

Microbe visualization and phenotype identification of mussel gills using SpatialOMx

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Introduction: SpatialOMx workflow for host microbe interaction

Host-microorganism associations range from marine symbioses to the human gut. In these environments where symbiotic bacteria provide nutrition for the host, extreme bacterial genomic strain-variation can lead to immense heterogeneity of bacterial phenotypes. Current methods do not allow for the differentiation of phenotypically different strains. On the other hand, SpatialOMx methods, such as spatial metabolomic and spatial proteomics, provide a unique opportunity to reveal the varied genotypes and the heterogeneous distribution of these different strains. In this study, we successfully applied the SpatialOMx workflow to spatially differentiate the phenotypes of the major symbiotic bacterial strains found on mussels.

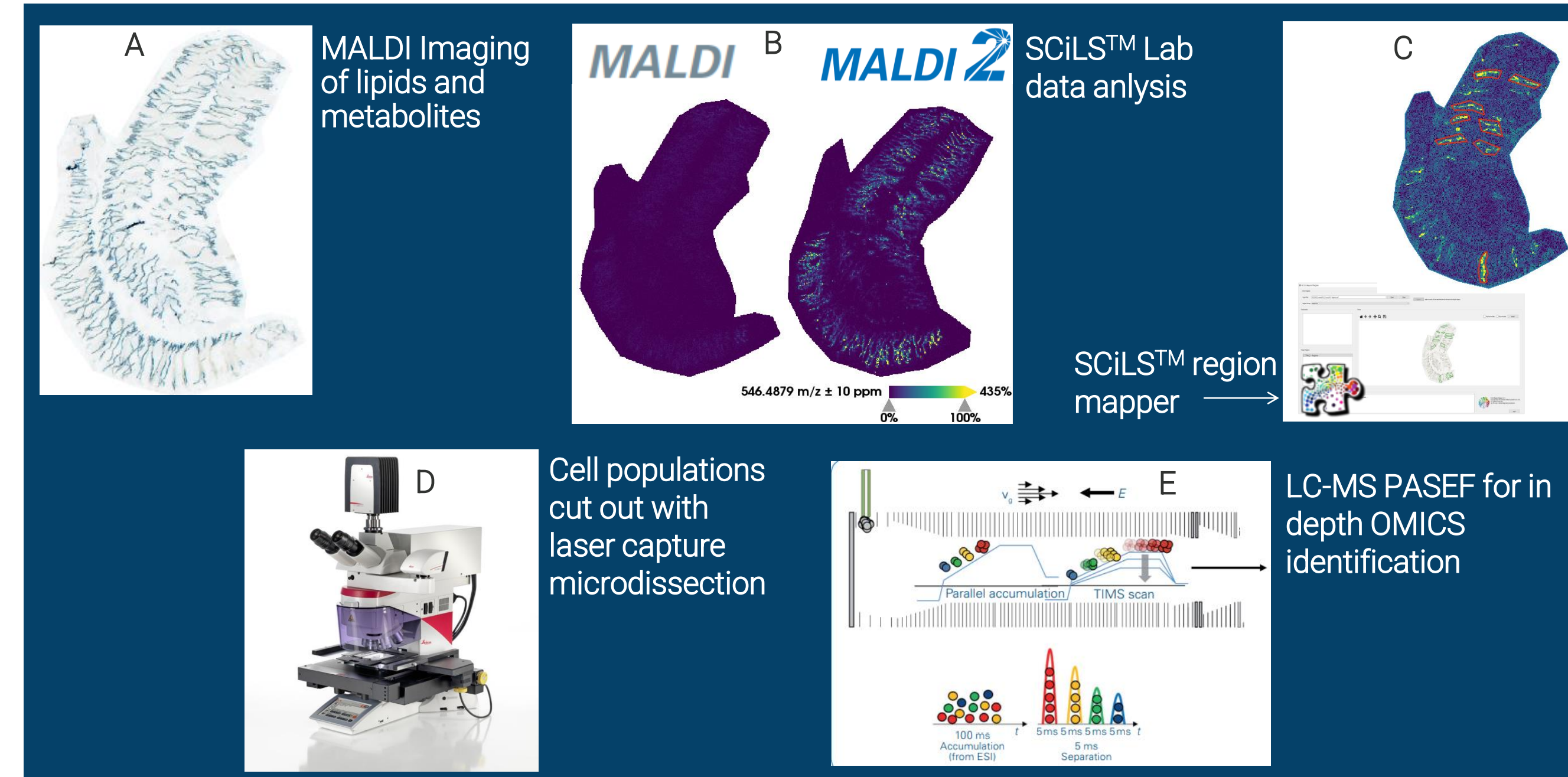


Fig. 1: A: MALDI imaging was performed on a sliced mussel gill sample mounted on an IntelliSlides. The timsTOF fleX MALDI-2 instrument was used for metabolite imaging. Target lists were generated with results from a LC-MS metabolomics approach (MetaboScape) using the same mussel as homogenate. B: SCiLS lab example image, comparison MALDI vs. MALDI 2 C: Interesting areas were transferred to the Region mapper tool as coordinate transfer option for the Laser Capture Microdissection (LCM) instrument. D: LCM was performed. E: In depth 4D proteomics

Methods

Frozen mussel sections of *Bathymodiolus azoricus* were sliced at 10 μm thickness and mounted on IntelliSlides (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). Slides were coated with MALDI DHAP matrix using a TM-sprayer (HTX Technologies, Chapel Hill, USA) and measured on a timsTOF fleX MALDI-2 (Bruker