Analyzing the Diversity and Abundance of Airborne Microorganisms in Different Environments

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Bacterial presence in airborne particles has been known for decades, but investigations into diversity and abundance of these microorganisms is limited. Samples of airborne bacteria were collected at four locations in Arcata and Eureka, California, to investigate the diversity and abundance of microorganisms in the air. Three samples were obtained from outdoor environments and one from an indoor environment. Bacterial counts were elevated on the plates sampled from the indoor environment. Suggesting a higher concentration of cultivable bacteria in the air of indoor environments. Our detected concentrations of bacteria in the air samples may have been skewed by factors such as wind and sampler performance. Colony morphology, cell morphology, and gram morphology were all considered when making observations on our collection plates. The most dominant bacteria growing on all of our collection plates were (un)pigmented gram positive cocci, suggesting that this bacterial morphology is present in high concentration in airborne environments.

INTRODUCTION

The abundance and diversity of airborne organisms are important indicators for examining pollutants from various sources of contamination (i.e. car exhaust, smoke stacks, run-off pollutants in oceanic environments, etc.) (1). Investigation of airborne microorganisms also provides insight into the versatility and flexibility of these microorganisms with respect to temperature, UV exposure, increasing elevation, pressure, humidity, etc. Airborne bacteria are expected to mirror the nature of environmental conditions under which they are found (10).

Bioaerosols are suspensions or collections of airborne particles (i.e. dust, water droplets, or pollen) that contain microorganisms. These microorganisms can be either living (viable aerosols) or non-living (non-viable aerosols). Communities of various species or populations of single species can be found in bioaerosols (10). Sources of bioaerosols include plants, soil, water (marine and fresh), and animals, including humans. Bioaerosols are characterized either by the source of the bioaerosol, its physical characteristics, or by the array of microorganisms found in them (8, 13). These properties interact with the environmental conditions that the bioaerosol is exposed to and ultimately determine the fate of the bioaerosol.

Environmental conditions have the greatest impact on the eventual fate of bioaerosols (11, 12). Conditions such as air currents (speed, direction, magnitude, frequency), humidity, temperature, and aerosol abundance affect the way and the location of settlement. For example, at a higher relative humidity, a bioaerosol might be able to stay suspended in the air current for

a longer period of time as opposed to a particle of the same size suspended in a relatively low humidity environment. Relative humidity has also been shown to cause species-specific effects. For example, *B. subtilus* was eliminated at a much slower rate than *S. marsescans* at a relative humidity of 85% (11). It has also been reported that the presence of various chemicals in the atmosphere, e.g. CO, has an effect on the survivorship of bacterial populations on aerosols (11). Increases in such atmospheric compounds negatively affect the abundance and survival of various microbial species within bioaerosols. As conditions fluctuate, different organisms survive at different rates.

Though environmental conditions play an important role in the nature of the bioaerosol, physical aspects of the bioaerosol also influence bioaerosol particle settling. Particle size, shape, quantity, and density are often determining factors in the fate of a bioaerosol particle (10). The more dense a particle, for example, the shorter amount of time it will be allowed to be suspended in an air current due to drag forces and the relative viscosity of the air. In a more humid environment, however, that same particle may be able to be airborne longer because of the density of the air relative to the density of the particle. Size of a bioaerosol particle can also have an effect on the ability of a macroorganism to uptake the microorganism (14). It has been reported that size distribution of an aerosol had a small effect on the infectivity of Aujeszky's disease in pigs, though no absolute correlation was made (15). Transmission via respiration relies on the size of the particle traveling through the respiratory tract to determine its infectious nature (14). Shape of the particle may also have an effect on the survival of the organisms found on it. A population of cells is sheltered from the environmental conditions by the contours of the microscopic surface of the dust particle or protected by a water film in a droplet.

An evaluation of the abundance and diversity of the airborne bacteria coming from various environments is a good indicator of potential health risks (2, 3). The amount of viable bacteria of fecal origin in air samples can help us predict the potential health risks associated with the inhalation of these organisms. Previous studies on this subject have looked into the airborne bacterial levels before a sewage treatment plant was in operation versus after the plant began running (2). These studies showed dramatic increases in overall bacterial counts as well as greater variety of airborne bacteria after the plants began operation. This can have a harmful effect on the overall health risks posed to people, animals, and plants living nearby. Additionally, indoor and outdoor concentrations of infectious microorganisms have been evaluated to show the effect of traveling aerosols and the spread of disease in areas affected by hurricanes Katrina and Rita (13). This study showed a large number of infectious organisms in areas experiencing higher humidity and water concentrations due to the spreading of airborne organisms. The spreading of infectious diseases as a result of bacterial exposure via bioaerosols has caused many dangerous and sometimes fatal outbreaks. For example, the spreading of *Streptomycetes* from moldy houses has been shown to increase adverse health effects (l3, 15).

