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Distinct community structures of the fungal microbiome and respiratory health in adults with cystic fibrosis

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Abstract

Background: The respiratory tract fungal microbiome in cystic fibrosis (CF) has been understudied despite increasing recognition of fungal pathogens in CF lung disease. We sought to better understand the fungal communities in adults with CF, and to define relationships between fungal profiles and clinical characteristics.

Methods: We enrolled 66 adults with CF and collected expectorated sputum, spirometry, Cystic Fibrosis Questionnaire-revised, and clinical data. Fungi were molecularly profiled by sequencing of the internal transcribed spacer (ITS) region. Total fungal abundance was measured by quantitative PCR. Relative abundance and qPCR-corrected abundances were determined. Selective fungus culture identified cultivable fungi. Alpha diversity and beta diversity were measured and relationships with clinical parameters were interrogated.

Results: Median age was 29 years and median FEV_1 percent predicted 58%. Members of the *Candida* genus were the most frequent dominant taxa in CF sputum. *Apiotrichum, Trichosporon, Saccharomyces cerevisiae, and Scedosporium* were present in high relative abundance in few samples; whereas, *Aspergillus* species were detected at low levels. Higher FEV_1% predicted and CFTR modulator use were associated with greater alpha-diversity. Chronic azithromycin use was associated with lower alpha-diversity. Patients with acute pulmonary had distinct fungal

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2023.02.003.

Credit author statement

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community composition compared to clinically stable subjects. Differing yeast species were mainly responsible for the community differences.

Conclusion: The respiratory tract fungal microbiome in adults with CF is associated with lung function, pulmonary exacerbation status, macrolide use, and CFTR modulator use. Future work to better understand fungal diversity in the CF airway and its impact on lung health is necessary.

Keywords

Fungi; Microbiome; Infection; Aspergillus; Candida

1. Introduction

The early description of cystic fibrosis (CF) was marked by malnutrition and lung infections. The diagnosis and treatment of infection has transformed disease prognosis to a median predicted survival over 50 years [1]. Yet, chronic infections continue to significantly impact morbidity and mortality in CF [1,2]. Over time, the microbial epidemiology has changed, with a rise of filamentous fungi and yeast recovered by culture from the CF respiratory tract [3]. The pressures of cumulative and chronic exposure to antibiotics in the course of CF likely contributes to the observed changes in fungal detection [4].

Conventional culture-based detection methods are the primary modality to identify microorganisms in CF sputum. However, the adoption of DNA sequence-based analyses has greatly expanded our understanding of bacterial diversity in CF. Unbiased 16S rRNA gene sequencing of CF respiratory samples have yielded insight into age-specific bacterial diversity and resiliency of the bacterial community during pulmonary exacerbations [5-7]. However, mycologic profiling of the CF airway has remained limited [8-13]. Our study aimed to molecularly define the fungal composition and community structure in CF sputa and evaluate their associations with clinical characteristics and outcomes.

2. Materials and methods

2.1. Study design and participants

We conducted a prospective study of adults 18 years and older with CF followed in the adult CF center at the University of Pennsylvania from March through November 2017. We included subjects with a diagnosis of CF according to the CF Foundation diagnosis guidelines and excluded those with a history of solid organ transplantation. All eligible participants, regardless of health state, were consecutively recruited from the clinic and written informed consent was obtained. The study was approved by the University of Pennsylvania institutional review board (protocol 825979).

Demographic and clinical data were collected at the time of sampling and included comorbidities, medication use within the past three months (cystic fibrosis transmembrane conductance regulator [CFTR] modulators, chronic non-macrolide oral antibiotics, azithromycin, chronic prednisone, inhaled anti-pseudomonal antibiotics, inhaled corticosteroid, and antifungals), spirometry, clinical microbiological data in the past 24 months, and presence of pulmonary exacerbation at the time of sampling. Pulmonary

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exacerbation was defined as a physician diagnosis of an acute worsening of respiratory symptoms and/or lung function with or without anorexia or weight loss, fatigue, and pulmonary exam findings. Chronic use of antibiotics was defined as continuous use for at least one month during the past 3 months. Spirometry was performed according to the American Thoracic Society guidelines and FEV_1% predicted reported according to the National Health and Nutrition Examination Survey III prediction equations. Respiratory-related quality-of-life scores were collected with the Cystic Fibrosis Questionnaire-revised (CFQ-R). A higher CFQ-R respiratory domain score (range 0 to 100) reflects better respiratory-related quality-of-life [14].

