



## Perspective

# The application of proteomic methods (MALDI-toff MS) for studying protein profiles of some nematodes (dirofilaria and ascaris) for differentiating species



Sergey Andreevich Nagorny<sup>a</sup>, Anna Valentinovna Aleshukina<sup>a</sup>,  
Iraida Sergeevna Aleshukina<sup>a</sup>, Larisa Alexandrovna Ermakova<sup>a,b</sup>,  
Natalia Yurievna Pshenichnaya<sup>c,\*</sup>

<sup>a</sup>Rostov Scientific Research Institute of Microbiology and Parasitology, Rostov-on-Don, Russia

<sup>b</sup>Rostov State Medical University

<sup>c</sup>Central Research Institute of Epidemiology, Moscow, Russia

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## ABSTRACT

**Introduction:** Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-toff MS) is a reliable method for diagnosing a number of bacterial and fungal infections. It is also effective as a method of rapid diagnosis of several parasitic agents. We used MALDI-toff MS to study the protein profiles of four nematodes: *Dirofilaria repens*, *Dirofilaria immitis*, *Ascaris suum* and *Ascaris lumbricoides*. **Methods:** We studied the protein profiles of dirofilaria (five of each species: *D. repens* and *D. immitis*) and ascaris (five of each species: *A. suum* and *A. lumbricoides*), using a proteomic analysis based on MALDI-toff MS.

**Results:** Analysis of protein extracts of dirofilaria and ascaris showed spectra with high-intensity peaks in the range of 2–20 kDa. The quality of the spectra (clear graphical reflection of mass/charge to luminous intensity, consistent in repeated analyzes) and the intensity of the spectral peaks were consistent in all samples of the same species. The spectra profiles of *D. repens* and *D. immitis* differed in eight major peaks which makes it possible to differentiate species according to the protein profile. The spectra profiles obtained from *A. suum* and *A. lumbricoides* proteins differed slightly in 3 major peaks in both species and were discovered in m/z 13000; 13400 and 14400. The protein peaks in diapason 3000 kD–7300 kD specific for all genus ascaris are constant.

**Conclusions:** MALDI-toff MS-based proteomic analysis can serve as an effective taxonomic tool for parasitological studies.

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## Introduction

Proteomic analysis is a relatively new direct method in the laboratory diagnosis of infectious diseases. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-toff MS) became a revolutionary method of direct diagnosis of bacterial and fungal pathogens (Lavigne et al., 2013).

In comparison with traditional bacteriological methods, the identification of microorganisms with MALDI-toff MS is uncomplicated and does not require a significant amount of technically qualified staff (Chong et al., 2018). This leads to a reduction in

analysis time, early results of the identification of pathogens and, as a consequence, improvement in clinical care (Lavigne et al., 2013; Theparee et al., 2018; Carbonnelle et al., 2011). This method of identification is comparable in accuracy with the full genome studies (Teng et al., 2018). The important advantage of MALDI-toff MS is its implementability to determine the resistance of microorganisms to antibiotics, disinfectants and the toxin production of pathogens (Hrabák et al., 2013; Burckhardt and Zimmermann, 2011; Lasserre et al., 2015; Carbonnelle et al., 2011).

In recent years MALDI-toff MS has established itself as highly reliable method for the identification of bacteria and fungi (Lavigne et al., 2013; Chong et al., 2018; Chong et al., 2018). Despite its advantages, the study of the protein profiles of parasitic pathogens has not yet become widespread (Singhal et al., 2016).

\* Corresponding author.

E-mail address: [pshenichnaya.natalia@gmail.com](mailto:pshenichnaya.natalia@gmail.com) (N.Y. Pshenichnaya).

It should be noted that the possibilities of this method are limited by the database of the mass spectra of microorganisms, which needs to be constantly updated (Lavigne et al., 2013; Chong et al., 2018 Chong et al., 2018).

