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Recovery and biogeography of Pseudomonas and Burkholderia species from the human home.

Megan E. Purdy
University of Louisville

RECOVERY AND BIOGEOGRAPHY OF *PSEUDOMONAS* AND *BURKHOLDERIA* SPECIES FROM THE HUMAN HOME

By

Megan E. Purdy
B.A. University of Louisville, 2008

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A Dissertation Approved on

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by the following Dissertation Committee:

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Dissertation Director
Susanna Remold

_____

Margaret Carreiro

_____

Sarah Emery

_____

James Graham

_____

Michael Perlin

DEDICATION

This dissertation is dedicated to my parents, Robert and Cheryl Purdy, for their unending support, unconditional love, and positive example in their own educational pursuits.

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ABSTRACT

RECOVERY AND BIOGEOGRAPHY OF *PSEUDOMONAS* AND *BURKHOLDERIA* SPECIES FROM THE HUMAN HOME

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15 households in the Louisville Metro area were sampled, 7 had a patient with cystic fibrosis (CF), and 8 did not. Houses were sampled between 3 and 8 times each, and samples were collected from 123 different sites within and around the homes. These sites were categorized into the variable environment type based on ecological similarity of sites. Between 75 and 168 samples were collected from each home per visit.

Recovery was examined for biogeographical patterns by environment type and season at multiple taxonomic levels. Approximately 10% of samples taken yielded *Pseudomonas*, and of these 61% were *P. putida* group, 23% *P. fluorescens* group, and 15% *P. aeruginosa* group. Environment type and season influenced patterns of *Pseudomonas* species recovery at all taxonomic levels (genus, species groups, and species), and house of recovery influenced recovery for all species groups but *P. aeruginosa*. Soils and drains were the environment types with the highest recovery. Soils had the highest recovery rates for *P. fluorescens* group and *P. putida* group, while drains had the highest recovery rates for *P. aeruginosa* group. This indicates that

household *Pseudomonas* distribution is influenced by dispersal limitation as well as adaption to the environment.

Recovery from the opportunistic pathogen, *P. aeruginosa*, was examined in greater detail with the aim of determining hotspots of recovery in the home. Drains were identified as hotspots for *P. aeruginosa* recovery were identified and no differences in recovery from drains were found in houses with CF patients compared to those without CF patients. No *P. aeruginosa* was recovered from animals, and only a single isolate was recovered from each soils and equipment used to treat CF. Indicating that *P. aeruginosa* is a drain specialist regardless of patient presence.

The human home was investigated as a potential source of *Burkholderia spp.* by looking at recovery from various types of environments. Overall, the recovery rate of *Burkholderia sp.* was very low (0.22%). Isolates that were recovered were primarily from soil environments and drains. Isolates evenly clustered within two phylogenetic clades: the plant-associated beneficial environmental group described by Suárez-Moreno et al. (2012) and the *B. cepacia* complex.

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CHAPTER 1

INTRODUCTION

Human Home as an Ecosystem of Study

In this dissertation I examined the recovery and biogeography of the bacterial genera *Pseudomonas* (Chapters 2 and 3) and *Burkholderia* (Chapter 4) from the human home. Homes have characteristics making them interesting and important sites to study. First, houses are an ecosystem for which we have *a priori* knowledge. We already have a basic understanding regarding possible dispersal mechanisms through the home; we understand how people, animals and objects move throughout the home. This knowledge allows us to evaluate and generate hypotheses about known patterns of use and movement as they relate to recovery of microbes.

Second, the human home provides a study site that is directly relevant to human exposure and interaction with microorganisms. We are in constant contact with our home environments, including the animal and human residents and objects both in and around the home, all of which are sources of microorganisms. Opportunistic pathogens, such as *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex, present in the home environment, are of special concern to individuals with immune-compromising diseases.

Understanding the distribution of opportunistic pathogens throughout the home will provide information necessary for decisions regarding the removal of pets or objects from the home, or avoidance of areas where opportunistic pathogens are frequently recovered.

Last, and perhaps most important, is that the human home provides many habitat types within a small geographical space. Homes also have a replication structure that allows for examining the effect that different types of environments and location have on the distribution of organisms. Rooms within a house can be thought of as one level of geographical location; while, houses themselves are an additional level. Sites within the house can be categorized by ecological similarity or type of environment; for example, drains may be located within different rooms of the house (kitchen, bathroom, etc.) but are more likely to be ecologically similar to each other than they are to a child's toy. It is possible for each home to have replication of both room (i.e., multiple bathrooms in a house) and environment type (i.e., multiple drains in a house). Figure 1 provides a schematic of environments and rooms within a single home. Studying multiple houses allows for replication at an additional geographic scale, the level of the house.

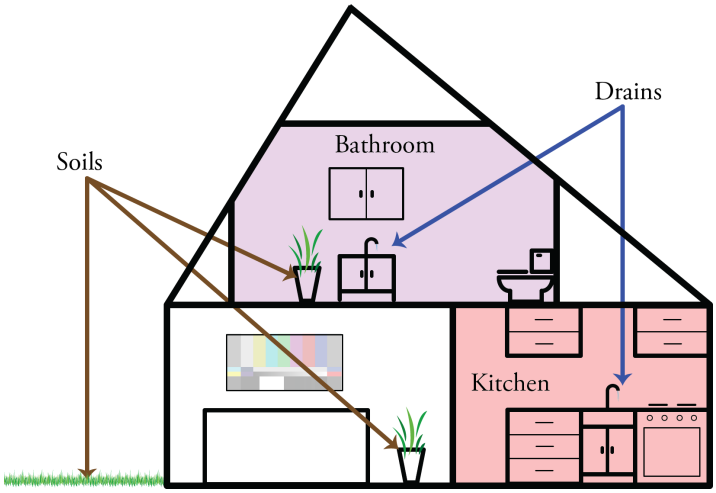

Figure 1. Different environment types with replication (brown lines for soils, blue lines for drains) and different rooms within a single house. Sampling multiple houses add an additional layer of replication (that of house) beyond what is pictured in this schematic.

Microbiology of the Built Environment

Humans spend, on average, nearly 90% of the day indoors (Klepeis, et al. 2001). This time is split between residential spaces, offices/work places, vehicles, shopping centers, etc. These indoor spaces are important ecosystems to study, as they are the ecosystems we interact with most. Traditionally, the public health concern with built environments has focused around broad topics of sanitation, fire and damage prevention, and safety (Jackson 2003). However, the air we breathe and the surfaces we touch, etc. are all environments for microorganisms, but only recently have researchers started to investigate the microbiome of indoor spaces and its potential effects on human health (Corsi et al. 2012).

Several studies have been conducted examining the microbiome of various indoor sites including: airplanes (Osman, et al. 2008), daycares/schools (Andersson, et al. 1999; Liu, et al. 2000), kitchens (Flores, et al. 2012), office spaces (Hewitt, et al. 2012), restrooms (Flores, et al. 2011), and homes (Täubel, et al. 2009). As a results of these

studies and others, we now know that human skin appears to be the main source of bacterial contamination of frequently touched surfaces, such as computer keyboards and computer mice (Fierer, et al. 2010). Bacteria associated with humans, particularly skin (i.e., *Propionibacteriacaae*), were also most abundantly recovered from surfaces inside office buildings (Hewitt, et al. 2012). Human skin, gut, and vagina-associated bacteria were found on varying surfaces of public restrooms (Flores, et al. 2011). Bacteria commonly associated with soils were also recovered from office buildings and public restrooms (particular samples from the floor). A study by Flores and colleagues (2012) of kitchens also found an abundance of human-associated bacteria on kitchen surfaces; interestingly, the abundance of bacteria commonly associated with food was widespread but recovered at much lower frequency. In addition to humans, pets are also sources for microbial communities in built environments (Fujimura, et al. 2010). In a study examining microbial communities in dust, Fujimura and colleagues found that homes with pets, particularly dogs, had significantly more diverse microbial dust communities, and hypothesize that this could be due to dogs being permitted indoors and outdoors.

