STRUCTURAL CHARACTERIZATION OF MYCOBACTERIUM BOVIS RECOMBINANT ANTIGENS BY MALDI-TOF MASS SPECTROMETRY

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Introduction

Mycobacterium bovis bacteria is the agent of bovine tuberculosis, an important zoonosis that still causes significant economic losses in beef and dairy cattle production in several countries.[1] High-resolution analytical methods are desirable to determine the composition of mycobacteria antigens,[2] in order to improve the production and quality control of antigens used for tuberculosis diagnostics and prevention.[3] Matrix assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOF) is a helpful methodology to accomplish precise peptide analysis since it renders accurate mass and enables amino acid *de novo* sequencing.[4] This work focused on structural analysis of seven antigens, previously reported in the literature as relevant immunodiagnostic tools for tuberculosis, which are being produced as recombinant proteins at Embrapa Beef Cattle. PE5, CFP-10, ESX-1, ESAT-6, PE13, TB10.4 and Mb0143 *M. bovis* peptides were expressed in *E. coli* as histidine-tagged proteins, purified by Ni^{2+} affinity chromatography and analyzed by MALDI-TOF mass spectrometry.

Methodology

Sinapinic acid and 2,5-dihydroxybenzoic acid were used in dried droplet method for MALDI-TOF mass spectra (MS) acquisition over a 2000–50,000 Da mass range after 1000 laser shots per sample on linear positive mode in an Autoflex III Smartbeam equipment (Bruker Daltonics). 1,5-diaminonaphtalen was used for CFP-10 N-terminal sequencing by In-Source-Decay method.[5]

Results

Expected masses of the cloned molecules were confirmed by observed mass values of the expressed recombinant peptides, except for PE5 e TB10.4 , which expressed 3.3 kDa and 2.3 kDa above the expected (theoretical) masses, respectively (Figure 1). This mass discrepancy may be due to some technical error during cloning procedures or even an artifact caused by bacterial expression machinery. On the other hand, we could determine the sequence of 18 amino acid residues at CFP-10 N-terminus, confirming the integrity of that portion of the expressed molecule (Figure 2).

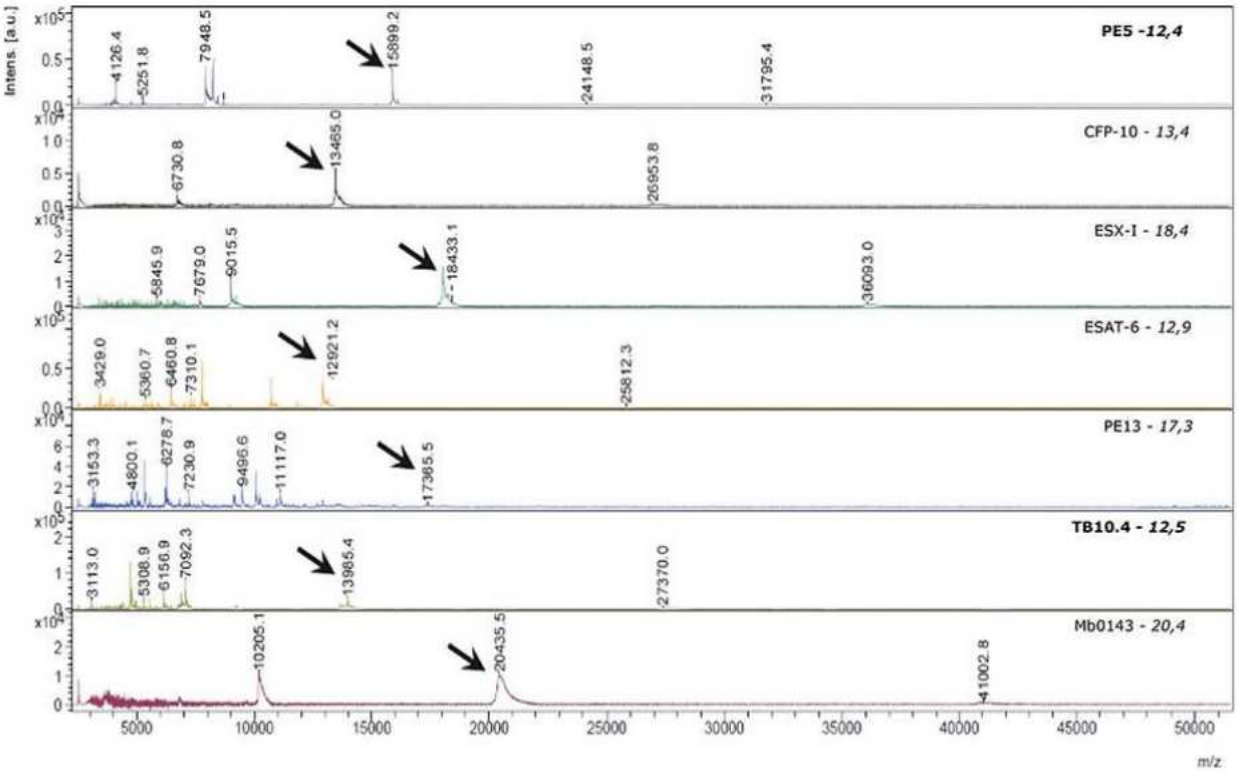
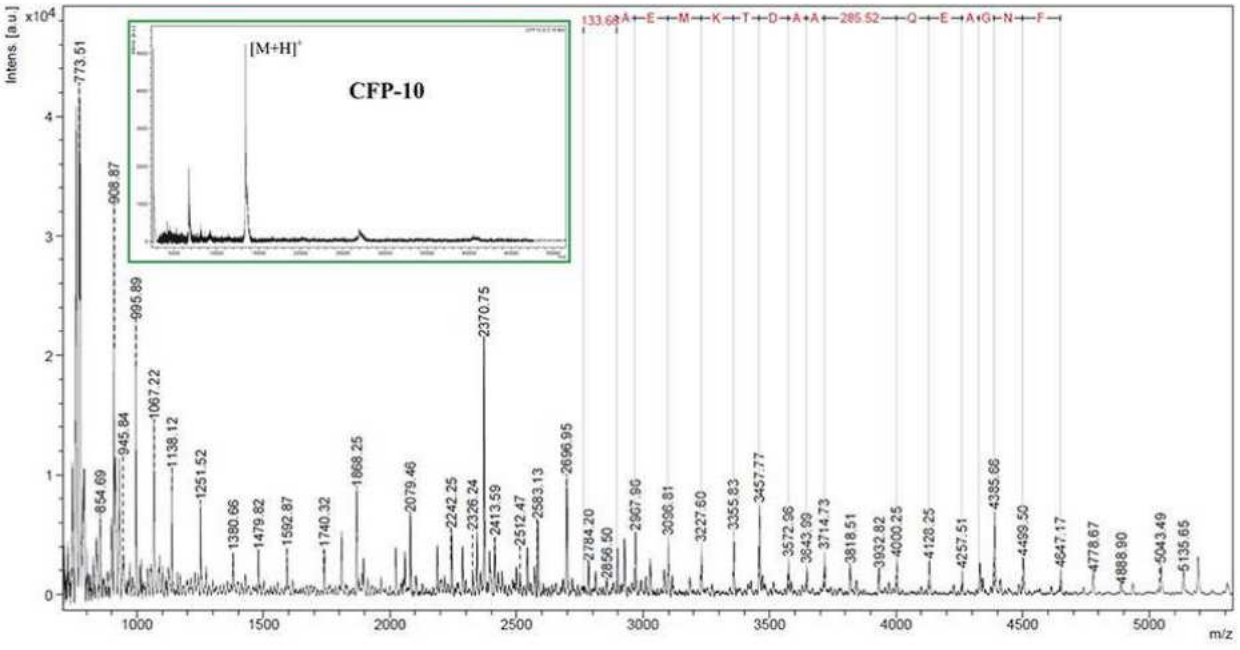


