Cloning and Characterization of Signal Peptidases Type I and II of *Mycoplasma hyopneumoniae*

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Mycoplasma hyopneumoniae is a swine pathogen that adheres to respiratory epithelial cells, causing enzootic pneumonia. Cell adhesion and other processes important for infection depend on exported proteins, most of which have signal peptides (SP) that is supposed to be cleaved by a signal peptidase (Spase). In the *M. hyopneumoniae* genome, there are two Spase encoding genes, one for a Spase type I (*sipS*) and other for a Spase type II (*lsp*). We have demonstrated the co-transcription of *sipS* and *lsp* with 4 and 3 other unrelated coding DNA sequences (CDS), respectively, confirming the organization of these genes in two independent operons. In order to functionally characterize the *M. hyopneumoniae* Spases I and II, the *sipS* and *lsp* CDSs were cloned into the expression vector pGEX-4T3 and expressed in *Escherichia coli* as a fusion with glutathione-S-transferase. Recombinant sipS was purified and used to immunize mice. The anti-sipS polyclonal antiserum is now being used to verify sipS expression in protein extracts of different *M. hyopneumoniae* strains and in growth and metabolic inhibition assays. Putative SPs cleaved by sipS and/or lsp were identified in *M. hyopneumoniae* genome by *in silico* analysis. Based on that, synthetic polypeptides will be synthesized for use in cleavage assays with recombinant sipS and lsp.

Keywords: *Mycoplasma hyopneumoniae*, signal peptidase, signal peptide, virulence factor.
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