Employment of a PCR-Based Monitoring System to Detect Bacteria and Fungi from Indoor Air Samples at Indoor Work Places: A Pilot Study in Ponce, Puerto Rico

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We analyzed a total of 125 air samples from 25 offices during five consecutive weeks. The sampling area was established as one air sample per 15m2. Collected samples (cassettes) were stored at −20°C until testing. DNA from air samples was extracted using the SoilMaster DNA Extraction Kit, DNA amplification of fungal and bacterial DNA (with universal primers P4/P5 and NS1/NS2 respectively) was performed using a Triple Master Kit. PCR conditions were set in a Eppendorf Gradient Mastercycler. The PCR products were analyzed in a 3% agarose gel, stained with ethidium bromide and visualized under UV light. Our data showed that fungal fauna other than *A. niger* or *A. flavus* is present in 87.2% of the samples. Statistical analysis demonstrated that none of the variables affects the PCR products. According to the weekly questionnaire, 50% of the negative PCR results can be attributed to cleaning measures that were performed within the weekly monitoring. The PCR-based detection method was proven to have an excellent potential for the detection of fungi and bacteria in the work place.

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Introduction

To decrease the risk of exposure to indoor air pollutants, federal agencies have established specific guidelines for monitoring such substances in air samples. However, other products, which have the potential for triggering respiratory diseases and are found at indoor air settings (endotoxins, fungal spores and mites allergens) are not included in the standard air-monitoring protocols. The bacteria and fungi found in the indoor air are known to cause human respiratory system diseases including infections and hypersensitivity responses.

Research Aims

We conducted this research to: 1) design and apply a PCR-based air screening panel to detect bacteria and fungi; and 2) evaluate indoor-environmental factors (temperature, humidity, ventilation) that may influence infestation levels of fungi and bacteria during a five-week period at indoor-work places. The analysis of air sampling using the PCR Air Screening Panel on periodical bases may be employed as an early detection tool in the case of potential epidemics and to prevent dissemination of contagious respiratory agents at indoor work places. Additionally, this system may allow a bioterrorism attack/attempt to be detected earlier. This project also identified and monitored bacteria and fungi that have not been documented as possible causes of the reported respiratory diseases at work/indoor settings in Puerto Rico.

Methods

Air Sample Collection

Air sampling was performed using the Zefon air pump with a 37 mm blue styrene 3-piece cassette (0.4um polycarbonate). The sampling time was 15 minutes at a rate of 15L/min (recommended rate by EPA/OSHA guidelines). The sampling area was established as one air sample per 15m2 of indoor area. Collected samples (cassettes) were stored at −20°C until testing. The air sampling was performed weekly on each location, during a period of five weeks. Initial conditions were documented in a questionnaire, which included the season, temperature, humidity, number of windows, number of mechanical fans, presence of air conditioning system and type (console, portable or window), presence of rugs and type of construction of the building. Variable conditions such as the season, temperature and humidity were recorded at every sample collection time. As well, changes in ventilation or air conditioning system were documented in the weekly report. Field blank samples were conducted in parallel to each air sample.

DNA Extraction, PCR and Electrophoresis

DNA from air samples was extracted using the SoilMaster DNA Extraction Kit (Epicentre Inc.) following manufacture's instructions. DNA amplification of fungal and bacterial DNA was per-

formed using a Triple Master Kit (Eppendorf Co.). This was accomplished utilizing a total PCR reaction volume of 20μl: 5.0μl of previously extracted air DNA, 0.5μl of each 10μM primer, 3.2μl of 10× HighFidelity Buffer with Mg^{2+}, 0.4μl of dntps mix (10mM each), 0.3μl of TripleMaster Enzyme Mix (1.5U), and 10.1μl of molecular grade distilled water. PCR conditions were set in an Eppendorf Gradient Mastercycler as follows: initial denaturation for 5 min at 94°C and 30 cycles of amplification using a step program of 20 sec at 94°C, 20 sec at 52.0°C (P4/P5 and NS1/NS2) and 5 min at 72°C. This was followed by a final extension of 10 min at 72°C and held at 4°C. Different conditions were used for the primers. The PCR parameters for these primers were as follows: denaturation at 96°C for 2 min; 30 cycles at 96°C for 30 sec, 57°C for 10 sec and 74°C for 60 sec. This was followed by a final extension of 10 min at 72°C and held at 4°C. PCR products were stored at −20°C until the electrophoresis was performed. Positive and negative controls were included in each reaction. PCR products were run in a 3% agarose gel, stained with ethidium bromide and visualized under UV light.

Statistical analysis was performed using a Stata (Stata Corporation TX). The chi square and the Student's *t* test were conducted.

CONCLUSIONS

No significant differences were observed between the temperature or humidity and number of windows, number of persons working per sampling area, or the presence of rugs or curtains to influence the PCR result for fungi or bacteria. According to the weekly questionnaire, 50% of the negative PCR results could be attributed to cleaning measures that were performed within the weekly monitoring. Fungal fauna other than *A. niger* or *A. flavus* were present in 87.2% of the samples. The PCR-based detection method was proven to have an excellent potential for the detection of fungi and bacteria.