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Complimentary use of MALDI FTICR-MS and TOF-SIMS Imaging Approaches in an Invertebrate

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In a previous untargeted metabolomics study, the metabolite 2-hexyl-5-ethyl-3-furansulfonic acid (HEFS) showed a toxicity induced reduction in earthworms. The results presented here focus on the specific localization of this metabolite in the invertebrate *Lumbricus rubellus* (widely distributed earthworm species) to gain a better understanding of its biological function.

The application of Imaging Mass Spectrometry (IMS) techniques allows for whole-organism to sub-cellular metabolite localization. The use of MALDI in conjunction with an ultra-high resolution mass spectrometer with MS/MS capabilities makes in-situ identification of known and unknown metabolites feasible. In addition, TOF-SIMS with much higher spatial resolution but with a limited mass range gives information on detailed cellular distribution, making both methods complementary.

We report here the combined use of Matrix Assisted Laser Desorption Ionization (MALDI) FTICR-MS to image tissue cross-sections (0.5cm2, 50µm spatial resolution) and TOF-SIMS to further elucidate the cellular distribution of selected small molecules (125 – 500µm2, 250 nm spatial resolution). Both IMS techniques revealed robust cross-platform tissue specific metabolite distributions. The MS/MS capabilities of the FTICR-MS were used to identify metabolites directly on the tissue section. LC-MSn separation of tissue extracts showed that co-localized mass spectral features represent individual metabolites with similar MS/MS characteristics.

The molecule of interest, 2-hexyl-5-ethyl-3-furansulfonic acid (HEFS) was identified and localized in earthworm intestine utilizing two different MSI technologies. High mass accuracy MALDI-FTMS yielded the localization at a 50µm spatial resolution. In addition, unexpected HEFS-related sulfonated compounds were identified in the earthworm gut by MS/MS approaches. The detection of structural analogs implicates the presence of a new group of gut surfactants in these invertebrates.

An even more refined localization was enabled by the TOF-SIMS technology at a spatial resolution of 250nm and revealed a main HEFS concentration in the gut lumen and neighboring cells where this class of compounds may be produced or stored.

The combined use of different MSI technologies for similar tissue sections gave a more comprehensive dataset than one technique alone. Especially the MS/MS capabilities of the MALDI FTICR are an essential part to identify co-localized molecules detected by TOF-SIMS.

The now established link between the toxicity-induced reduction of HEFS and the tissue of interest is key to designing biological assays which may unravel the biological role of the here discovered group of sulfonated compounds. Early experiments to elucidate surface active properties of HEFS in conjunction with compound uptake through the earthworm gut revealed an interaction with several naturally occurring nutrients. A test to elucidate a possible gut enzyme protective property of HEFS was successful in vitro and is demonstrating the importance of HEFS for earthworm metabolism/digestion.

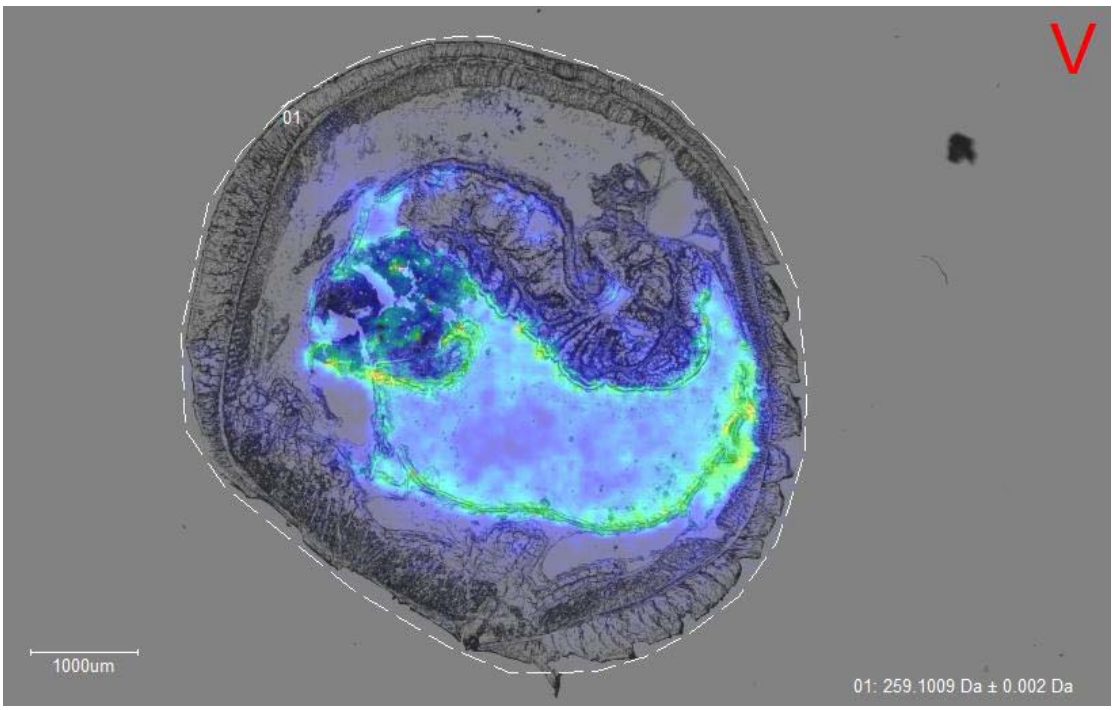

Figure 1. MALDI-FTMS image of an earthworm cross-section showing the localization of HEFS in the gut.

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