TITLE: IN-SILICO DESIGN AND EXPRESSION OF CHIMERIC PROTEINS FOR THE PRODUCTION MULTI-DETECTION KIT OF MOLICUTES CONTAMINATION IN CELL CULTURE

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ABSTRACT

Cell culture contamination caused by mycoplasma species has a major role around all laboratories in the world. These microorganisms can easily contaminate and disseminate in a high-level frequency. Their concentration in a culture medium can reach 10^8 cells per milliliter without causing perceptible changes such as turbidity and pH change, and visible influences on cell growth, which makes it difficult to be detected. Thus, the biological consequences on contaminated cells are devastating and it can cause severe scientific and economic impact. Therefore, an effective detection for this microorganism in contaminated cultures is very important. Thus, the objective of this study was to select, by in silico methods, conserved epitopes in the major species of culture contaminating molicutes, to express them in E. coli under multiepitope protein structure and to test them for reactivity with sera from experimentally infected animals with the molicutes of interest. The proteomes of *Mycoplasma hyorhinis SK76*, *Mycoplasma hyorhinis HUB-1*, *Mycoplasma arginini*, *Mycoplasma hominis ATCC 23114*, *Mycoplasma hominis ATCC 27545* and *Acholeplasma laidilawii PG-8A* were analyzed by bioinformatics methods in order to obtain multiepitopic proteins capable of composing kit production detection. Two multiepitopic proteins were constructed. Both proteins were constructed from B and T cell epitopes, interconnected by the EAAAK sequence. The targets were expressed in *E. coli* BL21 (DE3) cells, using the pET-28a (+) vector. Expressions were induced by isopropyl-β-D-thiogalactopyranoside (IPTG). The reactivity test was performed by enzyme-linked immunosorbent assay (ELISA), using *Mycoplasma hypohinis*, *Mycoplasma hominis* type 1, *Mycoplasma fermentans* (PG-18), *Mycoplasma arginini* (G-230), *Mycoplasma orale* type 1 (CH-19299) and *Mycoplasma salivarium* (PG-20). The results of the reactivity tests indicated that the multiepitope protein 1 reacts with all sera tested and the multiepitope protein 2 reacts with four of the six sera tested. In this way, both consist of potential antigens for the production of antibodies that compose a kit of detection of contaminating molicutes of culture.

Keywords: cell culture contaminant, *Mollicutes*, recombinant protein.