IDENTIFICATION OF ARTHROPODS BY THE MALDI TOF TEHNIQUE

Larisa IVĂNESCU[1], Gabriela-Victoria MARTINESCU[1], Simona MĂTIUȚ[2],
Raluca MÎNDRU[1], Lavinia ANDRONIC[1], Olimpia Iacob[1], Dumitru Acatrinei[1], Liviu MIRON[1]

e-mail: l.ivanescu@uaiasi.ro

Abstract

MALDI-TOF mass spectrometry is a relatively new diagnostic technique that has revolutionised clinical microbiology by accurately identifying species of bacteria, filamentous fungi and yeasts. Recently, new applications to identify parasites and arthropods of medical interest have been developed, but few have developed MALDI-TOF analysis protocols for characterizing arthropod species. Thus, there is a need for a standardization in terms of the anatomical part(s) to be used according to arthropod families (head, thorax, legs) and the steps for protein extraction and homogenization. In this study we created a bank of 47 specimens including the following species: *Aedes caspius, Anopheles hyrcanus, Anopheles maculipennis, Coquillettidia richiardii, Culiseta annulata, Culex pipiens.* Thus, the aim of this study was to compare the quality of spectra and of results in the identification between different anatomical parts of mosquitoes, head, thorax (with wings) and legs, in order to optimize the use of the MALDI-TOF spectrometry tool. We evaluated the bank using the "bank versus bank" test (database provided by the laboratory of parasitology and mycology of Paris, Sorbonne University), each specimen had 4 deposits of protein extracts and the identification threshold log(score) was set to 1.7. Identifications were confirmed by morphological identification keys. There were differences in the protein profiles between each anatomical part. Leg spectra had the lowest number of high intensity peaks compared to those of the head or thorax.

Key words: Maldi Tof mass spectrometry; database; arthropod;

MALDI-TOF (*Matrix assisted laser desorption and ionisation-Time of flight*) mass spectrometry is a method that has revolutionised clinical microbiology by identifying species of yeast, bacteria and filamentous fungi against a bank of reference spectra (Sanguinetti and Posteraro, 2017; Angeletti, 2017; Wolk and Clark, 2018). More recent applications have also been developed, such as the identification of parasites (Murugaiyan and Roesler, 2017) and more recently arthropods of medical interest (Yssouf *et al*, 2016; Laroche *et al*., 2017; Murugaiyan and Roesler, 2017).

The mass spectrometry method is based on the detection of protein fingerprints (characteristics of a species, strain or physiological state) constituted by mass spectra and/or protein profiling in search of biomarkers.

This method is widely used in northern countries becoming of interest and increasingly accessible in countries endemic for infectious and tropical diseases. The process is automated, simple and fast to use, providing highly accurate results with a minimum of technical expertise. Thus, for entomological field studies, the use of the MALDI-TOF spectrometry instrument is being considered. Indeed, studies have shown that it is possible to identify by mass spectrometry species of mosquitoes (*Diptera: Culicidae*), Culicidae (*Diptera*: *Ceratopogonidae*), ticks (*Acari: Ixodidae, Argasidae*), but also phlebotominae (*Diptera: Psychodidae: Phlebotominae*), fleas (*Siphonaptera*), tsetse flies (*Diptera: Glossinidae*) (Yssouf *et al*., 2016), and bedbugs or "triatomines" (*Hemiptera: Reduvidae*) (Laroche *et al*., 2018).

The method has been validated for the identification of all adult, egg and larval stages, representing a great advantage, especially for the imago stages that are more difficult to identify. Protein profiling studies have also shown biomarkers of infection in arthropods, such as infection with *Rickettsia spp.* or *Borrelia spp.* in ticks (Diarra *et al*., 2017; Fotso Fotso *et al*., 2014; Yssouf *et al*., 2015), with *Bartonella spp.* in fleas (El Hamzaoui, Laroche, & Parola, 2018) and with *Plasmodium spp.* in *Anopheles* (Laroche *et al*.,

---

2017). Other studies have shown the presence of biomarkers that make it possible to identify the host from which the blood mass was taken (Niare *et al*., 2016) or to identify closely related species of *Anopheles* (Müller *et al*., 2013). However, few have developed MALDI-TOF test protocols for characterizing arthropod species. Thus, there is a need for standardization in terms of the anatomical part(s) to be used according to arthropod families (head, thorax, legs) and of protein extraction and homogenization steps.

