

TITLE: CHARACTERIZATION OF OVERREPRESENTED PROTEINS OF UNKNOWN FUNCTION DISPLAYED ON *Mycoplasma hyopneumoniae* SURFACE

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ABSTRACT:

Mycoplasma hyopneumoniae is the causative agent of porcine enzootic pneumonia (PEP), a chronic respiratory disease that causes major economic losses to the pig industry. It has been demonstrated that several proteins localized on *M. hyopneumoniae* surface are associated to pathogenicity mechanisms, such as adhesion to swine respiratory epithelium, cytotoxicity, and modulation of host immune response. However, these pathogenicity mechanisms are not completely understood. About 30% of *M. hyopneumoniae* surface proteins are of unknown function (PUF), of which several were overrepresented on the pathogenic strain *M. hyopneumoniae* 7448 surface compared to their corresponding orthologs from non-pathogenic strain J and from non-pathogenic *Mycoplasma flocculare*. In this context, the aim of this study is to characterize the 5 most abundant PUF found in *M. hyopneumoniae* 7448 surfaceome. For that, these proteins and their corresponding orthologs from *M. hyopneumoniae* J and *M. flocculare* were comparatively analyzed *in silico* considering their topology, function and antigenicity. It was demonstrated that two proteins bear domains of unknown function, which are conserved among ortholog PUFs. Moreover, epitope coverage ranged from 36% to 68%, showing the antigenic potential of analyzed PUFs. Comparative analyses among the amino acid sequences of ortholog PUFs from *M. hyopneumoniae* strains and *M. flocculare* allowed the identification of at least 10 differential domains, which suggest that these PUFs may play differential roles on these mycoplasma surfaces. Potential proteolytic processing of these PUFs was also comparatively analyzed, demonstrating that some of them were differentially processed on *M. hyopneumoniae* and/or *M. flocculare* surfaces. It suggests that different antigenic PUF proteoforms are exposed on surface of these mycoplasmas. The conservation of the *M. hyopneumoniae* 7448 PUFs was investigated by phylogenetic analyses, showing the evolutionary relationship among *Mycoplasma* orthologs, including proteins of *Mycoplasma dispar* and *Mycoplasma ovipneumoniae*, which are related to respiratory diseases in bovine and ovine, respectively. Thus, these data provide relevant information for the characterization of *M. hyopneumoniae* PUFs that may be useful for future studies of diagnostics and/or vaccines for PEP control.

Keywords: *Mycoplasma hyopneumoniae*, porcine enzootic pneumonia, proteins of unknown function

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