By connecting bacteria found indoors to sites whose those taxa are typically isolated from other locations (i.e., outdoors) or sources (i.e., humans), these studies demonstrate potential sources of bacteria found in indoor environments, and also indicate the importance of humans as both sources for built environment microbial communities, and as possible vectors of microbes into the built environment. A source is defined by where bacteria in the home were originated, while a vector is defined as the method of transport for that organism. In the home there are a diversity of sources and vectors, and some things play both roles (Figure 2). Figure 2 is a schematic of possible sources and

vectors of microbial contamination of built environment sites, showing how humans, animals, etc. can influence microbial communities (adapted from Kelley and Gilbert 2013).

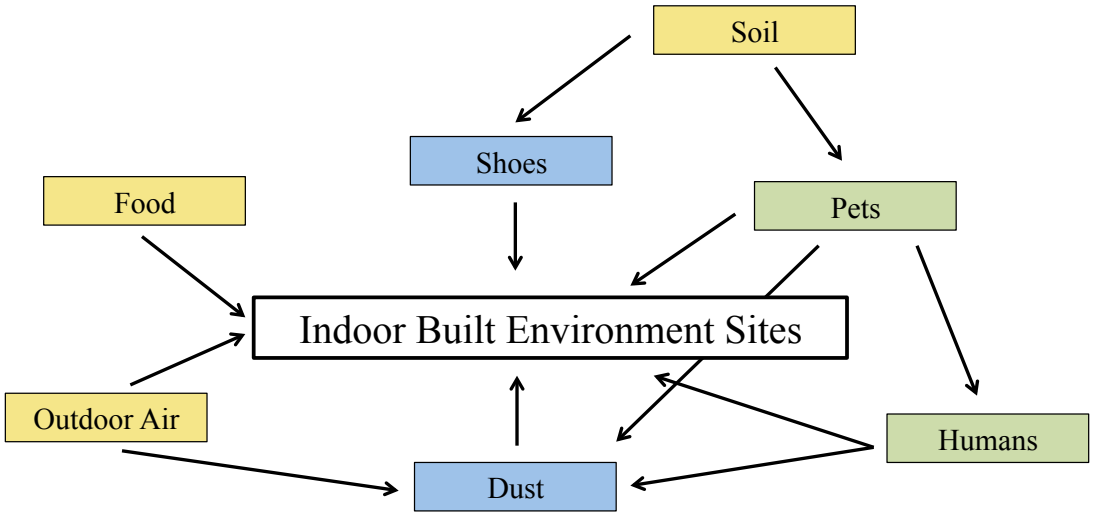

Figure 2. Possible sources and vectors of different microbial community members of the built environment. Yellow colored boxes represent possible external sources of microbial community members; blue colored boxes represent possible vectors for microbial dispersal to built environment; and, green colored boxes present possible vectors and sources of microbial community members (figure adapted from Kelley and Gilbert 2013).

Environmental conditions can also play a role in the microbiome of the built environment. Studies of indoor air and dust have shown variation in microbial communities across seasons (Moschandreas, et al. 2003; Rintala et al. 2008). In addition to seasonal variability, Moschandreas and colleagues (2003) examined indoor air of Chicago-area homes for differences by room of the home, and found no differences at this spatial scale. Microbial communities differ between different office buildings of the same city (Rinatala, et al. 2008) and between office buildings in different cities (Hewitt, et al. 2012), indicating that environmental factors, such as climate, may be important in influencing microbial community composition of indoor sites. In addition, differences

seen between buildings suggest founder effects and/or dispersal limitation may play a role in shaping indoor microbial communities. By studying the human home ecosystem in this dissertation I am able to examine the distribution of two organisms of interest (*Pseudomonas* and *Burkholderia* species), and explore seasonal variability, spatial variability by examining house-to-house variability in recovery, and variation in recovery by type of environment.

Culture-Based Methods of Recovery

The technique of culturing microbial organisms has existed for well over a century. Culture media is defined as "a solid or liquid preparation used to grow, transport and store microorganisms" (Willey et al. 2008, p. 110). Culture-based techniques are considered the standard for determining cell viability (Kelley and Gilbert 2013). Additionally, culture-based techniques used for the recovery of microbial organisms allow for archival of isolates collected for further study. However, there are a number of limitations of culture-based studies. Many organisms are not culturable or a have non-culturable state, or organisms for which there are no known media or conditions under which they can be cultured, and are thus missed by culture-based studies of diversity (DeLong and Pace 2001).

Culture-independent studies, or studies that use molecular techniques (e.g., 16s rDNA), for examining diversity can capture more organisms that culture-based studies miss, several orders of magnitude more diversity are found from these types of studies (Woese et al. 1990; Kelley and Gilbert 2013). However, there are also limitations to culture-independent studies. Culture-independent methods are biased by the efficacy of

the DNA extraction methods and the primers used (Kelley and Gilbert 2013). Additionally, culture-independent methods address only if the DNA of an organism is present, rather than if the organisms is viable under the sampled conditions.

The studies described in this dissertation use culture-based techniques to address distribution patterns of bacteria in the genera *Pseudomonas* and *Burkholderia*. Samples were plated on to *Pseudomonas* Isolation Agar (PIA). *Pseudomonas* Isolation Agar is a solid media that selects for the isolation of *Pseudomonas spp.* PIA contains glycerol as a carbon source, peptone for sustaining growth, and Irgasan as a selective agent (Hardy Diagnostics: PIA 1996). Irgasan works by preventing fatty-acid synthesis and causes membrane damage (Russell 2004). In addition to distribution information, the use of culture-based techniques allows for downstream studies and phenotypic and genotypic characterization of isolated strains.

Microbial Biogeography

Biogeography addresses one of the most intriguing biological questions: what factors explain the distribution of organisms in space and in time. The answer(s) to this fundamental biological question would provide a body of knowledge impacting a variety of additional topics, including: colonization, dispersal, extinction, inter- and intra-species interactions, speciation, and succession (Martiny et al. 2006). Biogeography has long been a topic of interest for "macro" ecology; however, only recently have these questions been applied to microorganisms. Ramette and colleagues (2009) propose that a combination of molecular advances and improvements in the fields of multivariate analyses, phylogenetics, and time-scale analyses have allowed the study of microbial biogeography and microbial ecology to increase at a dramatic rate.

Hutchinson (1959) introduced the idea of a fundamental niche and a realized niche. Generally speaking, the fundamental niche consists of everywhere the environmental conditions are suitable for an organism to grow and reproduce, and the realized niche can be defined as where, or under what conditions, an organism is actually found. There is some debate about what defines the fundamental niche and what determines the realized niche. Hutchinson's fundamental niche was defined by the abiotic environmental factors, such as temperature or disturbance, an organism can withstand, and the realized niche is further constrained by biotic factors, such as competition or disease (Hutchinson 1959; Kylafis and Loreau 2011). Opposing this is the biogeographical viewpoint of the realized niche discussed by Colwell and Rangel (2009). Colwell and Rangel (2009) discuss how the Hutchinson's fundamental niche is defined by a biotype, or geographical area and abiotic environmental conditions, but that the modern fundamental niche cannot be defined this way. They state that no geographical distribution of organisms should be assumed and that relying a biotype would require careful demonstration that the said factors are unaffected by the interactions of species. Colwell and Rangel (2009) propose that limitations of the fundamental niche be determined based on experiments and models of organism physiology, and that the realized niche is constrained not only by biotic interactions, but also by dispersal capabilities and abiotic environmental conditions. Regardless of whether abiotic environmental conditions define the fundamental niche of an organism or whether these conditions determine the size of the realized niche, abiotic environmental conditions are clearly important for determining the distribution of organisms.

