Identifying Microbial Proteins in Lower Airways of Infants and Young Children with Cystic Fibrosis

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Introduction

- Obstructive lung disease is the most prominent cause of morbidity and early mortality in cystic fibrosis (CF) (1). Organisms, such as *Pseudomonas aeruginosa* and *Burkholderia cepacia*, have been identified as "traditional" pathogens that drive inflammation and structural damage in CF (3).
- Bronchoalveolar lavage fluid (BALF) has been proposed as the ideal airway sample because upper airway cultures could not accurately predict lower airway microbiota (1,2). We are interested in lower airway microbiota because they have not been previously investigated.
- Recently, anaerobic bacteria have been identified as the dominant bacterial community in BALF of asymptomatic infants with CF during their first year of life. However, it is unclear how these microbial communities may contribute to early CF lung disease in infants and young children (1).
- To determine whether microbial communities in the lower airways contribute to early CF lung disease, BALF samples were analyzed using mass spectrometry (MS)-based metaproteomics, specifically tandem MS (MS/MS) and metaproteomic bioinformatic tools on the Galaxy platform.

Table 1. Identified genera and species of distinct peptides detected in control BALF samples A) 24 and B) 26.

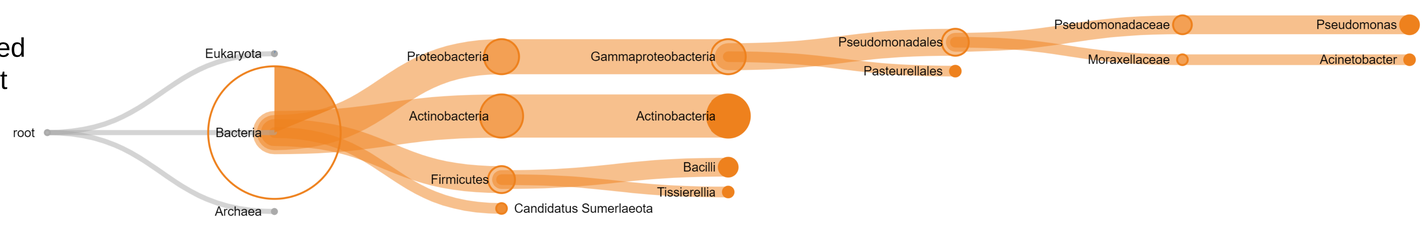
1A.

Sample 24					
Genus Name	**PSMs**	**Distinct Peptides**	**Species Name**	**PSMs**	**Distinct Peptides**
Nilaparvata	17	1	*N. lugens*	17	1
Streptococcus	3	3	*S. pneumoniae*	1	1
Actinobacillus	2	1	*A. delphinicola*	2	1
Capitella	2	1	*C. teleta*	2	1
Crenomytilus	2	1	*C. grayanus*	2	1
Fusarium	2	1			
Mycolicibacterium	2	2	*M. peregrinum*	1	1

1B.

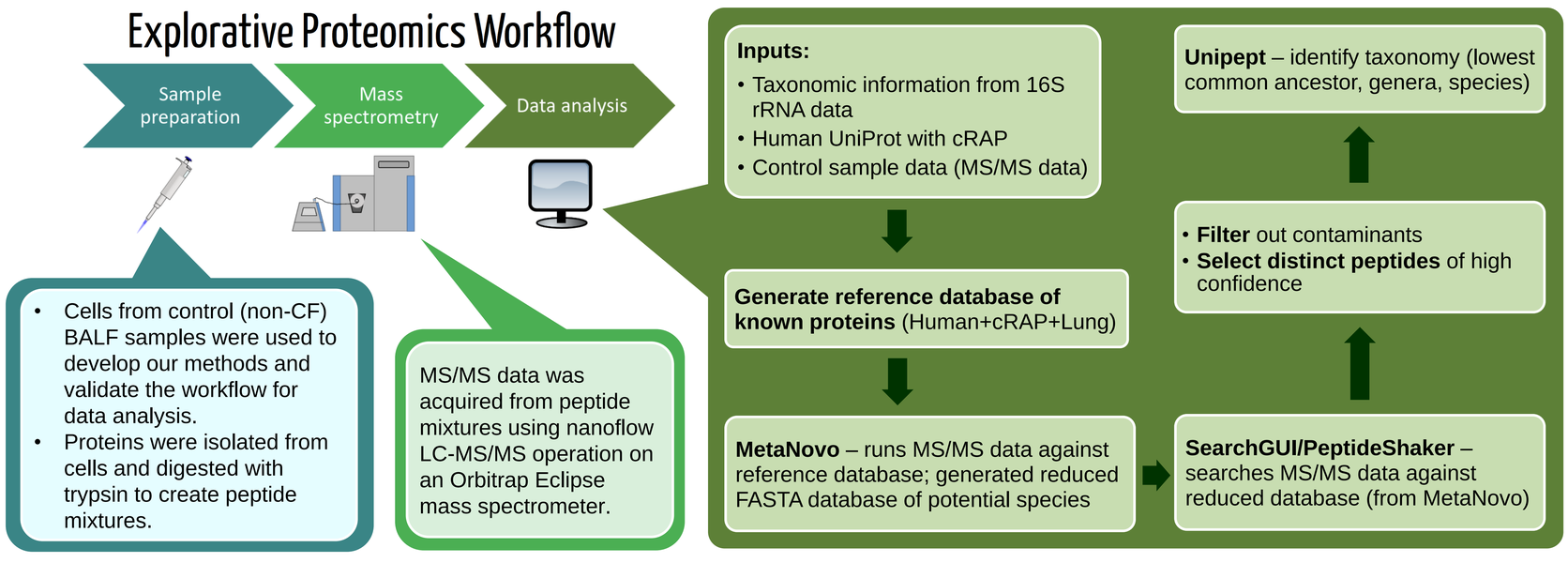
Sample 26					
Genus Name	**PSMs**	**Distinct Peptides**	**Species Name**	**PSMs**	**Distinct Peptides**
Nilaparvata	10	1	*N. lugens*	10	1
Actinobacillus	3	1	*A. delphinicola*	3	1
Cuerna	2	1	*C. arida*	2	1
Mycobacterium	2	1			
Pseudomonas	2	2	*P. stutzeri*	2	2
Rhizophagus	2	1	*R. clarus*	2	1
Streptococcus	2	2	*S. oralis*	1	1

