

TACKLING CHALLENGES IN CLINICAL METAPROTEOMIC ANALYSIS OF BRONCHOALVEOLAR LAVAGE FLUID TO CHARACTERIZE MICROBIAL CONTRIBUTORS TO CYSTIC FIBROSIS



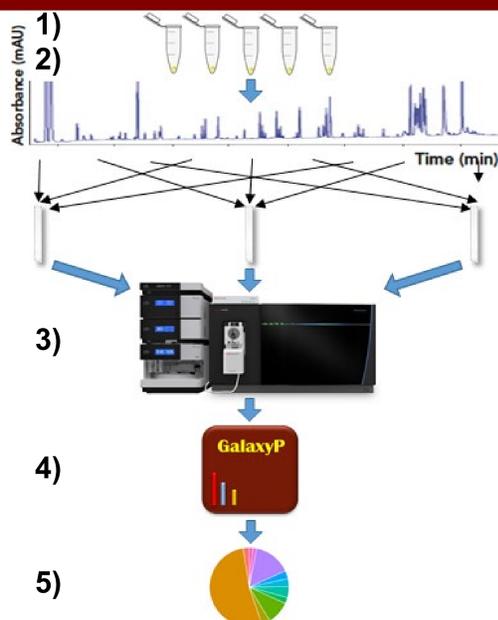
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Introduction

- The role of airway microbiota in the development and progression of cystic fibrosis (CF) disease remains unclear.
- MS-based metaproteomics of bronchoalveolar lavage fluid (BALF) can provide a unique look into the functional role of the lower airway microbiota in CF.
- We used liquid chromatography (LC)-based peptide fractionation, ion-mobility-based gas-phase fractionation (FAIMS) and high-resolution MS/MS, coupled with cutting-edge metaproteomic bioinformatic tools to characterize the metaproteome of cells isolated from BALF of CF patients.
- Our results, though preliminary, demonstrate insights into the CF microbiome, microbial contributions, and functional-taxonomic relationships.

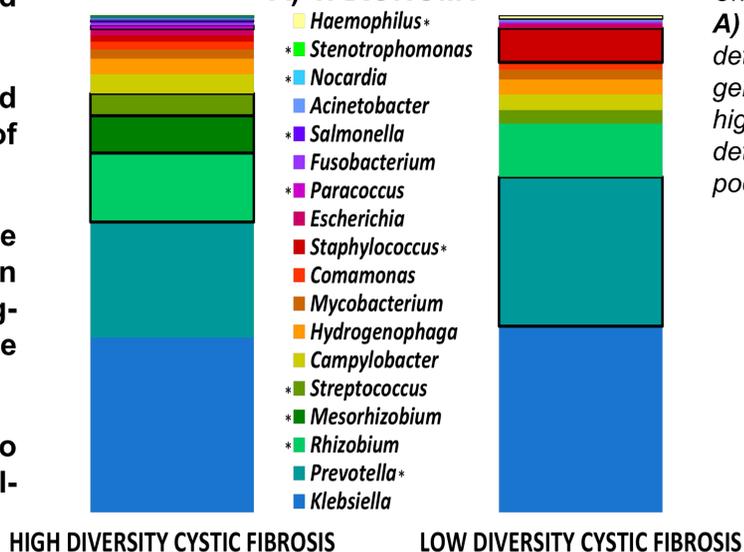
Methods



Generating comprehensive databases of CF and DC microbiomes:

- BALF pelleting, lysis, and digestion;
- High pH RPLC offline fractionation and concatenation (step omitted for individual sample analysis)
- LC-FAIMS-MS/MS on an Orbitrap Eclipse
- Galaxy-P/MetaNovo for matching MS/MS;
- Generation of a compact microbial database with taxonomic and functional information from Galaxy-P/Unipept + metaQuantome.