2.2. Sputum sample collection and analysis

Subjects spontaneously expectorated consecutive sputum samples into two sterile specimen cups during a single encounter; one for culture and one underwent DNA extraction and sequencing. Six samples of DNA-free water were collected in specimen cups at the clinic sites of sample collection to serve as environmental controls. Sputum underwent bacterial culture and semi-selective fungal culture within 8 h of collection as previously described [15]. Morphologic identification of macroscopic and microscopic features of fungal isolates was done following standard clinical microbiological procedure. Sputum for DNA extraction was placed immediately at 4 °C after collection and processed within 24 h. Dithiothreitol (DTT) was added to liquefy mucous, which was then aliquoted under sterile conditions and stored at −80 °C until sequencing. Genomic DNA was extracted from sputum in a single batch using the DNeasy PowerSoil kit (Qiagen) with an additional 10-minute heating step at 95 °C before bead beating to enhance fungal recovery. The fungal internal transcribed spacer (ITS) region was amplified using barcoded ITS1F/ITS2 primers (Table S1). The resulting library was sequences on the Illumina MiSeq instrument yielding paired end reads that were 250 bp in length. Total fungal abundance was measured by quantitative PCR (qPCR) using the FungiQuant assay, a Taqman qPCR assay targeting a 351 bp region in the fungal 18S rRNA gene [16]. Values were calculated as copy number per μL of sputum. A detailed description of the methods is outlined in the Supplement.

2.3. Bioinformatic analysis

Paired-end ITS sequence reads were analyzed using the PIPITS pipeline. Taxonomic assignments of representative ITS sequences were generated by BROCC (BLAST Read and Operational Taxonomic Unit Consensus Classifier), a validated pipeline using the nt database from NCBI. BROCC uses local alignments to classify sequences by consensus-based algorithm to accurately characterize single cell eukaryotes [17]. We determined the relative abundances of fungal taxa from ITS sequencing. To approximate absolute fungal quantities at a taxonomic level, the qPCR-corrected abundance was computed by multiplying the relative abundance of each taxon in the sample by the absolute fungal abundance as estimated by qPCR. For samples that did not have quantifiable qPCR values, we imputed the values by calculating half of the limit of detection with threshold of 40 cycles (33.4 copies per μL of sputum) as 16.7 copies. Detailed methods are included in the Supplement.

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2.4. Statistical analysis

Community composition was characterized by the richness (number of different taxa) and alpha (within sample) diversity based on the Shannon index, which accounts for the number of taxa and evenness of distribution. Bray-Curtis dissimilarity was used to measure beta diversity, or the dissimilarity between fungal communities in each pair of samples. We compared the diversity measures to clinical characteristics at the time of sampling, including age, lung function (defined as FEV_1 percent predicted), CFQ-R respiratory domain score, chronic inhaled antibiotic use, chronic azithromycin use, use of CFTR modulator drugs, antifungals, pulmonary exacerbation status, and culture-positive *Pseudomonas aeruginosa.* Least squares linear model was used to compare differences in richness and Shannon diversity. Bray-Curtis was analyzed using permutational multivariate analysis of variance (PERMANOVA) of uncorrected abundances, as implemented by the R function *adonis()* in the vegan package version 2.3–5. We included covariates age, sex, and FEV_1 percent predicted in the PER-MANOVA model. Sensitivity analysis using beta dispersion analyses were conducted. We calculated the ratio of qPCR-corrected abundances of *Candida dubliniensis* and *Candida albicans* and determined the association between the ratio and pulmonary exacerbation state using *t*-test.

Statistical analyses were conducted using R version 4.0.3.

3. Results

3.1. Subject characteristics

We recruited 66 patients with CF (Table 1). Median age was 29 years and 52% were female. Pancreatic insufficiency was present in 92%. Homozygosity for the F508del mutation was present in 47% and the use of CFTR modulators (ivacaftor or lumacaftor/ivacaftor) in 36%. Median forced expiratory volume in one second percent (FEV_1%) percent predicted was 58%. Thirty-five individuals (53%) were experiencing an acute pulmonary exacerbation at time of sampling.

Table S2 describes the culture-based microbiology at the time of sampling and over the prior two years. Two subjects lacked contemporaneous fungus culture results, neither of whom had a history of fungal isolation in the prior 24 months. Fifty-seven (86.4%) participants had history of *Pseudomonas aeruginosa* and nine (13.6%) had a history of *Aspergillus fumigatus.*

3.2. Taxonomonic identification of fungi in CF sputum

A total of 9.6 million fungal ITS sequence reads were generated from 66 sputum samples. After quality filtering, 8.4 million reads were used for analysis. A total of 249 taxa were identified in the cohort, including 128 at the species level. We randomly selected four subjects and tested two replicate samples from each subject for quality-control, which confirmed that beta diversity was significantly greater between than within subjects (p = 0.01; Fig. S1a and S1b).

