Iranian Journal of Microbiology
Volume 6 Number 4 (August 2014) 281-284

ORIGINAL ARTICLE

Development of a PCR assay to detect mycoplasma contamination in cord blood hematopoietic stem cells

Reza Tabatabaei-Qomi, Mohsen Sheykh-Hasan, Hoda Fazaely, Naser Kalhor, Mahdieh Ghiasi*

Jihad Daneshgahi Infertility Treatment Center, Stem Cell laboratory, Qom, Iran.

Received: January 2014, **Accepted:** May 2014

ABSTRACT

Background and Objectives: Contamination of cell lines and biological products is one of the major problems of cell culture techniques. Rapid detection of mycoplasma contamination in cell culture is an important part of quality control standards in related laboratories. The aim of this study was to evaluate the efficacy of PCR in detection of myroplasma as contaminants in cell cultures and other biological products.

Method: PCR assays were optimized for 16 S rRNA target gene. Also the utilized PCR method was evaluated in terms of sensitivity and specificity. Finally, a simple DNA extraction and PCR analysis of 164 cell culture of adipose tissue derived mesenchymal stem cells were performed.

Results: A 715 bp product was amplified and subsequently was confirmed by sequencing. The technique could detect 10 copies of the target DNA. No cross-reactivity with genomic DNA of other microorganisms was observed.

Conclusions: The PCR technique in this study was based on 16S rRNA gene. It was highly sensitive and specific since it was able to detected Mycoplasma contamination in cell cultures

Keywords: Mycoplasma, PCR, contamination, molecular detection, cell culture

INTRODUCTION

Mycoplasma belongs to the Mollicutes class and is one of the smallest free-living microorganisms capable of self- replicating. These bacteria are one of the contaminants of cell culture and biological products, and are considered as the destructive factor in bio-processing (1, 2). They consist of just a cell membrane (without a cell wall), ribosomes and a 580kb genome. Because of the small size, *Mycoplasmas* can pass through 0.22 µm and 0.45 filters which normally are used for sterilizing cell culture reagents (3). Mycoplasmas are often slow-growing contaminants normally presenting in small numbers, but they may cause some problems in cultured cells. It has been shown that *Mycoplasma* contamination alter the rate of cell growth, nucleic acid and amino acid metabolism and cell antigenicity changes, and in addition they may lead to cell membrane changes and chromosomal defects (3, 5).

In most cases, visual or microscopic identification of the contamination is impossible. Although Mycoplasmas do not cause visible damages in cells, they affect cell metabolism and growth in culture medium, protein synthesis, secretion of cytokines, and even damage to DNA and RNA. Various studies show that the percentage of cell cultures infected with Mycoplasma in cell banks is between 10 to 85 %. *Mycoplasma* contamination can be transmitted from bovine serum, laboratory staff, other contaminated cultures or cells taken from animals (1, 2). The most identified *Mycoplasma* species in contaminated cell cultures are: *Mycoplasma fermentans*, *Mycoplasma hyorinis*, *Mycoplasma arginini*, *Mycoplasma orale*

*Corresponding author: Mahdieh Ghiasi
Address: Jihad Daneshgahi Infertility Center, Shabnam Avenue, Isar square, Qom, Iran.
Email: mahdieh.ghiasi@yahoo.com
Telephone: +982532700152
Fax: +982532700154

and *Achoplasma laidlawi* (6). Mycoplasmas can be detected by direct culture techniques of the organisms and some indirect assays including DNA staining with the fluorescent dye, hybridization of nucleic acid, biochemical tests and polymerase chain reaction (PCR) (4). In most PCR methods, 16SrRNA sequences are used as the template sequences because this gene has some regions with commonly conserved sequences among Mycoplasmas (7-9). The aim of this study was to identify the *mycoplasma* genus in Hematopoietic stem cells (HSCs) by PCR based 16S rRNA.

MATERIALS AND METHODS

Bacterial strains. The following organisms were used in this study to evaluate the designed PCR: *Mycoplasma pneumoniae* (NCTC 10119), *Mycoplasma arginini, Mycoplasma hyorinis, Mycoplasma orale, Mycoplasma synoviae, Mycoplasma gallinarum* (Razi 1346,1350), *Mycoplasma gallisepticum* (Razi 1355), *Achoplasma laidlawi, Mycoplasma agalactiae* (Razi 1343), *Mycoplasma ovipneumoniae* (Razi 1364) and *Mycoplasma ureaplasma urealyticum* (Razi 1369).