Figure 1. MALDI-TOF MS of *M. bovis* proteins. *m/z* spectra of seven (PE5, CFP-10, ESX-1, ESAT-6, PE13, TB10.4 and Mb0143) proteins are shown. Arrows indicate observed masses of $[M + H]^+$ molecules. Expected masses are in italics (kDa) and the unmatched findings are in bold.



MAHHHHHHSAALEVLFQGPGYQDPNSIAEMKTDAATLAQEAGNFERISGDLKTQIDQVESTAGSLQGQWRG
AAGTAAQAAVVRFQE ANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF

Figure 2. MALDI-TOF in-source-decay confirms CFP-10 integrity. Observed N-terminal sequence (red) read from mass spectrum of fragments seen after CFP-10 protein ionisation with 1,5-diaminonaphtalen matrix. Expected sequence of the expressed (full) molecule is written below (plasmidial moiety in blue), with antigen sequence underlined. Inset shows the *m/z* of the protein ionised during the experiment.

Conclusion

MALDI-TOF mass spectrometry results contribute to the characterisation of these *M. bovis* recombinant proteins aiding to establish the production of new immunodiagnostics for bovine tuberculosis with improved quality control.

Support

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References

1. T. Garnier, K. Eiglmeier, J. Camus *et al.*, "The complete genome sequence of *Mycobacterium bovis*", *PNAS* **100,** 7877–7882 (2003). doi: 10.1073/pnas.1130426100
2. S. Bhamidi, L. Shi, D. Chatterjee *et al.*, "A bioanalytical method to determine the cell wall composition of *Mycobacterium tuberculosis* grown *in vivo*", *Anal. Biochem.* **421,** 240–249 (2012). doi: 10.1016/j.ab.2011.10.046
3. M. Vordermeier, S. Gordon and R. Hewinson, "*Mycobacterium bovis* antigens for the differential diagnosis of vaccinated and infected cattle", *Vet. Microbiol.* **151,** 8–13 (2011). doi: 10.1016/j.vetmic.2011.02.020
4. K. Gevaert and J. Vandekerckhove, "Protein identification methods in proteomics", *Electrophoresis* **21,** 1145–1154 (2000). doi: 10.1002/(SICI)1522-2683(20000401)21:6<1145::AID-ELPS1145>3.3.CO;2-Q
5. K. Demeure, L. Quinton, V. Gabelica and E. de Pauw, "Rational selection of the optimum MALDI matrix for top-down proteomics by in-source decay", *Anal. Chem.* **79,** 8678–8685 (2007). doi: 10.1021/ac070849z

Techniques:
Mass Spectrometry