Daltonics) in positive polarity at 10 and 20 μm spatial resolution for lipids and metabolites. Afterwards, regions of interest (ROIs) were selected in SCiLS lab by comparing the segmentation map and m/z features which showed endosymbiont specific metabolites. These ROIs were assigned in SCiLS™ Lab and transferred to the region mapper tool to export readable coordinates for laser capture microdissection (LCM). Tissue extracted by LCM with the Leica LMD 7000 microscope was then used for downstream 4D-Proteomics™ (retention time, m/z and MS/MS fingerprint and the addition of intrinsic CCS values) was performed on a TimsTOF fleX.

Results

Two different bacteria were expected to be present: the sulfur oxidizing symbionts (SOX) and the methane oxidizing symbionts (MOX). Mass spectrometry imaging results revealed these two different, major, spatially segregated bacterial phenotypes (chemotypes) on the metabolite level (Fig. 2). To link the molecular machinery behind the heterogeneous metabolite production to the intracellular microbes, we further analyzed both chemotypes with spatially targeted proteomics. Applying our novel pipeline of spatial metabolomics-guided laser capture microdissection (SpatialOMx), we extracted samples from the immediate host-microbe interface representing each of the chemotypes and successfully detected key proteins from the same bacterial genome as well as key host proteins. In total, we found about 400 bacteria-specific protein groups in both analyses. Additionally, we identified about 1200 mussel specific protein groups. A methane oxidation related cofactor metabolite was visible with MSI (m/z 869)- and chosen for LCM. The proteomics data identified the Methane Mono Oxygenase (MMO) which is part of the same pathway and provided a direct link from the metabolite of interest to the pathway.