Fig. 1 shows relative abundances of fungal taxa. *Candida albicans* was the dominant taxon in the largest number of samples, followed by *Candida dubliniensis* and *Candida*

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parapsilosis. Aspergillus species was dominant taxon in one sputum sample and detected as the sub-dominant taxa in 36 additional samples (Table S4). Other fungi were present in high relative abundances in small numbers of specimens including *Apiotrichum, Trichosporon, Saccharomyces cerevisiae, Cladosporium, Scedosporium, Malassezia restricta,* and *Diutina*.

Samples underwent qPCR to measure the total fungal abundance. A total of 47 samples had fungal DNA above the limit of detection (33.43 copies per μL), most of which had low fungal load (Fig. 2A). Because the absolute abundance of fungi differed markedly among samples, which is not reflected in relative abundance measures, we calculated the qPCR-corrected fungal abundances to estimate the absolute abundance of individual fungal taxa in the ITS sequencing results (Fig. 2B; qPCR-corrected fungal abundances including background controls is shown in Fig. S2) [18]. For example, in subject 10092, the relative abundance of *Aspergillus fumigatus* was 3×10^{-5} and total fungal abundance measured by FungiQuant was 15,900 copy number/uL. Therefore, estimated qPCR-corrected abundance of *A. fumigatus* was 0.44852.

Fig. 2B shows the qPCR-corrected fungal abundances in a heatmap, with the total fungal abundance for each sample. Samples to the left side of the dashed line represent the samples with fungal DNA below the limit of detection. We observed dominance of *Candida* species in high fungus-load specimens. To highlight correlation between sequencing and culture, we displayed the corresponding culture result for clinically relevant fungi (*Aspergillus, Trichosporon,* and *Scedosporium* species) in Fig. 2B. Of the 64 subjects that had concomitant culture evaluation, the concordance between fungal species identified on culture and the most abundant taxon identified by DNA sequencing was 64% (Table S3). However, we found 81% concordance between fungal species identified on culture and identification of fungal taxa by sequencing when including non-dominant taxa. *Aspergillus* species were detected in 37 samples (56%) but at mostly low levels. For the seven *Aspergillus species* culture-positive samples, *Aspergillus* genus was detected by DNA sequencing in all seven samples (Table S4). Correlation between qPCR corrected *Aspergillus* abundance and *Aspergillus* culture positivity was not observed (data not shown).

Scedosporium species were detected by sequencing in 26 (39%) individuals; but was the most abundant taxon in only two. *Scedosporium apiospermum* was detected by culture in subject 10086, but ITS sequencing identified *S. aurantiacum* in this subject. We found high *Trichosporon* abundance in three (4.5%) subjects and low level detection in 33 others. *Trichosporon asahii* was the dominant taxon in the sputum sample with the highest fungal DNA concentration, subject 10068 (Figs. 1 and 2); this subject grew *Candida* only on culture. *Trichosporon mycotoxinivorans* was cultivated in two samples (Table S3), but *Apiotrichum* genus was found to be abundant, while *Trichosporon* was not identified by ITS in those samples. *Apiotrichum* was found in high qPCR-corrected abundance in three (4.5%) subjects and low levels in 30 samples and eight environmental controls (Fig. S2). Overall, ITS sequencing identified the presence of clinically relevant fungi in nearly all culture-positive and several culture-negative samples.

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3.3. Relationship between fungal communities in CF sputum and clinical status

Higher FEV_1% predicted was directly associated with greater fungal richness and alpha-diversity (Shannon index; $p = 0.016$ and 0.003, respectively; Fig. 3A). Chronic azithromycin use was significantly associated with both lower fungal richness and alpha-diversity ($p = 4.77 \times 10^{-4}$ and 5.16×10^{-4}, respectively, Fig. 3B). CFTR modulator use was associated with higher fungal alpha-diversity, but not richness ($p = 0.04$ and 0.06, respectively, Fig. 3C). In contrast, there was no correlation between fungal richness or alpha-diversity and age, chronic anti-pseudomonal inhaled antibiotics, antifungal use, *Pseudomonas aeruginosa* colonization, or pulmonary exacerbation status (data not shown). Furthermore, fungal DNA copy number by qPCR did not correlate with clinical features (data not shown).

