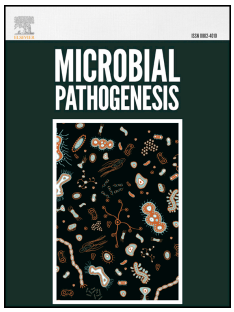
Accepted Manuscript



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

Xiang Gao, Shijun Bao, Xiaoyong Xing, Xiaoping Fu, Yi Zhang, Huiwen Xue, Fengqin Wen, Yanming Wei

PII: S0882-4010(18)30946-X

DOI: 10.1016/j.micpath.2018.08.032

Reference: YMPAT 3117

To appear in: *Microbial Pathogenesis*

Received Date: 23 May 2018

Revised Date: 14 August 2018

Accepted Date: 18 August 2018

Please cite this article as: Gao X, Bao S, Xing X, Fu X, Zhang Y, Xue H, Wen F, Wei Y, Fructose-1,6-bisphosphate aldolase of *Mycoplasma bovis* is a plasminogen-binding adhesin, *Microbial Pathogenesis* (2018), doi: 10.1016/j.micpath.2018.08.032.

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 μM and 43.8 μmol/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.