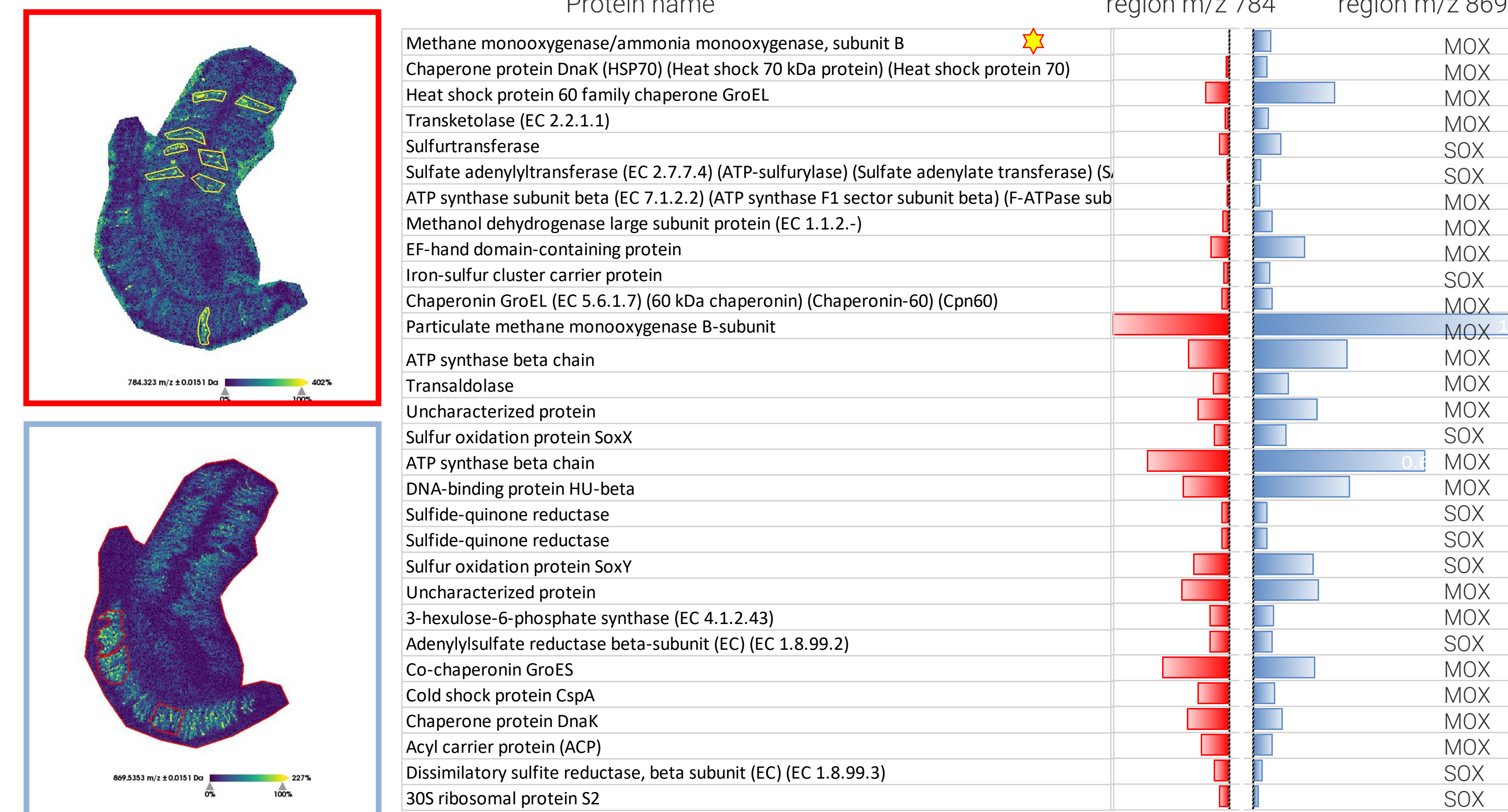


Fig. 2: Two different chemotypes were defined by the region of m/z 784 (images surrounded in red) and m/z 869 (images surrounded in blue). Some protein names and their intensities in the two different chemotypes is shown. A majority of the proteins showed equal intensities in both regions but some of them were differentially expressed. We can assume that the SOX and MOX are present in both areas with slightly more MOX symbionts in the area m/z 869. Methane Mono Oxygenase (MMO) is indicated with a star as this protein was set in the context of its pathway.