We observed a correlation between fungal community composition and pulmonary exacerbation status. CF patients experiencing an acute pulmonary exacerbation had distinct fungal profiles compared to clinically stable CF adults, adjusting for age, sex and FEV_1% predicted, as measured by Bray Curtis metric (PERMANOVA R^2=0.041, $p = 0.01$, Fig. 4A). Fungal community structure was also different in people with lower CFQ-R respiratory domain score, representing worse patient-reported respiratory health, compared to those with better respiratory health, accounting for age, sex, and FEV_1% predicted (PERMANOVA R^2 =0.036, $p = 0.02$, Fig. 4B). We followed our PERMANOVA-based analysis of beta diversity with an analysis of beta dispersion, or average within-group community distance. We observed no difference in beta dispersion according to pulmonary exacerbation ($p = 0.056$) but found that the community-level dispersion was slightly higher in samples with high CFQ-R score ($p = 0.04$, Fig. S3). Overall, differences in beta dispersion were small relative to the range of community-level distances between samples. Estimates for difference in beta dispersion were less than 20% of the IQR among within-group distances in both comparisons, suggesting that even statistically significant differences in beta dispersion were unlikely to have accounted for differences in community composition observed in our PERMANOVA comparisons.

Candida albicans represented the dominant taxon in 16 (51.6%) subjects with stable clinical state (Figs. 4C and S4). In contrast, *Candida dubliniensis* was abundant in subjects with acute pulmonary exacerbations (Fig. S4). We therefore calculated the ratio of *Candida dubliniensis* and *Candida albicans* (*C. dubliniensis/C. albicans*) to assess the relationship between *Candida* species profile and clinical status. We observed higher *C. dubliniensis/C. albicans* was associated with pulmonary exacerbation state whereas a lower ratio was associated with stable state (Fig. 5, $p < 0.001$). Furthermore, higher *C. dubliniensis/C. albicans* correlated with lower CFQ-R respiratory domain score, representing worse respiratory health (data not shown).

4. Discussion

The changing microbial epidemiology of CF airway has pro-voked investigation of the prevalence and clinical impact of filamentous fungi and yeast [3,19]. Culture-based detection has reported increasing prevalence of fungi in CF respiratory samples, which may contribute to infection and inflammation in the airways of children and adults [20,21]. By applying next-generation sequencing methods, we define the fungal microbiome in sputum

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and its relationship to host-specific factors and patient-reported respiratory quality-of-life in a well-characterized CF population.

Members of the *Candida* genus were the most prevalent and abundant fungi in CF sputum. These findings are consistent with previous descriptions of the CF fungal mycobiome [9,11,12,22]. *Candida* species are rarely implicated as the cause of acute lower respiratory infections and are present in the oral cavity of healthy and diseased individuals [23,24]. It is plausible that the *Candida* recovered in the sputum could represent contamination from passage through the mouth during sampling, but we understand that microbes in the oral compartment inform and influence the composition of the lung microbiome [25,26]. For example, *Candida albicans* was in the oral wash and also in stringently-collected bronchoalveolar lavage (BAL) of lung transplant recipients, some of whom had CF [10]. The pathogenicity of *Candida* species in the CF airways is controversial. Limited observational data have reported a potential association between *C. albicans* colonization and FEV_1 decline and pulmonary exacerbation rates in CF, but this has yet to be reproduced [27]. Indeed, we found that fungal composition was associated with disease status and respiratory symptom burden (Fig. 4). *C. dubliniensis* was seen in greater abundance in sick CF patients with acute exacerbation. On the other, *C. albicans* was found to be more abundant in stable individuals without pulmonary exacerbation. Willger et al. investigated six CF subjects experiencing pulmonary exacerbation and similar to our study, *C. albicans, C. parapsilosis,* and *C. dubliniensis* accounted for 75–99% of the reads [11]. The mechanism for this relationship is uncertain, though inter-kingdom interactions between *C. albicans* and *Pseudomonas aeruginosa* in the airway might play a role in our findings [28].

While *Aspergillus* species were widely present, it was rarely the dominant taxon in sputa. For all samples culture-positive for *Aspergillus*, ITS also detected *Aspergillus* DNA; yet, we did not observe a correlation between *Aspergillus* DNA count and culture status. Detection of *Aspergillus fumigatus* in the CF airway is important for *Aspergillus* related lung disease. The clinical diagnostic value of *Aspergillus* DNA detection has yet to be understood for fungal lung disease in CF. Although a positive *Aspergillus* PCR cannot differentiate colonization versus infection, these more sensitive molecular assays could have a potential diagnostic role in CF patients.

