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Impact of DNA extraction methods and culture-independent approaches on canine lung mycobiota analysis

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Introduction

There is growing evidence, at least in humans, that fungi play a role in lung health preservation and in disease development and progression. Our understanding of fungal implication in such conditions relies on accurate and reproducible data acquisition. One of the critical steps in mycobiota analysis concerns DNA extraction as fungi are protected by complex cell wall that resists to classical lysis protocol. There is also a need to limit biases introduced by contaminant DNA, susceptible to result in a wrong mycobiota representation. This concern is of particular importance in healthy lungs where fungi are rare.

In this study, we compared 2 protocols of DNA extraction and 2 sequencing approaches to analyze the lung mycobiota (LMy) of 8 healthy dogs of the terrier breed (i.e. Yorkshire and Jack Russel terrier).

Material & methods

DNA from bronchoalveolar lavage fluid samples were extracted using either the DNeasy Blood and Tissue kit with the pre-treatment for Gram-positive bacteria preceded by a mechanical lysis on FastPrep-24 (Protocol A), or the QIAsymphony DSP DNA Midi kit preceded by a mechanical lysis on TissueLyser and an enzymatic lysis (Protocol B). DNA were then analyzed by amplicon sequencing targeting the internal transcribed spacer (ITS) 2. DNA extracted with protocol B were also analyzed by shotgun metagenomics analysis (MetaMIC®).
Except for the step of DNA extraction, sequencing and data analysis were performed for all samples at the same time and in the same laboratory.

Results

Results of the LMy were highly variable depending on the dog. Comparison between extraction protocols using ITS amplicon profiling revealed that β-diversity was significantly different (P = 0.013) with a greater inverse Simpson diversity index in protocol A compared to B (Figure 1).

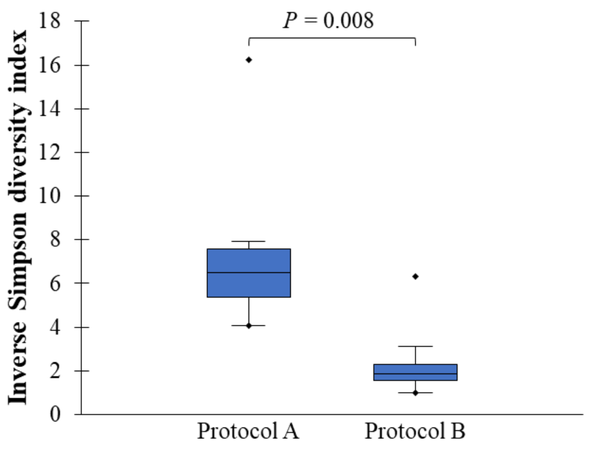

Figure 1: *Box plot graph showing the inverse Simpson diversity index between the 2 extraction protocols. The medians are represented by the central horizontal bars. The lower and upper limits of the box are the first and third quartiles, respectively. Points are considered as outliers.*

Only 2 phyla, Ascomycota and Basidiomycota, were found with protocol B versus 7 with protocol A (Figure 2). In only 2 samples, a similar predominant genus (Malassezia) was identified with the 2 protocols (Figure 3). Shotgun analysis resulted in a small number of fungal DNA fragment identification. It might partly be due to bioinformatics techniques used to process sequences that were designed for human samples. No real correlation was found between ITS amplicon profiling and shotgun results.

Conclusion:

The DNA extraction protocol and the techniques used to sequence DNA and process sequences have a great impact on LMy determination. Accordingly, LMy comparison between studies using different extraction and sequencing techniques is not recommended. The use of bioinformatic tools design for dogs is warranted. The rarity of the LMy of healthy dogs may explain the difficulty in obtaining accurate and reproducible data.