Preparation of samples. The Cell cultures were prepared from cord blood bank of Jihad Daneshgahi Research Cener in Qom city. Totally, 164 samples of *Hematopoietic stem cells* have been checked for *mycoplasma* contamination.

DNA extraction and nucleotide sequences of primers. Boiling method was used to extract DNA. Thus, 100 µl of the cell suspension with mineral oil placed in boiling heat and after centrifuging in 12000 X g for 10 minutes, supernatant was used as template in PCR testing (10). The target gene was *16S rRNA* of mycoplasma (11) using the primers [GPO-1 5'-ACTCCTACGGGAGGCAGCATAG and MGSO 5'- TGCACCATCTGTCACTCTGTTAACCTC-3.'

The PCR reaction consisted of 5 µl of template DNA, 1 µl of each one of forward and reverse primers, 2.5 µl of PCR buffer (10X) , 0.75 µl $MgCl_2$ (50mM concentration) , 0.5 µl of dNTP mixture (10mM) and 0.4 µl of Taq Enzyme and 14 µl of twice distilled sterile deionized water. The thermocycler was programmed for 40 cycles at 93 ° C for 20 s, 60 ° C for 20 s, and finally 72° C for 30 s. The PCR products were electrophoresed in agarose gel and visualized by UV transilluminator after staining the gel with ethidium bromide.

Serial dilutions of *Mycoplasma arginini* suspension with specific CFU was assayed to check the sensitivity of PCR. The specificity test was done using DNA samples from human, mouse, *Mycobacterium tuberculosis, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus.*

RESULTS

Of 164 samples obtained from Hematopoietic stem cells 6 (3.6%) were contaminated with Mycoplasma. PCR could detect 10 CFU/ml. The primer GPO-1 and MGSO Primers could produced an amplicon (715bp) from *Mycoplasma* types like *Mycoplasma pneumoniae, Mycoplasma arginini, Mycoplasma hyorinis, Mycoplasma orale, Achoplasma laidlawi* and *Mycoplasma aplasma urealyticum* (Figs. 1- 4). The results of PCR was negative for organisms other than Mycoplasmas in this study.

DISCUSSION

Mycoplasmas can easily pass through 220 and 450 nm filters holes and contaminate cell cultures. Genetic and biochemical changes are the problems caused by infecting mycoplasmas which will lead to unreliable results (12). There are various methods to detect *mycoplasma* in cell culture. In order to minimizing pseudo-results, in many studies the combination of two methods is used to detect mycoplasma in cell culture. Combination of PCR with cell culturing is widely used to identify infected samples (4, 9, 12). Combination of DNA staining technique and cultivation has also been used. Cultivation is a time-consuming method and gives high pseudo-negative results. The interpretation of DNA staining technique results is also difficult because of bacterial contamination (11, 13), but it is a quick, highly specified and sensitive method (13). Microbial culture methods require a 1-4 weeks period in the laboratory. In addition, there are still a number of strains of Mycoplasma which are non-growing in microbial cultures or too hard to grow like *Mycoplasma hyorinis* (13).

Finding a rapid and sensitive method for the detection of Mycoplasma contamination in cell cultures and biological products is a particular important issue. This method should be able to identify at least five

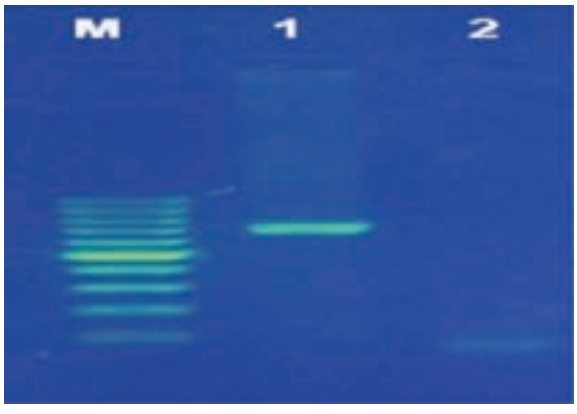

Fig. 1. The optimized PCR test using GPO-1 and MGSO primers. Column M: DNA ladder 100bp (Fermentas). lanes 1: positive control (DNA of mycoplasma arginini), 2: negative control: (sterile water) (Agarose 1.5% and TBE 0.5 × buffer)