Overall, MALDI-TOF is a promising tool for characterizing mosquito vectors. If used for mosquito monitoring, this proteomic method will avoid molecular test, with a gain in terms of the speed and cost of the test. Like DNA sequence databases, accessibility via online applications is essential for the widespread use of MALDI-TOF MS databases. Such online platforms have already been proposed for fungi (Diarra *et al.,* 2017) and *Leishmania* species (Laroche *et al.,* 2017) and are currently being set up for mosquito species identification (El Hamzaoui *et al*., 2018).

During the period 03-09.2022 mosquitoes were caught in the Danube Delta and Iasi City, using CDC Light Traps, by using dry ice as attractant. The captured mosquito specimens were stored at -20°C, in dry condition, being the preservation method with the best results. (Halada, Hlavackova, Dvorak, *et al.*, 2018) For the identification of mosquitoes by the MaldiTof technique, legs, head, thorax and wings were used. Recent studies have shown a significant sensitivity of legs, as the results may be influenced by capture, transport and storage conditions (Diarra *et al.*, 2019; Loaiza *et al.*, 2019; Rakotonirina *et al.*, 2020). Also, the cephalothorax should be cut with great care, as there is a high risk of blood contamination (Müller *et al.*, 2013).

Thus, the aim of this study was to compare the quality of spectra and identification results between the different anatomical parts of mosquitoes, head, thorax (with wings) and legs, in order to optimize the use of the MALDI-TOF spectrometry instrument. The automated MALDI-TOF mass spectrometry system, Microflex LT Bruker Daltonics®, was used for the analysis.

MATERIAL AND METHOD

Figure 1 **Automated MALDI-TOF mass spectrometry system, Microflex LT Bruker Daltonics®**

RESULTS AND DISCUSSIONS

To carry out this study, we created a bank of 47 specimens including the following species: *Aedes caspius, Anopheles hyrcanus, Anopheles maculipennis, Coquillettidia richiardii, Culiseta annulata, Culex pipiens.*

We evaluated the bank by the "bank versus bank" test (database provided by the laboratory of parasitology and mycology of Paris, Sorbonne University), each specimen had 4 protein extract

deposits and the log (score) identification threshold was set to 1.7. Identifications were confirmed by morphological identification keys. Between each anatomical part, there were differences in terms of the protein profiles. Leg spectra had the lowest number of high intensity peaks as compared to those of the head or thorax. The reproducibility of the spectra varied between anatomical parts. Leg spectra were the least reproducible and head spectra showed the best reproducibility, regardless of any experimental conditions. The distribution of

log (scores) varied significantly between anatomical parts, but the head part provided the highest log (scores).

