Enhanced ocean metaproteomic profiling with Orbitrap Astral MS in data independent acquisition

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Abstract

Herein we present a comprehensive metaproteomic profiling of a marine microbial community using the Thermo Scientific™ Orbitrap™ Astral™ Mass Spectrometer. A highly complex ocean sample from North Atlantic Ocean was profiled using multiple data acquisition strategies including Data-Dependent Acquisition (DDA) and Data-Independent Acquisition (DIA) to determine which approach enhances depth of coverage.

Tryptic digest of the sample was analyzed using chromatographic gradients spanning 14, 30 and 60 min. in both DDA and DIA modes. Data processing was performed using Thermo Scientific™ Proteome Discoverer™ 3.2. software and Spectronaut® 19 with a custom-built database.

DIA offered remarkable depth with 25,892 proteins and 81,815 peptides, with a dynamic range of protein abundances expanding approximately six orders of magnitude. In comparison, DDA data identified 18,363 protein groups and 53,316 peptides, offering a less extensive coverage. Taxonomic analysis covered 52 bacterial phyla, representing a threefold increase compared to previously reported results[1]. In addition, high functional diversity was observed in the DIA results from KEGG Orthology (KO) pathway analysis.

Introduction

Ocean metaproteomic studies play a crucial role in advancing our understanding of marine microbiomes and their influence on global biogeochemical processes. The extreme complexity of marine samples makes a significant challenge for achieving deep proteome coverage. Recent advancements in Mass Spectrometry (MS), particularly with the Thermo Scientific™ Astral™ analyzer, have significantly enhanced sensitivity and scan rate, thereby improving proteomic depth. While DDA remains the most widely used approach in the oceanic and other metaproteomic studies, DIA has gained traction for its ability to deliver deep protein profiling in complex samples. In the present study, we generated comprehensive metaproteomic datasets utilizing DIA mode. High sensitivity and rapid acquisition of MS/MS spectra by the Astral analyzer led to a significantly higher number of identified proteins and peptides.

Materials and methods

Sample Preparation

Ocean metaproteomic samples were collected from the euphotic zone of the North Atlantic Ocean (31.66 N 64.166 W, depth 80m, on June 16th, 2018) as a part of an interlaboratory LC-MS comparison project[1]. Samples were prepared by Waldbauer lab at University of Chicago. First, the filtered oceanic sample was treated with 2% Sodium Dodecyl Sulfate, sonicated at 95° C and acetone precipitation was performed. The protein pellet was then cleaned up and digested using a standard FASP protocol.

LC-MS/MS Analysis

A Thermo Scientific™ Vanquish™ Neo UHPLC system equipped with Aurora Ultimate™ XT 25 cm x 75 µm C18 UHPLC column coupled to an Orbitrap Astral mass spectrometer was used for sample analysis. Data acquired at two different gradient lengths including 30 and 60 minute, with and without Thermo Scientific ™ FAIMS Pro Duo Interface, employing both DDA and DIA modes. In both DIA and DDA, MS1 spectra were acquired in Orbitrap™ at 240,000 resolution with 5ms injection time and MS2 spectra acquired in Astral at 80,000 resolution with 2 *m/z* isolation width and 3.5 ms injection time.

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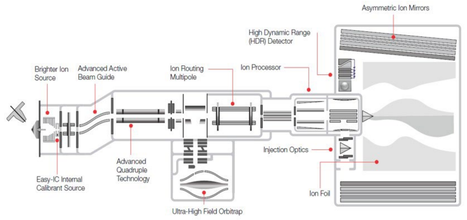
Data Analysis

Raw files were searched against the metagenomic database[1] and analyzed using Proteome Discoverer 3.2. using CHIMERYS™ intelligent search algorithm and Spectronaut® 19

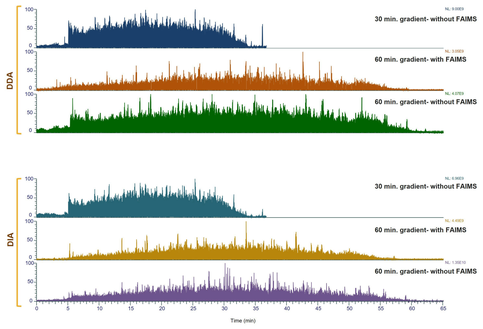
Results

Figure 1. Schematic diagram of Orbitrap Astral Mass Spectrometer



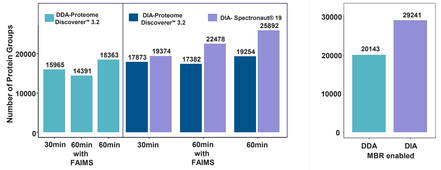
To assess the depth of metaproteomic analysis using the Orbitrap Astral MS in DIA mode versus DDA mode, two LC-MS/MS gradients were evaluated, both with and without the use of the FAIMS Pro Duo interface.

Figure 2. Total ion chromatograms of ocean metaproteomic sample analyzed by DDA and DIA



The number of protein groups identified using 30- and 60-minute gradients with 500 ng sample injections were significantly higher in DIA mode compared to DDA mode(Fig.3). DIA data analyzed using Spectronaut® 19 yielded the highest number of protein groups identified, with the 60-minute method identifying 22,478 protein groups with FAIMS and 25,892 protein groups without FAIMS. In comparison to the protein groups identified by all three methods in DDA, DIA increased protein identifications by 45%.