Microbial species are inherently more difficult to define than those of macro-organisms. Classical species concepts of interbreeding cannot be easily applied to organisms with the free exchange of genetic material through plasmids, transposons, etc. The difficultly in defining species has lead to an ongoing debate about whether it is better to use a more traditional phylogenetic or taxonomic approach to studying biogeography of microorganisms, or whether using a phenotype or trait based approach would be more appropriate (Fenchel and Finlay 2006; Green et al. 2008; Øvreås 2000). In this dissertation, I examined recovery at multiple taxonomic scales, including genus, species groups (as defined by Anzai et al. 2000), and species. Species assignments were made based on 16s rDNA sequences.

Biogeography has traditionally been studied by looking at the influence of past historical events on the current distribution of species alongside the role that current environmental conditions play on these species distributions (Martiny et al 2006). The study of microorganisms is no different. Martiny and colleagues (2006) outline four hypotheses, regarding the distribution of organisms in space. The first hypothesis, the null hypothesis, is that nothing explains the distribution of organisms, because their distribution is random. The second hypothesis is that organisms are dispersed everywhere and that the environmental conditions determine the distribution; this hypothesis is also known as the Bass-Becking (1934) hypothesis 'everything is everywhere – the environment selects'. The third is that distribution of organisms states a result of historical events, such as dispersal capabilities and historical environmental conditions. The last hypothesis, is a combination of the second and third, and is that both

environmental conditions and historical event shape the distributions of organisms (Martiny et al. 2006).

The two hypotheses that have generated the most interest and debate in microbial biogeography are the 'everything is everywhere – the environment selects' or that there is no dispersal limitation and the hypothesis that both past historical events and current environmental conditions are important in explaining species distribution (Bass-Becking 1934; Whitfield 2005; Martiny et al. 2006). The focus on these two hypotheses is because many studies have demonstrated some effect of environmental conditions or adaption to local environment types for microbial biogeography (Martiny et al. 2006). Studies that include both spatial and temporal variability are used to address biogeographical questions. In addition, taxonomic scale is important when evaluating distribution of organisms, what is considered globally distributed at one taxonomic level could be endemic to certain regions at a finer taxonomic resolution. At the taxonomic level of domain Bacteria, distribution is global; while, at finer taxonomic resolutions global distribution may not be the case (Ramette and Tiedje 2007). At a genus level some organisms are considered pandemic, or global in nature; fluorescent *Pseudomonas* from soils are an example of this (Chao and Tiedje 2000; Ramette and Tiedje 2007). At finer taxonomic levels this is not the case. In the case of fluorescent *Pseudomonas* studied by Chao and Tiedje (2000), genetic fingerprinting techniques showed endemic patterns of distribution.

Studies of spatial variation are able to identify possible 'hotspots' of diversity, correlate recovery and distribution with environmental conditions, and evaluate hypotheses regarding dispersal limitation (Gonzalez et al. 2012). Studies of temporal

variation can address many of the same questions as studies of spatial variation, but can also address questions of cyclical or static patterns of recovery and distribution (Gonzalez et al. 2012). This dissertation includes a study addressing both spatial and temporal variation, with replicate households sampled repeatedly across seasons. By analyzing recovery from different environment types (i.e., soils, drains) and houses, I evaluated biogeographical hypotheses at multiple taxonomic and spatial scales using household *Pseudomonas* species.

Cystic Fibrosis and Microbiology of the CF Lung

The incidence of cystic fibrosis in the United States is approximately 1 in every 3,500 live births, and is more common in Caucasian individuals than other demographic groups (Davis et al. 1996; CF Foundation Patient Registry 2011; Weiler and Drumm 2013). There are approximately 30,000 individuals living with CF in the United States, and it is estimated one-half of those are children under the age of 18 (CF Foundation Patient Registry 2011). Great strides have been made in the last few decades at increasing the length and quality of life for CF patients. According to the CF Foundation Patient Registry (2011), the median life expectancy of a patient 25 years ago was approximately 28 years old. Now it is nearly 37 years old.

Cystic fibrosis is an autosomal recessive disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which was identified in 1989 (Kerem, et al. 1989; Riordan et al. 1989; Rommens et al. 1989). Currently there are over 1900 known mutations to the CFTR (Cystic fibrosis mutation database http://www.genet.sickkids.on.ca/StatisticsPage.html). However, one mutation (Δ508) is

much more common than any of the others, with over 85% of patients possessing at least one copy (CF Foundation Patient Registry 2011). It has become clear that both the type and position of the mutation(s) to the CFTR gene affect the severity and presentation of the disease (Zielenski 2000). These mutations alter the main function of the CFTR protein, which is to transport chloride ions across epithelial cells (responsible for producing sweat, mucous, digestive enzyme, etc.) and regulate sodium transport (Zielenski 2000; Rowe et al. 2005; Weiler and Drumm 2013). Mutations causing a loss or decrease of function of the CFTR gene often manifest clinically as a thickening of the mucosal linings of the lungs, creating an environment very susceptible to infection (Weiler and Drumm 2013). These infections are associated with further decline in lung function, inflammation, and ultimately early mortality (Koch 2002; Weiler and Drumm 2013).

The CF lung is a complex ecosystem for establishing microbial communities, conditions and resources vary with disturbance events that include antibiotic treatment, immune system invasion and attack, and inputs from the external environment (Yang et al. 2011). Therefore microbial community composition is expected to vary with changing conditions. There are a handful of bacteria well-documented and classically associated with lung infections of the CF patient: *Staphylococcus aureus, Haemophilus influenzae; Pseudomonas aeruginosa; Burkholderia cepacia*. Of these organisms, *S. aureus* and *H. influenzae* are found more commonly in children with CF, while *P. aeruginosa* and *B. cepacia* are more commonly associated with adult patients (Yang et al. 2011). Traditionally, these organisms have been considered the normal infections in the CF lung environment, but in the last decade or so there has been an increased awareness

of the complex nature of the human microbiome and its interaction with the human body (Lynch and Bruce 2013).

Because of this awareness and advances in culture-independent techniques and sequencing technology, the entire microbiome of the CF lung is now being explored. The CF airways contain microbial communities including organisms traditionally associated with CF and anaerobes (Zemanick et al. 2011). In addition, the bacteria *Stenotrophomonas maltophila, Achromobacter xylosoxidans,* other non-*cepacia Burkholderia* species, and the fungus *Aspergillus fumigatus* are increasingly causing infections in CF patients and can cause a decline in lung function (Hauser et al. 2011; Zemanick et al. 2011). There is debate regarding the increased prevalence of these organisms and whether they are truly increasing in frequency or are products of changing patient care guidelines, differences in sample processing techniques between laboratories, and better identification techniques (LiPuma 2010).