Sampling of bioaerosols is performed through active air sampling. There are three main methods for bioaerosol sampling, which are impaction, impingement and filtration. The impaction method extracts particles from the air by utilizing the inertia of particles to make them adhere to a solid collection surface (10). The impingement method utilizes the same inertial forces of airborne particles as impaction but the collection medium is a liquid. The filtration method extracts particles from the air by passing them through a collection medium

with pores (10). The collection of bacterial samples depends on the size of the airborne particles, the adhesion properties of the airborne particles, Brownian motion of particles < 100 nm, thermal gradients, and the inertia of the particle (10). Smaller airborne particles have an increased chance of coming in contact with a surface and adhering to it because of the effects of Brownian motion. Filtration methods are useful for collecting small particles. Larger airborne particles (over 100 nm) are less affected by Brownian motion, but have greater gravitational attraction that causes them to settle on surfaces. This allows for effective collection of large particles via impaction/impingement methods.

The performance of an air sampler is an important part in collecting bioaerosols. Collection efficiency is the ability of the sampler to remove particles from the air stream and transfer them to the collection medium. The collection efficiency needs to be high to be sure that you have not collected a decreased particle concentration relative to the true particle concentration of the environment. There are many factors that may influence a particular air sampler's performance, including but not limited to, wind direction and speed, inlet sampling efficiency, and length of sampling time. Inlet sampling efficiency is the ability of the sampler's inlet to extract particles from the air current without excluding particles based on shape, size, or density. Sample collection time is an integral part of bioaerosol sampling. Collection time is complicated because bioaerosol concentrations may vary greatly over time in one environment. Realistically, air samples only provide a brief spatial and temporal insight into an environment's bioaerosol community. Based on the fact that bioaerosols vary temporally and many air sampling methods have short collection times, several samples are required to conduct an accurate investigation of the diversity and abundance of microorganisms in an environment.

The particular method and analysis used also plays a role in determining the variety of detected airborne bacteria. There are two categories of methods to identify airborne microorganisms, which include culture-dependent, and culture -independent. Culture dependent methods involve culturing organisms in some sort of media either by directly sampling onto a collection plate or onto a slide. Some culture independent methods are rRNA clone libraries, and metagenomic analysis. Metagenomic analysis involves isolating DNA from an environmental sample, cloning the DNA into a suitable vector, and sequencing all genes obtained in the sample. The clones are then screened for phylogenetic markers like 16SrRNA for identification. Taking the isolated DNA directly from the environment allows you to avoid culturing the organisms of study. Also, there are vast communities of microorganisms that are non-culturable and can only be identified through metagenomic analyses or rRNA approaches.

In this study, we used an impaction air sampler to sample airborne microorganisms from the Woodley Island Marina and the Bayshore Mall food court in Eureka, CA, and the Arcata Marsh and Redwood Community Forest in Arcata, CA. At each location, we determined the abundance and diversity of the cultivable species based on colony and cell morphology of organisms that grew on the impaction media.

MATERIALS AND METHODS

Media Preparation. Tryptic soy agar (TSA) plates were used as the growth medium for CFU enumerations. Low nutrient plates and high nutrient plates were both used. High nutrient agar plates were prepared from 17 g/L casitone, 3 g/L soytone, 5 g/L NaCL, 2.5 g/L potassium phosphate dibasic, 2.5 g/L glucose, and 15 g/L agar. A low

nutrient version of this medium was prepared by using 1/10 mass of all ingredients except the agar was maintained at 15 g/L. The pH of the solution was adjusted to 7.0 with 1N HCL. Media were sterilized by autoclaving at 121°C, 15 PSI for 20 minutes. Media were then cooled to 50°C before being poured into our plates.

Sampling. Airborne samples of bacterial diversity were taken at four different locations on the northwest coast of California. Sites included the Woodley Island Marina and the Bayshore Mall in Eureka, CA, and the Marsh and the Redwood Community Forest in Arcata, CA. Three impaction air samplers were ran for 15 minutes at each location to collect microorganisms. This was done for low nutrient TSA plates and high nutrient TSA plates. There were 3 replicates of each treatment ran at each site; 3 high nutrient plates and 3 low nutrient plates. Each replicate was run for 15 minutes. Inoculated plates were then transported in zip-lock bags until placed into incubation. Incubation of the plates was done at 30°C for 48 hours.

CFU Counts. Counts were conducted at 48 hours and again at 96 hours of incubation. Colony morphologies was visually recorded, and cell morphologies of morphologically distinct colonies were observed under phase contrast microscopy at 1000x magnification. Gram stain morphology was also observed under bright field at 1000x magnification. The data from these counts were used to determine means and standard deviations of bacterial abundance.

