This PhD thesis applies MALDI-TOF mass spectrometry to identification of highly pathogenic bacteria.

Three major topics were studied in this thesis. The aim of the first project was to propose a universal workflow of sample preparation method for the identification of highly virulent pathogenes by MALDI-TOF MS. Fifteen bacterial species, including highly virulent Gram-positive and Gram-negative bacteria were employed in the comparative study of four sample preparation methods compatible with MALDI-TOF MS. Based on the values of protein yield and spectral quality, the method using combination of ethanol treatment followed by extraction with formic acid and acetonitrile shows the highest extraction efficacy and the spectral quality with no detrimental effect caused by storage. Thus, this can be considered as a universal sample preparation method for the identification of highly virulent microorganisms by MALDI-TOF mass spectrometry.

In the second part, a reference database of MALDI-TOF MS profiles of high risk pathogens, containing mass spectra of 392 strains belonging to 12 bacterial species, was created. The database was validated in international proficiency tests. Based on the validation studies, the use of the database for identification of high risk pathogens was accredited by Czech Institute for Accreditation and a part of the database has been commecialized. For the purposes of European database of biological agents, a MALDI-TOF analysis of *F. tularensis*, *Brucella* spp. and *V. cholerae* was performed and the data included into the database. A reference database of *Legionella* genus was created and used for identification of *Legionella* species undistinguishable by serological methods. The use of the database revealed 9 new species belonging to *Legionella* genus; the novelty was confirmed by *mip* gene sequencing.

The third project, focused on differentiation of closely related *Yersinia* species, a total of 146 strains of different *Yersinia* species and 35 strains of other relevant genera of the *Enterobacteriaceae* family have been studied using MALDI-TOF MS and chemometrics. The mass spectral profiles were subsequently analyzed by statistical methods to reveal specifically identifying biomarker proteins (SIBP). Bioinformatic approaches and tandem mass spectrometry were employed to reveal the molecular identity of biomarker ions. For example, the *Y. pestis*-specific biomarker at m/z 3065 could be identified as a fragment of the plasmid-encoded plasminogen activator (Pla), which is one of the major virulence factors in plague infections.