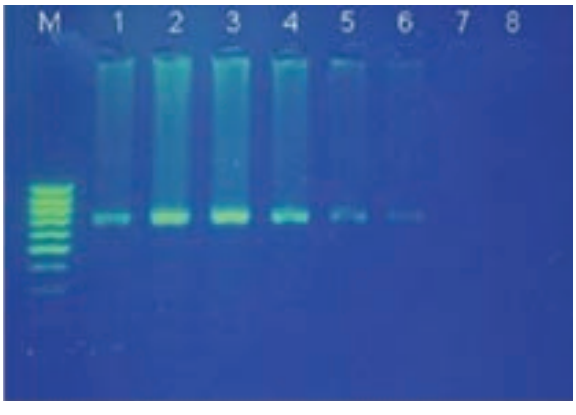

Fig. 2. Assessing the sensitivity of optimized PCR. Lanes; M: DNA ladder 100bp (fermentase). 1: positive control (DNA of mycoplasma arginini), 2: 10^6 CFU/ml, 3: 10^5 CFU/ml. 4: 10^3 CFU/ml, 5: 100 CFU/ml, 6: 10 CFU/ml, 7: 1 CFU/ ml, 8: negative control

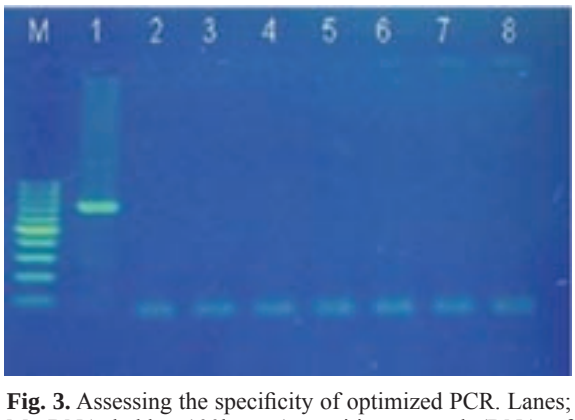

Fig. 3. Assessing the specificity of optimized PCR. Lanes; M: DNA ladder 100bp, 1: positive control (DNA of mycoplasma arginini), 2: human DNA, 3: mouse DNA, 4: *Mycobacterium tuberculosis* DNA, 5: *Pesudomonase aeroginosa* DNA, 6: *Staphylococcus aureus* DNA, 7: *Salmonella typhi* DNA, 8: Negative control.

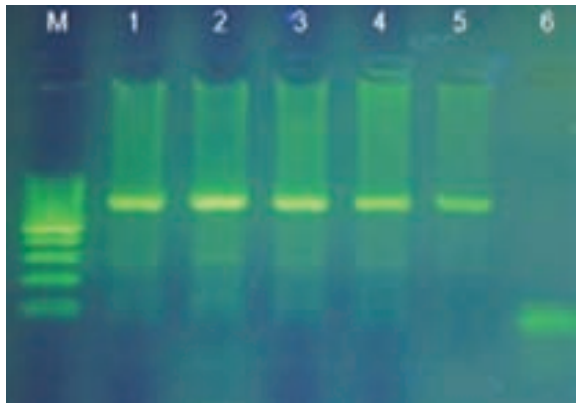

Fig. 4. PCR amplification results of examined cell culture samples. Lanes; M: DNA ladder 100 bp (fermentase). 1: positive control (DNA of *Mycoplasma arginini*), 2-5: contaminated samples, 6: negative control .

Typical species of Mycoplasma contaminating cell cultures: *Mycoplasma fermentans, Mycoplasma hyorinis, Mycoplasma arginini, Mycoplasma orale* and *Achoplasma laidlawi* that cause more than 95% of infection in cell cultures and biological products. The results of this study confirm that conserved and common sequences in 16S rRNA are appropriate target genes for the detection of Mycoplasma from cell cultures and biological products. The sensitivity of our PCR was high enough to detect 10 copies of target DNA molecule. In other studies the identification ranges of Mycoplasma has shown to be between 1 to 100 (14, 15).