The first studies of the MALDI-toff MS as a tool for obtaining the protein profile of nematodes with an unidentified genome were undertaken in 2007 by León et al. (León et al. (2007)). Later on, this method was used to identify proteins of the *Echinococcus multilocularis* protoscolex (Wang et al., 2009). A study from Korea paid attention to differences in the protein profiles of cells of cholangiocarcinoma (CCA) and protein cells of CCA, treated with *Clonorchis sinensis* excretory-secretory antigens (Pak et al., 2009).

Other studies observed the differentiation of protein profiles of males and females of the pathogen of *Schistosoma japonicum* (Yuan et al., 2009). MALDI-toff MS has shown its effectiveness as a method for the rapid diagnosis of nematodes parasitic on agricultural crops (Ahmad et al., 2012).

We used the MALDI-toff MS to study the protein profiles of four species of nematodes: *D. repens*, *D. immitis*, *A. suum* and *A. lumbricoides*.

## Materials and methods

### Nematode samples

Head ends of ten immature female *Dirofilaria* (five of each species: *D. repens* and *D. immitis*). Samples of *D. repens* were surgically removed from subcutaneous tissue of humans, while species *D. immitis* were taken from the hearts of dogs. Head ends of ten young, immature ascaris were obtained and used in the study: five *A. lumbricoides*, which were excreted from patients in a parasitic and infectious diseases clinic, and five young immature *A. suum*, removed from the intestines of pigs in slaughterhouses. Identification of species and sexual maturity was carried out on the basis of morphological features.

### Preparation of nematode homogenization

Specimens were washed several times in 0.9% NaCl solution and placed for 24 h in 0.9% NaCl solution with the addition of 100 units/ml phenoxymethylpenicillin (penicillin V) and 100 µg/ml streptomycin (Wang et al., 2009). (To achieve high-quality immunochemical experiments, samples are treated with broad-spectrum bactericidal antibiotics to prevent colonization by other microorganisms).

The head end of the body (20 mm) was separated, or, in the case of *D. repens*, if the length of nematode did not exceed 60 mm we studied the entire helminth. Material was placed in a sterile 0.9% NaCl solution in a ratio of 1:3 (parasite weight: volume of an isotonic solution). The resulting mass was frozen at  $-18^{\circ}\text{C}$  for 30 min. Mechanical homogenization of the frozen material was carried out in an ice bath to obtain a liquid mass, followed by repeated freezing. This stage is performed 5 times.

The resulting biomass is placed in an Eppendorf tube, which is placed in a frozen 70% alcohol bath ( $-30^{\circ}\text{C}$ ) and ultrasonic homogenization is carried out at 70 kHz for 30 s five times. 200 µl of lysis buffer from the MALDI Sepsityper Kit 50 (Bruker Daltonics) was added to the resulting biomass and mixed in a vortex. After centrifugation for 2 min at 13,000 rpm, the supernatant was removed and the antigen was pipetted in the wash buffer. The precipitate was then suspended in 300 µl of deionized water and 900 µl of ethanol. 10 µl of 70% formic acid and 10 µl acetonitrile was added to the tube and mixed. 1 µl of the supernatant of the sample was applied on a steel plate (Bruker Daltonics) in two repetitions. The target was dried for several (5–15) minutes at  $20^{\circ}\text{C}$ . Then, 1 µl of the CHCA matrix ( $\alpha$ -Cyano-4-hydroxycinnamic acid) was applied to each sample, dried, and placed in a mass spectrometer for analysis.

Mass profiles of homogenized protein were obtained using Microflex LT MALDI-toff MS (Bruker Daltonics) with Flex Control software (Bruker Daltonics), visualized using Flex analysis 3.3

