MolDeMould – Molecular detection of airborne mould by microfluidic qPCR

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The presence of indoor mould increases the potential risk to develop adverse health effects like respiratory symptoms and infections, skin symptoms, asthma or allergies (Heseltine, 2009; Twaroch, 2015). Such impairment to health can be caused by viable as well as non-viable fungal spores and mycelia fragments (Simon-Nobbe, 2008). The majority of commonly available detection systems are based on cultivation to determine the amount of fungal particles in settled dust or air.

Aim of this study is to establish a molecular detection system for airborne mould to provide qualitative and quantitative information of indoor fungal particle exposure. A broad coverage of the airborne fungal biodiversity is envisaged, to also allow efficient detection of unusual indoor contaminants. Biodiversity together with a comparison of indoor and outdoor fungal communities are essential parameters for evaluation of indoor mould situations and are used for interpretation of the measured samples.

The analytical method is based on quantitative PCR (qPCR) which is able to reveal the fungal load independently from viability and therefore permits a more precise determination of the biological air quality. The microfluidic system, for which this detection tool is designed, combines conventional quantitative PCR with partial automation and high throughput analysis. Dependent on the chosen format, up to several thousand qPCR reactions can be performed in parallel. This technique enables the samples to be analysed for a broad diversity of relevant fungal communities within one run. For the microfluidic system, a preamplification PCR step has to be introduced prior to the actual specific measurements of the fungal groups to enlarge the DNA contents of the samples.

So far 20 specific qPCR assays have been developed to detect and quantify relevant indoor moulds as well as important fungal outdoor reference species. The assays are designed to cover mainly higher phylogenetic levels such as orders or genera to cover a wide spectrum of airborne fungi present in the samples (Table 1.).

Table 1. Phylogenetic levels of developed qPCR assays.

Genera	9 specific assays
Order	8 specific assays[a]
Phyla	2 specific assays[a]
Higher	1 Assay Fungi common[a]

[a] some assays contain previously published primers

Bioaerosols from a bioaerosol chamber (Konlechner et al., 2013) produced under standardized conditions as well as bioaerosols from different sampling sites (high fungal contamination, no expected fungal load, outdoor references) have been collected. Besides the molecular biological analysis, culture-based samples of different commonly used sampling methods (impaction, impingement, filtration sampling) have been compared to check for the reliability and stability of the newly developed molecular detection system. For the molecular biological analysis, the wet-wall cyclone sampler Coriolis µ, (Bertin Technologies, France) was used. An advantage of this sampling device is that particles are collected in liquid, allowing cultivation on the one hand and molecular identification and quantification of the same sample on the other hand.

The comparison of the different sampling devices shows huge differences in collection efficiencies. Preliminary data of the molecular biological detection system agree with the findings of the culture-based systems to distinguish between outdoor references and contaminated sampling sites. Further optimization of the designed assays (specificity, stability) and optimization of sampling and sample preparation (detection limit) will provide a useful molecular biological tool to detect actual airborne fungal exposure.

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