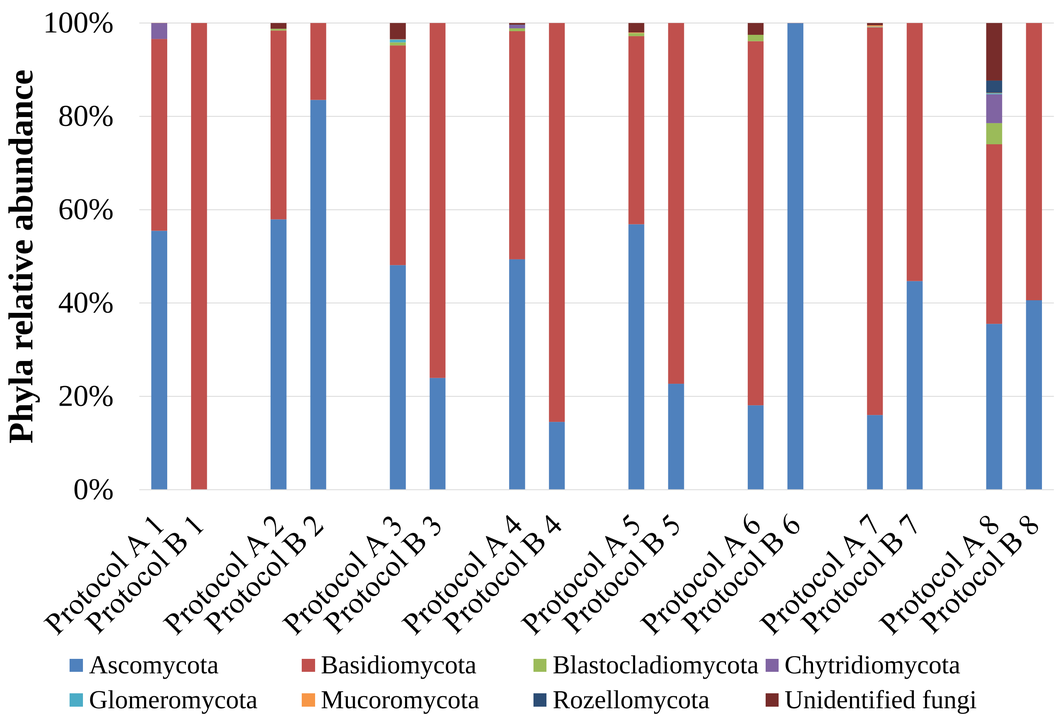

Figure 2: *Bar charts showing relative abundance of all fungal taxa detected in bronchoalveolar lavage fluid collected from 8 healthy dogs according to 2 different extraction protocol (A and B), annotated to the taxonomic level of phylum*

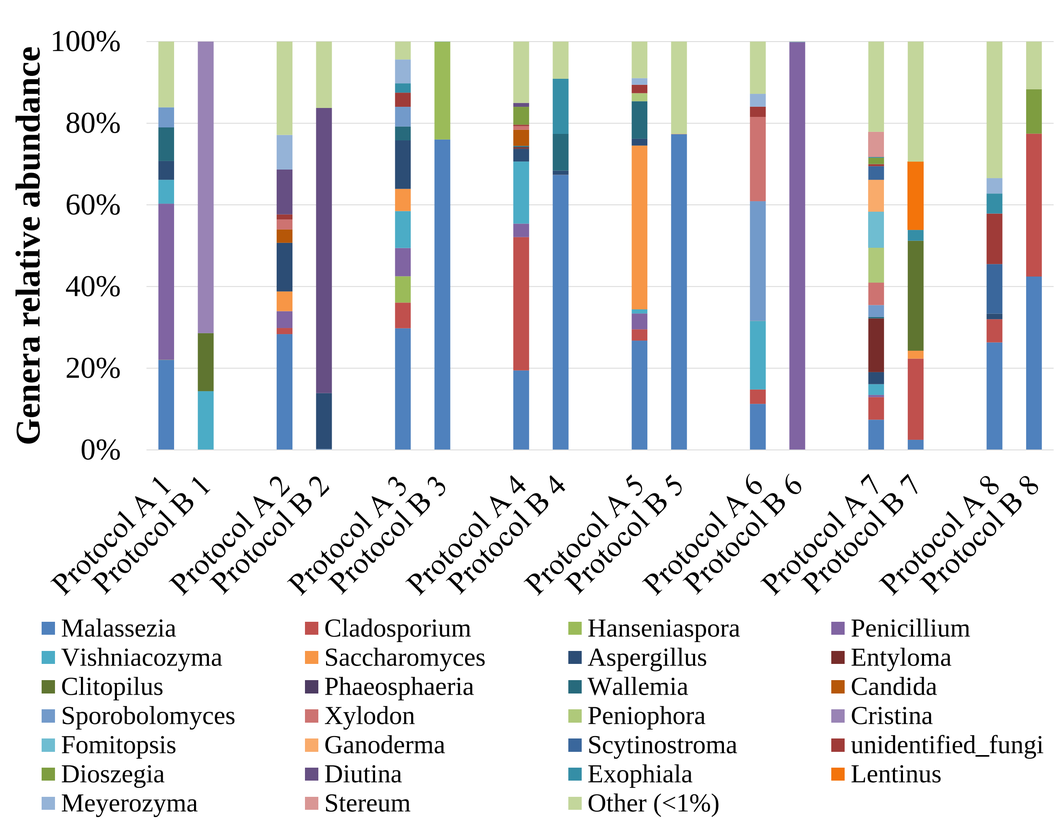

Figure 3: *Bar charts showing relative abundance of all fungal taxa detected in bronchoalveolar lavage fluid collected from 8 healthy dogs according to 2 different extraction protocol (A and B), annotated to the taxonomic level of genus*