Effect of DNA extraction protocol on the fungal microbiota identified from respiratory samples through high-throughput DNA sequencing methods.

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INTRODUCTION AND PURPOSE

The respiratory tract contains a vast community of fungi that remains largely unknown, especially due to the limitations of culture-based methods. Metagenomic studies are of great interest in this area to further apprehend the complexity of the fungal respiratory microbiot[a,b].

However, and by contrast with bacterial metagenomic analyses, standardized methods for the DNA extraction protocols or the PCR amplification targets are yet to be determined for fungi[c,d].

The aim of our study was (i) to investigate the use of **two different DNA extraction methods** and (ii) to analyse **two different amplification targets**, ITS1 and ITS2, on the fungal communities identified in human sputum.

METHODS

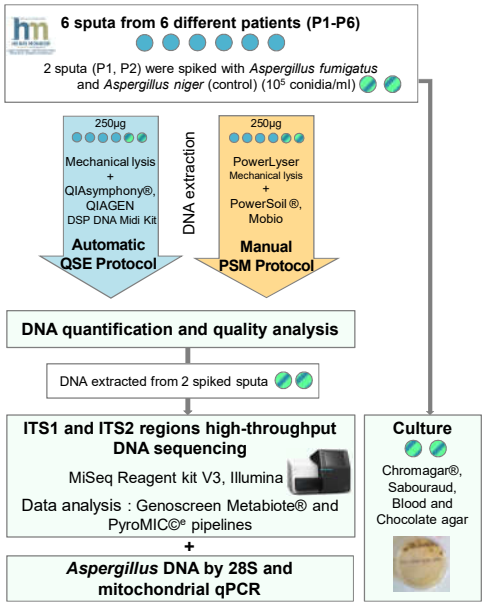

Fig 1: Workflow of the study.

RESULTS AND DISCUSSION

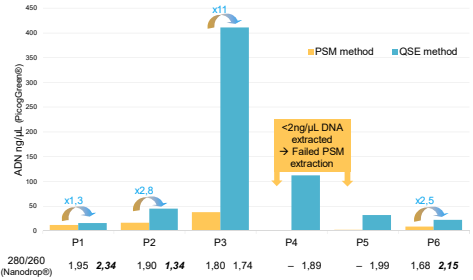

Fig 2: DNA quantification and quality analysis according to the extraction method.

Comparison of DNA extraction techniques for DNA yield and purity on six sputa (P1-P6)

✓Automatic QSE method + prior mechanical lysis:
- Highest DNA yield (1.3 to 16-fold)
- But lower DNA purity
- More reliable (automatic)

30min technique

✓Manual PSM extraction with mechanical lysis included:
- Less reliable (no DNA in 2/6 samples)
- Manual technique
- Time-consuming

2h technique

***Aspergillus* DNA detection by qPCR in the 2 spiked sputa (P1, P2)**

✓Both extraction methods → detection of similar yield of *Aspergillus* DNA.

Patient	Extraction	28S (Ct)	Mitochondrial (Ct)
P1	PSM	22.7	23.8
P1	QSE	22.4	23.6
P2	PSM	23.2	24.4
P2	QSE	23.2	23.9

Table 1: *Aspergillus* DNA expressed in Ct for 28S and mitochondrial qPCR from two spiked samples (P1, P2).

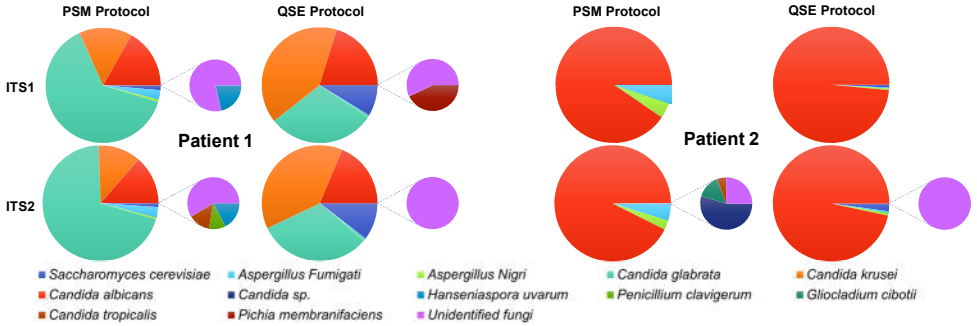

Fig 3: Relative abundances of fungal species identified by metagenomic analysis according to extraction protocol and amplification target.

Metagenomic analysis (31874 reads/sample) of 2 spiked sputa (P1, P2) compared to culture

✓ Whatever the extraction protocol used → identification of more fungal species than culture (4 and 5 species more for P1 and P2, respectively)
✓ All extra species detected by metagenomic → minor taxa with relative abundance <0,05%
✓ Except for one: *Saccharomyces cerevisiae* (P1) → major taxon with relative abundance > 1% (not retrieved in culture)

Impact of extraction protocol on the result of metagenomic analysis

✓Both extraction → identification of the major taxa
✓Relative abundances varied significantly following the extraction protocol
- QSE : the relative abundances of major taxa are closer to the culture results
- PSM: distortion with a significant increase of *C. glabrata* and *Aspergillus* reads

Impact of amplification targets (ITS1 and ITS2) on the result of metagenomic analysis

✓No significant differences in terms of diversity or abundance of major taxa
✓With ITS2 → more minor taxa than ITS1

CONCLUSION

Our data highlighted the superiority of the targeted metagenemic approach over culture for the assessment of fungal diversity in respiratory samples.

It also emphasized the importance of **carefully choosing the method of DNA extraction** for fungal metagenomic analysis, because it highly impacts the yield and purity of DNA extracted, which secondarily impacts the success of the NGS amplification and influences the fungal communities that can be identified, especially in terms of relative abundance of the major and minor taxa.

By contrast, **the choice of ITS1 or ITS2 target** for metagenomics analysis **does not impact significantly** the diversity of **fungal microbiota**.

These points, little described in the literature for respiratory specimens[c,d], are of interest for further use of metagenomics approaches in microbiological labs. However, further studies on the subject should be carried out to define standardized procedures, such as those proposed by IHMS[f] for bacterial analysis. Moreover, studies focusing on accurate protocols for both bacterial and fungal extraction and metagenomics analyses should be needed. In that respect, the results of the metagenomic bacterial analysis of our study are in progress.

REFERENCES

a- Nguyen *et al.* 2015. Front Microbiol. 10.3389/fmicb.2015.00089
b- Willger *et al.* 2014. Microbiome. 10.1186/2049-2618-2-40
c- Dollive *et al.* 2012. Genome Biol. 10.1186/gb-2012-13-7-r60
d- Vesty *et al.* 2017. PLoS One. 10.1371/journal.pone.0169877
e- Sitterlé *et al.* 2017. Front Microbiol. 10.3389/fmicb.2017.00334
f-Dore *et al.* 2015. IHMS Consortium. http://www.microbiome-standards.org

DISCLOSURE

The authors have no conflict of interest to declare.