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Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region

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HIGHLIGHTS

- Microbial activity has no statistical correlation with total microbial quantity.
- Temperature and wind speed had significant correlation with microbial activity.
- Microbial activity showed a seasonal variation of summer > autumn > winter > spring.
- Microbial activity was affected by foggy and hazy days.

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ABSTRACT

Microbial activities in the atmosphere can indicate the physiological processes of microorganisms and can indirectly affect cloud formation and environmental health. In this study, the microbial activity in bioaerosols collected in the Qingdao coastal region was investigated using the fluorescein diacetate (FDA) hydrolysis method to detect the enzyme activity of microorganisms. The results showed that the microbial activity ranged from 5.49 to 102 ng/m^3 sodium fluorescein from March 2013 to February 2014; the average value was 34.4 ng/m^3. Microbial activity has no statistical correlation with total microbial quantity. Multiple linear regression analysis showed that meteorological factors such as atmospheric temperature, relative humidity and wind speed accounted for approximately 35.7% of the variation of the microbial activity, although their individual impacts on microbial activity varied. According to the correlation analysis, atmospheric temperature and wind speed had a significant positive and negative influence on microbial activity, respectively, whereas relative humidity and wind direction had no significant influence. The seasonal distribution of microbial activity in bioaerosols was in the order of summer > autumn > winter > spring, with high fluctuations in the summer and autumn. Microbial activity in bioaerosols differed in different weather conditions such as the sunny, foggy, and hazy days of different seasons. Further in situ observations in different weather conditions at different times and places are needed to understand the seasonal distribution characteristics of microbial activity in bioaerosols and the influence factors of microbial activity.

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1. Introduction

Bioaerosols are airborne particles that are derived from biological activities in the terrestrial and aquatic environments; they include fungi, bacteria, viruses, pollens, disintegration products of plants and animals, and other substances from life activities (Ariya and Amyot, 2004). Airborne microorganisms are an important component of bioaerosols; their concentration varies at different times and sites (Bowers et al., 2013; Jaenicke, 2005; Kaarakainen et al., 2008; Qi et al., 2014). The abundance of microbial activity in bioaerosols indicates the ability of microorganisms to exhibit metabolic and other normal physiological activities. Microbial activity depends on various physical, chemical and biological factors, among which the nutrient condition in the environment is especially important because it reflects the microbial contribution to the energy flow and nutrient cycling within an ecosystem (Maier et al.,

Abbreviations: fluorescein diacetate, FDA; microbial activity, MA; 4′,6-diamidino-2-phenylindole, DAPI; Meteorological Information Comprehensive Analysis and Process System, MICAPS; analysis of variance, ANOVA; relative humidity, RH; ultraviolet, UV.

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2010).

Microbial activity, such as ubiquitous lipase, protease, and esterase enzyme activities (Green et al., 2006), oxidoreductase enzyme activity and hydrolase activity (Jorge-Mardomingo et al., 2013), and dehydrogenase and urease activity (Megharaj et al., 2000; Thavamani et al., 2012), has typically been used as an indicator to show whether an environment is healthy or whether the remediation of pollutants has been effective in soils. However, this indicator has rarely been used in the atmospheric environment. Microbial activity is different from total amounts of airborne microorganisms, which is the sum of microbe quantity (usually expressed in cells/m^3). No attempt has been made to relate the microbial activity to microbial quantity in the atmosphere. A previous study showed that 76% of the bacteria in atmospheric cloud water samples were metabolically active and that their activities influenced the climate and the ecosystem processes (Kourtev et al., 2011). Actively metabolizing cells can change the chemical composition of a cloud by transforming the speciation of organic carbon (Amato et al., 2007; Deguillaume et al., 2008) or nitrogen (Hill et al., 2007); therefore, microbial activity may indirectly affect cloud formation and global climate. Moreover, high abundance of microbial metabolic and reproductive activity in an environmental microbial community will result in potential environmental health risks when respiratory microbial allergens and pathogens increase in the atmosphere under special weather conditions (Gao et al., 2015). Therefore, it is necessary to study microbial activity to understand the impact of bioaerosols on environmental quality, atmospheric processes and human health. Qi et al. (2015) recently proposed a fluorescein diacetate (FDA) hydrolysis method of measuring microbial activity in bioaerosols in which the enzyme activity of microbes in bioaerosols was determined and its concentration was used as the indicator of the abundance of microbial activity.

