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Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Monsieur LIN Weidong,

Titulaire d'un diplôme de *Veterinary master*,

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 15 janvier 2021, à 10h00 précises (personne ne sera admis après cette heure),

en visioconférence :

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Cette dissertation originale a pour titre :

« Contribution of *Mycobacterium bovis* gene *Rv3671c* in acid resistance and replication ».

Le jury est composé comme suit :

Président : Prof. Y. BECKERS, Professeur ordinaire,
Membres : Prof. L. WILLEMS (Promoteur), Prof. H. ZHU (Copromoteur - CAAS, Chine), Prof. N. EVERAERT, Prof. S. MASSART, Dr J.-C. TWIZERE, Prof. X. DE BOLLE (UNamur), Prof. T. TCHIN (CAAS, Chine).

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Summary

The survival of Mycobacterium in the host is largely attributed to its ability to resist the acidic environment. Recently, it has been found that a Mycobacteria tuberculosis mutant cannot maintain intracellular pH in phagosomes in the absence of *Rv3671c*, which encodes Mycobacterial acid resistance protease (Marp). The aim of this study is to study the effect of Marp in acid resistance and the growth performance of *Mycobacterium bovis* and decipher the underlying working mechanisms.

We have expressed the periplasmic domain of Marp in *Escherichia coli* and prepared monoclonal antibodies (MAb) against Marp. The serine proteinase activity of Marp was verified by using β-casein as a substrate. Monoclonal antibodies were tested with recombinant or and natural Marp protein from *M. bovis*.

Meanwhile, we have constructed a knockout mutant (*ΔMarp*) using a mycobacteriophage, and validated its integrity by PCR and western blot. Besides, a complemented strain (ΔMarpComp) and a strain overexpressing the *Rv3671c* gene (PmvRv3671) were built by using plasmid pMV261. The growth rates of these strains were compared to that of the wild type *M. bovis* An5. Raw264.7 macrophages were infected at multiplicities of infections of 10 (MOI=10). After culture in standard 7H9 medium to the early logarithmic phase, the strains were transferred into 7H9 medium at pH 6.6 and pH 5.0 and maintenance solution at pH 6.6 and pH 4.5. The results show that while they all survive in 7H9 medium at pH 5.0 or pH 6.6 and maintenance buffer at pH 4.5, the viability at day 14^{th} of the over-expressing strain PmvRv3671 is significantly higher than that of wild-type strain *M. bovis*, ΔMarpComp and *ΔMarp*. After comparing their corrected survival rates, it can be observed that the acid resistance abilities of all strains significantly decrease between days 5 and 14. When exposed to pH 4.5 citrate maintenance solution for a long time, their acid-resistance abilities become weaker. In Raw264.7 macrophages stimulated with IFNγ, the replication rate of the PmvRv3671 is significantly higher than that of An5, *ΔMarp* and ΔMarpComp. So, *Rv3671c* over-expressing strain shows a better growth ability than the other three strains under acidic environments. In conclusion, the *Rv3671c* gene is not only related to acid resistance but also affects the growth of *M. bovis*.

To understand the panorama of the working mechanisms of Marp, we investigated proteins interacting with Marp. We used Mycobacteria tuberculosis Protein Chip to screen the proteins binding to Marp. The bacterial two-hybrid system was then used to validate the interacting proteins. The results show that among 295 interacting proteins, 52 displayed stronger binding. Interactions of seven candidates were verified by using the bacterial two-hybrid system. The biological functions were analyzed with String, Uniprot, BioCyc, and Gene Ontology databases.

We concluded that these 7 proteins are mainly responsible for two biological functions: the growth of M. bovis and the binding of GDP or ATP. Therefore, we hypothesize that the mechanisms of the acid resistance are associated with these Marp interactants. The results of the present study provide a basis for further deciphering the complete model and working mechanisms of Marp in M. bovis.