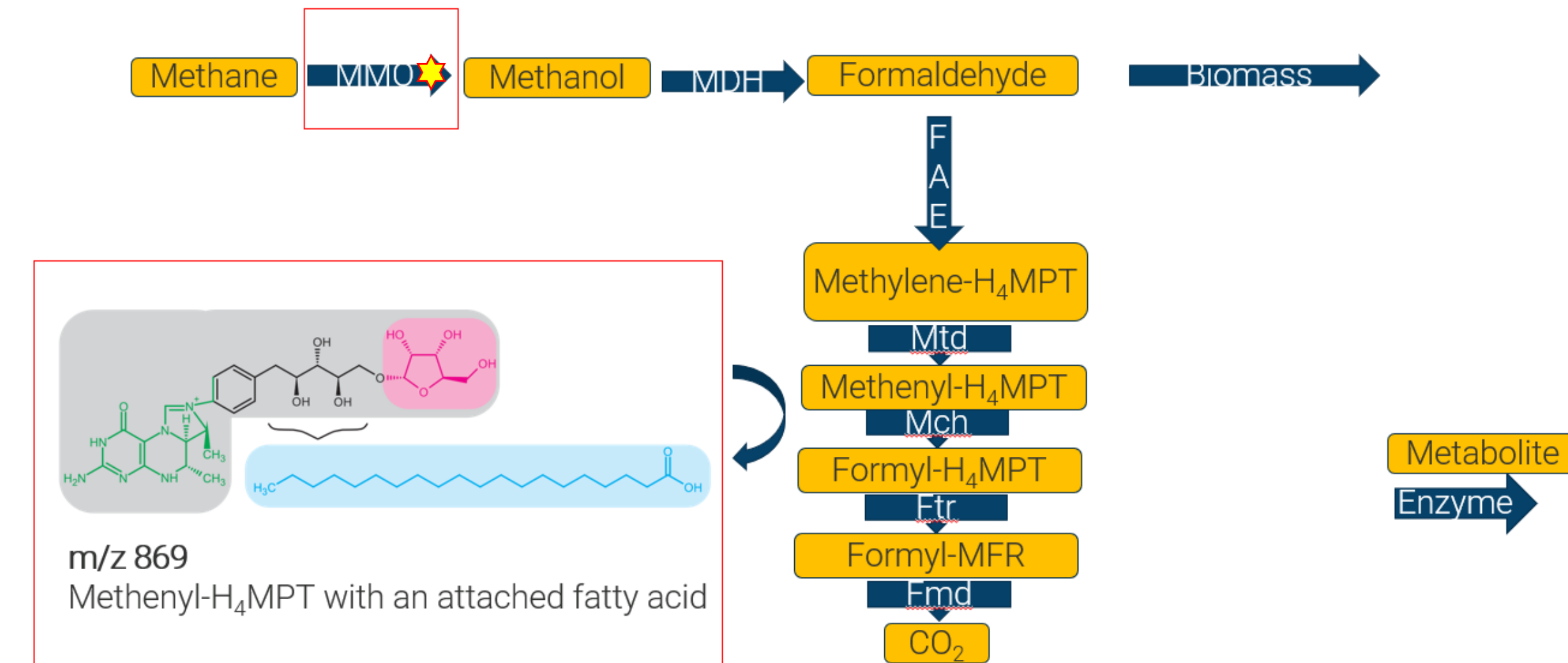


Fig. 3: Pathway illustration of the link between the identified Methane Mono Oxygenase (MMO) to the methane oxidation related cofactor metabolite. (isurrounded in red)

Conclusion

- The SpatialOMx workflow can investigate the microbiome – host interaction in a new dimension
- Biologically relevant ROIs were defined by MALDI imaging and symbiont specific metabolites get visualized
- In combination with a proteome-based characterization of those symbionts, deeper information on growth and active pathways could get identified
- Symbiont-specific areas were cut out using LCM to avoid signal dilution due to the more abundant mussel proteins present
- Differentially expressed proteins are shown and support the theory of different bacteria populations in the mussel

Reference

Spatial metabolomics of in situ host-microbe interactions at the micrometre scale. Geier B et al., Nat Microbiol. 2020 Mar;5(3):498-510. PMID: 32015496

<https://www.leica-microsystems.com/de/produkte/lichtmikroskope/p/leica-lmd7>

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