TITLE: SELECTION AND STANDARDIZATION OF EPITOPES FOR THE PRODUCTION OF MYCOPLASMA MULTI-DETECTION KIT 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 mL 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 causes severe scientific and economical impact. Therefore, an effective detection for this microorganism in contaminated cultures is very important. In this way, the aim of this work was to perform *in silico* selection of epitopes preserved in mycoplasmas for standardization and application in immunodiagnostic tests for contamination of cell cultures. 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 to obtain multiepitope proteins capable to compose the production of the detection kit. Subsequently, the target will be cloned and expressed in *E. coli* cells and, after purification, will be evaluated for antigenicity and standardized from immunoenzymatic assays with serum from rabbits previously immunized with the recombinant proteins. The results of this study returned nine proteins containing at least two distinct B-cell epitopes, conserved in six species of mycoplasmas and absent in mice, humans, swine and *Chlamydia trachomanis*; and MHC class II ligand epitopes. Finally, two chimeric proteins were produced, containing B and T cell epitopes, interconnected by the EAAAK sequence. According the predictions performed, we concluded that the chimeric proteins produced has potential to be stimulate the humoral immune response, producing polyclonal antibodies that will compose the kit of detection.

Keywords: *cell culture contaminant, Mycoplasma sp., recombinant protein.*