Blainey and colleagues' (2012) culture-independent study comparing sputum of healthy people to sputum of CF patients found significant differences in the micriobiota of the two groups, with healthy people having more diverse microbiota than the CF patients. They also found a unique signature of microorganisms for the sputum samples of the CF patients, which included a much greater presence of the phylum *Bacteroidetes* in CF sputum. Cox and colleagues (2010) examined the bacterial diversity of the CF lung and found that diversity increases for the first decade of life, and then decreases as patients age, presumably as organisms establish dominance in the community. In this study they found that only *Stenotrophomonas maltophilia*, *Haemophilus influenza*, and *P. aeruginosa* were significantly correlated with an increased patient age. Other studies

have indicated that the incidence of *P. aeruginosa* infection is as high as 80% by the age of 20 (FitzSimmons 1993; Koch 2002). In this dissertation I address the recovery of *P. aeruginosa* from homes with and without a CF patient (see Chapter 3). *Burkholderia* infections, while less common, can rapidly lead to a decline in lung function, and the recovery of *Burkholderia* species from the home is addressed in Chapter 4.

The Genus *Pseudomonas*

Pseudomonas species are gram-negative, motile rods. They are isolated from a wide variety of environments, causing the genus to often be described as ubiquitous or global (Golderberg 2000; Stover et al. 2000). *Pseudomonas* species have diverse metabolic profiles and large genomes (~6Mb), allowing them to use a large number of compounds. *Pseudomonas* species tend to grow preferentially on amino acids or organic acids compared to glucose or other sugars (Rojo 2010). This versatility contributes to *Pseudomonas'* ability to inhabit such diverse environments. *Pseudomonas* species have been described as human pathogens (predominantly *P. aeruginosa,* and occasionally other species), plant pathogens (*P. syringae*), insect pathogens (*P. entomophilia*), plant growth promoters (*P. fluorescens*), nitrogen-fixers (*P. stutzeri*), and bioremediators (*P. putida*) (Silby et al. 2011).

Pseudomonas species can produce bacteriocins, called pyocins, which are proteinaceous antimicrobial compounds, evolved from phage tails, that target closely related species (Parret and DeMot 2002). Pyocin production by *Pseudomonas* species alters the composition of mixed-culture biofilms (Waite and Curtis 2009). In addition, pyocins can provide a competitive advantage between *P. aeruginosa* strains (Heo et al.

2007). These studies indicate that pyocins could influence *Pseudomonas'* ability to compete with each other and other organisms. In addition, *Pseudomonas* species (especially the opportunistic pathogen *P. aeruginosa*) are often described as being resistant to antibiotics. *P. aeruginosa* infect susceptible patients, including burn victims, diabetics, and patients with cystic fibrosis. *P. aeruginosa* strains have been described as having an intrinsic resistance to antibiotics such as aminoglycosides, quinolones, carbapenems, and penicillins (Gellatly and Hancock 2013). *P. putida* has also been reported to have antibiotic resistance, but may also serve as an environmental reservoir for resistance genes, particularly to β-lactam antibiotics (Meireles et al. 2013).

In addition to their antibiotic resistance capacities, *Pseudomonas* species can also form biofilms. Biofilms are an aggregate of microbial organisms, usually consisting of multiple species, contained within a sticky, extracellular matrix of polysaccharides, proteins, and DNA, facilitating adherence to surfaces (Høiby et al 2010). Bacteria in biofilms are up to 1,000 times more resistant to antibiotics than planktonic, or free-living, bacteria (Wagner and Iglewski 2008). Not only have *Pseudomonas* biofilms been recovered from sites of clinical relevance, such as the lungs of CF patients (Wagner and Iglewski 2008), but also from environmental sites, such as plumbing (Eboigbidin et al. 2008).

Pseudomonas species have many characteristics that would be consistent with having a broad fundamental niche: relatively large genomes, strong competitive abilities, versatility in resource use, and tolerance to varied habitat conditions. In Chapter 2 and 3 of this dissertation *Pseudomonas* recovery from the human home is investigated. Chapter

2 focuses on all species of *Pseudomonas* and their biogeography, while Chapter 3 focuses on the opportunistic pathogen, *P. aeruginosa*.

The Genus *Burkholderia*

The genus *Burkholderia* was formally named in 1992 and was then comprised of a small number of species previously described as *Pseudomonas* (Yabuuchi, et al. 1992). Today, the number of species in the genus is much larger and more diverse. Much of this diversity is attributed to their large (4-9Mb) multireplicon genomes. *Burkholderia* species are versatile and commonly isolated from soils. There are *Burkholderia* species that are capable of fixing nitrogen, associating with plant roots to promote plant growth, protecting plants from fungal and other infections (Parke and Gurian-Sherman 2001), degrading a variety of pollutants, including trichloroethylene (TCE), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). (O'Sullivan and Mahenthiralingam 2005), and causing disease in plants, animals and humans (Coenye and Vandamme 2003; Mahenthiralingam et al. 2005; Baldwin et al. 2007). Most *Burkholderia* found in association with plants are considered beneficial (Mahenthiralingam et al. 2005; Suárez-Moreno et al. 2012). *Burkholderia* species have been used in agriculture, as well as for bioremediation; however, because some species are also considered opportunistic pathogens, this has caused concern that immune-compromised patients may be exposed to these species because of their use in agriculture and for bioremediation (Chiarini et al. 2006). It is because of this concern that the United States Environmental Protection Agency restricted use of *B. cepacia*, a species

commonly associated with human infection, for bioremediation and agricultural uses (Federal Register 2003).

One of the most studied groups of the *Burkholderia* genus is the *Burkholderia cepacia* (formerly *Pseudomonas cepacia)* complex. The *B. cepacia* complex consists of 17 formally named, phenotypically different species (LiPuma 2005; Nørskov-Lauritsen, et al. 2010; Suárez-Moreno et al. 2012). Many of the members of the *B. cepacia* complex are capable of the abilities listed previously and have been isolated from both environmental and clinical environments. *B. cepacia* complex species are considered opportunistic pathogens of particular interest to the cystic fibrosis (CF) community, as infection with these species can lead to a rapid decline in lung function and prognosis (Mahenthiralingam et al. 2008). The first reports of *B. cepacia* complex species infections in CF patients were in the 1970's and 1980's (LiPuma 2010). Causing additional concern to the CF community is the fact that *B. cepacia* complex species are transmissible from person-to-person (LiPuma 1990; Baldwin et al. 2008). Clinical efforts such as generating patient cohorts, where patients with *B. cepacia* complex infections are seen on different days than uninfected patients, and other infection control measures have greatly reduced the incidence of infection (LiPuma 2010). Clinical measures have not eliminated the incidence of *B. cepacia* complex infections, indicating that there could be an environmental source of infection. In Chapter 4, recovery of *Burkholderia* species from the human home is examined.

Research Interests and Hypotheses

What influences the biogeography of household Pseudomonas *species?*

I examined the biogeographical patterns and seasonal variation for *Pseudomonas* species collected from the human home environment, using 15 homes to provide replication at the spatial scale, and sampling each repeatedly over different seasons provide temporal variability. I hypothesized that both spatial and temporal factors affect the distribution of *Pseudomonas* species. Recovery was examined at multiple taxonomic levels, and varied significantly by within home environment types at all levels, indicating the ability of *Pseudomonas* species to adapt to varied conditions. Recovery varied significantly by season at the level of genus, and for two of the species groups (*P. fluorescens* group and *P. putida* group), indicating temporal variability in recovery for *Pseudomonas* species. Interestingly, the patterns of recovery by both environment and season were different between the species groups. Both season and environment type influenced distribution patterns of species within the genus *Pseudomonas*, suggesting niche partitioning within the genus. Overall, I found that *Pseudomonas* species exhibited both spatial and temporal variability, indicating that both adaption and dispersal limitation drive biogeographical patterns.

