Analysis of Novel Bioproduct Pathways in Populations of *Arthrospira platensis* at Microorganism Resolution

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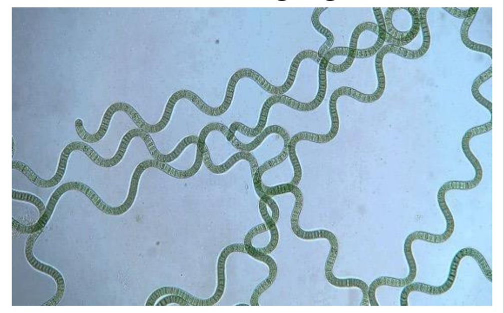
OVERVIEW

Arthrospira platensis (Spirulina) is a filamentous cyanobacterium that has received much attention thanks to its potential as a renewable source of fuels and bioproducts, and current commercial-scale cultivation. Recent progress in metabolic engineering of Spirulina allows for the rapid and directed tailoring of both growth improvements and synthesis of novel bioproducts. Identifying which pathways are responsible for lipid production under stressed and mixed population conditions presents a unique analytical challenge. Here we demonstrate that ultrahigh-resolution imaging mass spectrometry (IMS) is suitable for global lipidome profiling in single microorganisms. MALDI imaging coupled with Fourier transform ion cyclotron mass spectrometry was applied for the analysis of population heterogeneity in bioproduct yield and investigation of lipid rearrangements within wax ester producing strains.



RELEVANCE / BACKGROUND

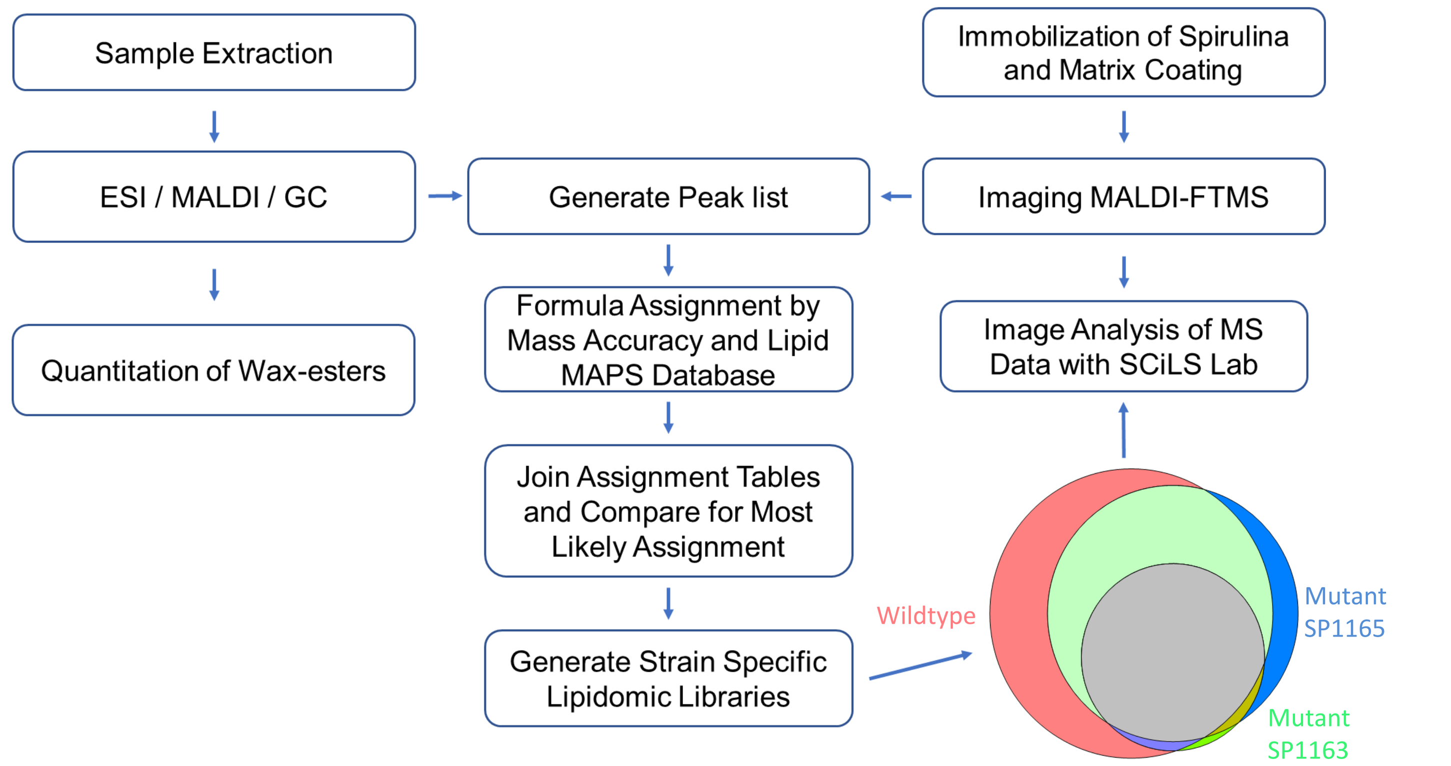
- A high-throughput monitoring strategy that recognizes lipid fluctuations due to stress or lipid speciation events without sample preprocessing could provide timely information on e.g. population heterogeneity and physiological adaptation to environmental stressors
- Variability in single filaments can skew results and give falsely high (or low) values to a particular cell type during culture evaluations
- Matrix assisted laser desorption ionization, imaging mass spectrometry (MALDI-IMS) is powerful technique that is useful for characterization of lipids
- We report, for the first-time, high-resolution MALDI-IMS for unambiguous visualization and identification of lipids associated with a single-algae-filament.
- Lipidomic investigation is used to direct trait engineering and model the conversion of biomass to high value products.

