



Complex Microbial Community Structure in Adult Cystic Fibrosis Airways as Revealed by 16S rRNA PhyloChip

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Background

The airways of cystic fibrosis (CF) patients are persistently colonized with a number of bacterial species. This polymicrobial, chronic infection commonly results in recurrent pulmonary exacerbations and represents the primary cause of death of CF patients. Currently little is known of the true depth of bacterial diversity in CF sputum and the shifts in community structure that underlie lung function stability or exacerbation.

Objective:

To investigate the community structure of the microbial assemblage in CF airways and to relate this to clinical data such as lung function, clinical stability or exacerbation and particular mutations of the CFTR (CF transmembrane conductance regulator).

Methods

- Induced sputum samples were collected after routine spirometry at the UCSF Adult Cystic Fibrosis Center and DNA was immediately extracted to limit alterations to the community structure.
- The 16 S rRNA PhyloChip, capable of identifying ~8500 individual bacterial taxa was used to profile the microbial community structure of each sample.
- Normalized PhyloChip data was analyzed by a variety of methods including NMDS (Fig. 1) and perMANOVA (Fig. 2) in the R statistical package.

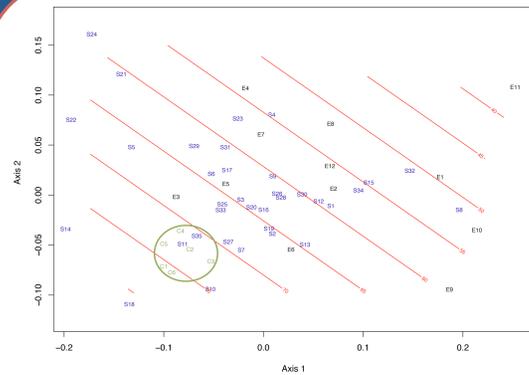


Fig 1. Non-metric multidimensional scaling of CF microbial communities. Expectorated sputum from healthy control individuals are green, stable CF patients blue and exacerbated patients black. The red isotherms represents an overlaid ordination of lung function (FEV₁) which is significantly associated with microbial community structure.

Association of Clinical Data with Microbial Community Structure

A number of clinical variables could be significantly associated with the community structure using perMANOVA (permutational multivariate analysis of variance). Intravenous antibiotic treatment, severity of disease (based on the FEV₁ - forced expiratory volume in one second - a measure of lung function) and whether the individual was homozygous or heterozygous for the severe CFTR mutation ΔF508 were all significantly ($p < 0.05$) associated with community structure. In this dataset, age or sex of the patient and complications of CF such as pancreatic insufficiency were not associated with community structure. The association between ΔF508 mutation and community structure is an interesting one, further work is needed to indicate whether this phenomenon is specific to this mutation or can also be associated with other CFTR mutations.

FEV₁ was correlated with the abundance of each taxon to determine individual taxa that were significantly positively or negatively associated with lung function. A diverse range of taxa were positively correlated with lung function. The taxa that were negatively correlated with lung function were much more taxonomically restricted and included many members of the Pseudomonadaceae (including *Pseudomonas aeruginosa*), *Burkholderia* spp., *Stenotrophomonas maltophilia* and *Acinetobacter* spp.

Finally, a number of taxa were detected exclusively in the airways of exacerbating patients who had not yet received IV antibiotics. Of these 45 taxa, few have previously been associated with cystic fibrosis and they include oral, intestinal and environmental species, many of which are only represented by environmental clone sequences. Examples include, *Corynebacterium segmentans*, *Propionibacterium acnes* and *Serratia fonticola*. Further investigation is required to ascertain whether these taxa can truly be implicated in cystic fibrosis exacerbations or whether the slightly more restricted diversity of the exacerbation samples compared to the stable samples results in the detection of rarer members of the microbial community.