Which environments in the human home are most likely to harbor Pseudomonas aeruginosa*, and are there differences in* P. aeruginosa *recovery between homes with and without a cystic fibrosis (CF) patient?*

I identified environments in the human home that harbored *Pseudomonas aeruginosa*, examined recovery seasonally, and compared recovery rates from homes

with *P. aeruginosa* culture-positive CF patients to those without. I hypothesized that *P. aeruginosa* recovery does not vary between home with and without a CF patient, when recovery from patient-associated sites is excluded. *P. aeruginosa* recovery patterns from homes were examined by comparing 10 different environment types (drains, soils, surfaces, etc.), and drains were identified as the environment type with the greatest *P. aeruginosa* recovery. This indicates that drains could pose a risk to CF patients. Season of isolation significantly influenced the recovery of *P. aeruginosa*, but season did not significantly influence recovery from drains. *P. aeruginosa* recovery did not differ from drains in CF vs. non-CF homes, indicating that high recovery from drains is not explained by spread from patients to drains.

How common are Burkholderia *species in the human home, and are there patterns of recovery by location or by environment type?*

I investigated the human home as a potential source of *Burkholderia sp.* for CF patients by looking at the recovery rate from various environment types, hypothesizing a low recovery rate. Overall, the recovery rate of *Burkholderia sp.* was very low, and isolates were recovered primarily from soil and soil-like environments and drains. However, even with low recovery, sites within the house cannot be eliminated as potential sources of new infection because of their recovery from within the home.

CHAPTER 2

BIOGEOGRAPHY PATTERNS AND SEASONAL VARIATION IN RECOVERY OF HOUSEHOLD *PSEUDOMONAS*

Summary

Pseudomonas species are often described as "ubiquitous" (Stover et al. 2000) and have been isolated from many different environments. In this study, I examined the biogeographical patterns and seasonal variation of *Pseudomonas* species collected from different environments in the human home; 15 homes were sampled 3-8 times each, with samplings being approximately 3 months apart. Recovery was examined at multiple taxonomic levels: genus, species groups (as described in Anzai et al. 2000), and species. The majority of *Pseudomonas* recovered was from the *P. putida* group (60.8%), followed by the *P. fluorescens* group (22.9%), and *P. aeruginosa* group (15.2%). Recovery varied by environment type (Appendix I) at the level of genus, with soils being most likely to yield *Pseudomonas*. Recovery for each of the three species groups varied, with drains being most likely to yield *P. aeruginosa* group, and soils being most likely to yield *P. fluorescens* and *P. putida* group. Seasonal variation was found at the level of genus, with winter having the lowest recovery. At the level of species group, recovery also varied seasonally for the *P. fluorescens* group and *P. putida* group. Distribution patterns of species within the genus *Pseudomonas* were found to vary by environment type and season. Soils and drains were the environment types with the highest recovery at the

taxonomic level of genus and at the level of species groups, so they were examined in greater detail by comparing different types soils and drains. Recovery patterns at the level of species group for indoor soils (houseplants) were significantly higher from outdoor soils. Overall, this study shows that variability between environment types and seasons can be seen at multiple taxonomic scales, that season influences recovery for household sites, that dispersal may be limited between houses, and that within the genus *Pseudomonas* species exhibit patterns of niche partitioning.

Introduction

Interactions with other organisms including competitive abilities, predation and disease, as well as resource use, abiotic conditions, and an organism's ability to disperse can all influence the distribution of organisms in space and time. As a genus *Pseudomonas* is highly versatile and capable of using a wide range of resources and conditions (Goldberg 2000; Madigan et al. 2000). In addition, *Pseudomonas* species are known to have advantages such as resistance to a wide array of antibiotics (Heuer et al 2002; Johansen et al. 2008; Ratjen et al. 2009; Meireles et al. 2013). These characteristics would indicate that as a genus *Pseudomonas* has a broad fundamental niche as defined by Hutchinson (1959). Consistent with this, *Pseudomonas* species have been isolated from a variety of habitats such as: soils and rhizospheres (Green et al 1974; Berg et al. 2005), drains (Regnath et al. 2004; Remold et al. 2011), fresh water rivers (Pirnay et al. 2005), open oceans (Kahn et al. 2008), "petroleum sludge" (Bharali and Konwar 2011), insects (Saitou et al. 2009), medical equipment (Srinivasan et al. 2003),

and the human body (Bodey et al. 1983), and *Pseudomonas* species have been described as "ubiquitous" (Stover et al. 2000).

However, an organism's realized niche is often smaller than that of its fundamental niches (Hutchinson 1959). *Pseudomonas* species, including *P. aeruginosa* (Knezevic et al. 2011), *P. fluorescens* (Sillankora et al. 2008), and *P. putida* group (Shaburova et al. 2009) are subject to disease, in the form of infection by phage. Presence of phage in an environment could affect the distribution of *Pseudomonas*. *Pseudomonas* can be prey for protozoan in the environment (Mazzola et al. 2009), which could also affect the distribution of *Pseudomonas.* Lastly, production of bacteriocins, which are antimicrobial compounds produced by a bacterium to kill other closely related bacteria (Parret and De Mot 2002), could also alter the distribution of *Pseudomonas*. It is because of these biotic factors that we expect not to find ubiquitous distribution of household *Pseudomonas.* Depending on the way the realized niche is defined, abiotic factors (i.e., temperature, pH) could also influences its size (Colwell and Rangel 2009).

Season of recovery, which encompasses a variety of environmental characteristics (i.e., temperature, humidity), has been shown to be important in soil microbiology (Smit et al. 2001; Lipson and Schmidt 2004; Fierer and Jackson 2006), and microbial communities of lakes (Jasser et al. 2013) and microbial communities of the oceans (Giovannoni and Vergin 2012). However, little is known about seasonal variability for *Pseudomonas* species or indoor microorganisms. A study by Rodríguez and colleagues (2012) found significant seasonal variability in the recovery of *Pseudomonas* species in the Cautro Ciengas Basin in Chihuahuan desert of Mexico, which consists of a small valley and multiple water systems. Rintala and colleagues (2008) found recovery of

microbial communities from indoor dust, mainly composed of gram-positive organisms, did vary by season. In this study I examined the distribution of species by environment type and seasonal variation of the genus *Pseudomonas* from sites in and around the human home in order to contribute to knowledge about the realized niche of *Pseudomonas* species.

Competing hypotheses in microbial biogeography examine whether dispersal limitation is what dictates an organism's distribution, or whether an organism's ability to adapt to a particular environment dictates its distribution, or if it is a combination of both (Martiny et al. 2006). The human home provides a place to study these questions with multiple microhabitats within and around a single building that are replicated within and between homes (i.e., bathroom sink drains). Analyzing isolate recovery from samples taken using culture-based methods in a longitudinal study of 15 households over 4.5 years, I describe variability of *Pseudomonas* recovery from 8 environment types within and around the home (Appendix I) to address biogeographical patterns of adaptation at multiple taxonomic scales: genus, species groups (as describe in Anzai et al. 2000), and species. Variability in *Pseudomonas* recovery between different houses, which are spatially distant, was analyzed to address biogeographical patterns of dispersal limitation. Seasonal variability is also examined at multiple taxonomic scales (genus, species group, and species). Lastly, I examined the distribution of *Pseudomonas* species across both seasons and environment types to determine if patterns of temporal and spatial niche partitioning occurred within this genus.

Materials and Methods

Sample Collection

I collected samples from 15 households in the Louisville, KY (USA) metropolitan area. Two types of households were sampled: houses with a young cystic fibrosis (CF) patient in residence and houses without CF patients. Each household was sampled between October 2007 and March 2012 at intervals of approximately three months. Houses were each sampled between 3 and 8 times, with over half (n=8) being sampled 8 times; the average number of samplings per household was 6. A total of 11,726 samples were taken. Within each household, between 75 and 168 samples were collected (depending on the number of bathrooms, people, pets, etc.) from 123 types of sites in and around the home (Appendix I). Choice of sites was not biased toward environments previously reported to harbor *Pseudomonas*; rather sites that might be important for human contact and the dispersal of microbial organisms were chosen. Subjects enrolled in the study were instructed not to clean the home the week before the sampling date. To minimize the risk of cross-contamination, no two households were sampled on the same day. Federal and institutional guidelines and policies regarding the use of human and animal subjects were followed including signed informed consent forms (and assent forms, where applicable).

