# #138: Pulmonary mycobiome of patients with suspicion of respiratory fungal infection – an exploratory study

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## ABSTRACT

This pilot study aimed to characterize the pulmonary mycobiome of patients with suspicion of fungal infection of the respiratory tract as well as to identify potentially pathogenic fungi infecting their lungs.

DNA was extracted from the respiratory samples of a cohort of 10 patients with suspicion of respiratory fungal infection. The internal transcribed spacer 1 (ITS1) region and the calmodulin (CMD) gene were amplified by PCR and the resulting amplicons were sequenced through next generation sequencing (NGS) techniques. The DNA sequences obtained were taxonomically identified using the PIPITS and bowtie2 platforms.

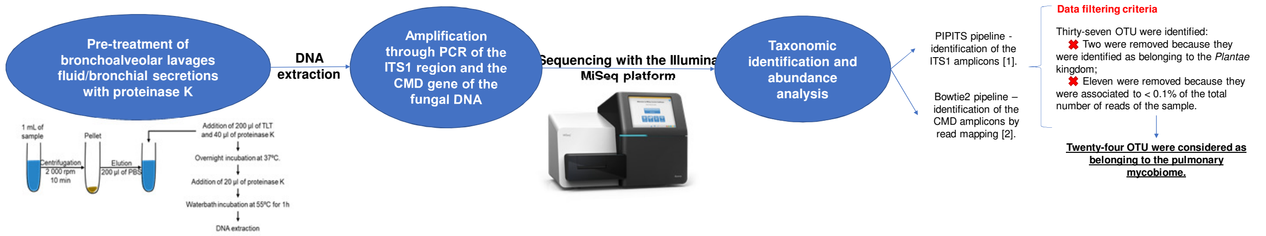
Twenty-four different OTU (grouped in 17 phylotypes) were considered as part of the pulmonary mycobiome. Twelve genera of fungi were identified. The genus *Papilotrema* and the potentially pathogenic genera *Cryptococcus* and *Pneumocystis* were exclusively found in the pulmonary mycobiome of HIV+ patients. Other potentially pathogenic fungi such as *Aspergillus* spp., *Trichosporon* spp., *Saccharomyces* spp. and *Schizophylum* spp. were also detected.

The pulmonary mycobiome of this cohort was rich, highly diverse and variable. This metagenomic analysis showed a great ability to quickly and effectively detect potentially pathogenic fungi in the mycobiome of patients, making it a promising diagnostic tool for fungal infections.

## INTRODUCTION

The possibility of knowing and comparing the mycobiome of healthy individuals with the mycobiome of patients with different pathologies, as well as the capacity to quickly and specifically detect and identify potentially pathogenic fungi present in the pulmonary mycobiome of patients makes NGS techniques useful for the laboratory diagnosis of fungal infections. Thus, the aim of this exploratory study was to optimize the procedure for the detection of fungi through NGS techniques. A metagenomic analysis was performed in order to characterize the pulmonary mycobiome of patients with suspicion of respiratory fungal infection of the respiratory tract (and with different comorbidities such as active or previous mycobacterial infection or HIV+) aiming to identify potential pathogenic fungi colonizing/infecting their lungs.

## METHODS



Pre-treatment of bronchoalveolar lavages fluid/bronchial secretions with proteinase K

DNA extraction

Amplification through PCR of the ITS1 region and the CMD gene of the fungal DNA

Sequencing with the Illumina MiSeq platform

Taxonomic identification and abundance analysis

PIPITS pipeline - identification of the ITS1 amplicons [1].

Bowtie2 pipeline – identification of the CMD amplicons by read mapping [2].

**Data filtering criteria**

Thirty-seven OTU were identified:

✖ Two were removed because they were identified as belonging to the *Plantae* kingdom;

✖ Eleven were removed because they were associated to < 0.1% of the total number of reads of the sample.

**Twenty-four OTU were considered as belonging to the pulmonary mycobiome.**

## RESULTS

Two phyla were identified within the lung mycobiome of these patients: Basidiomycota and Ascomycota (Figure 1A):

- Regarding the **Basidiomycota phylum**, reads were identified in three classes - Agaricomycetes, Tremellomycetes and Walleomycetes (Figure 1B). In this phylum, five orders, seven families and seven genera were identified.
- Regarding the **Ascomycota phylum**, four different taxonomic classes were identified: Pneumocystidomycetes, Dothideomycetes, Eurotiomycetes and Saccharomycetes, with the latter being the most frequent (Figure 1C). In this phylum, five orders, six families and five genera were identified.