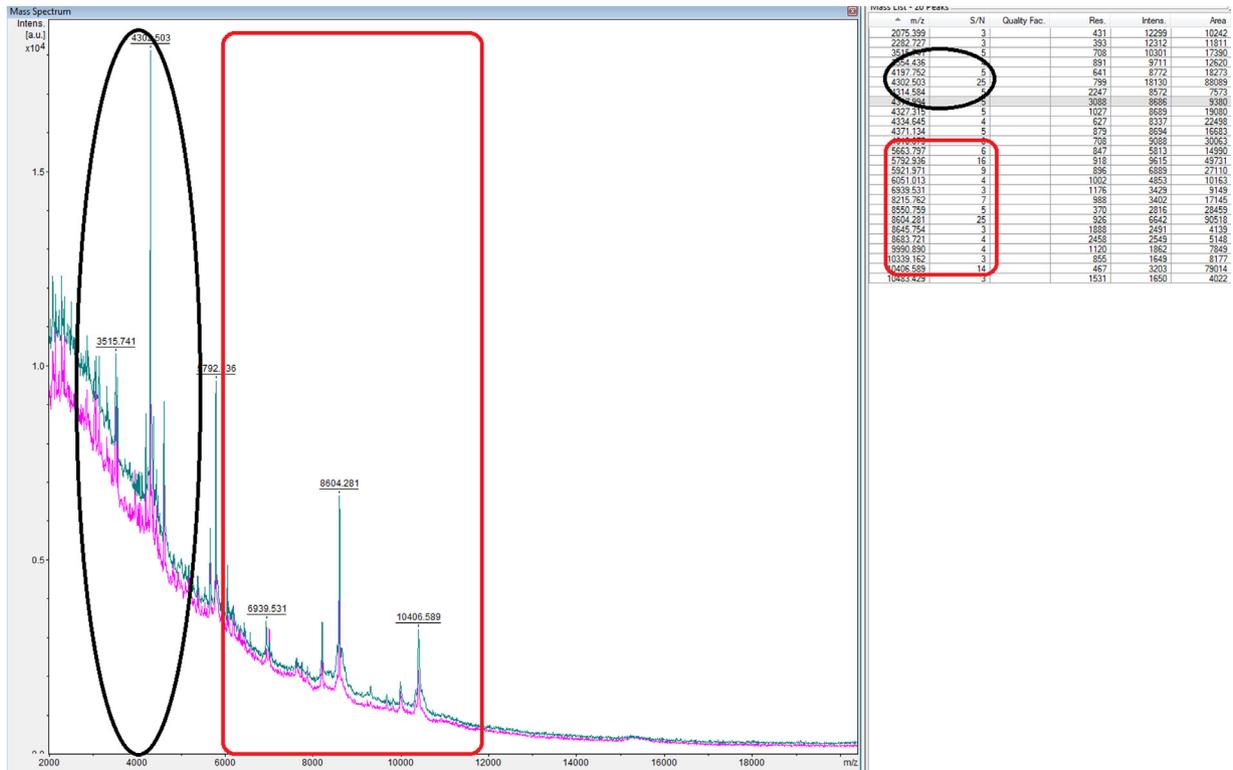


Figure 1. Protein profile *D. immitis*.

software (Bruker Daltonics). The spectral obtaining operation is fully computer-controlled. Spectral data and other monitoring parameters are displayed in real-time during the spectral acquisition process for quality assurance and system diagnostics purposes. Automatic spectral matching and identification is performed by the software accompanying the device, outputting best match organism identity and an objective score value.

## Results

Mass spectrometry analysis of protein extracts of dirofilariae showed spectra with high-intensity peaks in the range of 2–20 kDa (Figures 1 and 2). The quality of the spectra (clear graphical reflection of mass/charge to luminous intensity, consistent in repeated analyses) and the intensity of the spectral peaks were consistent in all samples of the same species. When using Flex analysis 3.3 software, it was noted that in the spectra obtained from different dirofilaria (*D. repens* and *D. immitis*) within the range from 3400 to 6000 kD, reliably frequent peaks were observed, which probably characterize the whole genus of nematodes. In the range above 6000 kD spectra differed in representative peaks, which allowed differentiating one species from another in the protein profile. In the protein spectrum of *D. immitis* (Figure 1), the peaks in the m/z range from 8600 to 10400 kD are reliably noted, while in spectra *D. repens* was detected as a peak in 11400 kD (Figure 2).

