Rapid Screening and Dereplication of Microbial Natural Products Using Data Independent Acquisition (DIA) UPLC-QTOF-MS Coupled with Streamlined Informatics

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INTRODUCTION

- Liquid chromatography (LC) coupled to a high-resolution mass spectrometry (MS) such as quadrupole time-of-flight (QToF) is becoming the most widely employed analytical platform for identification and molecular characterization of natural products
- Data independent acquisition (DIA) approaches, such as SONAR and MSE, can simultaneously provide the exact mass precursor and corresponding fragmentation pattern for identification without little prior knowledge of analytes
- Here we present a high-throughput and automated identification of marine microbial compounds using high-resolution UPLC/QTof MS technology and Natural Product Application Solution (NPAS) with UNIFI. The custom microbial database, developed in collaboration with Prof. Roger Linington from Simon Fraser University

METHODS

MICROBE PREPARATION

Microbes were isolated from marine sediment and grown under standard fermentation conditions with XAD-16 resin, extracted with 1:1 methanol/ dichloromethane, and fractionated on a reverse phase C18 column with an eleutropic series of water and methanol (20%, 40%, 60%, 80%, 100% methanol, and EtOAc) after first washing off polar molecules with 10% methanol in water. These fractionated extracts or prefractions were dried and re-suspended in 1 mL of dimethylsulfoxide (approximately 100 mg/mL). They were then diluted 1 to 40 into DMSO. This 1 to 40 solution was diluted 1 to 25 into 50:50 (MEOH:H2O). This 1 to 1000 solution was diluted 1 to 20 into 50:50 (MEOH:H2O) for a final dilution factor of 20,000 (approximately 5 µg/mL).

LC PARAMETERS

LC system: ACQUITY UPLC I-Class with FTN Sample Manager

Column: ACQUITY UPLC BEH 2.1 x 50 mm, 1.8 µm, 50 °C

Sample temperature: 10 °C

Mobile Phase: A: water (0.1% FA); B: acetonitrile (0.1% FA)

Flow Rate: 0.8 mL/min

MS CONDITIONS

MS system: Xevo G2-XS QTof MS

Acquisition range: 50-1800 Da (0.1 s scan rate)

Acquisition mode: MS^E, ESI^- and ESI^+ in resolution mode

Capillary voltage: 3 kV (ESI^+)/2.5 kV (ESI^-)

Cone voltage: 30 V

Collision energy (eV): Low CE: 6; High CE: 25-45

Source temp.:120 °C

Desolvation temp.: 500 °C

DATA PROCESSING PLATFORM

The data processing platform integrates image-based phenotypic profiling data from our recently reported cytological profiling platform with untargeted metabolomics data from UPLC/QTof MS platform. MS data was collected using data independent acquisition (MS^E) which simultaneously provides exact mass precursor and corresponding fragment ions for identification and structural elucidation. Using a custom informatics platform, image-based phenotypic and UPLC/QTof MS datasets are integrated to identify candidate molecules that are consistently positively correlated with specific phenotypes. Using network display, the bioactive metabolome from the natural product library is then displayed as an annotated network diagram that identifies all sets of bioactive molecules from within this set, allowing the selection and development of high priority lead compounds.

RESULTS & DISCUSSIONS

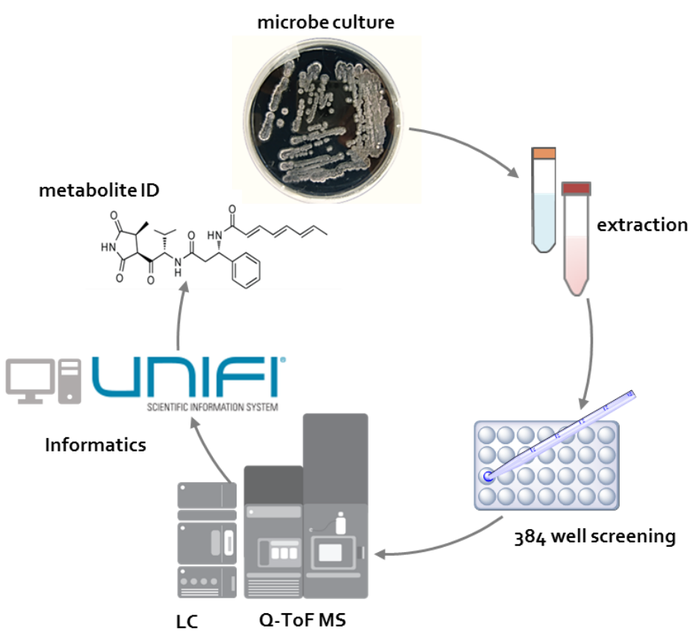

Figure 1. *High resolution quadrupole mass spectrometry based screening workflow platform for natural product discovery from microbial culture*

