Development of a Platform for Molecular Detection of Mycoplasma in Cell Cultures

Mayra Dorigan Macedo[1,2], Simone Kashima[1,2,3], Kamilla Swiech[1,2,3], Dimas Tadeu Covas[1,2,4]

[1] Regional Blood Center of Ribeirão Preto / [2] National Institute of Science and Technology in Stem Cell and Cell Therapy

[3] Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo / [4] Medical School of Ribeirão Preto, University of São Paulo.

mayra.dorigan@hemocentro.fmrp.usp.br

INTRODUCTION

The safety of mycoplasma-free cell culture is essential for cell therapy. Since its first isolation, mycoplasma contamination still remains a serious concern. *Mycoplasma* genera is one of the smallest free-living bacteria which presents specific growth characteristics such as the ability to propagate slowly in tissue culture medium without destroying it and keeping a small size that is not eye detectable. In addition, the mycoplasma contamination could affect the cells since it causes alteration in protein levels and RNA/DNA synthesis, cell morphology and metabolism, induction of chromosomal aberrations (numerical and structural alterations), changes in cell membrane composition (surface antigen and receptor expression), induction (or inhibition) of lymphocyte activation, cytokine expression and signaling transduction, increase (or decrease) of virus propagation, interference with various biochemical and biological assays, promotion of cellular transformation. Consequently, it affects the development of academic research and the production of biopharmaceuticals, and although many species do not cause diseases, it is currently the main problem that can derail the infusion of cells in immunosuppressed patients. There are several analytical methods for mycoplasma detection and many authors recommended more than one method for confirming mycoplasma contamination in cell cultures. However, the detection methods currently used have low sensitivity, difficult interpretation, long and costly techniques. Real-time PCR (RT-PCR) is a simple methodology, inexpensive, sensitive, and highly specific. It combines some advantages over conventional PCR because it uses specific probes that act as molecular flags making the test highly specific. The reaction occurs in one step, which avoids post-PCR processes, reducing the possibility of contamination. Thus, the need to ensure the quality and safety to cells grown in Cellular Technology Centers encourages us to develop, standardize and implement a molecular platform using RT-PCR for detection of *Mycoplasma* in cell culture.

AIM

Developing a molecular platform to detect the presence of the major species of *Mycoplasmas* in cell cultures for both academic research and cell therapy issues.

EXPERIMENTAL STRATEGY

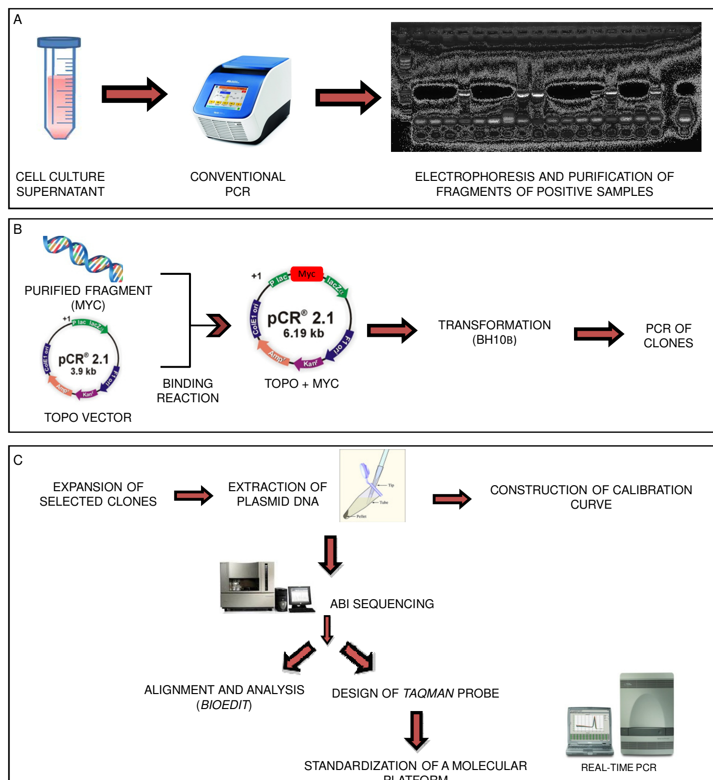

Figure 1: A) Amplification of *Mycoplasma* DNA fragment by conventional PCR from contaminated cell cultures, followed by electrophoresis and purification of the genetic material from the agarose gel. **B)** Cell cloning and recombinant colony selection. Conventional PCR of selected clones to confirm the presence of the insert in them. **C)** Obtaining the plasmid DNA which will be used for the development of molecular platform (real-time PCR).