Samples were collected with sterile swabs pre-moistened with phosphate-buffered saline. Surfaces were sampled at locations most likely to have frequent contact with human skin (i.e., knobs, buttons, etc.). Soils were sampled by inserting a swab 1-2 inches from the surface, collecting soil on the swab. All drains were swabbed within the first 1-

2 inches from the top of the opening. The subjects or their parents/guardians collected human fecal and genital samples. Swabs were streaked onto *Pseudomonas* isolation agar (PIA) at the homes. Plates were transported on ice and incubated for 48 hours at 28ºC immediately upon return to the laboratory. Where growth occurred, a single colony from each plate was picked randomly, re-streaked onto PIA, grown, and frozen for further analysis. Where multiple colony morphologies grew from a single sample, one of each was frozen (194 instances total) for further analysis. On twenty-eight instances, morphologically different isolates from the same sample were identified to be the same species; for these only one isolate was used in the analyses presented.

Sample Identification

Isolates were first identified as belonging to the genus *Pseudomonas* using previously described primers that selectively amplify members of the genus *Pseudomonas* (Spilker et al. 2004). *Pseudomonas* isolates were identified to a finer taxonomic level by using >500bp sequences of the isolates' 16s rDNA (8f and 1492r universal bacterial primer pair for PCR and the 1401r primer for sequencing (Weisburg et al. 1991). Assignments were made to the level of species using three separate databases: Bioinfo 1200 neucleotide, Bioinfo 2 sequences, and EzTaxon (Chun et al. 2007; Croce et al. 2010). Where the databases were not in agreement, Bioinfo 1200 nucleotide database was used for species assignment. Species were then classified into species groups, as defined by Anzai and colleagues (2000). While identification to the level of species was not always consistent for members of the *P. fluorescens* group and *P. putida* group, identification at the group level was.

Statistical Analysis

Preliminary analyses of data excluding CF-associated sites, human upper respiratory sites and equipment used to treat CF, show that there are no differences between homes with and without a CF patient. In order to increase the power of the analyses we therefore combined the two types of houses and continued analyses with this reduced dataset. For analysis, the 123 types of sites considered in this study were categorized to create a summary variable: "environment type" which describes ecological similarity (Appendix I). Using a mixed linear model with presence/absence of *Pseudomonas sp.* as the response variable, season and environment type as the fixed predictor variables. The interaction between season and environment type was not tested because of imbalance in the dataset. House and the interaction of house and environment type were random factors, and sampling as repeated measure nested with season of recovery (PROC GLIMMIX, SAS 9.3). It is important to note that absence of recovery does not preclude these species being present, and sampling techniques could have biased recovery from sites. For this, and all analyses involving seasons, the season of recovery was determined based on the meteorological seasons rather than astronomical seasons. The meteorological season considers factors such as average high and low temperatures for the area, Louisville Metro (Climatology – Louisville). Winter was classified as December through February, spring as March through May, summer as June through August, and fall as September through November.

In all analyses, for significant predictor variables, differences in least squares means among levels were calculated, and adjusted for multiple comparisons using Bonferroni corrections (PROC GLIMMIX, SAS 9.3). Wald Z tests were used to calculate p-values for the covariance parameters of house and house by environment type

interaction. In graphing these data and all other data presented in this chapter, odds ratios and least square means were calculated from the relevant mixed linear model and used to estimate probability of recovery for each season and environment type with 95% confidence intervals. This is a maximum likelihood approach to estimate the variance for multiple variables (i.e., environment type and house) simultaneously, and is used because of the intrinsic imbalance and multivariate nature of the dataset. For example, raw probabilities would not be able to account for factors such as having three times as many samples collected during a certain season compared to other seasons, while this approach accounts for such variation. Least square means are generated from parameter estimates of the model and are estimates of fixed effects.

Seasonal and environmental variability of recovery was examined for the three major species groups recovered in this study: *P. aeruginosa* group, *P. fluorescens* group, and *P. putida* group (Table 1; Anzai, et al. 2000) were tested. Mixed linear models with presence/absence of each species group were the response variables, season and environment type as the predictor variables, house and the interaction of house and environment type as random factors, and sampling as repeated measure nested with season of recovery were used to address these questions. (PROC GLIMMIX, SAS 9.3). Contrasts and graphical display of the data are as previously described for overall *Pseudomonas spp.* recovery.

Data were then analyzed for potential patterns of niche partitioning within the genus *Pseudomonas* by season and environment type. The relative rate of recovery was generated for each species across environment types for each season (number of isolates of a particular species divided by the total number of *Pseudomonas* isolates). Species for

which fewer than 8 isolates were recovered were pooled together by species group (i.e., Other *P. putida* group). A Bray-Curtis dissimilarity matrix was calculated for the relative recovery rates by species across environment type for each season using PRIMER (2006). A permutational ANOVA was used to evaluate the effect of environment type and season (as fixed factors) on the dissimilarity matrix generated from proportion data (Anderson 2005; PRIMER).

Since recovery was greatest from soils and drains they were examined further. Soils were further classified as indoor soils (including only houseplants) and outdoor soils (including only yard and garden soils). Drains were further classified into three types: kitchen sink drains (including garbage disposals), bathroom sink drains, and bathtub/shower drains. Recovery was then examined by species group for soil type and drain type using mixed linear models with presence/absence of each species group were the response variables, season and drain (or soil) type as the predictor variables, and sampling as repeated measure nested with season of recovery were used to address these questions. (PROC GLIMMIX, SAS 9.3). In addition, recovery from soils and drains were examined for patterns of niche partitioning within the genus *Pseudomonas*. Analyses preformed as previously described with soil types and drain types rather than environment types.

Results

Pseudomonas species were recovered from every season, every house, and every environment type sampled. A total of 1,152 *Pseudomonas* isolates were recovered from 11,726 samples taken for an overall recovery rate of 9.8%. Of the isolates collected,

60.8% were from the *P. putida* group, 22.9% were from the *P. fluorescens* group, 15.2% were from the *P. aeruginosa* group, and 1.1% were from other *Pseudomonas* species (Table 1).

Table 1. Species recovered in this study, as categorized into species groups. Species groups were defined by Anzai et al. (2000).

***P. aeruginosa* group**	***P. fluorescens* group**	***P. putida* group**	**Other *Pseudomonas sp.***
P. aeruginosa	*P. cedrina*	*P. cremoricolorata*	*P. abietaniphila*
P. alcaligenes	*P. extremaustralis*	*P. fulva*	*P. chlororaphis*
P. citronellolis	*P. fluorescens*	*P. monteilii*	*P. cichorii*
P. multiresinivorans	*P. libanensis*	*P. mosseliiss*	*P. rhizosphaerae*
P. nitroreducens	*P. lurida*	*P. oryzihabitans*	*P. stutzeri*
P. otitidis	*P. orientalis*	*P. plecoglossicida*	*P. syringae*
	P. poae	*P. putida*	
	P. proteolytica		
	P. rhodesiae		
	P. tolaasii		
	P. trivialis		
	P. veronii		

Environment type and season were examined at the level of genus to determine their influence on the recovery of *Pseudomonas.* Household *Pseudomonas* recovery varied significantly by environment type ($p<0.0001$), and was significantly higher from soil sites compared to all other types of environments (Figure 3A). Recovery from drains was also higher than all other types of environments excluding soils. Human skin sites had the lowest recovery of *Pseudomonas* species, followed by animal and surface sites. *Pseudomonas* recovery varied significantly by season ($p<0.0001$), with winter having significantly lower recovery than other seasons. The interaction between season and environment type was unable to be tested due to imbalance in the dataset. Recovery rates of *Pseudomonas* differed significantly among houses ($p=0.047$); additionally, there was a significant interaction between house and environment type ($p=0.0002$).