We found high qPCR corrected abundances of *Trichosporon asahii*, a basidiomycetous yeast, in three subjects, suggesting authentic *Trichosporon* presence, whereas low abundance samples cannot be distinguished from background. Notably, the *Trichosporon mycotoxinivorans* culture-positive samples in the cohort did not identify *Trichosporon* by ITS sequencing; yet *Apiotrichum* was identified (Fig. S3). This may be explained by the recent taxonomic reassignment of *Trichosporon mycotoxinivorans* to *Apiotrichum mycotoxinivorans* and the phylogenetic similarities between the two genera [29]. *Apiotrichum/Trichosporon* species has been identified as a potentially pathogenic member of the CF fungal community and may contribute to lung function decline [11,30]

ITS sequencing also detected fungal taxa, such as *Saccharomyces cerevisiae, Cystofilobasidium*, and *Malassezia,* supporting previous observations [9,11-31]. While these taxa have known roles as skin and gut flora, they are of unclear significance in the CF

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lung. We also observed high relative abundance of *Diutina* in samples with three samples with high fungal load. Yet, *Diutina* (formerly *Candida*) species is a rare pathogen in human infection and has not been well-described in the CF lung [32]. When comparing culture and sequence results, discordance between cultivable agents and dominant fungal taxa did occur for a minority of samples (Table S3). Sensitivity of molecular methods may identify DNA from dead microorganisms, which may contribute to the discrepancies between culture and culture-independent diagnostics.

As CF lung disease progresses, bacterial richness and diversity are known to decrease [6], concomitant with increased absolute and relative abundance of CF pathogens, such as *Pseudomonas aeruginosa*. We observed lower fungal diversity in more advanced lung disease [9]. The mechanism for this is unclear. The use of chronic inhaled anti-pseudomonal antibiotics has been thought to potentially drive enrichment of fungal communities [4]. Yet, we did not observe this association. Interestingly, lower fungal alpha diversity was observed in sputa of patients on chronic azithromycin compared to those who did not use azithromycin. Chronic macrolide use has been implicated in altering the airway bacterial microbiota in non-CF bronchiectasis and asthma, though azithromycin has not been associated with changes in bacterial diversity in CF [33-35]. The immunomodulatory mechanism of azithromycin or the interaction between fungi and bacteria may be playing a role in the observed fungal diversity differences. Alternatively, individuals on chronic azithromycin in our cohort may be represent a sicker population. Adults with CF on ivacaftor or lumacaftor/ivacaftor showed trends of higher fungal richness and alpha-diversity. Greater bacterial richness and alpha diversity have been reported in CFTR modulators, but fungal diversity has not been previously described to our knowledge [36,37]. CFTR modulators are known to exhibit antimicrobial properties, but the direct mechanism of how improved CFTR function alters the microbiome remains unknown.

A novel aspect of our study is that we investigated total fungal burden and estimated abundance of each taxon adjusted for the total fungal content rather than simply relative abundances. Microbiome analyses often focus only on relative abundances, which omits potentially important information on total microbial burden and burden of each taxon. This may be particularly critical in environments like the lung, where there are very large differences in total fungal burden between subjects. In addition, fungal qPCR-corrected abundance enables better comparison between samples of interest and background, given the risk of contamination in low biomass respiratory samples, adding methodological rigor.

Our study has several limitations. Sputum has the potential to be contaminated by organisms in the oral cavity, although bacterial studies suggest that may be less problematic in CF than other states due to a higher microbial burden in the lung [22]. Nevertheless, the absence of oral samples in this study leads to potential risk. The study was cross-sectional, limiting the ability to infer causality in the observed associations, and longitudinal examination must be the next step. The risk of confounding is present, but we adjusted our models for age, sex, and lung function to address this. We used ITS1F/ITS2 primers targeting the ITS1 region, one of the several fungal ribosomal target regions, which may impact the ability to identify certain fungal taxa [38]. We did not analyze sputum bacterial communities and understanding bacterialfungal interaction will be an important topic for future investigation.

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While total fungal burden adds considerable information to relative abundances, the extent to which it reflects airway burden may vary among patients based on levels of sputum production. The inability to distinguish dead or alive microbes with molecular testing remains a limitation in most human microbiome studies. Finally, this cohort was studied before the introduction of elexacaftor/tezacaftor/ivacaftor (ETI), which may alter the fungal community landscape. Studies investigating the microbiological changes in CF sputa related to ETI are ongoing (NCT04038047).