SUMMARY

- Spirulina samples were immobilized on plasma-cleaned glass microscope slides. A key innovation of this method was the removal of salt prior to immobilization and subsequent sublimation without the delocalization of filaments. DHB and super-DHB matrices were evaluated for lipidomic coverage. A Bruker Solarix 7 T FT-ICR MS was used for data collection, and SCiLS Lab software was used to process and analyze data. High resolution and mass accuracy were utilized to determine elemental formula assignments for lipids, and assignments were confirmed with a custom database matching algorithm. Statistical population analysis was utilized to understand the diversity of lipid production between and within algae populations. Sample extracts were collected for compositional analysis, lipid quantitation, and lipid assignment validation by MS/MS..

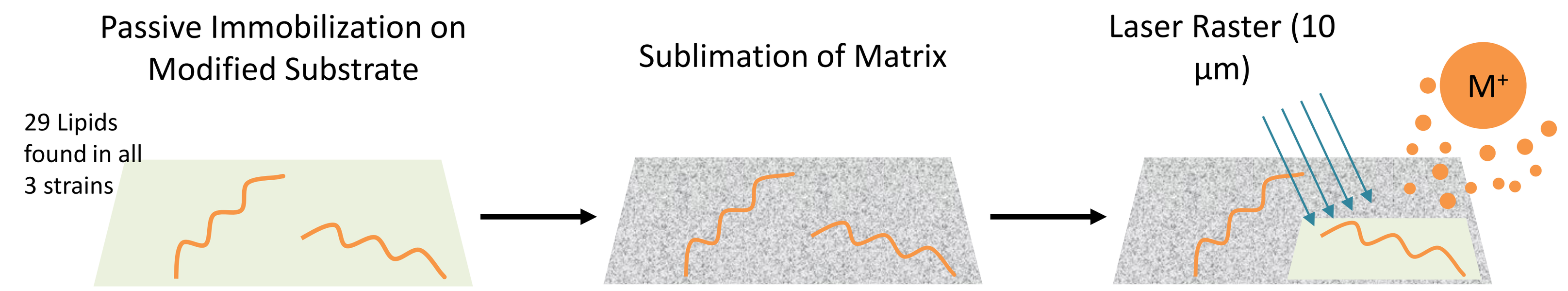
METHODS

Technical Workflow



Venn Diagram showing lipid IDs between different strains of A. *platensis*.

Imaging Mass Spectrometry



Scheme 1. Schematic representation of single filament immobilization on hydrophilic glass, matrix sublimation, and ion image acquisition.