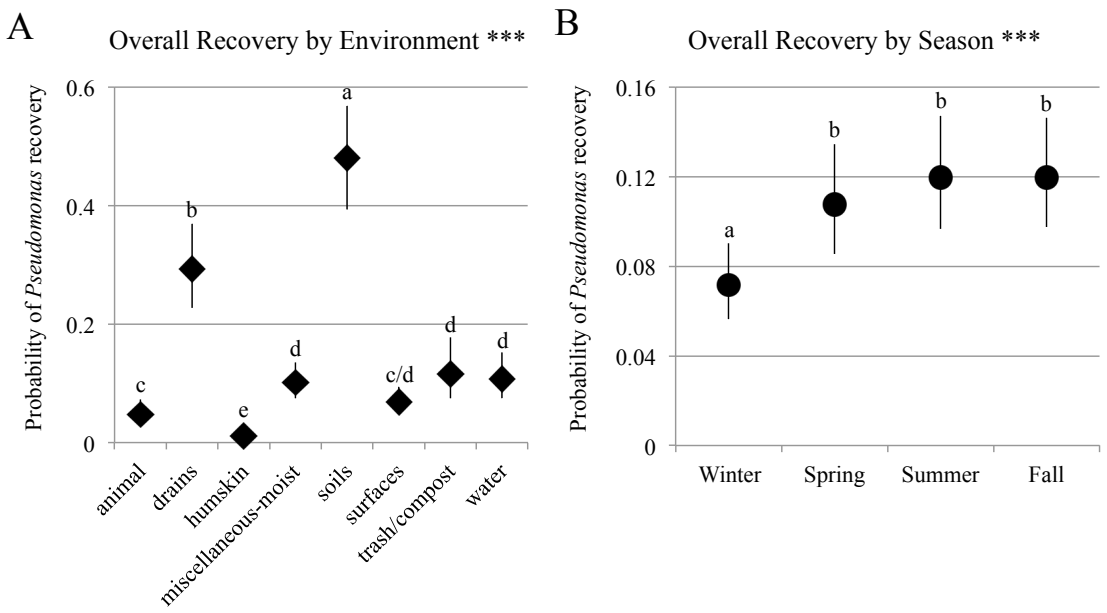

Figure 3. Predicted probability of *Pseudomonas* recovery as calculated from mixed general linear model with *Pseudomonas sp.* recovery as the response variable, environment type and season as predictor variables, house and the interaction of house and environment type as random factors, and sampling as a repeated measure nested within season of recovery by A) environment type B) season. 95% confidence intervals of the probability are shown. The letters over data-points indicate the results of differences of least square means comparisons of seasons. Each letter that appears multiple times designates pairs of seasons did not differ significantly; seasons that differed significantly ($p<0.05$) do not share letters. Contrasts were adjusted for multiple comparisons using a Bonferroni correction. Overall significance designated by: ***$p<0.0001$.

Next, variability in recovery by environment type was explored at the level of species group (*P. aeruginosa* group, *P. fluorescens* group, and *P. putida* group). For all three species groups recovery rates varied among the environment types, and interestingly, the species groups did not exhibit the same patterns of recovery (Figure 4). *P. aeruginosa* group recovery differed from the pattern of recovery at the genus level, with drain sites as the environment type with the highest recovery. Recovery for drains was significantly different from all other environment types including soils (Figure 4A). Due to small sample size animals were unable to be included in analyses examining recovery of *P. aeruginosa* group by environment type. For *P. fluorescens* groups, soil

sites were the environment type with the highest recovery. Due to high variation in recovery from *P. fluorescens* group differences were not statistically significantly different, from recovery from drain sites, trash/compost sites, water sites, or miscellaneous moist sites, which include items that are frequently wet or in contact with water but have opportunities to dry (i.e., dish scrubbing tools, bath toys, etc.) (Figure 4B). For *P. putida* group, recovery was significantly higher for soil sites than any of the other environment types (Figure 4C). Recovery from drain sites was also significantly higher than most other environment sites for *P. putida* group. Although results of pairwise tests differ, the recovery trends for *P. fluorescens* group and *P. putida* group are similar. All three groups had very low recovery from vertebrates (animal and human skin environment types).

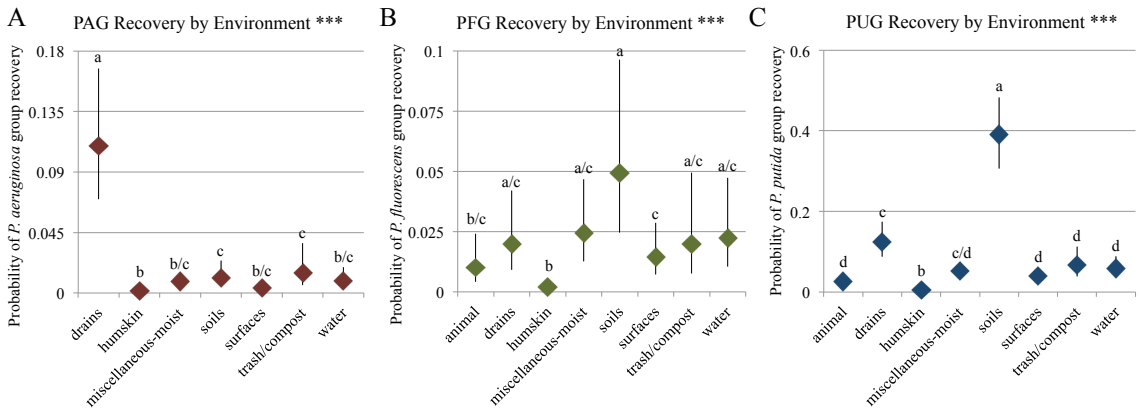

Figure 4. Predicted probability of recovery from environment types from mixed general linear model analygous to that described in Figure 3, expect that response variables are recovery from: A) PAG - *P. aeruginosa* group B) PFG - *P. fluorescens* group C) PUG - *P. putida* group 95% confidence intervals of the probability are shown. The letters over data-points indicate the results of differences of least square means comparisons of environment type. Each letter that appears multiple times designates pairs of seasons did not differ significantly; seasons that differed significantly ($p<0.05$) do not share letters. Contrasts were adjusted for multiple comparisons using a Bonferroni correction. Overall significance designated by: ***$p<0.0001$.

Recovery at the level of species groups was also examined for variability across season. *P. aeruginosa* group recovery did not differ by season (data not shown). *P. putida* group and *P. fluorescens* group show significant seasonal differences (Figure 5) and interestingly have different seasonal patterns from each other. *P. putida* group had a higher probability of recovery in the summer and fall (Figure 5B), and while not the same as exhibited at the level of genus, the pattern is similar. *P. fluorescens* group had significantly more recovery in the spring, followed by summer, and the lowest recovery in winter and fall (Figure 5A). This pattern of recovery, with spring having greatest recovery compared to all other seasons appears to differ from that of the genus as a whole. The effect of house on recovery variability was also examined for each of the three *Pseudomonas* species groups. Variability in recovery was not significantly explained by house-to-house variability for the *P. aeruginosa* group ($p=0.3626$); however the interaction between house and environment type was significant ($p=0.001$). Variability in recovery was significantly explained by house-to-house variability for both *P. fluorescens* group and *P. putida* group ($p=0.022$ and $p=0.035$ respectively); additionally, the interaction between house and environment type was significant for both *P. fluorescens* group and *P. putida* group ($p=0.0002$ and $p=0.001$ respectively).