In conclusion, our study contributes to the understanding of the understudied mycobiome in a CF adult population. The differences in the fungal community structure of CF sputa influenced by *C. albicans* and *C. dubliniensis* in pulmonary exacerbation states and high respiratory symptom burden requires further investigation to determine whether fungi may contribute. The associations between azithromycin, CFTR modulation, and lung function with fungal richness and diversity in CF sputa also merits closer examination. Longitudinal evaluation of the mycobiome, bacterial microbiome, and microbial interactions in a large cohort over time is necessary to elucidate these relationships further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

FEV_1	forced expiratory volume in one second
CFQ-R	cystic fibrosis questionnaire-revised
DTT	dithiothreitol
ITS	internal transcribed spacer
QPCR	quantitative polymerase chain reaction
OTU	operational taxonomic unit
CFTR	cystic fibrosis transmembrane conductance regulator
ABPA	allergic bronchopulmonary aspergillosis
IV	intravenous

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BAL	bronchoalveolar lavage

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Fig. 1.

Top ten fungal taxa by relative mean abundance, grouped by the most dominant taxon. Each row represents a sputum sample from unique subject. For subjects with “Other” species listed as dominant taxon, the most abundant taxon is listed in Table S3.

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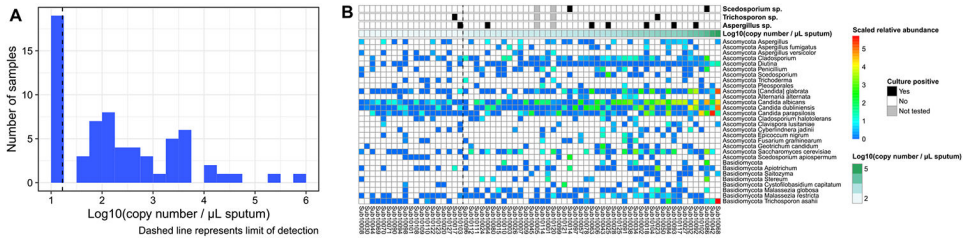

Fig. 2.

(A) Histogram of the log transformation of quantitative PCR of fungal DNA in CF samples. Dashed line represents to the limit of detection. (B) FungiQuant qPCR-corrected or absolute abundance of fungal taxa in sputum samples of 66 individual participants are shown. The corresponding culture result for *Scedosporium* species, *Trichosporon* species, and *Aspergillus* species are displayed in top three rows. The fourth row represents the log-transformed FungiQuant qPCR DNA count, keyed by teal-colored scale at the top right. In the samples to the left of the dashed line (representing the limit of DNA detection), total fungal abundance was imputed to 16.7 copies per µL of sputum.

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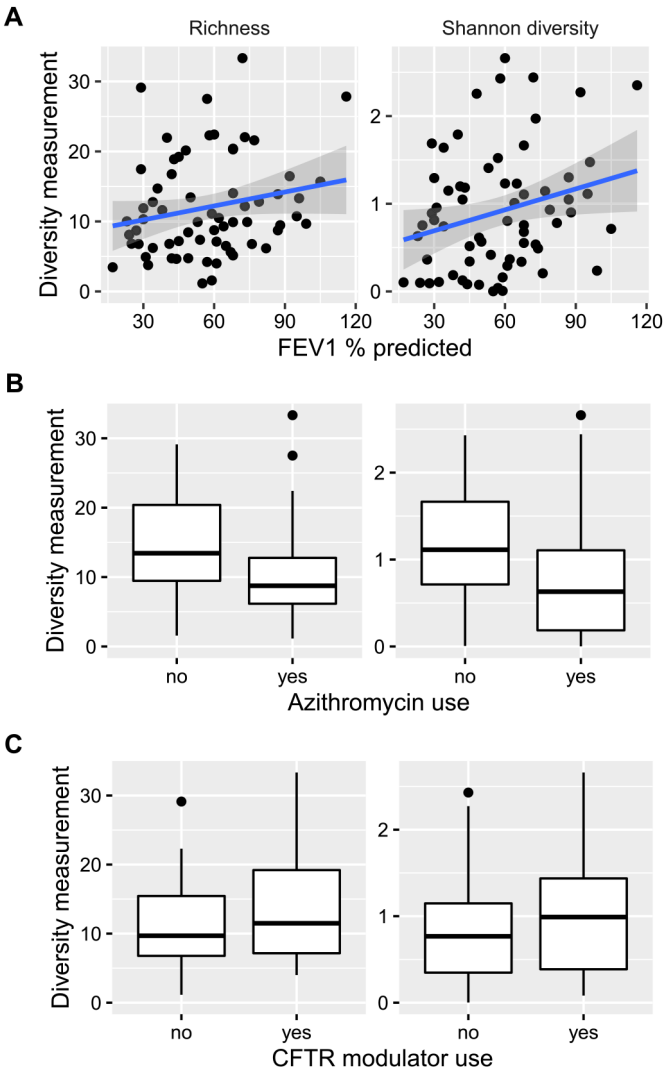

