

Molecular cloning of HSP70 in Mycoplasma ovipneumoniae and comparison with that of other mycoplasmas

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ABSTRACT. Mycoplasma ovipneumoniae, a bacterial species that specifically affects ovine and goat, is the cause of ovine infectious pleuropneumonia. We cloned, sequenced and analyzed heat shock protein 70 (HSP70) (dnaK) gene of M. ovipneumoniae. The full length open reading frame of the M. ovipneumoniae HSP70 gene consists of 1812 nucleotides, with a G+C content of 34.16%, encoding 604 amino acids. Comparative analysis with the HSP70 sequences of 15 Mycoplasma species revealed 59 to 87% DNA sequence identity, with an amino acid sequence identity range of 58 to 94%. M. ovipneumoniae and M. hyopneumoniae shared the highest DNA and amino acid sequence identity (87 and 94%, respectively). Based on phylogenetic analysis, both the DNA and amino acid identities of M. ovipneumoniae with other mycoplasmal HSP70 were correlated with the degree of relationship between the species.

The C-terminus of the HSP70 was cloned into a bacterial expression vector and expressed in *Escherichia coli* cells. The recombinant C-terminal portion of HSP70 protein strongly reacted with convalescent sera from *M. ovipneumoniae*-infected sheep, based on an immunoblotting assay. This indicates that HSP70 is immunogenic in a natural *M. ovipneumoniae* infection and may be a relevant antigen for vaccine development.

Key words: *Mycoplasma ovipneumoniae*; Heat shock protein 70; HSP70; Immune response; Recombinant protein; Bioinformatic analysis