Image Analysis Processing

CONCLUSIONS

- An optimized method for sample immobilization and matrix deposition enabled ion imaging of algae filaments at 10 µm resolution.
- A high-throughput monitoring strategy that recognizes lipid fluctuations due to stress or lipid speciation events without sample preprocessing could provide timely information on e.g. population heterogeneity.
- The novel approach we are developing here combines the use of a custom lipidomics database as a training tool for robust full lipidomics profiling of uncharacterized microbial species
- Metabolic engineering of A. platensis to produce novel lipids can be detected by high-throughput lipidomics and by MALDI – imaging mass spectrometry (MALDI-IMS)
- This approach has many potentially useful applications, including early signs of genetic drift within a cell population, metabolic engineering optimization and general culture health monitoring where univariate and multivariate analytical approaches can be applied to MALDI-imaging data

ACCOMPLISHMENTS AND PROGRESS

High Spatial Resolution Imaging

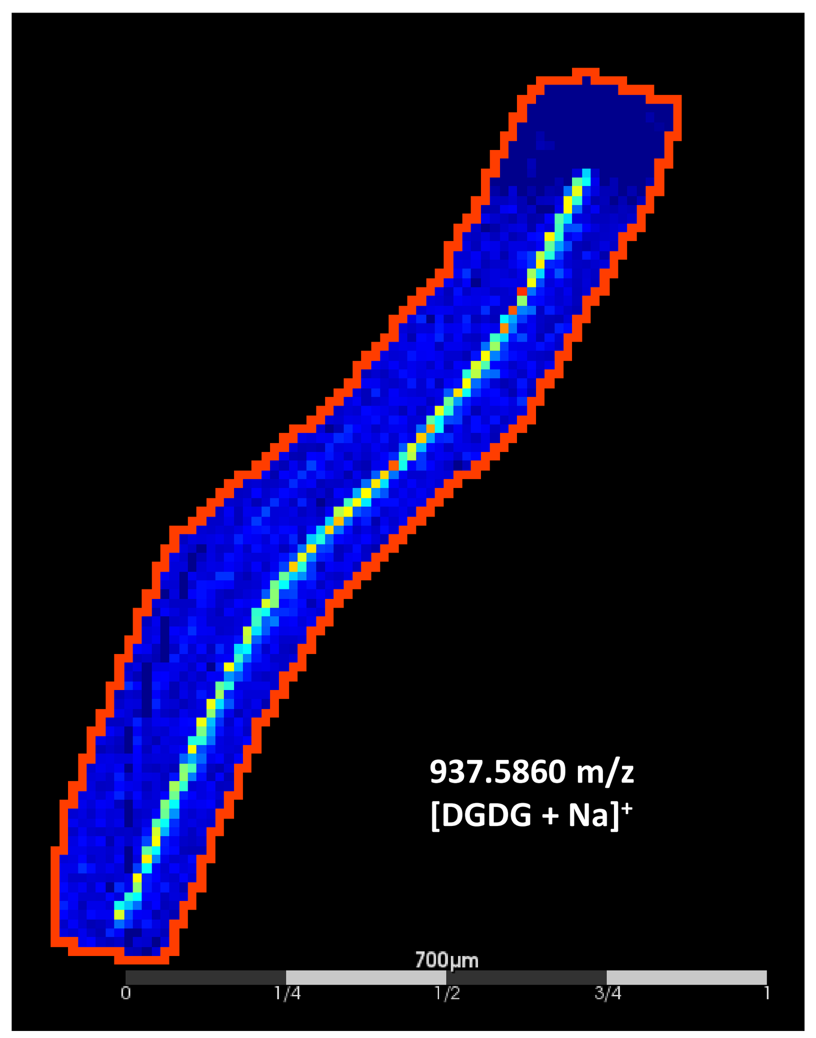


Figure 1. 10 µm spatial resolved MS images of single filaments.