Fig. 3.

Fungal within-sample (alpha) diversity of CF sputum specimens correlates with lung function (3A),chronic azithromycin use (3B), and CFTR modulator use (3C) in adults with cystic fibrosis. Alpha diversity was summarized using richness and Shannon diversity index, represented on the y-axes.

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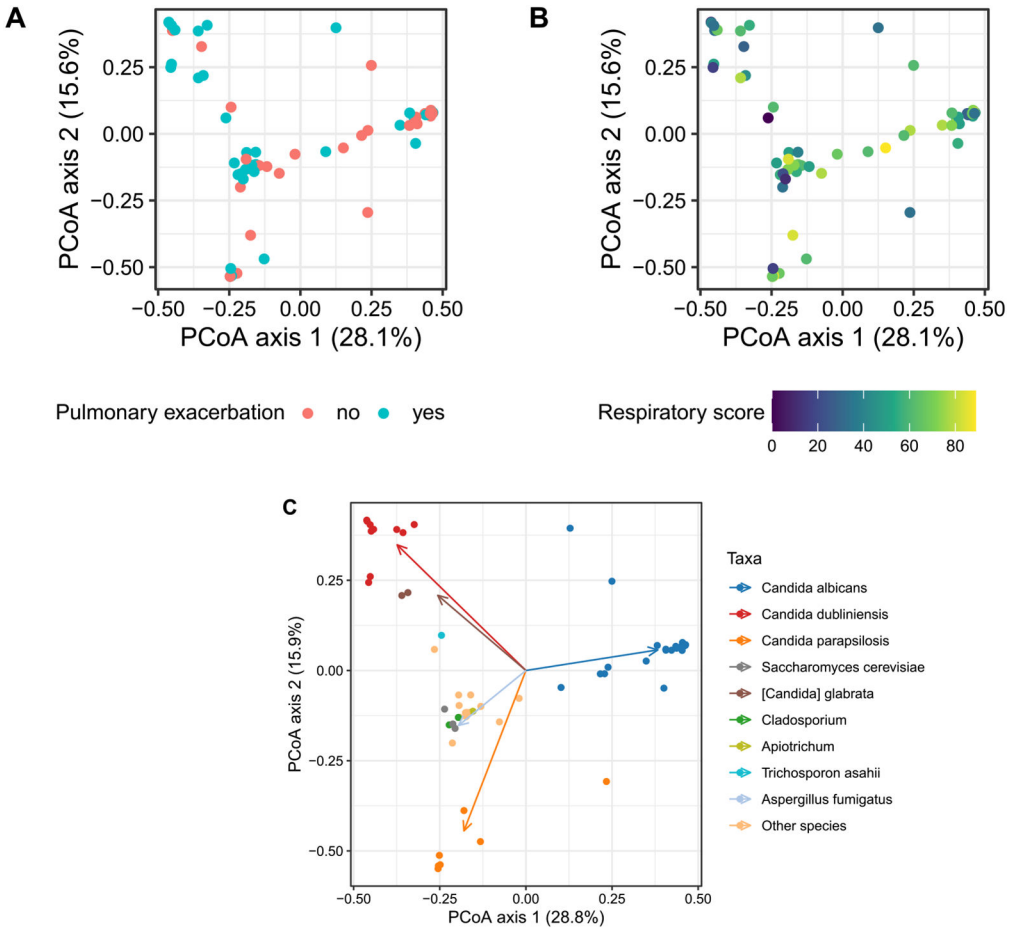

Fig. 4.

