Takaaki Fujii, Misaki Ohta, Midori Kono, Sadayori Hoshina, Keiso Fukuhara and Makoto Tsuruoka

Towa Kagaku Co. Ltd., Hiroshima 730-0841, Japan, The Jikei University School of Medicine, Minato-ku 105-8461, Japan and Advanced Science and Technology Laboratory, Hiroshima 731-3162, Japan

ABSTRACT

We attempted the rapid detection method of *Legionella pneumophila* by the asymmetric PCR and the fluorescence polarization. Eleven extracted DNAs from *L. pneumophila* serogroup 1—6, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. micdadei*, and *Pseudomonas aeruginosa* were amplified by asymmetric PCR, and the polarization of those products were measured. Only the polarization of *L. pneumophila* serogroup 1—6 rose within a few minutes after the beginning of measurement. The sensitivity to *L. pneumophila* using this method was $10^3$ cells.

INTRODUCTION

*Legionella pneumophila* is an aerobe gram-negative, facultative intracellular bacterium and the causative agent of both Legionnaires' disease and Pontiac fever. The traditional methods for the detection of *L. pneumophila* is the viable plate count. This method was able to detect viable cells, but it is tedious and time-consuming. Therefore we studied the rapid detection of the *L. pneumophila* using PCR and fluorescence polarization. The target of PCR primers and probes for fluorescence polarization was the intergenic 16S-23S ribosomal spacer regions that have been used for identification at the species level in various genera.

METHODS

*L. pneumophila* serogroup 1—6, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. micdadei* were kindly contributed by Shiba in the Jikei University School of Medicine, and *Pseudomonas aeruginosa* was kindly contributed by Ohtake in the Hiroshima University. These eleven isolations were incubated on a BCYE plates at 37°C for 3 days. Eleven extracted DNAs were then extracted from these colonies on the plates.

Oligonucleotide primers and probe were designed from the partial sequences of a 16S-23S rRNA spacer region of *L. pneumophila* in the Genbank database. Template DNAs were amplified by the asymmetric PCR, therefore the polarization sensitivity increased. A front primer (Lp2F: 5'-CATCCTCCTCGGCTCCACCA-3') and a reverse primer (Lp5R: 5'-CGCTCGTTTCCAGCTCCCC-3') were each used 20 pmol and 200 pmol to amplify a 272 bp fragment of the 16S-23S rRNA spacer region of *L. pneumophila*. Total volume of the reaction mixture was 100 μl, and template DNA volume was 50 ng.

The PCR mixtures were initially denatured at 94°C for 2 min and then subjected to 40 PCR cycles by using a three-temperature PCR system consisting of denaturalization at 94°C for 0.5 min, primer annealing for 0.15 min and extension for 1.15 min. Products were separated by electrophoresis using a 1% agarose gel and visualized under UV. The target fragment was then mixed with the probe and the fluorescence polarization was measured.

Fig. 1 Agarose gel (1.2%) electrophoretic patterns of asymmetric PCR products from various *Legionella* spp. and *P. aeruginosa* DNAs 1—6: *L. pneumophila* serogroup 1—6, 7: *L. bozemanii*, 8: *L. dumoffii*, 9: *L. gormanii*, 10: *L. micdadei*, 11: *P. aeruginosa* 12: negative control

Fig. 2 Polarization of Asymmetric PCR products from various *Legionella* spp. and *P. aeruginosa* DNAs, five minutes after mixing the probe. 1—6: *L. pneumophila* serogroup 1—6, 7: *L. bozemanii*, 8: *L. dumoffii*, 9: *L. gormanii*, 10: *L. micdadei*, 11: *P. aeruginosa* 12: salmon sperm DNA
at 60°C for 0.5 min, and extension at 72°C for 1 min. The last cycle was denaturation at 94°C for 0.5 min, primer annealing at 60°C for 0.5 min.

The method used to measure the polarization of PCR products was modified from one described by the previous report. The probe (LpP2-13: GAAGAAGAGG TAACACAAGCGATTGG) used in this study was diluted at 0.6 nM with TE buffer (pH 8.0) containing 0.8 M NaCl. This probe buffer (800 ul) was mixed with PCR products (80 µl), and the polarization was measured at 42°C, at fifteen times a minute by FP-777 (Japan Spectroscopic Co.Ltd.). The salmon sperm DNA (800 ng/ml) was used as negative control.

Sensitivity of L. pneumophila detection by polarization was investigated by using serial dilution of bacterial suspensions. The suspensions were boiled for fifteen minutes, then 10 µl of suspensions were put into PCR tubes for amplification. Asymmetric PCR was done as described above, and each PCR products were measured to determine the lowest detection by fluorescence polarization.

RESULTS

The bands were detected near 272 bp from all PCR products, except L. mannii and P. aeruginosa (Fig. 1). Therefore we could not distinguish L. pneumophila from the other Legionella spp. by agarose gel electrophoresis.

After a few minutes of incubation, only the polarization of L. pneumophila isolations increased and the others didn't. This enabled us to distinguish L. pneumophila isolations from the others on polarization within five minutes (fig. 2).

There was obvious difference between polarization of 10^3 cells and the control. Although the average of 10^2 cells was a little higher than the control, there was no obvious difference between two polarization values (Fig. 3). The minimum detectable count was 10^3 cells.

DISCUSSION

Bacterial culture was the standard method for Legionella detection, but the time required for culture and confirmation will extend to several days. The detection of amplified products by hybridization is sensitive, but the process is tedious and requires additional equipment and training, whereas the method using PCR and fluorescence polarization can easily and rapidly detect the L. pneumophila.

The minimum detectable count was 10^3 cells. In general, the cautionary concentration of L. pneumophila is more than 10^3 cell/1L, so we need to improve the sensitivity of this method. Also there are many PCR inhibitors in the environments. In this method, DNA amplification is very important for detectable sensitivity. Therefore we should investigate the influence by PCR inhibitors in the environment.

In this study, we showed that the specific and rapid detection of L. pneumophila could be achieved unequivocally by DNA amplified by asymmetric PCR and fluorescence polarization. This method can be adapted for detection of various microorganisms, and very useful to detect many samples from the environment.

REFERENCE