A METAPROTEOMICS BIOINFORMATICS WORKFLOW TO STUDY HOST-MICROBE DYNAMICS IN CLINICAL SAMPLES

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CLINICAL METAPROTEOMIC STUDIES DETECT LIMITED NUMBER OF MICROBIAL PEPTIDES

![Graph showing the number of distinct peptides in different datasets.](image)

- **Cystic Fibrosis dataset**
  - Total: 85713
  - Microbial: 2292
  - Human: 83421

- **Salivary dataset**
  - Total: 20808
  - Microbial: 1927
  - Human: 18881

- **Cervical vaginal dataset**
  - Total: 11009
  - Microbial: 1624
  - Human: 9385

**References:**
DATASETS

- BALF from Cystic Fibrosis or Disease Control Samples
- Nasal Swabs from COVID-19 patients

Sample Collection → Sample processing → Data Acquisition
Microbes of the Upper Respiratory Tract → UniProt

Comprehensive Protein Database → MetaNovo

Reduced Database → Search GUI

FragPipe → MaxQuant

Identification of Microbial Peptides

Unipept → PepQuery

Protein Database of PepQuery verified organisms

Targeted Analysis of Microbial Peptides

Skyline

Data Analysis

MS Datasets
MICROBIAL PROTEINS FROM COVID-19 PATIENTS

Severe
Negative
Log
2
(FC)

C
D
PC 2 (11.3%)
PC 1 (49%)

Log
10
(p)

Log
2
(FC)

Fusarium vanettenii
MARVEL domain-containing protein

Enterobacter
Thiamine biosynthesis protein ThiH

Clostridium
tRNA pseudouridine synthase B

Pseudomonas fluorescens
T2SSF domain-containing protein

Penicillium steckii
DNA repair and recombination protein

RAD26

Fusarium vanettenii
MARVEL domain-containing protein

Aryan Gupta
Targeted Analysis of Potential Pathogens in COVID Patients

**Streptococcus parasanguinis**
Dominant isolate of dental plaque
Opportunistic pathogen associated with subacute endocarditis

**Burkholderia cenocepacia**
Opportunistic pathogen
Cause systemic infections in immunocompromised individuals including cystic fibrosis patients.

**Cryptococcus floricola**
Infect immunocompromised hosts
Infection initiates in the lungs.
MICROBIAL PEPTIDES FROM CYSTIC FIBROSIS PATIENTS

**TAXONOMY:** Pseudomonas
**FUNCTION:** Type II secretion system protein

**TAXONOMY:** Streptococcus agalactiae
**FUNCTION:** DNA recombination enzyme

**TAXONOMY:** Bacteria (UniPept)
**FUNCTION:** Membrane kinase activity
Nucleoside triphosphate hydrolase (A0A119BW38)

**TAXONOMY:** Unassigned (UniPept)
**FUNCTION:** Uncharacterized protein

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**MKIGNLGGAYR**
**TAXONOMY:** Streptococcus agalactiae
**FUNCTION:** DNA recombination enzyme

**VLGARGHR**
**TAXONOMY:** Bacteria (UniPept)
**FUNCTION:** Membrane kinase activity
Nucleoside triphosphate hydrolase (A0A119BW38)

**AALGAYDLR**
**TAXONOMY:** Unassigned (UniPept)
**FUNCTION:** Uncharacterized protein (A0A1A2YS21)

**ATVEELHLEGI**
**TAXONOMY:** Unassigned (UniPept)
**FUNCTION:** Uncharacterized protein (A0A1H9MU03)
Targeted assay for host-microbial protein dynamics in clinical CF samples

- Lipoprotein particle remodeling
- Cholesterol transport
- Phospholipid efflux

- Inflammatory response
- Leukocyte migration
- Neutrophil degranulation
- Cytokine production
- Defense response to bacteria
Conclusions & Next Steps

• We have developed a MS-based Galaxy-driven bioinformatics workflow for processing of microbial and host proteins, generating verified microbial peptide candidates suitable for targeted analysis within individual patient samples.

• We have utilized this workflow to detect and validate microbial peptides during co-infection during the COVID-19 pandemic.

• The workflow was also used to generate a promising microbial and host peptide panel for application to CF disease progression studies by comparing it with disease control (DC).

• We intend to use this workflow in other clinical metaproteomic studies to detect differentially expressed host and microbial proteins in disease state.
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