

# Generation of a fungal consortium: impact of analytic tools on biodiversity analysis

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The biodiversity in bioaerosols is an important issue to investigate for a better understanding of occupational risks due to exposure to airborne particles. The methods used for studying biodiversity include morphology methods, molecular biology or biochemical techniques. While providing different information about the composition of bioaerosols, these methods have specific advantages and limits. High-throughput sequencing followed by bioinformatics analysis is presented as a powerful tool for biodiversity studies that overcome the main drawbacks of the culture-based method. However, these molecular methods may lead to erroneous measurements of microbial composition of complex environmental bioaerosols due to biases that were insufficiently investigated.

In the present study, we used bioaerosols with a known microbial composition and produced in laboratory conditions to investigate such biases. The aim was study the effect of 1) the DNA extraction kit; 2) the target gene for sequencing; 3) the method used for sequences assignment on results from biodiversity measurements in bioaerosols.

The complex fungal bioaerosol was generated from a liquid culture prepared with four different moulds species (*Aspergillus niger*, *Cladosporium cladosporoides*, *Penicillium brevicompactum* and *Wallemia sebi*). The bioaerosols were aerosolized using a bubbling generator (Simon et al. 2011). Aerosolised moulds were collected by filtration of the air through polycarbonate membrane (0.8 µm) using closed-face cassette (Millipore, France), at 2 L/min and for one hour. DNA extraction was performed using the two commercial kits, FastDNA<sup>®</sup>SPIN kit for soil (MP Biomedicals, USA) and PowerSoil<sup>®</sup> DNA isolation kit (MOBio, USA). Then, two genetic targets (18S and ITS2) were sequenced by an external provider (INRA Transfert, France) using Illumina<sup>®</sup> technology. Sequences were cleaned using Mothur software (Schloss et al. 2009). Assignment of 18S sequences was done using BLAST (Basic Local Alignment Search Tool) algorithm with NCBI database. ITS2 sequences were identified using BLAST algorithm or another algorithm executed in Mothur. Cultivable molds were identified according to macroscopic and microscopic criteria. Fungal biodiversity obtained thank that the different methods was compared.

Results show that the MP Biomedicals kit was the only one allowing the extraction a sufficient amount of DNA for sequencing (>3 ng/µL). This demonstrates

that DNA extraction is a crucial step when studying biodiversity by high-throughput sequencing.

Three fungal genera among the four ones composing the initial liquid culture were observed in the generated bioaerosols when biodiversity was assessed by identification of cultured fungi (figure 1). Indeed, *Wallemia sebi*, a small spore size (1-2 µm of diameter) and slow growing fungi, was not observed on the Petri dishes in the experimental conditions. At the opposite, when biodiversity was assessed by the molecular method, *W. sebi* accounted for near 60% of the sequences regardless the target gene and the algorithm used for the sequence assignment. For the other fungi, the proportion of each genus was dependent on the method used. *Aspergillus niger* was weakly detected by sequencing (near 0.10%), may be due to the bad efficiency of DNA extraction for this specie. Furthermore, data from sequencing revealed the occurrence of unidentified sequences at the genus rank, sequences assigned to other fungi and also to plants or animals for the 18S (which is a eukaryota genetic target and can detect all DNA of plants, animals, Fungi or protozoa). These results were not expected and may indicate errors due to sequencing or assignment.

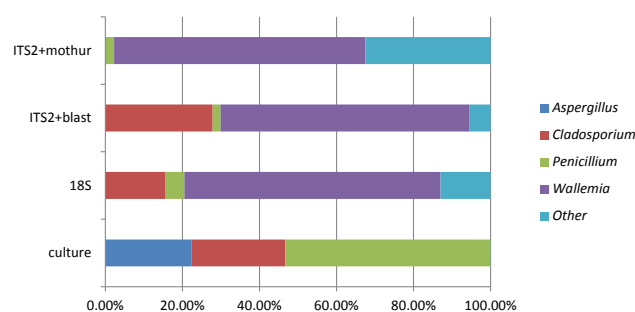


Figure 1: Proportion of the four aerosolized moulds according to several analysis methods

The study shows that results from biodiversity investigations are strongly depending on the analysis method used. In our case, using the MPBio kit for DNA extraction, ITS2 sequencing and BLAST assignment provided results that were the closest to those expected ones. Further studies are required to evaluate the impact of these findings when considering of biodiversity in complex and unknown environments.

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Simon, Xavier, et al. (2011), J. Aerosol Sci., 42 (8), 517-31.