RESULTS

CFU counts were estimated on 1X and 0.1X TSA agar plates. Samples were taken from 4 sites, 3 replicates per site, each replicate was run for 15 minutes. The high nutrient plates often yielded the highest number of CFU. The CFU number for the high nutrient plates ranged from 0.5×10^1 to 1.28×10^2 m^{-3} (table 1). Overall bacterial abundance and diversity was increased on high nutrient plates (table 1). The dominant colony morphology observed on high nutrient plates were pigmented, medium or small, shiny, raised, with defined edges. This morphology always showed gram positive, cocci shaped bacterial cells. Suggesting that these microorganisms flourish in an airborne system and grow well under the conditions established. The indoor environment (Bayshore Mall food court) showed the highest CFU counts for the high nutrient plates as well as the low nutrient plates (tables 1 & 2). The marine environment (Woodley Island Marina) showed the lowest CFU counts at a low nutrient concentration (table 2). The Marsh environment showed the lowest CFU counts at a high nutrient concentration (table 1). Low nutrient plates consistently yielded the lowest CFU counts. The CFU number for the low nutrient plates ranged from 0.3×10^1 to 1.11×10^2 m^{-3} (table 2).

***Table 1*: Description of CFU counts and dominant morphologies at high nutrient concentration**

Sample Location	CFU Counts (CFU/mL) Repetition			Mean	Standard Deviation	Number of Colony Types (Diversity)	Dominant Morphology Descriptions
	1	2	3				
Woodley Island Marina	37	7	35	26.33	16.77	5	white/opaque, shiny, defined edges, raised, gram positive, cocci
Community Forest	56	24	21	33.7	19.4	7	yellow, white, opaque, shiny, defined edges, raised, some fuzzy, gram positive, cocci
Bayshore Mall Food Court	128	107	125	120.0	11.36	9	yellow, white, shiny, defined edges, some acitnomycetes, gram positive, cocci
Arcata Marsh	7	33	33	24.33	15.01	9	white, shiny, some fuzzy, brown, soluble, defined/undefined edges, gram positive, cocci

***Table 2* : Description of CFU counts and dominant morphologies at low nutrient concentration**

Sample Location	CFU Counts (CFU/mL) Repetition			Mean	Standard Deviation	Number of Colony Types (Diversity)	Dominant Morphology Descriptions
	1	2	3				
Woodley Island Marina	8	15	8	10.33	4.04	6	white, yellow, shiny, defined edges, some furry and spreading, gram positive, cocci
Community Forest	6	37	25	22.67	15.63	3	white, shiny, defined edges, gram positive cocci
Bayshore Mall Food Court	116	114	113	114.3	1.53	5	white, yellow, shiny, defined edges, gram positive, cocci, some rod shaped
Arcata Marsh	31	37	26	31.33	5.51	5	white, opaque, shiny, defined edges and rough edges, gram positive, cocci

DISCUSSION

The Redwood Community Forest in Arcata, CA was used as a site to sample airborne microorganisms. Its elevation rises to about 33 feet above sea level and the average temperature has a range of 39°F-65°F. Humidity at this location may be as high as 80%. There is a high amount of precipitation at this site, which could have an effect on the aerosol abundance. This site was chosen because there is extreme diversity found at terrestrial sites due to relative distance exposure to certain pollutants and other various sources of contamination (5). From studying this site, we expected to find vast diversity in the community reflecting environmental conditions such as temperature, humidity and exposure to contaminants. Our results showed a relative high level of diversity, 7 different colony types, under high nutrient concentrations**.** Although the colony morphology showed diversity the dominant cell type did not. The dominant cell type was cocci, and the dominant gram type was positive. We believe that this dominant type was seen because the organism has adapted the ability to live in an airborne environment and cultures well on the media provided. Abundance of growth overall was relatively low **(**tables 1 & 2**)**, thus airborne bacterial concentrations were low. This result may reflect the true concentrations of airborne bacteria in the forest but there may be other factors playing a role in the low yield of colonies. Factors such as the location picked for sampling, wind speed/direction in a covered forest, amount of moisture in the air, and amount of human traffic through the particular location in the forest.