Mass spectrometry analysis of *A. lumbricoides* (Figure 3) and *A. suum* (Figure 4) protein profiles showed spectra with high-intensity peaks in the 2–20 kD range. The distribution of patterns and intensities of spectral (mass-passing) peaks with a same mass were identical in all samples of the same species of acaridae.

In contrast to dirofilariae, when obtaining mass spectra of ascaris, we detected an almost complete homology of graphic images in the m/z range from 3000 kD to 15000 kD.

At the same time, the protein peaks in diapason 3000 kD–7300 kD specific for all genus ascaris are constant. 3 major peaks in both species were discovered in m/z 13000, 13400 and 14400. We failed to establish a significant difference in patterns in our studies.

## Discussion

A few studies by foreign authors also indicate the effectiveness of the use of MALDI-toff MS for the diagnosis of parasitic pathogens, in particular plant nematodoses (Ahmad et al., 2012), animal helminth infections, human protozoal invasions (Hrabák et al., 2013; Wang et al., 2009 Wang et al., 2009). Human dirofilariasis is a topical issue in temperate countries. The “gold standard” for diagnosing human dirofilariasis is the morphological identification of the nematode, removed by surgery. However, in the case of damage to the nematode, the diagnostic value of this method is significantly reduced (Nagorny et al., 2018). The results of the application of MALDI-toff MS showed the zones of the spectra of dirofilaria in the range from 8 to 20 kDa have significant differences between the species of repens and immitis, which allows one to differentiate one type from another by mass spectrometry. It makes this method potentially diagnostic of dirofilariasis in cases of nematode damage.

The inconsiderable differences in the ascaris spectra in the same interval which, in our opinion, characterizes the species of nematodes, possibly connected with the fact that the causative agent of human ascariasis is an evolutionary close relative of the ascaris of pigs (descendant) (Shao et al., 2014; Leles et al., 2012; Betson and Stothard, 2016). The morphological and genomic similarity of these helminths suggests the obligate pathogenicity of the ascaris of pigs to humans. The results we obtained are confirmed by the data of colleagues from Denmark who described cases of human infection with *A. suum* (Nejsjum et al., 2005). MALDI-toff MS makes it possible to determine the species of not only whole helminths, but also their fragments (on a par with the

