

UC Berkeley

UC Berkeley Previously Published Works

Title

DNA-based protocol optimization for bioaerosol sampling in an urban tropical environment

Permalink

<https://escholarship.org/uc/item/1ck093q7>

Authors

Luhung, I
Wu, Y
Nazaroff, WW
[et al.](#)

Publication Date

2014

Peer reviewed

DNA-BASED PROTOCOL OPTIMIZATION FOR BIOAEROSOL SAMPLING IN AN URBAN TROPICAL ENVIRONMENT

Irvan LUHUNG^{1,2}, Yan WU^{1,2}, William W NAZAROFF^{1,3}, Victor W.-C. CHANG^{1,2*}

¹SinBerBEST Program, Berkeley Education Alliance for Research in Singapore (BEARS)

²School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue 639798, Singapore

³Department of Civil and Environmental Engineering, University of California, Berkeley, California 94720, United States

*Corresponding email: wcchang@ntu.edu.sg

Keywords: Tropics, Environmental samples, Bioaerosol, Bacteria, Fungi.

INTRODUCTION

One of the challenges in bioaerosol research is developing a reliable and repeatable protocol for indoor environmental sampling. Collecting environmental samples in warm and humid tropical climates pose distinct sampling and analysis challenges.

DNA extraction is an important step in DNA-based analyses. A large number of bioaerosol-related studies have reported the use of commercially available extraction kits (e.g., MOBIO, Qiagen, Invitrogen) with some modifications such as chemical additions (Noris et al., 2011), additional sonication (Smith et al., 2012) or thermal incubation (Meadow et al., 2014). However, none of these studies report how these changes affect the DNA yields.

The trend toward non-viable analysis methods is accelerating as culture-dependent methods are known to substantially underestimate environmental microbial abundances (Xu et al., 2011; Pietarinen et al., 2008). On the other hand, non-viable methods also pose challenges, among them being the naturally low biomass concentration in air (Peccia and Hernandez, 2006). Some advanced microbiological analysis techniques like metagenomic sequencing may not have the capability to handle the low DNA loadings typical of air samples. This study shows how DNA with adequate quantity and quality can be extracted from local environmental samples in the absence of any amplification measures prior to analysis.

METHODS

Two modifications for the extraction protocol are described here. First, the effect of additional thermal sonication to DNA yield was investigated. Second, two concentration approaches were proposed to circumvent the relatively low DNA abundance issue in bioaerosol samples. The methods were then tested on environmental samples with a range of biomass loadings

Samples were collected from various environments to represent high and low bioaerosol levels. High concentration samples included HVAC filters that were obtained from buildings at the Nanyang Technological University in Singapore. Low biomass samples were collected from ambient air by means of impacting air onto a PES filter membrane at a flow rate of 20 L/min for 8 hours.

Following collection, DNA was extracted from all samples with the MOBIO Power Water (PW) DNA extraction kit. An additional period of 30 minutes for thermal sonication (65 °C) was added to the cell lysis step of the original PW protocol for one set of samples and the DNA yield was compared to the one that followed the original MOBIO protocol.

In addition to lysis modification, two concentration approaches were attempted to investigate whether more biomass could be gathered from several low concentration samples without necessitating any replication, either by PCR or via culturing. The first approach was to concentrate during extraction. Two set of identical samples (HVAC filters and ambient air samples) consisting of 4 filters each were prepared. Three filters were then concentrated into one during the spin filter-binding step of the MOBIO PW protocol, whereas the other filter was extracted normally. The DNA yields were studied to see whether the expected 3 to 1 ratio was obtained for both high and low biomass samples. The second approach was concentration post-extraction. Several extracted DNA solutions were pooled and concentrated using an Amicon Ultra concentrating centrifuge. Quantitative PCR (Roche LC 480) and Qubit fluorometry (Invitrogen) were applied to quantify and verify the quality of the extracted DNA solutions.

RESULTS AND DISCUSSION

The MOBIO PW kit was preferred over other extraction kits mainly due to its inhibitor removal technology (IRT), flexibility and the fact that it is uniquely designed for filter-sample extraction. Extra thermal and sonication lysis were conveniently added prior to the bead-beating step of the original PW protocol. As shown in Figure 1, the alteration effectively increases the overall DNA yield from both samples by at least 2.5 times.