We obtained 98% and 96% correct identification for the head and legs respectively. For the thorax, correct identification rates were lower (81%), which could be partly explained by blood contamination from the abdomen after dissection of frozen specimens.
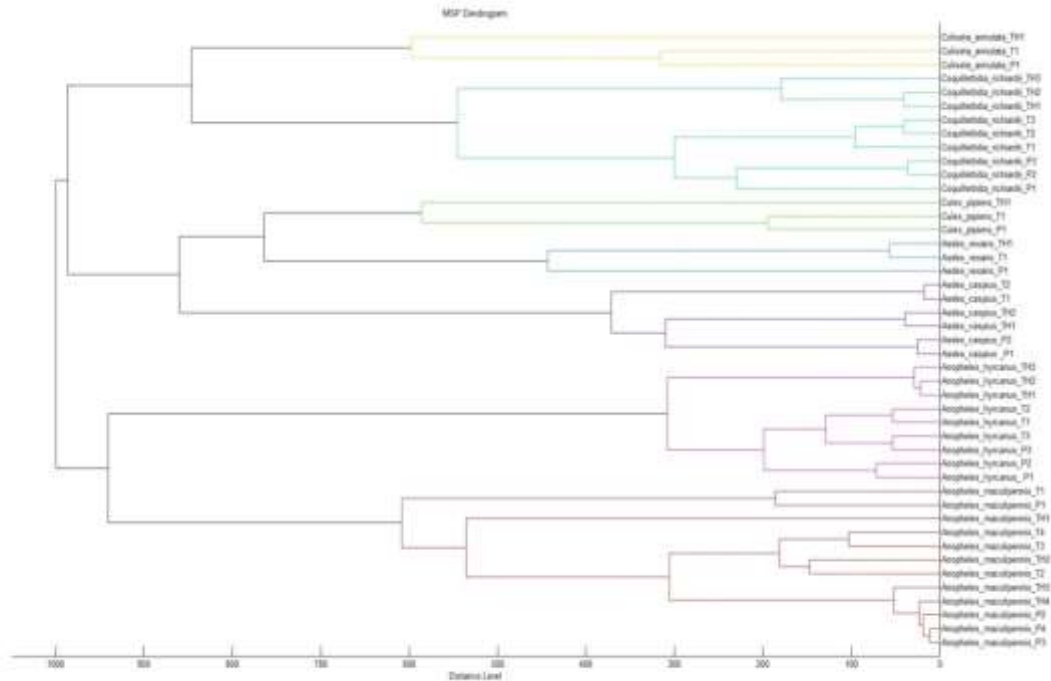

Figure 2 **Dendrogram of matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectra constructed with the 47-mass spectral library (MSL) specimens**

The dendrogram was calculated using Maldi Biotyper v4.1 software and the distance units correspond to the relative similarity of the mass spectra. Cluster analysis of the dendrogram (*figure 2*) showed that specimens belonged to 5 different species, either male or female (*Aedes caspius, Anopheles hyrcanus, Anopheles maculipennis, Coquillettidia richiardii, Culiseta annulata, Culex pipiens*).
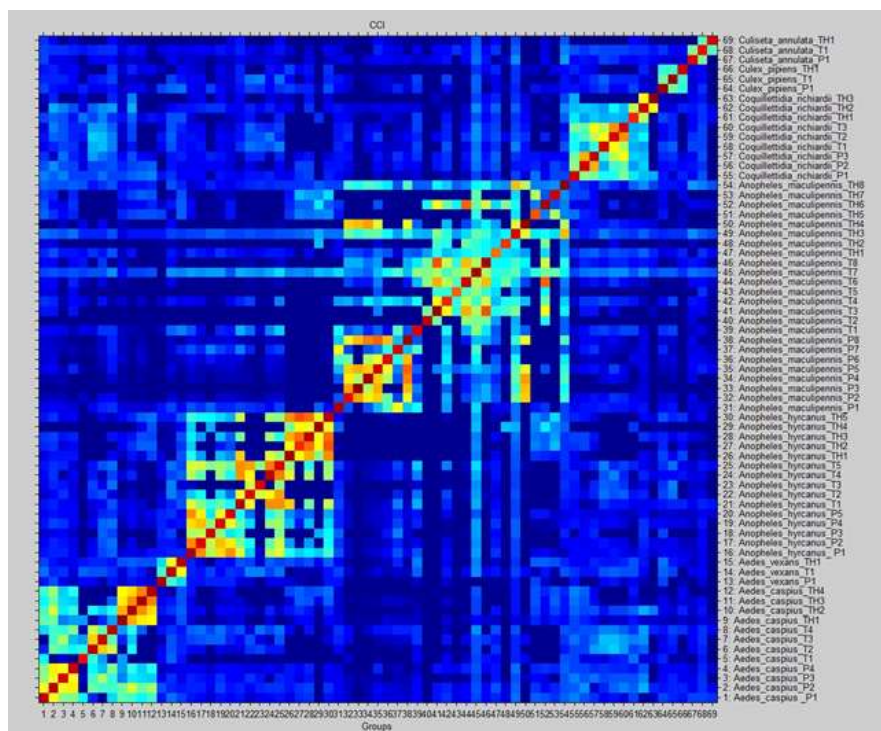
Figure 3 **Heatmap grid of the composite correlation index (CCI) of mass spectra-protein profiles**