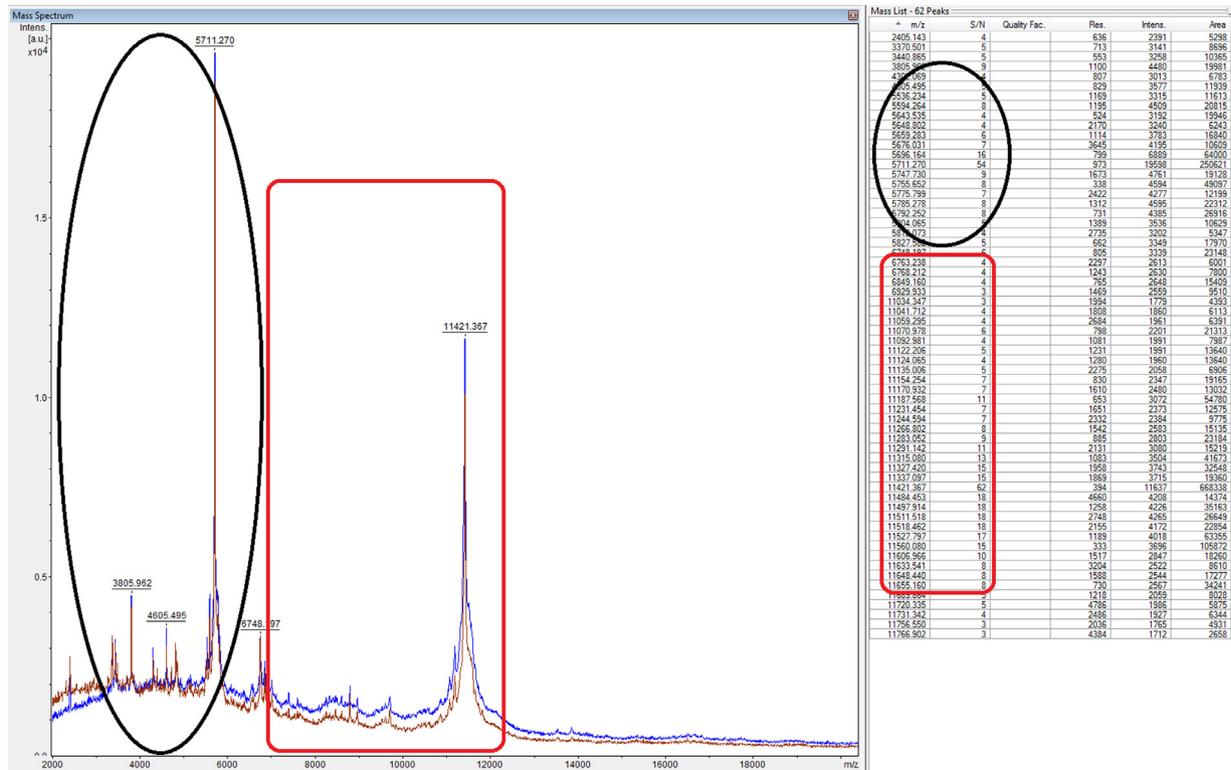


Figure 2. Protein profile *D. repens*.



molecular genetic method). MALDI-toff MS greatly optimizes the diagnosis of parasitic invasion. Creating a library of mass spectrometric profiles of nematodes based on the MALDI Sepsityper Kit 50 will allow the use of this method along with the “gold standard”.

MALDI-toff MS typing offers a rapid and reliable typing at species level of dirofilaria and ascaris and is a worthy alternative to molecular sequencing. Precise typing of the nematodes are important in epidemiology, for instance to understand the biology of *A. suum* in humans.

## Conclusion

Mass spectrometric biotyping is a direct method for diagnosing infectious diseases, and, in contrast to traditional bacteriology, is more economical in terms of time and labor. The obtained results show the possibility of species differentiation of nematodes using the method of mass spectrometry. It can serve as an effective taxonomic tool in parasitological studies.

## Ethical approval

Not applicable.

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## Conflict of interest declaration

The authors declare that they have no competing financial interests.

The content is solely the responsibility of the authors.