Figure 3. Comparison of the number of protein groups identified by each method



Similar trends were observed in peptide group identifications, with the 60-minute method identifying 63857 peptide groups with FAIMS and 81815 peptide groups without FAIMS (Fig.4). The peptide groups detected by the Orbitrap Astral MS is ~5 times greater compared to those reported in a prior inter-laboratory study using the same sample[1].

Figure 4. Number of peptide groups identified by each method

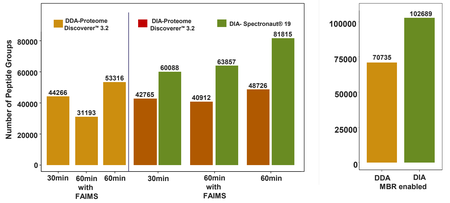
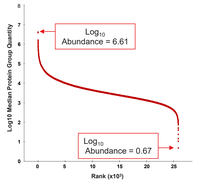


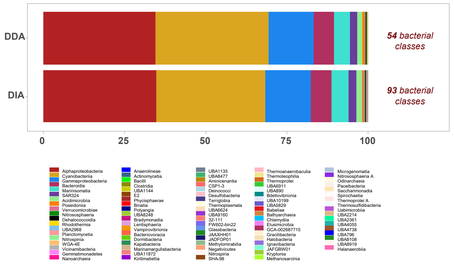
Figure 5. Ranked protein groups



The signal intensities of protein groups exhibit a dynamic range spanning approximately six orders of magnitude (Fig.5). This analysis was performed using data collected under DIA mode with 60-minute gradient without FAIMS. The results highlight the sensitivity of the DIA mode in Astral, as well as the depth of coverage, which enables detection of proteins expressed at very low levels.

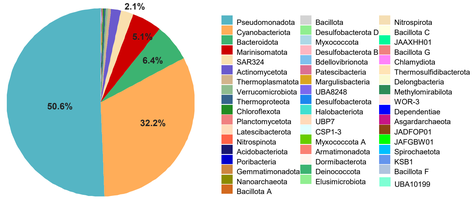
A taxonomic analysis was performed on total peptides identified through match-between-runs across three sample runs for both DDA and DIA (Fig.6). Consistent taxonomic distribution patterns were observed between the two acquisition types, with Alphaproteobacteria and Cyanobacteria being the most abundant classes. Overall, the DIA data covered 93 bacterial classes, while the DDA data covered only 54 bacterial classes.

Figure 6. Taxonomic distribution comparison between DIA and DDA (MBR)



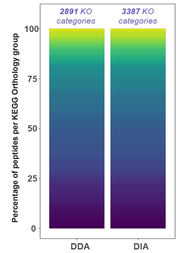
In ocean metagenome annotations, Proteobacteria, Bacteroidetes, and Cyanobacteria are among the most abundant bacterial phyla. Notably, Proteobacteria are especially prevalent in deep-sea environments, while Cyanobacteria are more abundant in surface waters. The 93 bacterial classes identified through DIA (MBR) analysis represented 52 phyla (Fig. 7), whereas DDA (MBR) analysis identified only 38 phyla. Among these, Pseudomonadota (Proteobacteria), Cyanobacteriota, and Bacteroidota were the most dominant, reflecting the common patterns observed in metagenomic annotations. Additionally, the Orbitrap Astral MS data showed three times higher phyla coverage compared to the combined results reported in the inter-laboratory project using the same sample[1].

Figure 7. Phylum distribution of data acquired by DIA



Similar trends were observed in the KEGG Orthology (KO) group analysis regarding functional diversity (Fig.8). The distribution of peptides across KO categories was comparable between DDA and DIA datasets. However, DIA demonstrated a clear advantage in the number of KO categories identified with 3,387 detected compared to 2,891 in the DDA dataset. The higher number of KO categories identified in DIA data could reflect its enhanced sensitivity in detecting lower-abundance peptides, which may be underrepresented in DDA.

Figure 8. KEGG Orthology (KO) group analysis: DIA vs. DDA (MBR)



Conclusions

The results clearly demonstrate the high potential of the Orbitrap Astral mass spectrometer in DIA mode for metaproteomic analysis, highlighting its effectiveness and reliability for comprehensive profiling.

- Over 25,000 protein groups were identified from a single sample run in DIA mode, sequencing 81815 peptides.
- High sensitivity and dynamic range enhance the detection of proteins expressed at very low abundances.
- The increased identifications in DIA suggest that this approach better suited for metaproteomic analysis, where high taxonomic and functional diversity is expected.

References

1. Saito, M. A., et al. 'Results from a Multi-Laboratory Ocean Metaproteomic Intercomparison: Effects of LC-MS Acquisition and Data Analysis Procedures'. Biogeosciences 21 (2024).
2. Dumas, T., Martinez Pinna, R., Lozano, C. et al. The astounding exhaustiveness and speed of the Astral mass analyzer for highly complex samples is a quantum leap in the functional analysis of microbiomes. Microbiome 12, 46 (2024).

Acknowledgements

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