

Analysis of bioaerosol diversity in Athens, Greece, by DNA barcoding

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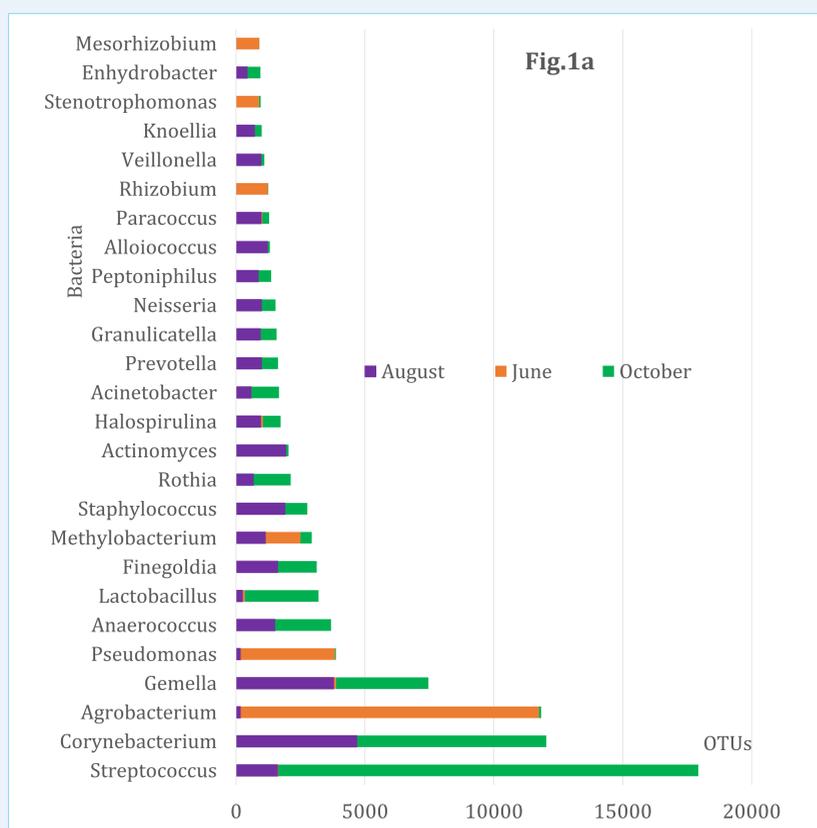
We collected air samples using Rutgers Electrostatic Passive Sampler (REPS) and studied the diversity of bioaerosols in Athens, Greece. REPS is a newly developed passive sampler that does not use any power supplies or air movers, but instead relies on permanently polarized ferroelectric film (Therkorn et al, 2017). REPS uses a specific spiral configuration of the film to capture biological aerosols using an electrostatic mechanism as well as gravitational settling.

Samples were collected in three campaigns in the summer and fall of 2019. REPS collected similar amounts of bacterial and fungal operational taxonomic units (OTUs) per sampling campaign. The internal transcribed spacer (ITS) region was our primary choice for the molecular identification of fungi (Metaxatos et al, 2021). Our air samples in Athens detected two major fungal phyla (Ascomycota and Basidiomycota).

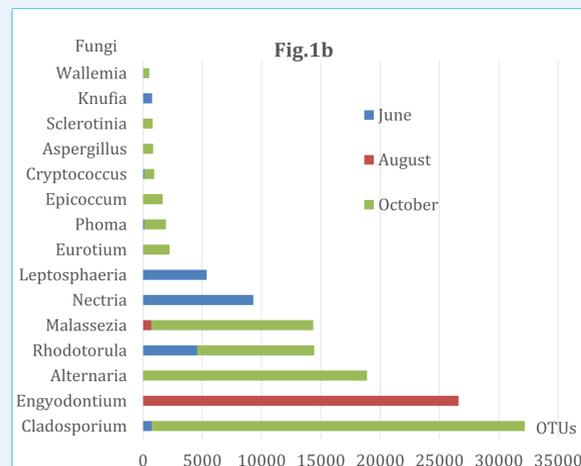
The diversity of bacterial aerosol was also examined by analyzing 16S rRNA genes (Metaxatos et al, 2022). Overall, we detected six bacterial phyla. We found a complex community of bacterial and fungal aerosols with several opportunistic or potential pathogens in Athens' urban air.

