SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: FA1408
STSM title: Training of MALDI-TOF MS for the identification of Trichinella isolates
STSM start and end date: 19/02/2018 to 23/02/2018
Grantee name: KARADJIAN Grégory

PURPOSE OF THE STSM/

The short term scientific mission "Training of MALDI-TOF MS for the identification of *Trichinella* isolates" took place in the National Reference Laboratory (NRL) of the Federal Institute for Risk Assessment (BfR), in Berlin, between 19/02/2018 and 26/02/2018. The training was conducted by Peter Bahn under the supervision of Dr. Anne Mayer-Scholl and Dr. Anette Jonhe.

To date, the differentiation of the larvae to the species and genotype level is based primarily on molecular methods, which can be relatively time consuming and labor intensive. The NRL of the BfR has developed and optimized the protein extraction protocol for investigation of the protein profile of the different *Trichinella* species and genotypes by mass spectrometry matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometer (MALDI-TOF) (Mayer-Scholl et al., *Plos One*, 2016). MALDI-TOF MS is a high throughput technology based on the comparison of the biomolecules fingerprint (mainly proteins) obtained from microorganisms with a database of reference spectra from known microorganisms.

The purpose of the training was to learn the technical aspects of MALDI-TOF, including protein extraction, spectra analysis and reference Mass Spectra (MSP) Library creation. Furthermore, an additional aim of the STSM was the analysis of spectra measured from different *Trichinella* species/strains maintained at the French NRL on "Foodborne Parasites" of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), including some strains isolated in different region on France and identified at the species level by multiplex PCR. The 55 isolates were sent to the BfR in 96% ethyl-alcohol previously to the STSM.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

The STSM took place according to the following Work plan provided by the host institution:

Day 1

Introduction to the principles of MALDI TOF

Protein extraction and samples preparation of Trichinella isolates

Sample preparation and MALDI TOF measurement

Day 2

Analysis under supervision: principles of analysis, introduction to Flex Analysis software