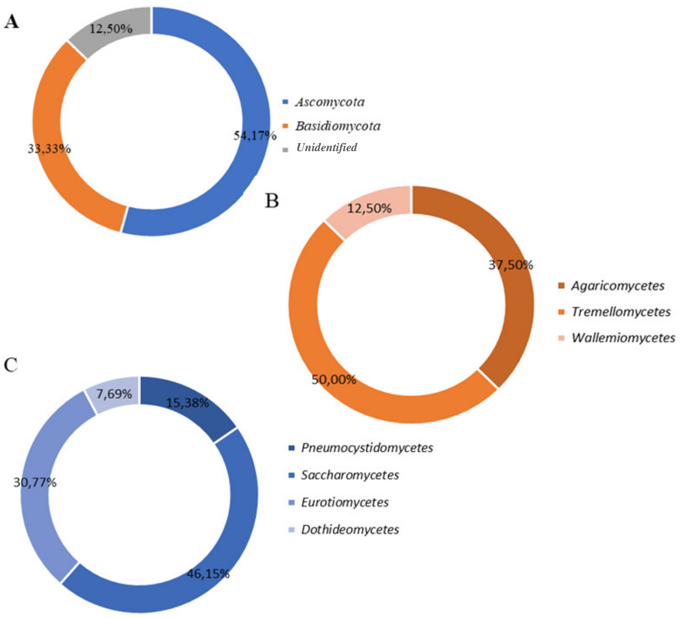


Figure 1 – Frequency of the phylum (A) and the orders of the Basidiomycota (B) and the Ascomycota (C) phylum.

Table 1 – Description of the comorbilities of each patient studied – if they were negative (-) or positive (+) for HIV and/or mycobacterial infection - and the fungal taxa detected in their respiratory samples. In **bold** are the genera of fungi which were exclusively detected in the pulmonary mycobiome of HIV+ patients.

| Patient | Comorbilities | | Fungal taxa detected by metagenomic analysis | |
|---|---|---|---|---|
| | HIV | Mycobacterial infection | Potentially pathogenic fungi | Other fungi |
| 1 | - | - | *Trichosporon* spp.<br>*Aspergillus fumigatus*<br>*Saccharomyces* spp. | *Candida* spp.<br>*Wallemia* spp.<br>*Trichophyton* spp.. |
| 2 | + | + | *Schizophylum* spp.<br>*Aspergillus tabacinus*<br>*Saccharomyces* spp.. | *Candida* spp.<br>*Trichophyton* spp.<br>*Wallemia* spp.<br>*Trametes* spp.<br>*Phlebia rufa*<br>Fungi from the order Eurotiales. |
| 3 | + | + | *Aspergillus fumigatus* | *Candida* spp. |
| 4 | - | + | *Schizophylum* spp.<br>*Trischosporon* spp.<br>*Aspergillus fumigatus* | *Candida* spp.<br>*Trichophyton* spp.<br>Fungi from the order Eurotiales. |
| 5 | + | - | ***Pneumocystis* spp.**<br>*Schizophylum* spp.<br>*Trischosporon* spp.<br>*Aspergillus fumigatus* | *Candida* spp.<br>*Trametes* spp. |
| 6 | + | - | ***Pneumocystis* spp.**<br>*Schizophylum* spp.<br>*Aspergillus fumigatus.* | *Candida* spp. |
| 7 | - | - | *Schizophylum* spp.<br>*Trichosporon* spp.<br>*Aspergillus* spp.. | *Candida* spp. |
| 8 | - | - | *Aspergillus fumigatus*<br>*Schizophylum* spp.<br>*Trichosporon* spp.. | *Candida* spp.<br>*Trichophyton* spp.<br>*Wallemia* spp.<br>*Trametes* spp.<br>*Phlebia rufa*<br>Fungi from the family Aureobasidiciaceae. |
| 9 | + | + | ***Pneumocystis* spp.**<br>***Cryptococcus* spp..** | *Candida* spp.<br>***Papilotrema* spp**.<br>*Trichophyton* spp.,<br>Fungi from the order Tremalalles. |
| 10 | - | - | *Aspergillus fumigatus*<br>*Aspergillus tubingensis.* | *Candida metapsilopsis*<br>*Candida* spp.<br>Unidentified fungi. |

Twelve fungal genera were identified in the pulmonary mycobiome of this cohort.

- *Candida* spp. was the most frequent genus.
- *Papilotrema* and *Cryptococcus* were genus exclusively found in the mycobiome of HIV+ patients (Table 1).
- Other potentially pathogenic fungi such as *Aspergillus* spp. *Saccharomyces* spp. and *Schizophylum* spp. were also detected (Table 1).

## FINAL REMARKS

- ✓ The pulmonary mycobiome is rich and diverse, comprising a variety of different fungal species which vary widely between patients.
- ✓ This metagenomic analysis alllowed the detection of potentially pathogenic fungi (such as *Aspergillus*, *Cryptococcus*, *Pneumocystis, Trichosporon, Saccharomyces and Scizophylum*) in the respiratory samples of patients with different comorbilities.
- ✓ Most of the fungi detected by NGS had not been previsouly detected in the analyzed samples, either because they did not grow in the culture media or because exams for its detection had not been required clinically, revealing that WGS might be a valuable tool in the diagnosis of fungal infections.
- ✓ It is fundamental to optimize and standardize this techniques in order for them to be implemented in the laboratory.

## REFERENCES

1. Gweon HS, Oliver A, Taylor J, *et al.* PIPITS: an automated pipeline for analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. *Methods Ecol Evol* 2015; **6**:973–980.
2. Langmead B, Salzberg SL. Fast gapped-*read* alignment with Bowtie 2. *Nat Methods* 2012; **9**:357-359.

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