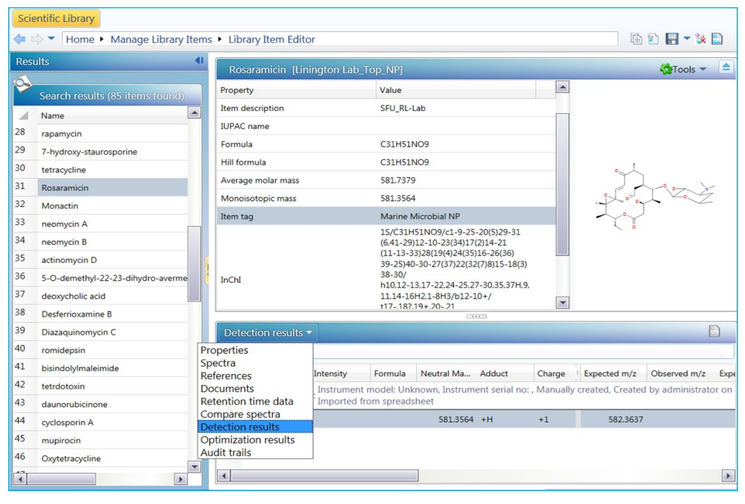

Figure 2. *A basic infrastructure of a marine natural products library in UNIFI.*

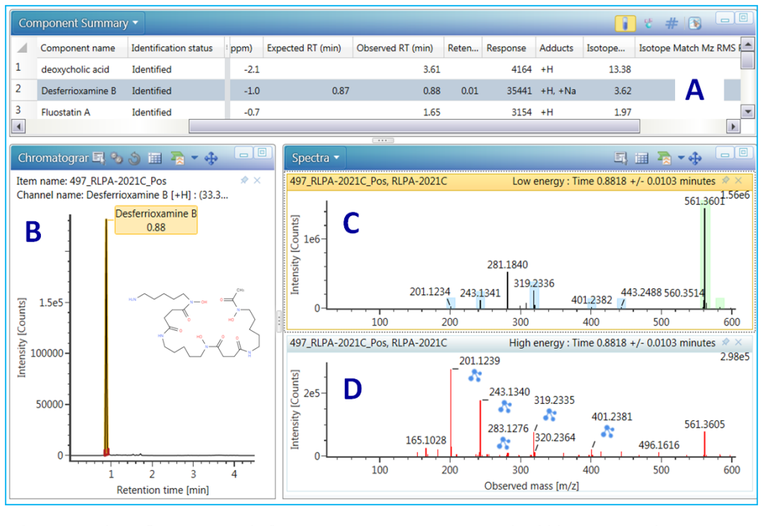

Figure 3. *Identification result from a custom marine microbial library. (A) The component summary interface; (B) Selected ion chromatogram of single component corresponding to panel A (C) The respective low energy precursor exact mass spectrum and (D) The corresponding high energy fragment ion spectrum. In the hig energy MSE spectrum, the blue "" mark indicates the experimental fragment ion that matches to the expected in silico fragment ions generated from the mol structure using MassFragment.*

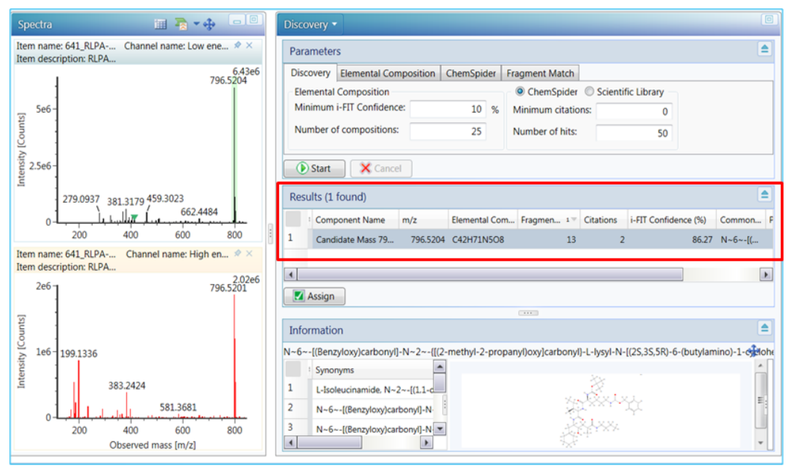

Figure 4. *Structural elucidation for the identification and confirmation of the unknown components.*

Structural elucidation tools in UNIFI was used for the identification of unknown high intensity peaks. Batch search can be performed using online databases such as Chemspider. The key steps for the unknown structural elucidation are (Figure 5):

1. Set the basic search parameters such as possible elemental composition, fragment match and isotopic distribution in the discover.
2. Search against Chemspider database (on-line) containing about 600 libraries.
3. The initial match was validated and confirmed by analyzing fragment ions obtained in MS^E scan via Embedded *in-silico* tool MassFragment™.
4. After final identification results are reviewed and confirmed, the identified compounds can be sent to the scientific library with all information including exact mass, retention time and fragment ion.

CONCLUSION

- The Natural Products Application Solution with UNIFI provides a single workflow for data acquisition, processing and confident compound identification based on low energy precursor exact mass, theoretical isotopic distribution and corresponding high energy fragment ion information from custom marine microbial scientific library or Chemspider.
- Integrating image-based screening and high-resolution UPLC/QTof MS provides a comprehensive annotation of the identities and biological attributes of all bioactive constituents.
- This technology provides natural products chemists with a new mechanism to convert complex metabolomics profiling of extract libraries into a Compound Activity Map, which clusters extracts and metabolites based on common chemical and biological properties and highlights those compounds predicted to be responsible for the observed phenotype of a particular extract.
- In the future we are planning to connect the Natural Product Atlas Library (https://www.npatlas.org) directly with UNIFI for rapid natural products dereplication in identifying novel active compounds.

REFERENCES

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3. Microbial Databases for Rapid Screening and Dereplication of Microbial Natural Products Using UPLC-QTof-MS Coupled to Novel Informatics Platform. Waters. 2018;720006412EN

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