Introduction to generation of Master Spectra and criteria for MALDI

TOF database development

Introduction to MALDI Biotyper software

Day 3

Repetition of the sample preparation and MALDI TOF measurement with own isolates

Sample preparation , MALDI TOF measurement with own isolates

Day4

Repetition of the sample preparation and MALDI TOF measurement with own isolates

Sample preparation , MALDI TOF measurement with own isolates

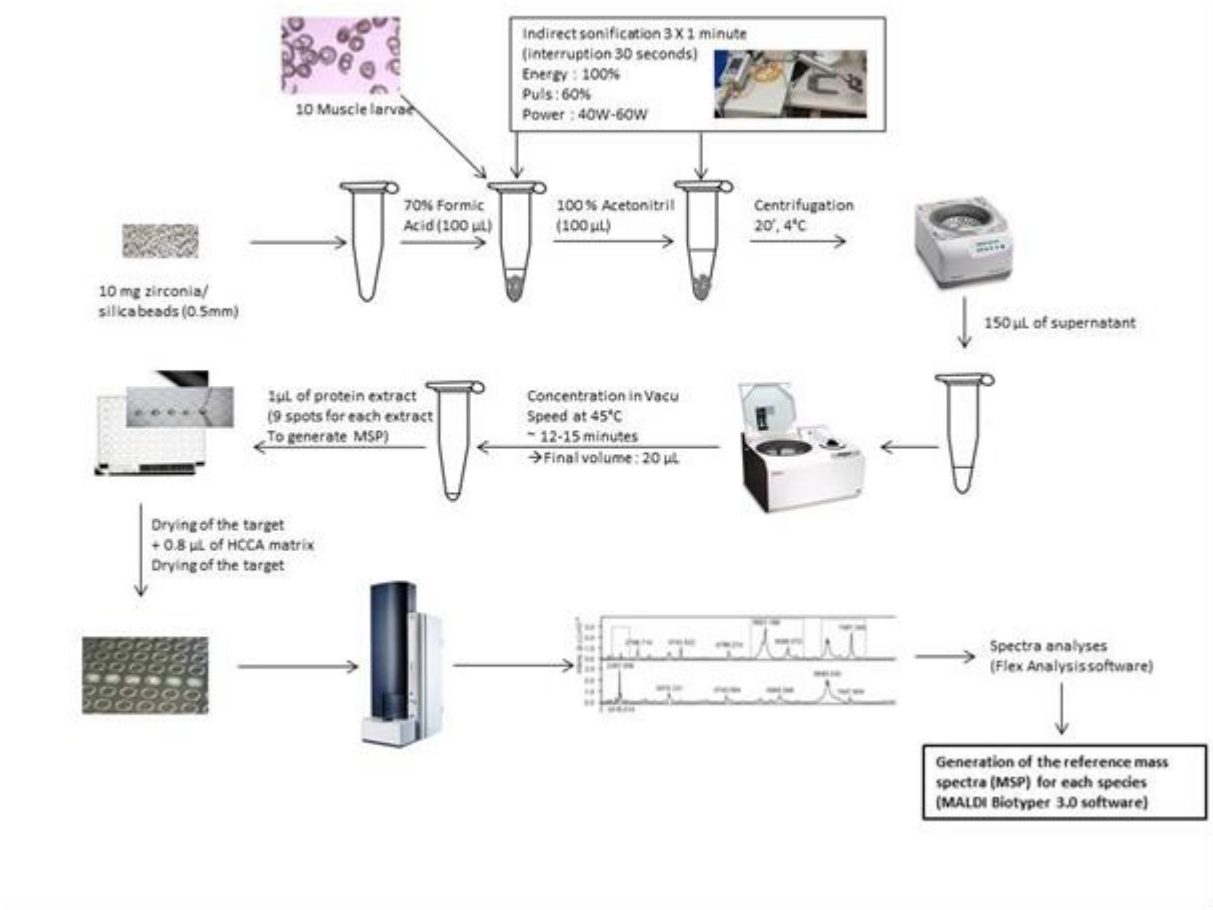
Day 5

Analysis with own isolates

Generation of Master Spectra

Protocol:

Proteins from the different isolates were extracted at the NRL for Trichinella (BfR). The proteins were extracted from ten pooled larvae per isolate with 10 mg zirconia/silica beads (0.5µm) and 100µl 70% formic acid in a 2 ml Eppendorf tube. The samples were sonicated on ice for 3 fold 1 minute with 30 seconds of interruption, and then 100µl acetonitrile (100%) were added and sonicated again under the same conditions. The suspension was centrifuged at 15.000g for 20 min at 4°C, and subsequently 150µl of supernatant was collected for protein concentration in a vacuum centrifuge at 45°C. One µl of each sample was spotted onto the target plate nine times (Bruker Daltonik, Bremen, Germany), air dried and then overlaid with 1µl of saturated α-cyano-4-hydroxy-cinnamic acid matrix solution. After complete drying, the target with spotted samples and the Bacterial Test Standard for calibration were inserted into the MALDI TOF MS equipment for measurement and acquisition of the mass spectra. Each specimen was spotted nine times and each spot was measured three times. The spectra of all the isolates were analyzed using Flex Analysis software. Briefly, the 27 spectra for one specimen were superimposed and the spectra with peaks out of the accepted range were deleted. The remaining 19-20 spectra were then used to generate a Master spectrum (MSP). This is the procedure allowing to generate MSP according to the manufacturer (Bruker).



Workflow followed to obtain the Mass Spectra from 10 Trichinella larvae

DESCRIPTION OF THE MAIN RESULTS OBTAINED

(max. 500 words)

The trainee was able to perform protein extraction and to set up the MALDI-TOF to acquire the mass spectra, and to analyze the spectra according to the Workflow. He learned also to generate a Master Spectrum (MSP).

A total of 1485 mass spectra were obtained corresponding to 27 spectra per specimen. And 55 MSP were generated.

The newly generated MSP were then compared with the MSP already generated at the BfR (Mayer-Scholl et al., 2016) to blindly test to identify at the species level using the MALDI Biotyper software. The results were in accordance with the results from the multiplex PCR (actual gold standard to identify *Trichinella* spp. at the species level). Interestingly, the comparison of the MSP allows to discriminate the different strains of the same species that were recovered in different isolates. This point could allow to follow the strains of the different outbreaks occurring in a same region, comparing the infectivity of different strains …

FUTURE COLLABORATIONS (if applicable)

(max.500 words)

In conclusion, the STSM training has been successful in terms of announced aims. It has successfully contributed to technical learning and improved collaboration between BfR and ANSES for the typing of *Trichinella* species/genotypes. MALDI-TOF technology will be transferred at the French NRL and the databases from the German and the French isolates will be pooled so that having a more complete library. In the future, this library will be implemented with the new reference spectra generated from the new samples that will be recovered by both ANSES and BfR. We think that in the future, the multiplex PCR will not be used anymore. The collaboration of scientists from ANSES and BfR will also extend to the identification of other nematodes by MALDI-TOF.