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

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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 mycoplasma as contaminants in cell cultures and other biological products.

Method: PCR assays were optimized for 16S 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.
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, 16S rRNA 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 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 Daneshghahi Research Center 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 MgCl₂ (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 pneumoniae*, *Mycoplasma arginini*, *Mycoplasma hyorinis*, *Mycoplasma orale*, *Achoplasma laidlawi* and *Mycoplasma ureaplasma 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
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.

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