High throughput Screening of Dense Cultures

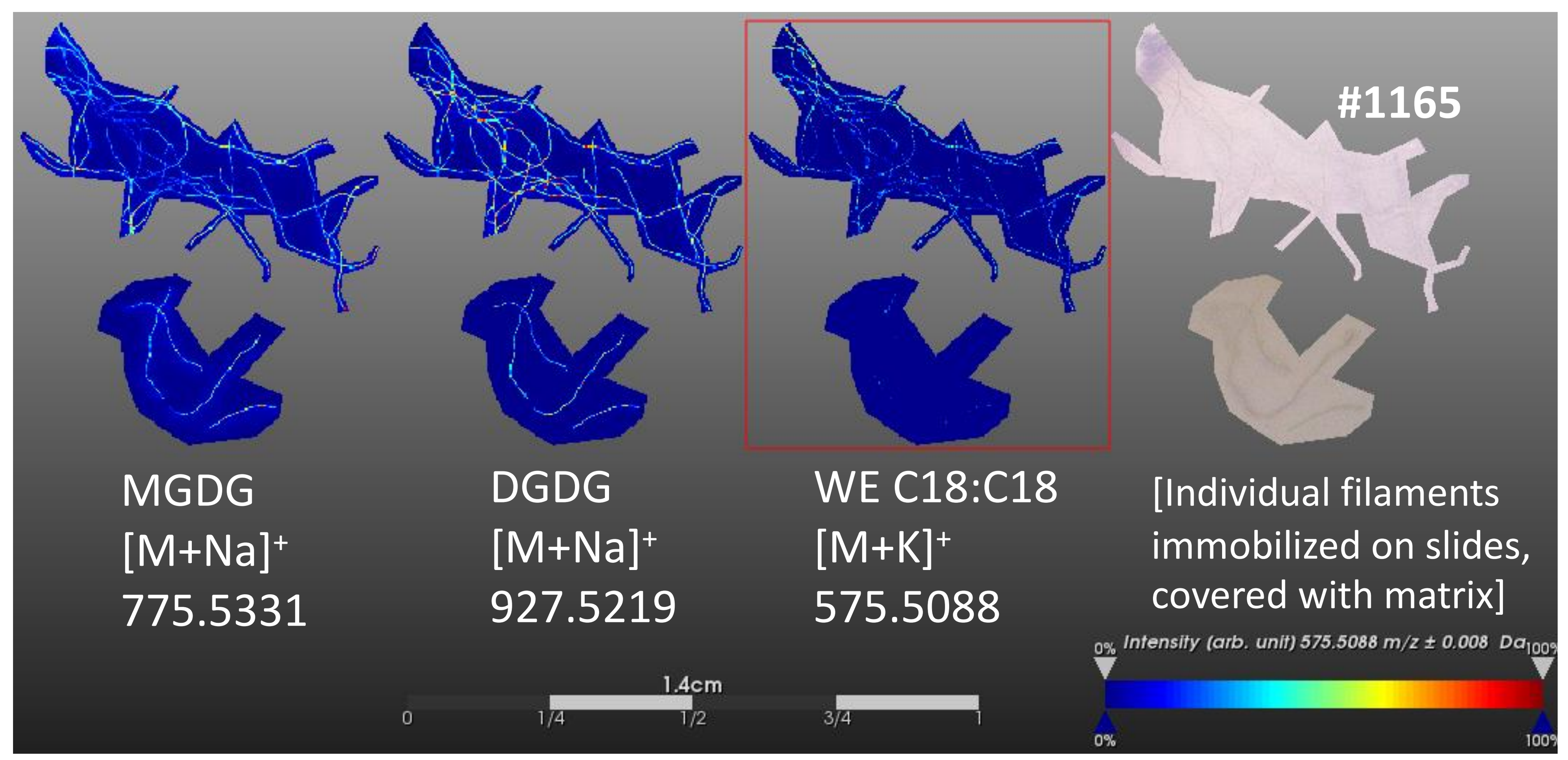


Figure 2. MALDI FTICR imaging analysis of replicate filaments from algae cultures demonstrating population heterogeneity at 10 µm resolution. Mutant strains selectively express wax esters. Ion images of assigned lipids, MGDG, DGDG and wax ester expressed uniquely in genetically engineered and wildtype strains of A. platensis.

Single Filament Separation and Interrogation for Discriminate Analysis of Population Heterogeneity