RESULTS

Firstly, we collected the supernatant of distinct cell cultures. Among the cells from which the supernatant was collected including: *BJ*, *CCD27SK*, *MEF*, *HEK-293T*, *MSC*, *MT-2 and HeLa.* Then, we performed conventional PCR of these supernatants using specific primers for 16S rRNA region of *Mycoplasma* species. By electrophoresis, we obtained fragments of 271 base pairs (bp) in the positive samples, of which three samples were selected to perform the purification and cloning into TOPO vector. The selected samples were: *CCD27SK, HeLa* and *HEK-293T* identified respectively by 19, 31, and 32 (Figure 2). The fragments were then removed from the agarose gel and purified with the appropriate kit .

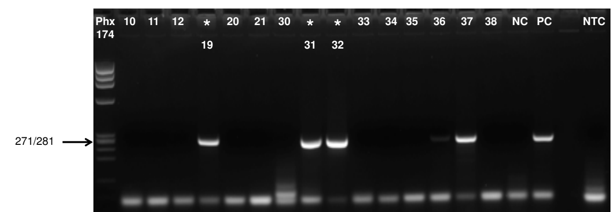

Figure 2: Electrophoresis of 271 bp fragment of 16S rRNA from cell cultures supernatants. The arrow indicates the fragment size of the molecular weight. *: positive samples (identified as 19, 31 and 32), NC: Negative Control; PC: Positive Control; NTC: Non Template Control.

After obtaining 271 bp-fragment, it was cloned into TOPO vector and white colonies were selected. By conventional PCR, we confirmed the insert cloning and selected 5 to 7 clones from each positive colony (identified as 19, 31, and 32) (Figure 3).

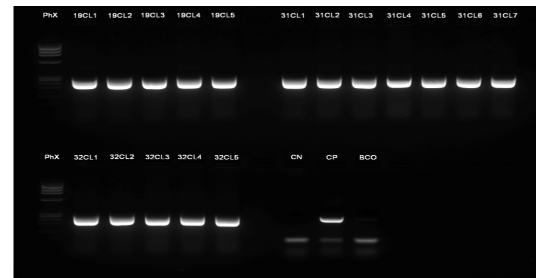

Figure 3: PCR demonstrating the efficiency of bacterial transformation (BH10β), which was performed by the method of thermal shock. All selected clones were compatible with the expected amplicon size (271bp).

Once confirmed the gene cloning, one clone of each sample was placed to expand and plasmid DNA was extracted. Then we performed the sequencing reaction (*ABI*) of plasmid DNA 19-CL1, 31-CL1, and 32-CL1. The sequences were aligned and analyzed using the *BioEdit* program. The result indicated 100% identity of the samples with *Mycoplasma hyorhinis*. Then, calibration curve was constructed (duplicated) with the following dilutions: 10^6, 10^5, 10^4, 10^3, 10^2, and 10 copies. As a result, the dilution points were followed by a gradual decrease in the intensity of the bands, which was visible up to 10^4 copies (Figure 4).

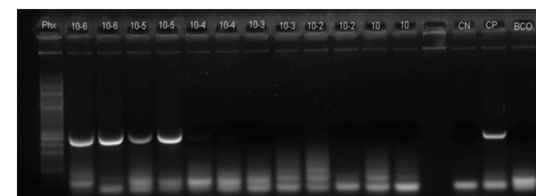

Figure 4: Calibration curve with a gradual decrease in the intensity of the bands. Fragments detected up to 10^4 copies.

The next steps are to design the *TaqMan* probe to standardize the RT-PCR and validate the platform through the evaluation of the parameters of analytical and diagnostic sensitivity, analytical and diagnostic specificity, accuracy and robustness.

CONCLUSION

Due to the importance and need for our Cellular Technology Center to develop a method for *Mycoplasma* detection in cell cultures, conventional PCR has been standardized. However, real-time PCR some advantages over conventional PCR, such as simplicity, rapidity, sensitivity, and specificity. So at this point, it is evident the importance of obtaining the design of the *TaqMan* probe to the standardization of a molecular platform that will be of great value to this Centre.