Species are indicated on the right side of the heat map. The reproducibility levels of the mass spectra are indicated in red and blue, revealing the relationship and respectively the incongruence between the spectra. The CCI matrix was calculated using Maldi Biotyper v4.1 software with default settings (*figure 3*).

The best results were obtained for specimens stored in the dry state at -20°C.

To increase the reproducibility of the spectra and the speed of testing, the homogenization of protein extracts was done with glass beads. The identification scores obtained were satisfactory (LS≥1.8) and therefore the method was considered suitable.

The extraction was carried out in a mixture of 70% formic acid and 100% acetonitrile. Transport of mosquitoes was done at room temperature in tubes containing silica gel then frozen at -20°C.

CONCLUSIONS

The entomological characterisation of vector mosquitoes is in some cases very complicated, especially when we talk about morphologically twin species, and is an expanding field of research. MALDI-TOF spectrometry is a proteomic tool that requires technical expertise but offers a speed of analysis compatible with its use for entomo-epidemiological monitoring. In this study we proved the speed of this

spectrophotometric technique, the accuracy of species identification, and it is a method of the future in medical entomology. Bioinformatics tools coupled with MALDI-TOF spectrometry have paved the way for promising new applications in medical entomology, such as differentiation of similar species, age determination, pathogen infection or blood meal history. Future applications remain to be explored, such as identifying the host from which the blood meal was taken or detecting insecticide resistance.

REFERENCES

Angeletti, S., 2017 - Matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) in clinical microbiology. Journal of Microbiological Methods, What's next in microbiology methods? Emerging methods 138, 20–29. https://doi.org/10.1016/j.mimet.2016.09.003

Diarra, A.Z., Almeras, L., Laroche, M., Berenger, J.-M., Koné, A.K., Bocoum, Z., Dabo, A., Doumbo, O., Raoult, D., Parola, P., 2017 - Molecular and MALDI-TOF identification of ticks and tick-associated bacteria in Mali. PLOS Neglected Tropical Diseases 11, e0005762. https://doi.org/10.1371/journal.pntd.0005762

Diarra, A.Z., Laroche, M., Berger, F., Parola, P., 2019 - Use of MALDI-TOF MS for the Identification of Chad Mosquitoes and the Origin of Their Blood Meal. Am J Trop Med Hyg 100, 47–53. https://doi.org/10.4269/ajtmh.18-0657

Fotso, A.F., Mediannikov, O., Diatta, G., Almeras, L., Flaudrops, C., Parola, P., Drancourt, M., 2014 - MALDI-TOF Mass Spectrometry Detection of Pathogens in Vectors: The Borrelia

crocidurae/Ornithodoros sonrai Paradigm. PLOS Neglected Tropical Diseases 8, e2984. https://doi.org/10.1371/journal.pntd.0002984

Halada, P., Hlavackova, K., Risueño, J., Berriatua, E., Volf, P., Dvorak, V., 2018 - Effect of trapping method on species identification of phlebotomine sandflies by MALDI-TOF MS protein profiling. Medical and Veterinary Entomology 32, 388–392. https://doi.org/10.1111/mve.12305

Hamzaoui, B.E., Laroche, M., Almeras, L., Bérenger, J.-M., Raoult, D., Parola, P., 2018 - Detection of Bartonella spp. in fleas by MALDI-TOF MS. PLOS Neglected Tropical Diseases 12, e0006189.
https://doi.org/10.1371/journal.pntd.0006189