Figure 1. Generated visualization output of taxonomy of microbial proteins detected in control sample 26.



Methods

- Metaproteomics directly identifies microbial proteins, indicating biochemical state and taxonomic relationships (4).
- Mass spectrometry enables many proteins to be reliably characterized and can identify thousands of proteins in large numbers of samples with a high degree of reproducibility (5).
- Galaxy is a free web-based user-friendly platform that provides easy access to complex bioinformatics tools and has been successfully employed in MS-based proteomics data analysis, such as the Galaxy-P project (6).



Results

- We successfully built a workflow for LC-MS/MS analysis of control (non-CF) BALF samples and microbial proteins that identified genera and species of distinct peptides (**Table 1**). Reported results include distinct peptides of which genera and species were detected with more than one peptide-spectrum-match (PSM), which is where a peptide is successfully matched to a sequence from a database.
- We generated visualization outputs that illustrated the taxonomic relationships of the detected microbial proteins (**Figure 1**).

Conclusion

- We were able to identify the taxonomy of lower airway microbial communities by building an operating workflow for LC-MS/MS analysis of control (non-CF) BALF samples and microbial proteins.
- While inconclusive as to whether lower airway microbial communities contribute to early CF lung disease, our preliminary results demonstrate that the identification of microbial communities in CF BALF samples is feasible using the same workflow.
- Additionally, we plan to test the viability of high field asymmetric waveform ion mobility spectrometry (FAIMS). Past research has suggested that FAIMS increases sensitivity for identifying peptides in complex samples, enabling us to extend further into the metaproteome (7). We will adapt the analysis approach we used for non-FAIMS data to determine if LC-FAIMS-MS/MS provides increased sensitivity in BALF samples. These findings could allow further investigation into the microbial role in advancing early CF lung disease.

References

1. Laguna TA, Wagner BD, Williams CB, Stevens MJ, Robertson CE, Welchlin CW, Moen CE, Zemanick ET, Harris JK. Airway microbiota in bronchoalveolar lavage fluid from clinically well infants with cystic fibrosis. PLoS One 2016;11:(12):e0167649.
2. Armstrong DS, Grimwood K, Carlin JB, Carzino R, Olinsky A, Phelan PD. Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. Pediatr.Pulmonol. 1996;21:(5):267-275.
3. Elborn J. S. (2008). Identification and management of unusual pathogens in cystic fibrosis. Journal of the Royal Society of Medicine, 101 Suppl 1(Suppl 1), S2–S5. https://doi.org/10.1258/jrsm.2008.s18002
4. Griffin TJ, Jagtap P, Johnson J, Blank C, Mehta S. (2016). Galaxy Training: Metaproteomics tutorial. Galaxy Training! https://training.galaxyproject.org/training-material/topics/proteomics/tutorials/metaproteomics/tutorial.html.
5. Aebersold, R., Mann, M. Mass-spectrometric exploration of proteome structure and function. Nature 537, 347–355 (2016). https://doi.org/10.1038/nature19949
6. Blank, C., Easterly, C., Gruening, B., Johnson, J., Kolmeder, C., Kumar, P., … Jagtap, P. (2018). Disseminating Metaproteomic Informatics Capabilities and Knowledge Using the Galaxy-P Framework. Proteomes, 6(1), 7. doi:10.3390/proteomes6010007
7. Pfammatter, S., Bonneil, E., McManus, F. P., Prasad, S., Bailey, D. J., Belford, M., Dunyach, J. J., & Thibault, P. (2018). A Novel Differential Ion Mobility Device Expands the Depth of Proteome Coverage and the Sensitivity of Multiplex Proteomic Measurements. Molecular & cellular proteomics : MCP, 17(10), 2051–2067. https://doi.org/10.1074/mcp.TIR118.000862

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