A site within close proximity to a sewage treatment plant at the Marsh in Arcata, CA offers a look in into airborne bacteria that may have a fecal origin. Some of these facilities are located near urban environments and the ejection of airborne bacteria may be the cause of health problems. The cell and gram type we expected to see were rod and gram-negative. This was expected because these characteristic of coliform microorganisms, of fecal origins. Our results, however found none of these, indicating that the organisms in the air were not of fecal origin **(**tables 1 & 2). The dominant cell and gram type observed were cocci and gram positive. The dominant colony morphology was pigmented, shiny, with defined edges. Although there were a brown water soluble bacterial colonies they did not dominate the sample plates. This result is consistent with all of the other sample sites, suggesting that there may be a common morphology that is most adaptive for an airborne life. Because the wind direction was not taken into account, origin of wind gusts and patterns could not be taken into consideration when determining the diversity of the species sampled. Thus there may have been a high concentration of bioaerosols of fecal origin but the wind was clearly not blowing in the direction of our samplers. These results of diversity would change, based on the wind origin (i.e. from the sea, from the town, or from another outside region). Samples taken from a location known to be downwind from the sewage site may have experienced more gram-negative rods, indicating more bacteria of fecal origin.

Airborne bacteria are extremely common in urban environments (11). The concentration of airborne bacteria can be a reflection the quality of air. Our chosen urban study site was the food court Bayshore Mall food court in Eureka, CA. Conditions here are common of many city-like environments, including car emissions, industrial outputs, human interaction, and common dust presence. Dust particles carry a high amount of microorganisms (8). At this site, we expected to observe an extreme diversity as well as

high species richness due to the increased levels of contaminants in the air. Results from this site showed a diversity of morphologically different colonies on both of the nutrient level treatments (Tables 1 & 2**).** Not only was diversity at an increased level, abundance was also increased. Colony numbers were greater than any other site (High nutrient mean 120 CFU/mL, low nutrient mean 114.3 CFU/mL). The diversity was very high at this location but the dominant colony/cell/gram morphology was the same as the other sample sites. Thus again suggesting that there may be a common morphology that is most adaptive for airborne life. The increased level of abundance and diversity also suggest an increased level of exposure sources in an indoor environment. Thus suggesting that the high concentration of humans in our indoor environment has facilitated the high concentration of airborne bacteria.

To investigate the airborne bacteria near a marine environment, we chose our sample site to be the Woodley Island Marina in Eureka, CA. We predicted that the weather would have a big impact on the number of bacteria collected. If winds are high one should expect to collect more bacteria than if the winds are calm. Also the size of the swell will also play a role in the number of bacteria collected. Yet on the day of sampling the winds were calm and the swell was low. We expected to find little diversity in morphology of colonies due to the passive conditions at the time of sampling. We also expected to see low abundance of colonies, as our incubation conditions are not optimal for the growth of marine microbes. From our results relatively little diversity was seen, especially on the high nutrient plates **(**table 1)**.** Abundance was low especially on low nutrient plates (table 2). This may have been observed because the conditions at which the plates were incubated were different than the natural conditions at which the organisms grow. On days where the swell is higher the waves crashing into rocks, or against the wind cause bubbles to be released into the air, which may become bioaerosols and increase the concentration of airborne bacteria in the marina. Also, our growth medium was solid, and may have inhibited bacterial growth of organism that would prefer a liquid medium.

For this study we used the impaction method to collect our samples. The impaction method utilizes the inertia of the particles to adhere to a solid media. This method is efficient for collecting large particles, thus it may exclude some small particles. Our results suggest that the dominant bacteria found adhere well to large particles. The liquid impingement and filtration methods can also be used when sampling airborne bacteria. Each method is efficient at different aspects of sampling. Thus to generate a complete investigation into airborne bacterial life then all three methods should be used at each location. All of these methods for obtaining airborne bacteria depend on if the bacteria collected are culturable. There are large populations of non-culturable airborne bacteria that would require different methods for collection/identification. Methods like metagenomic analysis or rRNA clone libraries work well for culture-independent assessment of abundance and diversity. These methods simply capture bacterial particles directly from the air, thus avoiding the culturing of organisms.

In order to generate more robust data, many changes could have been made to this study. An increased amount of replicates at each site, more growth mediums at high and low concentrations, would give a more precise mean, which would provide more confidence in CFU counts as well as better demonstrate levels of diversity. Considering that all samples were taken from the same location at each site relative to a fixed landmark (i.e. a tree, a building or a post), variation in the placement of the air samplers could yield a

more diverse sample. This would allow evaluation of each site to be more thorough and complete due to the changes in general location. For example, upwind samples along with downwind samples could have been taken at the marsh in order to be compared to one another. Sampling variation could also take the form of time of day, differing weather conditions, variation in exposure sites relative to sample sites, and types of air samplers used. Another alteration to improve this experiment would be the length of time over which the study was conducted. Extending the time of study would allow for changes and patterns to be seen in the results. These patterns might serve to represent changes in environmental conditions, variation in exposure to contaminants, or simply just shifts in community composition at a specific site. Examining the colonies microscopically would also serve to improve the experiment. Diversity could be examined more closely, which would allow for more inferences to be made regarding individual species and colonies.

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