(A) PERMANOVA reveals demonstrating microbial composition differs in CF adults with (aqua) and without (pink) pulmonary exacerbation, adjusted for age, sex, and FEV1 percent predicted, $R^2 = 0.041$, $p = 0.01$. (B) Fungal composition may be associated with respiratory quality-of-life (QOL) with the yellow (light) color representing higher respiratory domain score (scale 0–100, higher respiratory domain score corresponding with better respiratory QOL), adjusted for age, sex and FEV1 percent predicted, $R^2 = 0.036$, $p = 0.02$. (C) Dominant taxa groups are depicted in this clustered biplot of Bray-Curtis distance. Each dot represents unique sample color-coded by dominant taxon. Color-coded arrow (vector) represents the dominant taxon driving the differences.

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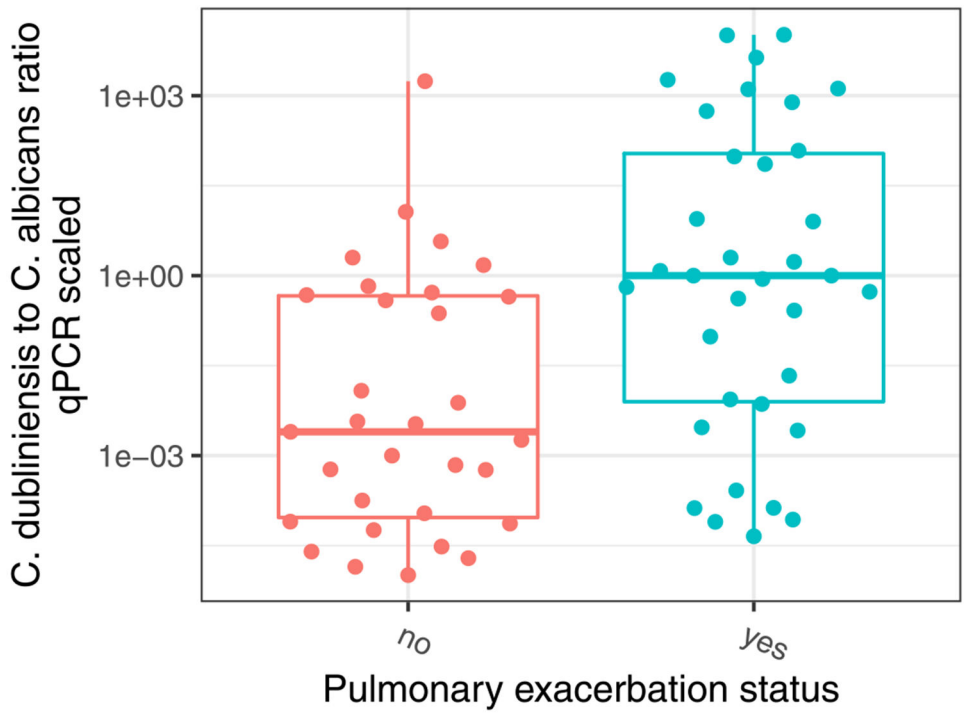

Fig. 5.

The ratio of *Candida dubliniensis* to *Candida albicans* qPCR corrected relative abundance is associated with pulmonary exacerbation status, $p<0.001$. Higher *C. dubliniensis/C. albicans* was observed in CF patients with pulmonary exacerbation.

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Table 1

Subject characteristics (n = 66).

	N (%) or median [interquartile range]
Demographics	
Age (years)	29 [23,41]
Female sex	34 (51.5)
White race	62 (93.9)
Disease characteristics	
F508del homozygous	31 (47.0)
Pancreatic insufficiency	61 (92.4)
Body mass index (kg/m^2)	22 [19.6, 23.8]
FEV_1% predicted	57.5 [40,72]
Cystic fibrosis related diabetes	29 (43.9)
ABPA	4 (6.1)
CFQ-R respiratory quality-of-life *	58.3 [38.9, 72.2]
Medications	
CFTR modulators ≠	24 (36.4)
Ivacaftor	6 (9.1)
Inhaled antibiotics	44 (66.7)
Azithromycin	41 (62.1)
Chronic oral antibiotics	22 (33.3)
Inhaled corticosteroids	54 (81.8)
Prednisone	13 (19.7)
Antifungal	7 (10.6)
Oral and intravenous antibiotic courses in previous 12 months	2 [1,4]
IV antibiotics courses in previous 12 months	0.5 [0,1]

Abbreviations:FEV_1 = forced expiratory volume in 1 second, ABPA=allergic bronchopulmonary aspergillosis, CFQ-R =Cystic Fibrosis Questionnaire-Revised, CFTR =cystic fibrosis transmembrane conductance regulator.

* CFQ-R missing data for one subject.

≠ includes Ivacaftor and Lumacaftor/Ivacaftor.