Laroche, M., Almeras, L., Pecchi, E., Bechah, Y., Raoult, D., Viola, A., Parola, P., 2017a - MALDI-TOF MS as an innovative tool for detection of Plasmodium parasites in Anopheles mosquitoes. Malar J 16, 5. https://doi.org/10.1186/s12936-016-1657-z

Laroche, M., Bérenger, J.-M., Delaunay, P., Charrel, R., Pradines, B., Berger, F., Ranque, S., Bitam, I., Davoust, B., Raoult, D., Parola, P., 2017b - Medical Entomology: A Reemerging Field of Research to Better Understand Vector-Borne Infectious Diseases. Clinical Infectious Diseases 65, S30–S38. https://doi.org/10.1093/cid/cix463

Laroche, M., Bérenger, J.-M., Gazelle, G., Blanchet, D., Raoult, D., Parola, P., 2018 - MALDI-TOF MS protein profiling for the rapid identification of Chagas disease triatomine vectors and application to the triatomine fauna of French Guiana. Parasitology 145, 665–675. https://doi.org/10.1017/S0031182017001342

Loaiza, J.R., Almanza, A., Rojas, J.C., Mejía, L., Cervantes, N.D., Sanchez-Galan, J.E., Merchán, F., Grillet, A., Miller, M.J., De León, L.F., Gittens, R.A., 2019 - Application of matrix-assisted laser desorption/ionization mass spectrometry to identify species of Neotropical Anopheles vectors of malaria. Malaria Journal 18, 95. https://doi.org/10.1186/s12936-019-2723-0

Müller, P., Pflüger, V., Wittwer, M., Ziegler, D., Chandre, F., Simard, F., Lengeler, C., 2013 - Identification of Cryptic Anopheles Mosquito Species by Molecular Protein Profiling. PLOS ONE 8, e57486. https://doi.org/10.1371/journal.pone.0057486

Murugaiyan, J., Roesler, U., 2017 - MALDI-TOF MS Profiling-Advances in Species Identification of Pests, Parasites, and Vectors. Frontiers in Cellular and Infection Microbiology 7.

Niare, S., Berenger, J.-M., Dieme, C., Doumbo, O., Raoult, D., Parola, P., Almeras, L., 2016 - Identification of blood meal sources in the main African malaria mosquito vector by MALDI-TOF MS. Malar J 15, 87. https://doi.org/10.1186/s12936-016-1152-6

Rakotonirina, A., Pol, M., Kainiu, M., Barsac, E., Tutagata, J., Kilama, S., O'Connor, O., Tarantola, A., Colot, J., Dupont-Rouzeyrol, M., Richard, V., Pocquet, N., 2020 - MALDI-TOF MS: optimization for future uses in entomological surveillance and identification of mosquitoes from New Caledonia. Parasites Vectors 13, 359. https://doi.org/10.1186/s13071-020-04234-8

Sanguinetti, M., Posteraro, B., 2017 - Identification of Molds by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. Journal of Clinical Microbiology 55, 369–379. https://doi.org/10.1128/jcm.01640-16

Wolk, D.M., Clark, A.E., 2018 - Matrix-Assisted Laser Desorption Time of Flight Mass Spectrometry. Clinics in Laboratory Medicine 38, 471–486. https://doi.org/10.1016/j.cll.2018.05.008

Yssouf, A., Almeras, L., Raoult, D., Parola, P., 2016 - Emerging tools for identification of arthropod vectors. Future Microbiology 11, 549–566. https://doi.org/10.2217/fmb.16.5

Yssouf, A., Almeras, L., Terras, J., Socolovschi, C., Raoult, D., Parola, P., 2015 - Detection of Rickettsia spp in Ticks by MALDI-TOF MS. PLOS Neglected Tropical Diseases 9, e0003473. https://doi.org/10.1371/journal.pntd.0003473