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Microbial ice nucleators scavenged from the atmosphere during simulated rain events

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HIGHLIGHTS

- Simulated rain events were conducted at ~55 m above ground to screen for culturable ice nucleators.
- Putative microbial ice nucleators were cultured from 94% (31/33) of the simulated rain events.
- Microbes confirmed as ice nucleators in repeated assays represented 0.4% (34/8331) of the total.
- Ice-nucleating genera included *Pseudomonas*, *Pantoea*, *Xanthomonas*, *Fusarium*, *Humicola*, and *Mortierella*.
- Some microbial ice nucleators in natural rainfall may have been scrubbed from the atmosphere.

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ABSTRACT

Rain and snow collected at ground level have been found to contain biological ice nucleators. These ice nucleators have been proposed to have originated in clouds, where they may have participated in the formation of precipitation via ice phase nucleation. We conducted a series of field experiments to test the hypothesis that at least some of the microbial ice nucleators (prokaryotes and eukaryotes) present in rain may not originate in clouds but instead be scavenged from the lower atmosphere by rainfall. Thirty-three simulated rain events were conducted over four months off the side of the Smart Road Bridge in Blacksburg, VA, USA. In each event, sterile water was dispensed over the side of the bridge and recovered in sterile containers in an open fallow agricultural field below (a distance of ~55 m). Microbes scavenged from the simulated rain events were cultured and their ice nucleation activity was examined. Putative microbial ice nucleators were cultured from 94% (31/33) of the simulated rain events, and represented 1.5% (121/8331) of the total colonies assayed. Putative ice nucleators were subjected to additional droplet freezing assays, and those confirmed through these repeated assays represented 0.4% (34/8331) of the total. Mean CFUs scavenged by simulated rain ranged from 2 to 267 CFUs/mL. Scavenged ice nucleators belong to a number of taxa including the bacterial genera *Pseudomonas*, *Pantoea*, and *Xanthomonas*, and the fungal genera *Fusarium*, *Humicola*, and *Mortierella*. An ice-nucleating strain of the fungal genus *Penicillium* was also recovered from a volumetric air sampler at the study site. This work expands our knowledge of the scavenging properties of rainfall, and suggests that at least some ice nucleators in natural precipitation events may have been scrubbed from the atmosphere during rainfall, and thus are not likely to be involved in precipitation.

1. Introduction

Scavenging is the process of particles being removed from the atmosphere by precipitation (Seinfeld and Pandis, 2012). The dynamics of particle scavenging during rainfall are determined by a series of complex interactions among raindrop size and intensity, particle size, and relative humidity (Chate et al., 2003; Chate and Pranesha, 2004). Larger droplets experience vortices that dissipate more quickly relative to smaller droplets, thus reducing the chance of wake collection (Engelmann, 1965). Inertial impaction of

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particles and wake effects allow for collection of larger atmospheric particles (Engelmann, 1965). Scavenging increases with rainfall intensity (Chate, 2005) and particle size (Chate et al., 2007). Little is known about the fundamental processes of particle entrainment in droplets falling at near terminal velocities (Rodhe and Grandell, 1972; Flossmann et al., 1985; Pruppacher and Klett, 1997), and empirical data are lacking to demonstrate how these processes might contribute to precipitation dynamics across a region. Research is needed to examine raindrop dynamics during natural descent and at relevant (near terminal) velocities, and to study fundamental processes associated with the entrainment of microorganisms in raindrops during deposition.

Pure water droplets in clouds can persist in a supercooled state to temperatures as low as −38 °C before freezing (Murray et al., 2012; Petters and Wright, 2015; Rosenfeld and Woodley, 2000). Metastable droplets of pure water in the upper troposphere freeze into ice crystals homogeneously below −38 °C. At temperatures warmer than −38 °C, ice crystals are formed by heterogeneous ice nucleation and can be initiated by an entity acting as an ice nucleating particle, or a collection of ice nucleating particles (Vali et al., 2015). A field study of scavenged aerosols, both natural and anthropogenic, captured various particle sizes from rainfall and confirmed the presence of particles that ranged from 0.013 to 0.75 μm (Chate and Pranesha, 2004). Heterogeneous nucleation can be catalyzed by immersion, condensation, contact, and deposition nucleation (Chou et al., 2011). High mineral concentrations in the atmosphere can play a significant role in shifting ice nucleation from a homogeneous to a heterogeneous event, and have implications on atmospheric conditions at the cirrus cloud level (Chou et al., 2011).