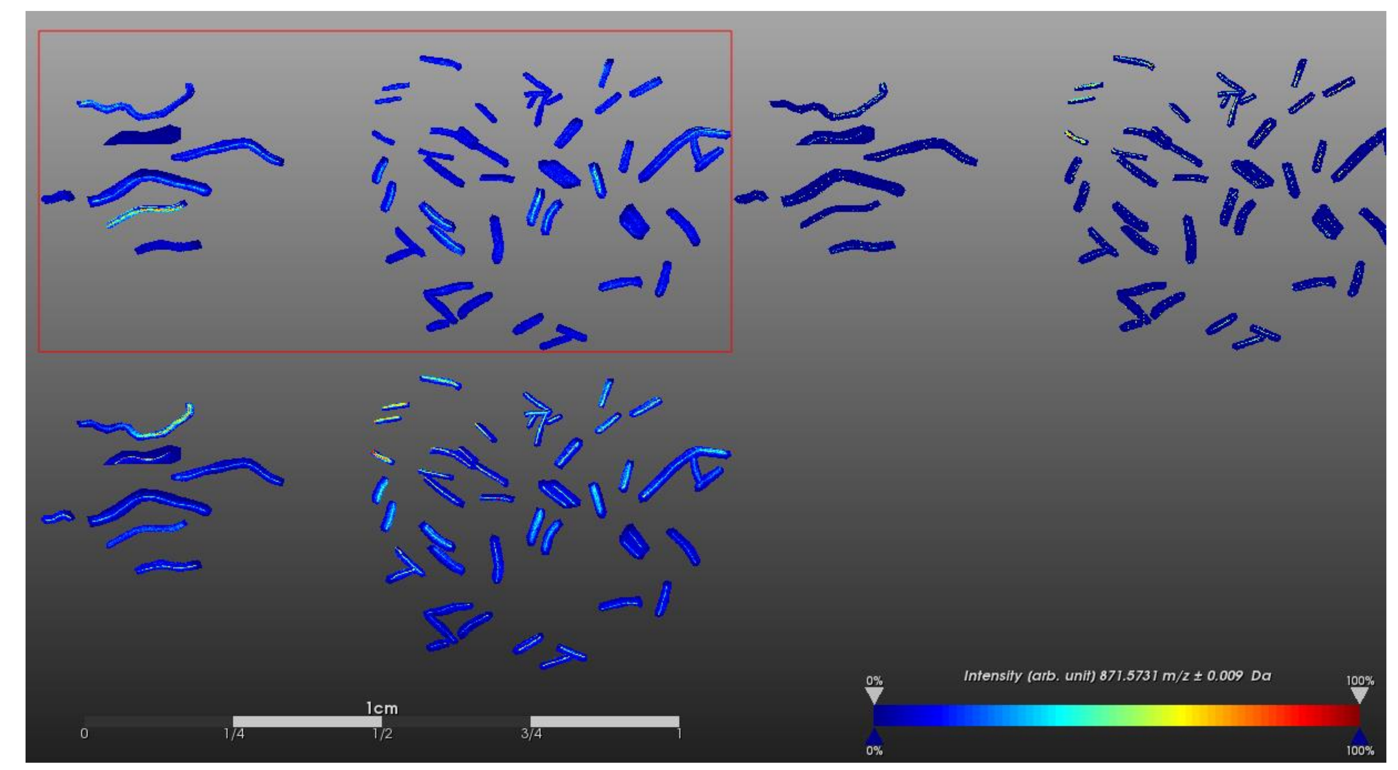


Figure 3. Single filaments were immobilized for IMS analysis Three ion images showing lipid heterogeneity within population.

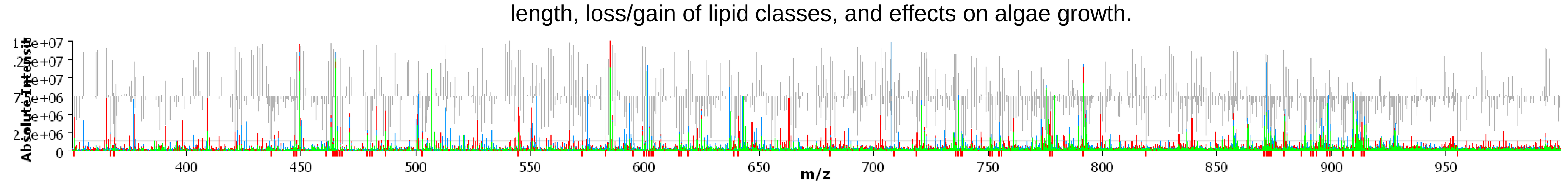


Figure 4. Discriminate PCA investigation of single filaments. Results show that transgenic algae express several spatially resolved phenotypic traits, including lipid rearrangements in acyl chain length, loss/gain of lipid classes, and effects on algae growth.

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