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Temporal variation in airborne microbial populations and microbially-derived allergens in a tropical urban landscape

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HIGHLIGHTS

- Tropical urban bacterial and fungal bio-aerosols displayed pronounced seasonality.
- Bio-aerosol composition was determined by climate rather than airborne pollutants.
- A range of bacterial and fungal pathogens was detected in bio-aerosols.
- Microbially-derived allergen levels exceeded safe levels during summer months.

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ABSTRACT

The microbial component of outdoor aerosols was assessed along a gradient of urban development from inner-city to rural in the seasonal-tropical metropolis of Hong Kong. Sampling over a continuous one-year period was conducted, with molecular analyses to characterize bacterial and eukaryal microbial populations, immuno-assays to detect microbially-derived allergens and extensive environmental and meteorological observations. The data revealed bio-aerosol populations were not significantly impacted by the level of urban development as measured by anthropogenic pollutants and human population levels, but instead exhibited a strong seasonal trend related to general climatic variables. We applied back-trajectory analysis to establish sources of air masses and this allowed further explanation of urban bio-aerosols largely in terms of summer-marine and winter-continental origins. We also evaluated bio-aerosols for the potential to detect human health threats. Many samples supported bacterial and fungal phylotypes indicative of known pathogenic taxa, together with common indicators of human presence. The occurrence of allergenic endotoxins and beta-glucans generally tracked trends in microbial populations, with levels known to induce symptoms detected during summer months when microbial loading was higher. This strengthens calls for bio-aerosols to be considered in future risk assessments and surveillance of air quality, along with existing chemical and particulate indices.

1. Introduction

Microorganisms disperse widely in the aerosphere (Griffin, 2007; Burrows et al., 2009). Numerous studies have demonstrated the recovery of cultivable taxa and/or DNA signatures, most notably from bacteria and fungi and including pathogenic species (Griffin, 2007). Biological materials accounts for up to 25% of aerosolized matter (Jaenicke, 2005), which includes allergens (e.g. endotoxins, glucans) of microbial origin (Horner et al., 1995). The emerging field of aerobiology, the study of these bio-aerosols, has been identified as a high priority and immediacy issue in relation to not only public health, but also climate, environment, ecology, epidemiology and environmental engineering (Peccia et al., 2008).

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The occurrence of bio-aerosols within indoor built environments has received much attention (Li et al., 2007; Rintala et al., 2008; Tringe et al., 2008) due to public health concern. In contrast, outdoor environments are often well-characterized in terms of particulate or chemical pollution as part of air-quality monitoring efforts (Pöschl, 2003), and yet relatively little is known about biodiversity or the spatio-temporal dynamics of outdoor bio-aerosols in urban areas. In particular the impact of urbanization on natural microbial populations, and potential to detect possible threats of outdoor bio-aerosols to human health, are not well understood. Early studies employed cultivation approaches to characterize outdoor urban bio-aerosols, including presence of pathogenic taxa (Mancinelli and Shulls, 1978). More recently, molecular surveys have provided critical insight as to the biodiversity of these aerosols (Maron et al., 2006; Brodie et al., 2007; Frohlich-Nowoisky, 2009; Bowers et al., 2011b), but also highlight new paradigms in their ecology that have yet to be resolved.

In an urban setting, an additional factor is the contribution of microbial allergens to public health issues. There is a lack of data on the occurrence of microbial allergens (e.g. bacterial endotoxins, fungal glucans and mycotoxins) in outdoor urban environments, although their contribution to allergies from indoor air studies is well documented (Horner et al., 1995). Evidence is also emerging that urban human populations may experience elevated incidence of allergy due to lack of exposure to 'natural' environmental microbiota (Hanski et al., 2012), and so understanding the reservoir and dynamics of microbial bio-aerosols in urban areas potentially has major public health implications.

The coastal metropolis of Hong Kong provides an ideal model system against which to test hypotheses related to the impact of urbanization on bio-aerosols since a large population (approx. 7 million people) inhabit a steep and relatively well-defined gradient of urbanization, with a comprehensive network of meteorological stations that measure a broad suite of climatic and pollutant variables. A high level of regional relevance exists since Hong Kong exhibits one of the world's highest rates of respiratory allergy as a result of poor air quality (Leung, 1993; Leung et al., 1997), although the relative contribution of abiotic and biotic components to allergy symptoms is unknown. Global relevance arises as Hong Kong represents an 'extreme' case that mimics the situation likely to be faced in other cities worldwide as human population (and hence urbanization) increases. Here, we present the first multi-domain assessment of urban microbial bio-aerosols over a continuous annual cycle. We use high-throughput molecular analyses and immunoassays to detect changes in microbial populations and microbially-derived allergens. Multivariate analyses indicate that local climate and regional air masses best explained changes within urban and rural areas, and urbanization *per-se* does not affect microbial dispersal in Hong Kong. We also highlight the potential for identification of pathogenic and allergenic signals from such data sets.