The genera *Streptococcus*, *Corynebacterium*, *Neisseria*, *Pseudomonas*, and *Staphylococcus*, are medically important species, and they were dominant in our samples. In addition, the genera *Finegoldia*, *Prevotella*, *Anaerococcus*, *Gemella*, and *Rothia* are part of the commensal human microbiota and opportunistic pathogens (Fig. 1a).

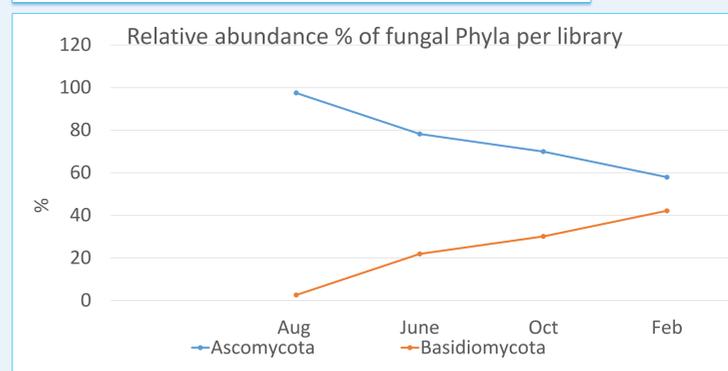
The dominant bacterial (Fig.1a) and fungal genera (Fig.1b) detected in Athens in June, August, and October of 2019, are shown below.



Pathogenic basidiomycetes *Rhodotorula* and *Engyodontium*, as well as allergenic ascomycetes *Aspergillus*, *Eurotium*, *Eurotiales*, *Alternaria*, *Cladosporium*, *Epicoccum*, *Leptosphaeria*, *Eurotium*, and *Epicoccum* were detected, and they feature a seasonal spore release (Fig. 1b).

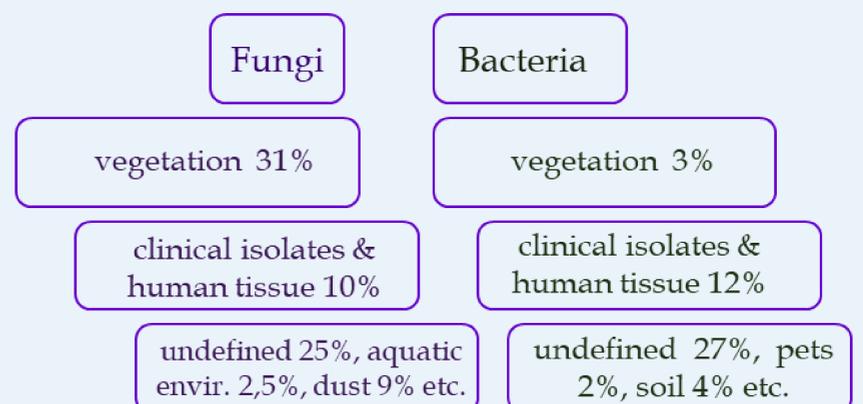


The Rutgers Electrostatic Passive Sampler (REPS)



Final OTUs were taxonomically classified using BLASTn database, where the source of each archived OTU is given. Thus, the main sources of aerosols in Athens, as previously defined (www.ncbi.nlm.nih.gov), are associated with vegetation (31%; 3%), clinical isolates, and human tissue (10%; 12%), while undefined sources reach (25%; 27%) of fungal and bacterial aerosols respectively.

Sources of OTUs detected in the air of Athens



Currently, there are limitations in establishing a reliable link between airborne microbiome diversity, exposure, and health effects. The lack of internationally accepted standards and protocols for bioaerosol sampling and analysis methods and the lack of reliable epidemiological records add to those limitations. New sampling technologies, monitoring regulations, and analytical techniques can help better understand the link between exposures and health effects.

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