

CLONING AND EXPRESSION OF A THIOL-PEROXIDASE FROM THE SWINE PATHOGEN *MYCOPLASMA HYOPNEUMONIAE*

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Mycoplasma hyopneumoniae is the etiological agent of the swine enzootic pneumonia, which affects swineherds around the world. In the infection process, the host immune system generates reactive oxygen species (ROS), known to cause a series of cell lesions, as one of the strategies to neutralize the pathogen. Pathogens, on the other hand, have developed defense mechanisms to avoid ROS-induced damage. *M. hyopneumoniae* has a defective protection system against ROS, lacking important antioxidant enzymes normally found in other pathogens. However, the *in silico* analysis of the genome sequence of the *M. hyopneumoniae* pathogenic strain 7448 allowed the identification of a gene coding for a thiol-peroxidase (TPx), possibly involved in protection from ROS-induced damage. The corresponding coding DNA sequence was mutated by the megaprimer PCR method to change a TGA stop codon (which codes for tryptophan in *M. hyopneumoniae*) to TGG and cloned in the pGEX-4T-1 vector for expression in *Escherichia coli*. A recombinant TPx was then expressed in the form of fusion with glutathione-S-transferase (GST), which was later confirmed by immunoblotting using anti-GST monoclonal antibody. After purification by affinity chromatography, the *M. hyopneumoniae* TPx is now under immunological and functional characterization.

Key Words: *Mycoplasma hyopneumoniae*, enzootic pneumonia, antioxidants, thiol-peroxidase.

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