2. Material and methods

2.1. Sampling locations, environmental data and bio-aerosol collection

Near-ground (2 m average elevation) air samples were collected at 4 outdoor meteorological station locations along a gradient of urbanization in Hong Kong, a seasonal-tropical metropolis with a population of approximately 7 million (Fig. 1, Supplementary Table S1). The gradient was defined according to criteria based upon long-term environmental and population monitoring, by the Environmental Protection Department of the HKSAR government. Aerosols were sampled on a weekly basis over one year during November 2008 until October 2009 with randomization within any given week to account for differences in urban inputs (i.e. weekends, public holidays). A comprehensive suite of environmental and climatic variables was measured during this period (Supplementary Table S1) for each sampling interval ($N = 192$, corresponding to 48 samples per location over a one year period). Aerosol samples were collected using a two-stage bioaerosol cyclone sampler (National Institute for Occupational Safety and Health, CDC, US) attached to universal sampling pumps (SKC Inc, 224-PCXR8, US). The sampling time was 12 h, and mean air volume collected was 2500 L for DNA and 1800 L for allergens. Filters inserted into cassette apparatus but not exposed to air-flow were used as controls. All filters and cassettes were UV-sterilized and rinsed with 70% alcohol before use.

Air fractions for endotoxin and β-glucan analysis were sampled in parallel to DNA sampling using endotoxin-free cassettes (Zefon International, 4 μm PC 37 mm 3 PC ENDOTEC). Two endotoxin/β-glucan samples were collected per month for each sampling site during an 8-month sampling period from March–October 2009 and analyzed in duplicate ($N = 64$), although a technical error (equipment malfunction during automated analysis) resulted in 25 glucan analyses being voided (final $N = 39$). All samples were stored at −80°Cuntil processed. Calibrations of the flow rate for the pumps were made every month to ensure sampling volume consistency. Meteorological data were obtained from the Hong Kong Observatory website: (http://www.hko.gov.hk/cis/data/awsext_e.htm). Pollution variables including Air Pollution Index, sulfur dioxide, respirable suspended particulates, nitrogen oxides, nitrogen dioxide, carbon monoxide and ozone were obtained from the Environmental Protection Department, Hong Kong SAR website: (http://epic.epd.gov.hk/ca/uid/airdata/p/1).

2.2. DNA recovery, PCR amplification, terminal RFLP and pyrosequencing

Total DNA was extracted directly from the filters and tubes using the DNeasy Plant Mini Kit (Qiagen, CA, US), after first washing with kit lysis buffer for 10 min. The remaining steps of the extraction were carried out according to the manufacturers instructions. Recovered DNA was quantified using Nanodrop (Thermo-scientific). The PCR reaction comprised a 25 μl PCR mixture containing 0.1–2 μl of DNA template, 0.5 μM of each primer, 2.5 units of high fidelity *Taq* polymerase (Takara), 1× PCR buffer provided by the manufacturer, 200 μM of each dNTP, and H_2O. Amplification of target genes was achieved using primer pair A341F and 1058R for Archaea (Baker et al., 2003; Nercessian et al., 2003), 341F and 907R for bacteria (16S rRNA) (Muyzer et al., 1993), NS1/NS2 (18S rRNA) and ITS1F/ITS4 (ITS) for eukarya (White et al., 1990) (Supplementary Tables S3, S4). For pyrosequencing, barcodes were assigned to forward primers to enable discrimination of samples after sequencing. The PCR reaction involved an initial denaturation time of 5 min (16S rRNA and 18S rRNA) or 3 min (ITS); 30 cycles at 95 °C for 1 min, 55 °C for 1 min (16S rRNA and 18S rRNA) or 51 °C for 1 min (ITS), 72 °C for 1 min, and a final extension at 72 °C for 10 min. Positive and negative controls were run for every PCR.

For t-RFLP analysis, PCR was performed with FAM-labeled forward 341F primer (Muyzer et al., 1993) and NS1 primer (White et al., 1990) targeting 16S rRNA and 18S rRNA respectively ($N = 192$). Restriction digests (*Msp*I and *Cfo*I for 16S/18S rRNA) of FAM-labeled PCR amplicons were analyzed by capillary electrophoresis (3730 Genetic Analyzer Biosystems). Samples were further interrogated via pyrosequencing using the Roche GS Junior System (454 Life Sciences Corp., Branford, CT, USA). For each amplicon library, purification was carried out with Agencourt AMPure XP Bead (Beckman Coulter, Inc., CA, USA) according to manufacturers instructions. The library was quantified with Quant-iT PicoGreen