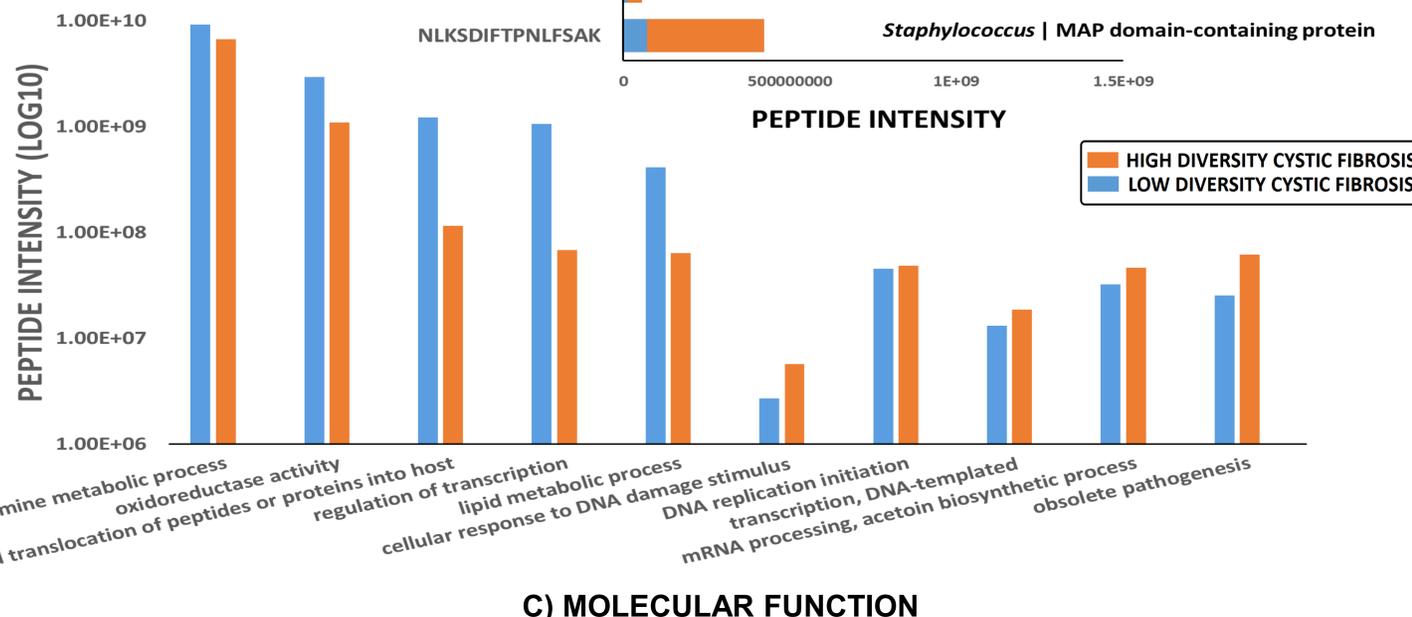
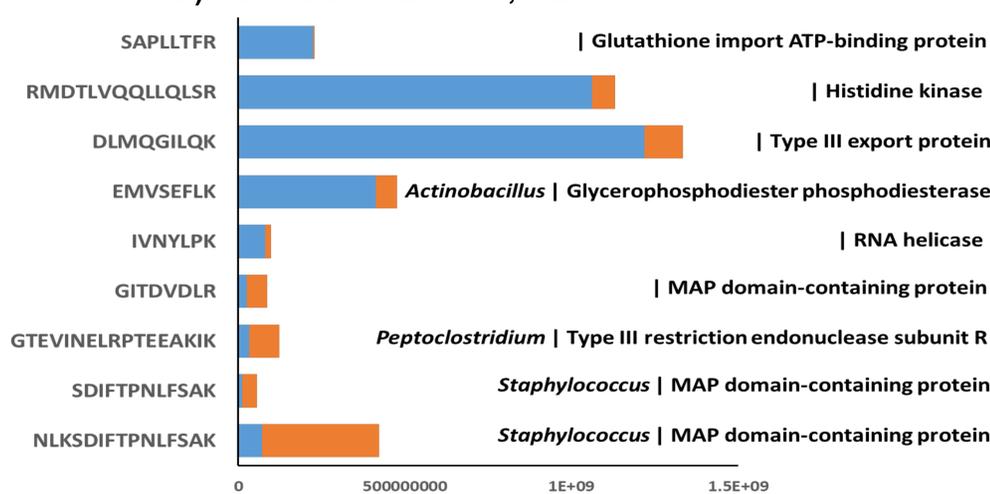
A) TAXONOMY



Characterization of the Cystic Fibrosis microbiome using bioinformatics tools.

A) Normalized microbial taxonomic intensities of assigned genera with at least 2 peptides detected; differences in taxa between high and low diversities indicated. **B)** Intensity, genus, and function of some peptides from 168 detected. **C)** Top 5 molecular functions in high diversity CF (left half) and top 5 in low diversity CF (right half) based on peptides detected. Data is of top 99th percentile genera detected in LC-FAIMS-MS/MS analysis of pooled patient samples, each with 16 patient samples per category.

B) PEPTIDE INTENSITY, GENUS & FUNCTION



Results

- More taxa detected in high diversity than low diversity CF (A).
- Mesorhizobium, Paracoccus, Salmonella, Nocardia, and Stenotrophomonas were found uniquely in high diversity CF and Haemophilus only in low diversity CF (A).
- Differentially expressed peptides were detected and associated with microbial taxa and functionality, such as Actinobacillus linked to GP-PDE activity in low diversity CF. In high diversity CF, Staphylococcus was linked to MAP proteins, enabling cellular internalization (B).
- Using Galaxy-P tools, the functionality of peptides and proteins can be determined and compared across our low and high diversity microbial databases. Pathways in pathogenesis have been identified (C).

Conclusion

Our work shows the initial steps toward generation of Cystic Fibrosis-specific metaproteomic sequence databases and peptide panels for both high diversity and low diversity disease contexts.

The peptide panel generated from the cystic fibrosis and disease control (not shown) samples will be used to analyze a large cohort of 182 clinical BALF samples. The cohort will be investigated using these comprehensive peptide libraries and databases, followed by integration with multi-omic data (16S rRNA, metabolomics, measurements of mucin integrity) while additionally assessing taxonomic-functional relationships in disease progression.

References

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