Studies have found that microbial abundance varies with the season and region due to geographical and climatic diversity (Hurtado et al., 2014; Li et al., 2011; Lighthart and Shaffer, 1994). Moreover, various meteorological factors such as air temperature, relative humidity (RH), wind speed, and wind direction can also make a difference (Dong et al., 2016; Jones and Harrison, 2004; Mouli et al., 2005), although their effects may vary by time or location (Kurkela, 1997). Because microbial activity depends on physical, chemical and biological factors in the environment, the abundance of microbial activity in bioaerosols is also affected by the season, region and meteorological factors. However, studies on microbial activity in bioaerosols have been very limited. The temporal and spatial variations of microbial activity in bioaerosols have scarcely been revealed, and the environmental factors affecting the activity need to be studied further. In this study, we analysed the relation between airborne microbial concentration and microbial activity and the influences of meteorological factors and weather conditions on bioaerosol microbial activity, and we also analysed the seasonal distribution of bioaerosol microbial activity in the Qingdao coastal region.

2. Materials and methods

2.1. Sampling site

The sampling site was located at the Laoshan campus of the Ocean University of China (36°10′N, 120°30′E) in Qingdao, China (Fig. 1). The bioaerosol samples were collected at approximately 9.0 m above the ground on the rooftop of an academic building. The sampling site is 7.0 km away from the shore and is surrounded by green space that covers up to 50% of the total area.

2.2. Sample collection

All bioaerosol samples were collected on polycarbonate membranes that were placed on each plate of six-stage microorganism FA-1 cascade impactors (Applied Technical Institute of Liaoyang, China) that were lifted on tripods at a height of 1.5 m above the floor level. The bioaerosol particle sizes were divided into six size ranges: 0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–7.0, and >7.0 μm. The polycarbonate membranes had a pore size of 0.22 μm and a diameter of 80 mm and were coated with a 40% polyethylene glycol solution to reduce the loss of viability during sampling (Qi et al., 2015). Before sampling, an autoclave was used to sterilize all membranes at 121 °C for 15 min. Two sets of FA-1 cascade impactors were used to collect duplicate samples at 8 a.m. each morning at 5-day intervals from March 2013 to February 2014. Sampling duration was 40 min and the air flow was 28.3 L/min. The sampling of bioaerosols was also performed when special weather conditions occurred.

The total airborne microbe samples were also simultaneously collected on sterilized polycarbonate membranes at the same site using the same samplers from October 2013 to February 2014. Sampling duration was 30 min for each sample and the air flow rate was 28.3 L/min (Dong et al., 2016).

2.3. Measurement

The microbial activity of bioaerosols was determined by the fluorescein diacetate (FDA) hydrolysis method described by Qi et al. (2015). All experimental apparatuses were sterilized at 121 °C for 15 min using an autoclave. Briefly, the membranes with bioaerosol samples were cut using a sterile scissors on a clean bench, resuspended in 20 mL 0.9% NaCl solution, and shaken at 37 °C for 30 min at 150 rpm. The bioaerosol particles then separated themselves from the membranes. After centrifugation (5000 rpm for 5 min), the supernatant was transferred to a conical flask and 200 μL of FDA solution were added, reacting at 30 °C for 150 min in the dark. Thereafter, 1 mL chloroform/methanol (2:1 v/v) was added to end the reaction, and all samples were then measured within 30 min after preparation by fluorescence spectrophotometry (λex = 488 nm, λem = 530 nm). As sodium fluorescein releases the same yellow-coloured fluorescein acid as FDA, sodium fluorescein salt was then used as the standard and indicator of the abundance of microbial activity in bioaerosols (Qi et al., 2015). The bioaerosol samples and blank membranes were treated with the identical procedure. After measured values of samples were corrected by subtracting the blank, the microbial activity (*MA*) in bioaerosols was calculated and represented by the concentration of sodium fluorescein per cubic metre of air (ng/m^3) given by:

$$MA\left(ng/m^3\right) = [(C \times V)]/(Q \times t)$$

where C is the concentration of fluorescein sodium that was determined in the experiment, V is the volume of the solution, Q is the flow speed of air sampling, and t is the duration of sampling.

The total of airborne microbes was determined using an epifluorescence microscope after staining using the 4′,6-diamidino-2-phenylindole (DAPI) method (Dong et al., 2016; Li et al., 2011; Xu et al., 2011).

2.4. Statistical analysis

SPSS 22.0 (trial version) software was used to statistically analyse the experimental data. Meteorological data were obtained from the Bureau of Meteorology in Qingdao (http://qdqx.qingdao.

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