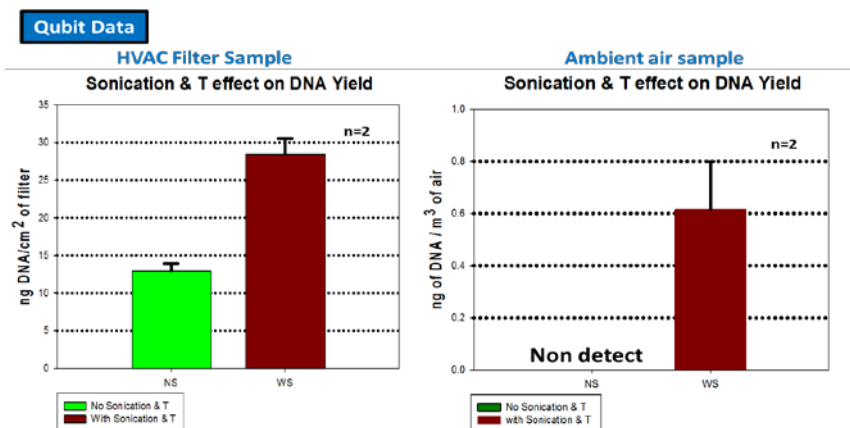


Figure 1. The effect of thermal sonication lysis on DNA yield for HVAC filter samples and ambient air samples.

We speculate that the higher DNA yield from the extra lysis originates from cells with thicker cell walls such as gram positive bacteria or fungal spores (Wu and Yao, 2010), which were not effectively lysed previously. The false negative result from the first ambient air sample suggests that such additional step is crucial for quantification of low biomass samples.

Next, considering concentration during extraction, Figure 2 displays how Qubit assay verified that the biomass from the three filters of both samples were successfully concentrated.

The results indicate that higher efficiency was achieved during the concentration approach as all samples exceeded the 3 to 1 ratio. DNA loss during the concentration process appears to be minimal. In addition, the spin filters of PW kit appear to trap more DNA as exposure times

to the solution increase. Finally, the post-extraction concentration approach looks to be less efficient for both types of environmental samples due to the observed DNA loss (30%).

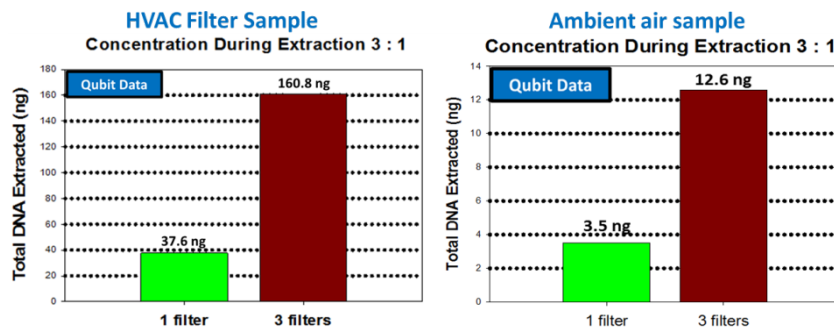


Figure 2. 3 to 1 concentration during extraction approach results on HVAC filter samples and air samples.

CONCLUSIONS

An amended sampling and extraction protocol was demonstrated to provide good results in dealing with tropical urban environmental samples. The modifications help indoor bioaerosol research to achieve what was previously unachievable. For instance, the DNA produced by our methods was then successfully processed for unamplified metagenomic sequencing. This achievement indicates that the optimized protocol is able to generate DNA from ordinary environments with sufficient quality and quantity for both amplicon and metagenomic workflows. It is a good foundation for investigations of the indoor microbiome in the tropics.

ACKNOWLEDGEMENT

This research is funded by the Republic of Singapore's National Research Foundation through a grant to the Berkeley Education Alliance for Research in Singapore (BEARS) for the Singapore-Berkeley Building Efficiency and Sustainability in the Tropics (SinBerBEST).

REFERENCES

- Meadow JF, Altrichter AE, Kembel SW et al (2014) Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air*, **24**, 41-48.
- Noris F, Siegel JA, and Kinney KA (2011) Evaluation of HVAC filters as a sampling mechanism for indoor microbial communities. *Atmospheric Environment*, **45**, 338-346.
- Peccia J and Hernandez M (2006), Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. *Atmospheric Environment*, **40**, 3941-3961.
- Pietarinen VM, Rintala H, Hyvärinen A, et al (2008) Quantitative PCR analysis of fungi and bacteria in building materials and comparison to culture-based analysis. *Journal of Environmental Monitoring*, **10**, 655-663.
- Smith DJ, Jaffe DA, Birmele MN et al (2012) Free tropospheric transport of microorganisms from Asia to North America. *Microbial Ecology*, **64**, 973-985.
- Wu Y and Yao M (2010) Inactivation of bacteria and fungus aerosols using microwave irradiation. *Journal of Aerosol Science*, **41**, 682-693.
- Xu Z, Wu Y, Shen F et al (2011) Bioaerosol science, technology, and engineering: past, present, and future. *Aerosol Science and Technology* **45**, 1337-1349.