Non-targeted exploration of metabolic processes and xenobiotic metabolism in plants exposed to micropolutants using mass spectrometry imaging (MSI)

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Introduction

The targeted screening of pollutants in biological samples using LC-MS has been extensively developed. This resulted in quick and easy identification and quantification of pesticides, drugs, toxins or narcotics. The next step is to follow the metabolism of such compounds in living organisms; discover the spatial distributions of compounds and their derivatives (metabolites) in tissues and identify the enzymatic processes which might be involved in the transformation of these compounds. Here we present a workflow based on mass spectrometry imaging coupled to in-silico prediction of metabolites (biological derivatives) and automatic annotation. This enabled the investigation of metabolic processes occurring when living organisms are exposed to pollutants, or more generally to xenobiotics (compounds from exogenous origin).

SCiLS and MetaboScape: Tailored molecular imaging workflow for ID and visualization

Fig. 1: Integrated workflow for compound identification and visualization of MALDI imaging data by SCiLS Lab and MetaboScape.

Methods

White willow (Salix alba) leaves, as well as water and sludge samples were collected near the exit of a wastewater treatment facility. LC-MS analyses were conducted using an impact II QTOF system (Bruker) [2]. Mass spectrometry imaging was performed on the leaf tissues using matrix assisted laser desorption ionization (MALDI) on a Solarix XR 7T (Bruker). Datasets were visualized in SCiLS Lab 2020a to determine and define tissue regions of interest with differentiating m/z localizations. Regions of interest generated in SCiLS Lab 2020a were exported to a pre-release version of MetaboScape 2021 (Bruker) for compound annotation (Fig. 1). For xenobiotic metabolite prediction the BioTransformer® tool “SuperBio” rule set was released to within MetaboScape on a local server (see Fig. 2). For applying the “Environmental BioTransformer” [3] rule set the online version was used. Generated SDF files enabled automatic assignment of xenobiotic metabolites (Fig. 3). These annotations were imported back to SCiLS Lab 2020a to study the tissue localization of the metabolites.

Results & Discussion

• By non-targeted LC-MS metabolite analysis of water, sludge and plant leaf extracts several xenobiotics including the drug Telmisartan could be annotated [1] (data not shown).
• To better understand the biological processes occurring in the white willow leaves exposed to Telmisartan and other xenobiotics, mass spectrometry imaging (MSI) was applied and datasets were investigated using an evaluation pipeline based on SCiLS Lab and MetaboScape softwares.
• Annotation of the dataset with BioTransformer predicted drug metabolites allowed for assignment of several xenobiotics.
• Annotation Quality Scoring enabled to quickly assess confidence for each automatic annotation (see Fig. 3).
• Predicted Phase 1 and 2 metabolites and their reaction mechanisms were investigated by intuitive visualizations (see Fig. 2 and 3).
• Investigation of annotated m/z signals in SCiLS Lab revealed leaf specific tissue localizations for xenobiotic metabolites.
• Tissue specific localizations of predicted Telmisartan metabolites including phase one oxidized forms (C19H29N7O5) and second-generation metabolites (C19H29N7O5) were detected (see Fig. 4).

Summary

The presented MSI workflow proved useful and complementary to LC-MS based environmental metabolomics studies for investigating spatial distribution, metabolic effects and metabolism of xenobiotics in plants (here S. alba) chronically exposed to micropolutants in environmental conditions.

Conclusions

• A software pipeline for targeted exploration of metabolic processes directly on plant tissue exposed to xenobiotics is shown for the first time.
• An integrated workflow based on SCiLS Lab and MetaboScape was applied for MSI data processing.
• The BioTransformer® tool is triggered from within MetaboScape for local, i.e. secure, in-silico metabolism prediction.
• BioTransformer based compound annotation was performed using m/z accurate isotope pattern fitting extracted by the T-ReX® algorithm.
• The presented workflow enables assignment of the "dark matter" of the metabolome – molecules that remained uncharacterized before.

References & Notes


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