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Protocol Optimization on Bioaerosols Analysis in Tropical Buildings

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Introduction

Bioaerosols refer to the airborne particles that contain living organisms or were released from living organisms (e.g. fungi, bacteria or viruses…etc). They are of concern due to their potential impacts on human health and productivity.

Bioeaerosols are typically abundant in tropical urban environment because of the warm and humid climates. The major sources of indoor bioaerosols include human occupants, indoor dust, organic waste, and the ventilation system. As more than 95% of local office buildings are air-conditioned, the ventilation system might served as an important reservoir for biological aerosol in the indoor environment.

Current Issues and Objectives

The main problem currently faced in bioaerosols studies is the lack of reliable protocols for both qualitative and quantitative examinations. The bioaerosols concentrations are typically very low, while most of the current analysis methods are designed for liquid based sampling which have considerably higher concentration compared to air samples.

The current work aims to compare, analyze and further optimize various bioaerosols analysis methods in order to develop a solid and reliable protocol for further evaluation of healthy indoor environment.

This tool is essential for creating the fundamental knowledge in the relationship between building operation, indoor environmental quality, indoor microbial community, human activities, human health and productivity. It helps to identify the source populations and provide information on the processes that suspend and disseminate microbes and microbial products in indoor environment.

Methodology Overview

Aerosolised into indoor env.
→ Trigger Sick Building Syndromes and other health concerns

Moldy and dusty air ducts, damp/humid corners
→ Potential microorganism reservoir
→ Might affect occupants' health and productivity

Bioaerosol Sampling :
- Filter based sampling
- Culture based sampling
- Other possible approaches

Bioaerosol Existence Check :
- Culture Incubation in 30°C & 37°C
- Filter Staining & Microscopic Image

Exist

Non Exist → **Keep Monitoring**

Sample Pre-Treatment
- Bead beating process
- Filter cutting/segmentation
- Other concentration method

DNA Extraction & Purification:
- MagNa Compactor (Automated DNA extraction)
- Isolation Kits (manual DNA extraction with some cell lysis buffer solutions)

Quantification & Speciation
- qPCR (DNA based quantification approach)
- Gene sequencing (identify species for health assessment and mitigation)

Bioaerosols Sampling

Fig. 1 bioimpactor

← Cultured Based Approach (Figure 1)
- LB agar plates is used in the bio-impactor with fixed air flowrate for sampling.
- 3-10 minutes sampling time depending on the amount of bioaerosols.
- Incubated in 30°C and 37°C for culture growing and quantification

Filter Based approach (Figure 2) →
- Filter is used to collect bioaerosols when air flow through the filter
- Filter sampling can go up to 24 – 48 hours in duration.
- Stained for microscopic imaging or processed for qPCR quantification.

Fig.2 Sampled filters

DNA Extraction and Bioaerosols Quantification

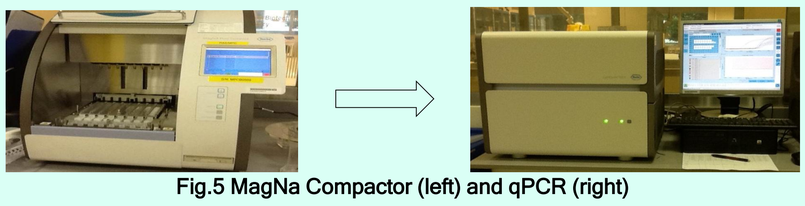

Fig.5 MagNa Compactor (left) and qPCR (right)

- Bioaerosols quantification is done by using quantitative polymerase chain reaction (qPCR) which amplifies the targeted DNA stains by temperature cycles.
- The preliminary results showed the inhibiting effects from the environmental samples, indication the needs for optimizing sample pre-treatment process as well as DNA purification & extraction methods.

Bioaerosols Existence Check

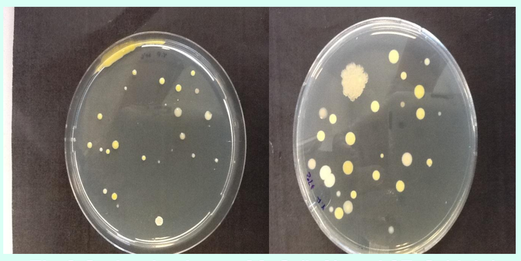

Fig.3 Stained cultures 30°C (left) and 37°C (right)

- The Cultures Incubated in both 30°C and 37°C confirmed the existence of indoor bioaerosols.
- Different morfologies in both cultures indicate different species of microbial.

- Sampled filter is treated with DAPI which stains the A-T rich region of DNA
- Epifluorescent microscope image shows the substantial amount of biological matters are attached on the filter. The biotic materials appears in blue, while the **black** parts are abiotic materials.

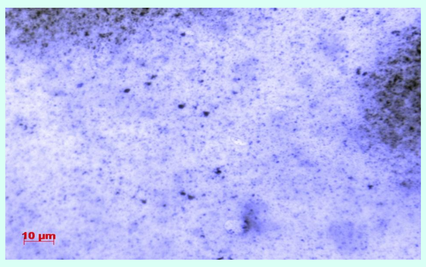

Fig. 4 Microscopic image of sampled filter

Current Findings

- Bioaerosols do exist in a substantial amount in local environment.
- Indoor environment has more bioaerosols than outdoor ambient environment. Some possible reasons include human activity, building envelop, air conditioning and mechanical ventilation (ACMC) system, and building materials.
- qPCR based analysis relies on a decent DNA isolation process. Some current automated purification method did not produce satisfactory results in bioaerosols studies. Further investigation is needed to optimize the detection protocol for local environment.

Future Plans

- Further optimizing various parameters in the detection protocols including filter materials, extraction methods, DNA isolation and purification approaches, and PCR amplification processes, etc.
- Develop capability for bioaerosol speciation