## References

- Ahmad F, Gopal J, Wu HF. Rapid and highly sensitive detection of single nematode via direct MALDI mass spectrometry. *Talanta* 2012;93:182–5, doi:http://dx.doi.org/10.1016/j.talanta.2012.02.009.
- Betson M, Stothard JR. *Ascaris lumbricoides* or *Ascaris suum*: what's in a Name?. *J Infect Dis* 2016;213(8):1355–6, doi:http://dx.doi.org/10.1186/1756-3305-5-42.
- Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. *J Clin Microbiol* 2011;49:3321–4, doi:http://dx.doi.org/10.1128/JCM.00287-11.
- Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti JL, et al. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem* 2011;44(1):104–9, doi:http://dx.doi.org/10.1016/j.clinbiochem.2010.06.017.
- Chong YK, Ho CC, Leung SY, Lau SKP, Woo PCY. Clinical mass spectrometry in the bioinformatics era: a Hitchhiker's guide. *Comput Struct Biotechnol J* 2018;16:316–34, doi:http://dx.doi.org/10.1016/j.csbj.2018.08.003.
- Hrabák J, Chudácková E, Walková R. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. *Clin Microbiol Rev* 2013;26:103–14, doi:http://dx.doi.org/10.1128/CMR.00058-12.
- Lasserre C, De Saint Martin L, Cuzon G, Bogaerts P, Lamar E, Glupczynski Y. Efficient detection of carbapenemase activity in Enterobacteriaceae by matrix-assisted laser desorption ionization-time of flight mass spectrometry in less than 30 minutes. *J Clin Microbiol* 2015;53(7):2163–71.
- Lavigne JP, Espinal P, Dunyach-Remy C, Messad N, Pantel A, Sotto A. Mass spectrometry: a revolution in clinical microbiology?. *Clin Chem Lab Med* 2013;51(2):257–70, doi:http://dx.doi.org/10.1515/cclm-2012-0291.
- Leles D, Gardner SL, Reinhard K, Iñiguez A, Araujo A. Are *Ascaris lumbricoides* and *Ascaris suum* a single species?. *Parasites Vectors* 2012;5:42, doi:http://dx.doi.org/10.1186/1756-3305-5-42.
- León IR, Neves-Ferreira AG, Valente RH, Mota EM, Lenzi HL, Perales J. Improved protein identification efficiency by mass spectrometry using N-terminal chemical derivatization of peptides from *Angiostrongylus costaricensis*, a nematode with unknown genome. *J Mass Spectrom* 2007;42(6):781–92, doi:http://dx.doi.org/10.1002/jms.1214.
- Nagorny S, Ermakova L, Pshenichnaya N, Krivorotova E, Zhuravlev A. Epidemiological features of human Dirofilaria in the South of Russia. *Epidemiological features of human Dirofilaria in the South of Russia. Int J Infect Dis* 2018;73 (Suppl):313, doi:http://dx.doi.org/10.1016/j.ijid.2018.04.4126.
- Nejsum P, Parker Jr ED, Frydenberg J, Roepstorff A, Boes J, Haque R, et al. Ascariasis is a zoonosis in Denmark. *J Clin Microbiol* 2005;43(3):1142–8, doi:http://dx.doi.org/10.1128/JCM.43.3.1142-1148.2005.
- Pak JH, Moon JH, Hwang SJ, Cho SH, Seo SB, Kim TS. Proteomic analysis of differentially expressed proteins in human cholangiocarcinoma cells treated with *Clonorchis sinensis* excretory-secretory products. *J Cell Biochem* 2009;108(6):1376–88, doi:http://dx.doi.org/10.1002/jcb.22368.
- Shao CC, Xu MJ, Alasaad S, Song HQ, Peng L, Tao JP, et al. Comparative analysis of microRNA profiles between adult *Ascaris lumbricoides* and *Ascaris suum*. *BMC Vet Res* 2014;10:99, doi:http://dx.doi.org/10.1186/1746-6148-10-99.
- Singhal N, Kumar M, Virdi JS. MALDI-TOF MS in clinical parasitology: applications, constraints and prospects. *Parasitology* 2016;143:1491–500, doi:http://dx.doi.org/10.1017/S0031182016001189.
- Teng JLL, Tang Y, Wong SSS, Fong JYH, Zhao Z, Wong C-P. MALDI-TOF MS for identification of *Tsukamurella* species: *Tsukamurella tyrosinosolvans* as the predominant species associated with ocular infections. *Emerg Microbes Infect* 2018;7(1):1–11, doi:http://dx.doi.org/10.1038/s41426-018-0083-4.
- Theparee T, Das S, Thomson RB. Total laboratory automation and matrix-assisted laser desorption ionization-time of flight mass spectrometry improve turnaround times in the clinical microbiology laboratory: a retrospective analysis. *J Clin Microbiol* 2018;56:e01242-17, doi:http://dx.doi.org/10.1128/JCM.01242-17.
- Wang Y, Cheng Z, Lu X, Tang C. *Echinococcus multilocularis*: proteomic analysis of the protoscolex by two-dimensional electrophoresis and mass spectrometry. *Exp Parasitol* 2009;123(2):162–7, doi:http://dx.doi.org/10.1016/j.exppara.2009.06.014.
- Yuan SS, Xing XM, Liu JJ, Huang QY, Yang SQ, Peng F. Screening and identification of differentially expressed proteins between adult female and male worms of *Schistosoma japonicum*. *Zhonghua Yu Fang Yi Xue Za Zhi* 2009;43(8):695–9 [PMID: 20021849].