In conclusion, *Mycoplasma* contamination is one of the threatening factor in cell cultures, which affects the biological characteristics of cells. The results of this study are based on accuracy, speed, sensibility and high specification of PCR technique according to conserved and common sequence of 16srRNA to detect *Mycoplasma* contamination in cell cultures.

ACKNOWLEDGEMENT

The authors would like to express their special thanks to director of the Academic Center for Education, Culture and Research (ACECR)- Qom Branch, Mr Mohammad Ebrahim Faghih Zadeh, for his supports.

Funding/Support: This survey was supported by Highly Specialized Jihad Daneshgahi Infertility Center, Stem Cell Laboratory, Qom Branch (ACECR), Qom, Iran

REFERENCES

1. Wang H, Kang F, Jelfs P, James G, Gilbert GL. Simultaneous detection and identification of common cell culture contaminant and pathogenic mollicutes strain by reverse line blot hybridization. *Appl Environ Microbiol* 2004; 70: 1483–1486.
2. Sung H, Kang SH, Bae YJ, Hong JT, Chung YB et al. PCR-based detection of Mycoplasma species. *J Microbiol* 2006; 44: 42–49.
3. Loens K, Uris D, Goossens H, Ieven M. Molecular diagnosis of *Mycoplasma pneumoniae* respiratory tract infections. *J Clin Microbiol* 2003; 41: 4915–4923.
4. Stakenborg T, Vicca J, Verhelst R, Butaya P, Maes D, et al. Evaluation of tRNA gene PCR for identification of Mollicutes. *J Clin Microbiol* 2005;43: 4558–4566.
5. Mardassi BB, Mohamad RB, Gueriri I, Boughattaas S, Mlik B. Duplex PCR to differentiate between *Mycoplasma* synoviae and *Mycoplasma gallisepticum* on the basis of conserved species-specific sequences of their hemagglutinin genes. *J Clin Microbiol* 2005; 43: 948–958.
6. Woubit S, Manso-Silvan L, Lorenzon S, Gaurivaud P, Poumarat F, et al. A PCR for the detection of mycoplasmas belonging to the *Mycoplasma* mycoides cluster: Application to the diagnosis of contagious agalactia. *MolCell Probes* 2007; 21: 391–399.
7. Quirk JT, Kupinski JM, Dicioccio RA. Detection of Mycoplasma ribosomal DNA sequences in ovarian tumors by nested PCR. *Gynecol Oncol* 2001; 83: 560–562.
8. Tang J, Hu M, Lee S, Robin RA. Polymerse chain reaction based method for detecting *Mycoplasma*/*Acholeplasma* contaminants in cell culture. *J Microbiol Methods* 2000; 39: 121–126.
9. Kong F, James G, Gordon S, Zelynski A, Gilbert GL. Species-specific PCR for identification of common contaminant Mollicutes in cell culture. *Appl Environ Microbiol* 2001; 67: 3195–3200.
10. Timenetsy J, Santos LM, Buzinhani M, Mettifogo E Detection of multiple mycoplasma infection in cell cultures by PCR. *Braz J Med Biol Res* 2006; 39: 907-914.
11. Van-kuppeveld FJ, Van-der-logt JT, Angulo AF, Van- Zoest MJ, Quint WG, et al. Genus- and speciesspecific identification of Mycoplasmas by 16S Rrna amplification. *Appl Environ Microbiol* 1992; 58: 2606–2615.
12. Harasawa R, Mizusawa H, Nozawa K, Nakagawa T, Asada K, et al. Detection and tentative identification of dominant Mycoplasma species in cell cultures by restriction analysis of the 16S-23S rRNA intergenic spacer regions. *Res Microbiol* 1993; 144: 489– 493.
13. Been-Abdelmoumen B, Roy RS. Antigenic relatedness between seven avian Mycoplasma species as recovered by western blot analysis *Avian. Dis* 1995; 39: 250– 262.
14. Grau O, Kovacic R, Griffais R, Montagnier L.Development of a selective and sensitive polymerase chain reaction assay for the detection of Mycoplasma pirum. *FEMS Microbiol Lett* 1993; 106: 327–334.
15. Kai M, Kamiya S, Yabe H, Takakura I, Shiozawa K, et al. Rapid detection of Mycoplasma pneumoniae in clinical samples by the polymerase chain reaction. *J Med Microbiol* 1993; 38: 166–170.