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Fructose-1,6-bisphosphate aldolase of *Mycoplasma bovis* is a plasminogen-binding adhesin

Xiang Gao^a, Shijun Bao^{a*}, Xiaoyong Xing^a, Xiaoping Fu^a, Yi Zhang^a, Huiwen Xue^a, Fengqin Wen^a, Yanming Wei^a

^a College of Veterinary Medicine, Gansu Agricultural University, 1 Yingmencun, Lanzhou 730070, China.

Abstract: *Mycoplasma bovis* is an extremely small cell wall-deficient pathogenic bacterium in the genus *Mycoplasma* that causes serious economic losses to the cattle industry worldwide. Fructose-1,6-bisphosphate aldolase (FBA), a key enzyme in the glycolytic pathway, is a multifunctional protein in several pathogenic bacterial species, but its role in *M. bovis* remains unknown. Herein, the FBA gene of the *M. bovis* was amplified by PCR, and subcloned into the prokaryotic expression vector pET28a(+) to generate the pET28a-FBA plasmid for recombinant expression in *Escherichia coli* Transetta. Expression of the 34 kDa recombinant rMbFBA protein was confirmed by electrophoresis, and enzymatic activity assays based on conversion of NADH to NAD⁺ revealed Km and Vmax values of 48 μ M and 43.8 μ mol/L/min, respectively. Rabbit anti-rMbFBA and anti-*M. bovis* serum were generated by inoculation with rMbFBA and *M. bovis*, and antigenicity and immunofluorescence assay demonstrated that FBA is an immunogenic protein expressed on the cell membrane in *M. bovis* cells. Enzyme-linked immunosorbent assays revealed equal distribution of FBA in the cell membrane and cytoplasm. Complement-dependent mycoplasmacidal assays showed that rabbit anti-rMbFBA serum killed 44.1% of *M. bovis* cells in the presence of complement. Binding and ELISA assays demonstrated that rMbFBA binds native bovine plasminogen and in a dose-dependent manner. Fluorescent microscopy revealed that pre-treatment with antibodies against rMbFBA decreased the adhesion of *M. bovis* to embryonic bovine lung (EBL) cells. Furthermore, adherence inhibition assays revealed 34.4% inhibition of *M. bovis* infection of EBL cells following

* Correspondence: bsjdy@126.com College of Veterinary Medicine, Gansu Agricultural University, 1 Yingmencun, Lanzhou 730070, China.

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