

# Rapid monitoring of bioaerosols in industrial, agricultural and urban environments

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Understanding the identities, distribution and abundance of airborne microorganisms remains in its infancy. Meanwhile, the impact of emissions of these microorganisms from urban, agricultural, and industrial areas on local air quality is a growing concern for public policy. Current monitoring technologies take days to generate results and do not address the identities of the microorganisms in detail. Rapid, real-time monitoring technologies are required to do this and to provide a solid evidence base for developing public policy. The aim of this research is to combine next-generation sequencing with fast sampling methodologies to enable real-time monitoring of bioaerosols.

Previously the bioaerosol microbial community was analysed downwind, upwind, and on sight at a composting sight in the UK (Pankhurst *et al.* 2012). Culture-independent analysis of the 16s rRNA gene and microbial lipid markers showed the compost sight had a significant effect on the structure of the downwind bioaerosol microbial community and that some genera, including some pathogens, had a greater propensity to disperse. The bioaerosol community however showed significant variation between sampling visits; to fully understand this variation greater phylogenetic resolution is required to link site activity and meteorological conditions to the bioaerosol community.

In the current project we are developing tools for the rapid monitoring of bioaerosols in industrial, agricultural and urban environments using high-throughput sampling and next-generation sequencing technologies. A number of sampling methods have been trialled, including air filtration, liquid impingement with Coriolis and SKS, and Andersen impactor. These have been tested in controlled chamber experiments, and in field experiments at urban, industrial, and agricultural sites.

First we optimised methods for DNA extraction from filters and liquids for use in bioaerosols detection. We tried two filters, gelatine and polycarbonate (PC), and three liquid impingement matrix; distilled water, Phosphate buffered saline (PBS), and Tris(HCL). To determine the detection limits for bacteria on filters and in liquid impingement, known numbers of either *Bacillus subtilis* or *Escherichia coli* were spiked onto filters or into 15 ml of liquid matrix. DNA was then extracted directly from the filter, or for the liquids a bacterial pellet was obtained by centrifugation at 4500 g for 45 mins. DNA extraction was carried out with incubation with SDS extraction buffer at 70°C for 45 mins followed by bead lysis and phenol-chloroform extraction. PCR of

the 16s rRNA was then carried out on the purified cDNA. We found that bacterial DNA was already present in the gelatine filters; therefore these are inappropriate for our applications. For the liquid matrix PBS performed the most consistently in field tests, however in the lab the difference between tris(HCL) and PBS was minimal. The detection limits for *E.coli* and *B.Subtilis* was 7500 and 70,000 cells per filter/15 ml of PBS respectively. There was no difference in detection limits for filters or in PBS, therefore the main limitation in sampling with filters or liquid impingement is not the DNA extraction but the amount of air sampled.

In the case of this research the aim to have as short a sampling period as possible, both so that sampling can be rapid and also so that the microbial community can be correlated with changes in climatic conditions or site activity. Field tests were carried out with sampling onto filters with a vacuum pump at 30 l/m and with a Coriolis  $\mu$  microbial air sampler. For the filters various sampling times from 2 mins up to 2 hrs were trialled. For the Coriolis  $\mu$  times from 2 mins up to 10 mins were trialled at 300 l/m. Results with the filters were very inconsistent with many samples not containing any amplifiable DNA, even at sampling periods > 1 hour, whereas the Coriolis  $\mu$  was able to capture amplifiable DNA consistently at 10-20 mins even in sites with the lowest bioaerosol concentrations. Therefore it was determined that the Coriolis  $\mu$  air sampler was the most suitable sampling method for our applications.

The next step in this research is to develop in field qPCR and loop mediated isothermal amplification of DNA for rapid detection and quantification of specific pathogens in the field with same day results. We are also using next-generation sequencing on MiSeq and HiSeq to examine the structure and function of the bioaerosol microbial communities at industrial, agricultural and urban environments in relation to climate and activity. Combining this with concurrent work on the chemical composition of bioaerosols and traditional bioaerosols monitoring technologies will enable us to predict when and where to target bioaerosol sampling.

References: Pankhurst *et al.* (2012) Temporal and spatial changes in the microbial bioaerosol communities in green-waste composting. FEMS Microbiol Ecol; 79(1):229-39.

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