IDENTIFICATION OF *FRANCISELLA TULARENSIS* PROTEINS

Irena KASALOVÁ, Lenka HERNYCHOVÁ, Věra NEUBAUEROVÁ, Jiří STULÍK, Jana HAVLASOVÁ, Aleš MACELA
Proteome Center for the Study of Intracellular Parasitism of Bacteria, Purkyně Military Medical Academy, Hradec Králové, Czech Republic

Introduction

Francisella tularensis is the Gram – negative, facultative intracellular bacterium which is able to cause tularemia. It can survive in macrophages or hepathocytes. *Francisella tularensis* is devided into four subspecies *F. tularensis* subsp. *tularensis*, *F. tularensis* subsp. *palearctica*, *F. tularensis* subsp. *mediaasiatica*, *F. tularensis* subsp. *palearctica japonica*. Live attenuated vaccine strain (LVS) selected from a mixture of attenuated strains is used as an experimental model for human infection in mice.

Materials and methods

Protein identification procedure include cultivation of *F. tularensis*, preparation of samples for one- or two-dimensional electrophoresis, separation of proteins by one- or two-dimensional gel electrophoresis, protein digestion, ZipTip purification, mass spectrometry measurement and searching into the databases.

Protein lysates of *F. tularensis* subsp. t*ularensis* (obtained from FOI, Sweden) and *F. tularensis* LVS were separated by two-dimensional gel electrophoresis. Protein spots were cut from the polyacrylamide gels after the separation of proteins with two – dimensional electrophoresis. Peptides are extracted by digestion with trypsin.

Mass spectrometry is primary technique for identifying proteins. Mass spectrometry permits higher sensitivity for peptides comparing to intact proteins. Fast atom bombardment (FAB) and ^{252}Cf plasma desorption mass spectrometry were used in the past. Recently, matrix assisted laser desorption ionisation (MALDI) mass spectrometry and electrospray ionisation (ESI) mass spectrometry are used for better ionisation efficiency

MALDI-TOF: Samples of peptides are mixed with matrix on the sample plate. The energy is transferred from the matrix to ions and they undergo ionisation. The ions are accelerated by a voltage pulse and fly through the evacuated tube. They can be detected in linear or reflectron mode. Reflectron is used for higher accuracy for peptides.

ESI: The ions are formed from the solution. Charged droplets are produced at capillary tip. Solvent evaporation from the charged droplets leads to the droplet shrinkage and Coulomb fission. Gas phase ions are formed from very small and highly charged droplets in the ion source. ESI can be coupled with quadrupole or TOF mass analyser. Q-T of Ultima API (Micromass, UK) is equipped with both mass analysers. Selected precursor ion filtered through the first quadrupole can collide with inert gas. Tandem mass spectrum (MS/MS) of this precursor is important for the analysis of amino acid sequence. ESI mass spectrometry can be coupled with liquid chromatography (LC) due to the working under the atmospheric pressure.

Protein identification compares the experimental data and data deposited in public protein databases. Most of all we use Profound web site http://129.85.19.192/prowl-cgi/Profound.exe?FORM=1, http://129.85.19.192/prowl-cgi/Profound.exe?FORM=2 and ExPASy Molecular Biology Server http://www.expasy.ch. We enter pI, molecular weight of the protein, masses of digested peptides, possible modifications. Result of searching includes the list of most probably proteins. The other possibility of identification of the proteins is comparison of experimental results to genome database of *F. tularensis* (FOI, Umea, Sweden).

In spite of the fact that mass spectrometry permits the sensitive and precise analyses of proteins, the other methods can be used, e.g. N-terminal sequencing [2], amino acid analysis, immunoblotting [3], ELISA [3], flow cytometric analysis [4], immunoelectron microscopy [5], protein arrays [6].

Results

116 protein spots of *F. tularensis* LVS and 400 proteins of *F. tularensis* subsp. *tularensis* were analysed by mass spectrometry. 76 proteins of *F. tularensis* LVS (see tab. 1, tab. 3) and recently 8 ones of *F. tularensis* subsp. *tularensis* were identified (see tab.2). The other proteins of *F. tularensis* subsp. *tularensis* should be identified.

References

1. PATTERSON, SC., AEBERSOLD, R. Mass Spectrometric Approaches for the Identification of Gel Separated

Proteins. *Electrophoresis*, 1995, 16, p. 1791–1814.
2. GOLOVIOV, I., et al. Identification of Proteins of *Francisella tularensis* Induced during Growth in Macrophages and Cloning of the Gene Encoding a Prominently Induced 23-Kilodalton Protein. *Infect. Immun.*, 1997, 65, p. 2183–2189.
3. GRUNOW, R., et al. Detection of *Francisella tularensis* in Biological Specimens Using a Capture Enzyme-Linked Immunosorbent Assay, an Immunochromatographic Handheld Assay and PCR. *Clin. Diagn. Lab. Immunol.*, 2000, 7, p. 86–90.
4. POQUET, Y., et al. Expansion of Vgamma9 Vdelta2 T Cells is Trigered by *Francisella tularensis*-Derived Phosphoantigens in Tularemia but not after Tularemia Vaccination. *Infect. Immun.*, 1998, 66, p. 2107–2114.
5. MACELLARO, A., et al. Identification of a 71-Kilodalton Surface-Associated Hsp70 Homologue in *Coxiella burnetii*. *Infect. Immun.*, 1998, 66, p. 5882–5888.
6. CAHILL, DJ.: Protein Arrays: A High-Throughput Solution for Proteomics Research?. *Proteomics: A Trends Guide*, 2000, 1, p. 47–51.

Acknowledgement: The presented study was fully supported by Ministry of Education, Youth and Sport, grant No. LN00A033.

Correspondence: Irena Kasalová
Institute of Nuclear and Biological Medical Defence
Purkyně Military Medical Academy
Třebešská 1575
500 01 Hradec Králové
Czech Republic
e-mail: kasalova@pmfhk.cz

Received 16. 9. 2002