Natural bioaerosols, including the bacterium *Pseudomonas syringae*, cause ice nuclei formation at warmer temperatures than mineral dust (Möhler et al., 2008; Morris et al., 2013; Murray et al., 2012). Microbial ice nucleators in clouds have the potential to initiate heterogeneous ice nucleation and contribute to the water cycle through precipitation (Delort et al., 2017; Morris et al., 2014). At the Jungfraujoch observatory, a higher abundance of ice nucleating particles was measured prior to significant rainfall, when a moist air mass occurred concurrently with high wind speed (Stopelli et al., 2015). Biological ice nucleators (sensitive to heat and/or lysozyme treatment) have been found to be prevalent in rain and snow collected in locations on different continents (Christner et al., 2008; Morris et al., 2008; Monteil et al., 2014; Sands et al., 1982). *Pseudomonas syringae* represented over 80% of the culturable, ice-nucleating prokaryotes sampled in 23 precipitation events in Virginia over 15 months (unpublished observations). The presence of multiple strains of *P. syringae* in different environments suggests that the bacterium interacts with the environment in ways that could influence atmospheric processes (Morris et al., 2011; Pietsch et al., 2017). Significant movement of *P. syringae* into the atmosphere from terrestrial sources has been demonstrated (Lindemann et al., 1982). A handful of fungi have also been reported to be ice nucleators, including different species of *Fusarium* and *Mortierella* (Pouleur et al., 1992; Richard et al., 1996; Fröhlich-Nowoisky et al., 2015). Other genera of ice-nucleating microorganisms have been reported (Gurian-Sherman and Lindow, 1993), but their relative abundance in the atmosphere and their potential role in atmospheric processes is presently unknown (Morris et al., 2013).

We hypothesized that prokaryotic and eukaryotic microbial ice nucleators are scavenged from the atmosphere by rainfall, and thus are not exclusively involved in precipitation. To test this hypothesis, we conducted 33 Simulated Rain Events (SREs) over four different months in two calendar years off the side of the Smart Road Bridge (SRB) in Blacksburg, VA, USA. In each event, sterile water was dispensed over the side of the bridge and recovered in sterile containers following gravitational settling from the bridge to an open fallow agricultural field below (a distance of ~55 m). This work focuses on microbial ice nucleators at −8 °C, and does not include non-cultured ice nucleating particles that could influence heterogeneous ice nucleation events at temperatures colder than −8 °C. The specific objectives of our work were to: (1) simulate natural rainfall events off the side of a bridge to study the scavenging of microbial ice nucleators from the atmosphere, (2) culture and identify microbial ice nucleators from simulated rain events, and (3) observe the number and size of particles scavenged during simulated rainfall.

2. Materials and methods

2.1. Experimental site

Collection of simulated rain events was conducted at the Virginia Tech Transportation Institute (VTTI) Smart Road Bridge (SRB) in Blacksburg, VA, USA (Fig. 1). The SRB extends about 700 m across Ellett Valley, and stands more than 53 m above Wilson Creek and surrounding farmland (GPS location 37.173099, −80.373299). An open, gently sloping, fallow field area below the bridge was utilized for sample collection. There were no physical objects or obstructions between the bridge pylon supports, and the simulated rain fell through the atmosphere from the top of the bridge to the ground without interference. Samples were processed and stored immediately following collection in a mobile laboratory (an instrumented stepvan) located at the perimeter of the collection site.

2.2. Simulated rain events

Simulated Rain Event (SRE) experiments were performed on November 13, 2014, December 12, 2014, April 23, 2015, and June 9, 2015 (Table 1). Autoclaved metal 2-gallon watering cans (Home Depot #878469) were used to dispense simulated rain from the top of the SRB. Commercial spring water (Primo Water Corporation, Winston-Salem, NC, USA) was sterilized and transported in Nalgene 4 L wide mouth round bottles (Nalgene 2121-0010-4l). Six liters of sterile water was poured into autoclaved watering cans for each SRE, and was dispensed over the side of the SRB as shown in Fig. 1. Simulated rain descended from ~55 m above ground level and was collected in 31 gallon galvanized cans (Home Depot #001223296) lined with sterile polypropylene bags (Fisher #01-830E, 122 × 94 cm), at ~2 m above ground level. The cans were held over the heads of individuals below the bridge coordinating the collection of the simulated rain (Fig. 1). Collection liners were removed immediately following collection inside the mobile laboratory at the field site, and samples were transferred to sterile 1 L, wide-mouth bottles (Nalgene #2187–0032) and placed on ice.

2.3. Control collection events

Control collection events were performed on three of the four collection campaign days. Rain cans with lids were lined with sterile polypropylene bags (identical to the experimental design for the scavenging experiments), and were placed at ground level about 50 m from the perimeter of the SRE collection area. At the start of the pour of an SRE (on top of the bridge), the lid was removed from control can (at ground level) and exposed to the atmosphere for the duration of the pour (less than 1 min). The lid was placed on the control can once the SRE was complete. The