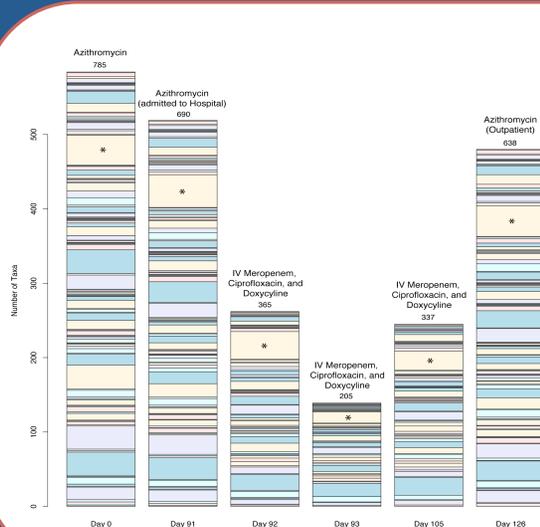


Fig. 2. Diversity changes upon antibiotic therapy in a single CF patient. Temporal samples from an individual CF patient demonstrate the change in diversity associated with periods of clinical stability, exacerbation (defined by and hospitalization), and return to clinical stability. The species richness (number of taxa) for each sample is displayed at the top of each bar, along with the treatment regimen at the time of sampling. Bars are divided at the family, rather than taxon level and the Pseudomonadaceae are marked with an asterisk. A total of 145 taxa are present in all 6 samples from this patient, despite prolonged, intravenous antibiotic therapy. This included important known pathogens listed in the table below.

Taxon Name	Notes
<i>Achromobacter subsp. denitrificans</i> str. DSM 30026 (T)	Commonly isolated CF pathogen
<i>Alkanindiges hongkongensis</i> str. HKU9	
<i>Arthrobacter ureafaciens</i> str. DSM 20126	
<i>Brevundimonas diminuta</i> str. DSM 1635	
<i>Burkholderia hospita</i> str. LMG 20598T	1 other <i>Burkholderia</i> sp. detected
<i>Comamonas testosteroni</i> str. SMCC B329	
<i>Helicobacter pullorum</i> str. NCTC 12826	6 other <i>Helicobacter</i> sp. detected
<i>Jonesia quinghalensis</i> str. DSM 15701	
<i>Kineococcus aurantiacus</i> str. IFO 15268	
<i>Kocuria roseus</i>	
<i>Lyrodia pedicellatus</i> symbiont	
<i>Micrococcus luteus</i> str. HNZ-11	
<i>Pseudalteromonas ruthenica</i> str. KMM300	
<i>Pseudomonas aeruginosa</i> str. PAO1	Commonly isolated CF pathogen
<i>Pseudomonas fluorescens</i> str. CHA0	8 other Pseudomonadaceae detected
<i>Pseudomonas stutzeri</i> HV-105	
<i>Pseudomonas syringae</i> pv. theae str. PT1	
<i>Rothia mucilaginosus</i> str. DSM	Emerging opportunistic pathogen
<i>Streptococcus constellatus</i> str. ATCC27823	<i>Streptococcus milleri</i> group CF Pathogen

Results

- The 16S rRNA PhyloChip provides an extremely detailed view of the microbial diversity in CF airways and allows community structure to be associated with clinical variables.
- Although microbial diversity is greatly reduced by antibiotic therapy, important CF pathogens persist despite this treatment, which form a core community in CF airways
- Lung function (FEV₁) is significantly associated with both overall community structure and the abundance of individual CF pathogens.
- Presence of homozygous or heterozygous ΔF508 mutation is correlated with community structure.
- The PhyloChip identified taxa present only in exacerbating patients that may be involved in the pathogenesis of pulmonary exacerbations.

Conclusion

Our data demonstrates the enormous diversity of the microbial community associated with CF airways and indicates that a wide range of bacteria, including a large number of known pathogens persist despite antimicrobial therapy. Microbial community structure is associated with various clinical factors including lung function and CFTR mutation. Further investigation of the variables driving the community structure may lead to alternative approaches for managing this disease.

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