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The *Caenorhabditis elegans* lipidome blueprint – established using UHPLC- QTOF-MS and UHPLC-IMS-QTOF-MS

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Introduction

The small nematode *Caenorhabditis elegans (C. elegans)* is a premier model organism in biomedical research and used in different research fields such as development, ageing, neurobiology or host-microbe interactions. As part of this research, lipids are nowadays analyzed on a routine basis. Despite the large knowledge collected, no reference database for the *C. elegans* lipidome exists. Here, we used data curated from literature and evaluated lipid extracts of *C. elegans* cultivated under different growth conditions. Data of these extracts was acquired on two different MS platforms and lipids were automatically annotated and manually validated in order to derive a blueprint for the *C. elegans* lipidome.

Methods

We curated lipids detected in *C. elegans* from multiple publications from 2007 to 2022. All lipids identified were adapted to the nomenclature recommended by the Lipidomics Standards Initiative (LSI) to allow for a comparison between previous publications and render the generated database future proof.

Multiple datasets previously generated on a maXis Q-TOF-MS as well as new reference sample measurements performed on a timsTOF Pro 2 IMS-QTOF-MS (Bruker Daltonics) were used for in-depth annotation of *C. elegans* lipids. Separation of lipids was achieved using a Waters Cortecs UPLC C18 column (150 mm x 2.1 mm, 1.6 µm) and 40% H2O / 60% ACN + 10 mM ammonium formate / 0.1% formic acid as eluent A and 10% ACN / 90% iPrOH + 10mM ammonium formate / 0.1% formic acid as eluent B. Data processing and investigation was performed using the MetaboScape 2023b software. Glycerolipids, glycerophospholipids and sphingolipids were identified by rule-based lipid annotation. All identifications were manually validated.

Results

In order to generate a first version of a *C. elegans* lipid database, lipids were curated from literature. Forty-five articles from the years 2007 to 2022 were included in this curation. Lipids were searched in figures, tables, and supplementary information. Since lipids are reported on different levels of detail, all of them were normalized using the most recent LSI notation. In total, 16347 lipid-literature associations were collected, with over 2700 unique lipid species from 38 lipid classes. In several articles, different isomeric species of lipids were potentially detected and reported.

In addition to lipids curated from literature and detected in the different datasets, potential lipid species were predicted based on fatty acids known to occur in *C. elegans*.

12 different data sets (10 from maXis and 2 from timsTOF) were analyzed using MetaboScape. The processing workflow included *m/z* recalibration, peak detection and grouping, alignment and lipid annotation. On average, around 600 lipids were identified per dataset. Generally, more than 90% of initially automatically annotated lipids could be confirmed by manual validation. No systematic trend in identification rates between lipid classes were observed. Here, only results from timsTOF Pro 2 are shown.

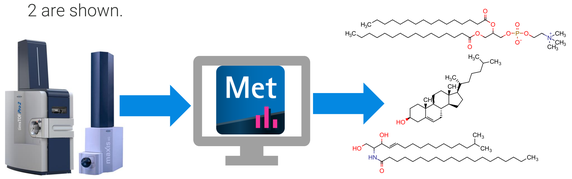

Fig 1. Workflow of data analysis

Rule-based lipid annotation was able to correctly annotate lipid species, which are not covered in public databases so far, e.g. in *C. elegans* specific sphingolipids containing C17-iso branched-chain sphingoid bases. Use of CCS trendlines and CCS prediction helped to reduce false positive annotations.

Covered lipid species in curated, predicted or detected datasets were compared on a species level. Coverage was evaluated by plotting the number of double bonds against the number of carbons in all side chains (Fig. 2). Lipids curated from the literature were also filtered to be present in 5 to 9, or more than 10 publications.

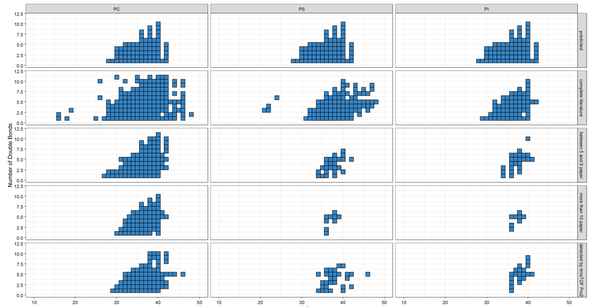

Fig. 2 PCs, PS and PIs as selected examples for the comparison of lipid species covered. Lipids detected by LC-IMS-MS/MS are used for comparison. Each tile represents a curated, predicted or detected lipid species composition, respectively.

The space covered between the prediction, curated literature and PCs detected on the timsTOF Pro 2 is comparable (Fig. 2). 82% of the detected PC species have been either described in the literature or predicted. Remaining 18% are new species, which need further investigation (mostly containing long and very long acyl chains). Predicted spaces for PS and PI are comparable to PC, since no lipid class specific fatty acyl profiles have been used. Taking into consideration all possible species from literature similar spaces are covered but are drastically reduced when filtering for species detected multiple times. These reduced spaces could be confirmed by measurements on the timsTOF Pro 2, indicating potential over-annotation in some of the used references.

Several lipid classes, previously described in *C. elegans* are not covered in the rule-based annotation database of MetaboScape so far, such as mono-methyl phosphoethanolamine glucosyl ceramides (mmPEGC), which to our knowledge are so far only described by Boland *et al. [Nat Chem Biol. 2017 Jun;13(6):647-654.].* The main species detected in this previous study were mmPEGC-C22, -C23 and -C24. These mmPEGCs were also identified in several of the *C. elegans* extracts investigated here based on manual search and interpretation of MS and MS/MS data. By using Kendrick mass defect plots and visualization of retention time vs. CCS trend lines for the LC-ion mobility MS data, additional species were annotated including mmPEGC-C21 and mmPEGC-C25 (Fig. 3). These lipids show different abundance in different life stages of *C. elegans* (Fig. 4)

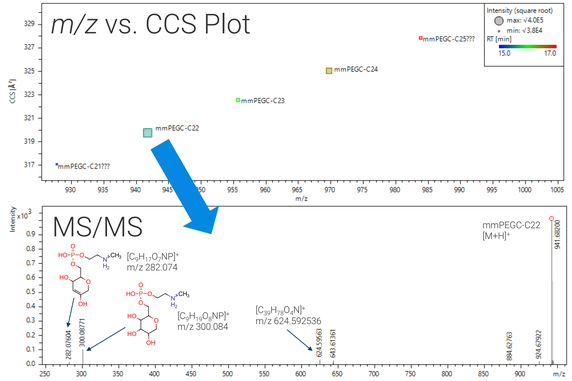

Fig. 3. Annotation of mmPEGCs in *C. elegans* samples using CCS trend lines and MS/MS. As example the MS/MS of mmPEGC-C22 is shown

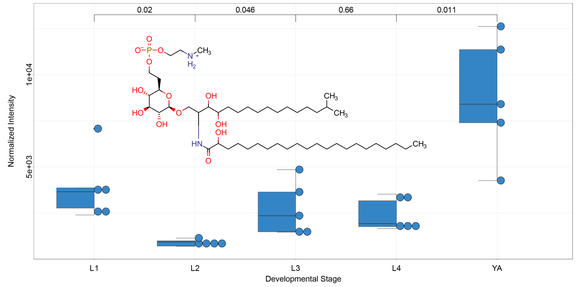

Fig. 4 Intensities of mmPEGC-C22 across different life stages of *C. elegans*

Conclusion

- MetaboScape enables the non-targeted annotation of lipid species in *C. elegans*
- More than 90% of the automatically generated lipid annotations (n ~600) could be validated manually
- Use of CCS trend lines and CCS prediction helped to remove false positive annotations
- MetaboScape allows for annotation of novel lipid species not contained in predefined rules for automatic annotation

LC-TIMS-MS