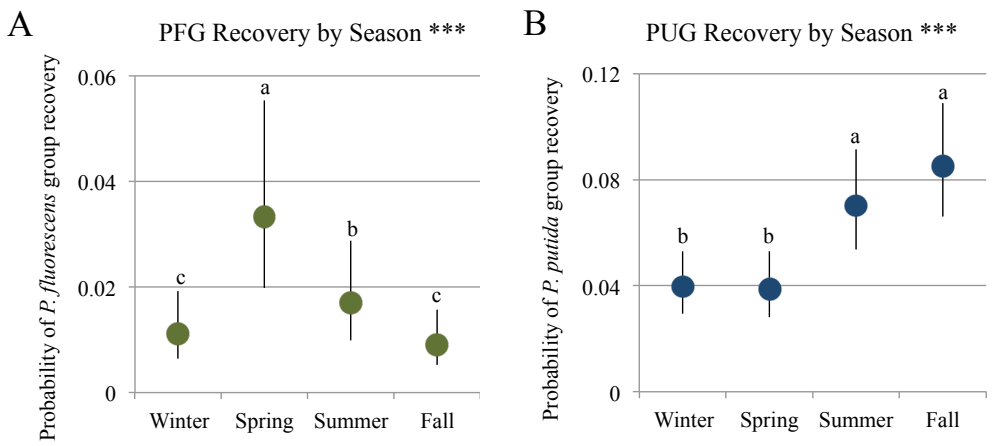

Figure 5. Predicted probability of recovery from seasons by *Pseudomonas* species groups: A) *P. fluorescens* group B) *P. putida* group from mixed general linear models as described in Figure 4. 95% confidence intervals of the probability are shown. The letters over data-points indicate the results of differences of least square means comparisons of seasons. Each letter that appears multiple times designates pairs of seasons did not differ significantly; seasons that differed significantly ($p<0.05$) do not share letters. Contrasts were adjusted for multiple comparisons using a Bonferroni correction. Overall significance designated by: ***$p<0.0001$.

The relative rates of recovery for *Pseudomonas* species across seasons and environment types was examined for patterns of niche partitioning within the genus by comparing proportions for each species by season/environment type. The relative recovery rate of species in the genus *Pseudomonas* differs significantly by season ($p=0.017$), with spring differing from winter and fall (Figure 6A). Members of the *P. fluorescens* group make up a greater proportion of the *Pseudomonas* collected in the spring compared to other seasons, which is consistent with what is found examining recovery at the level of species group (Figure 4A). Figures depicting the seasonal relative rates of recovery for *Pseudomonas* species by environment are contained in Appendix III, and show that in the spring *P. fluorescens* group members dominate recovery from surfaces, while *P. putida* group is most prevalent for other seasons. For the summer season it appears that *P. fluorescens* group recovery is high for human skin

sites. While fall had nearly twice as many *Pseudomonas* collected as winter, the distribution of species present did not significantly differ

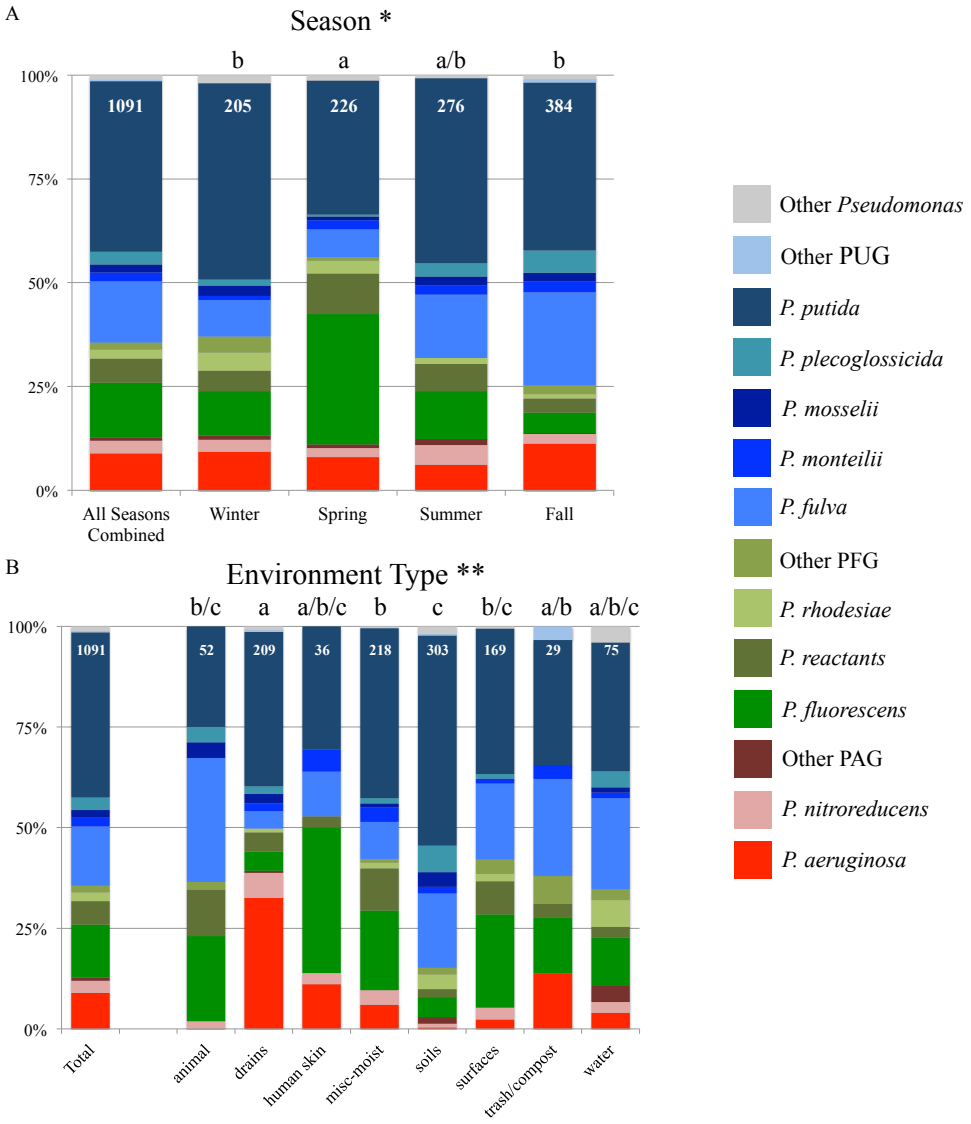

Figure 6. Proportion of recovered strains by species for all *Pseudomonas* by A) seasons B) environment types. Coloring represents species (see legend) and group: reds - *P. aeruginosa* group, greens - *P. fluorescens* group; blues – *P. putida* group. Numbers on the tops of bars indicate sample size (# *Pseudomonas* collected) for each category. A permutational ANOVA was used to evaluate the effect of environment type and season (as fixed factors) and for pairwise comparison between environment type and season. The letters over bars indicate the results of pairwise comparisons. Each letter that appears multiple times designates pairs of seasons or environments that did not differ significantly; seasons that differed significantly ($p<0.05$) do not share letters. Contrasts were not adjusted for multiple comparisons and are shown to explore trends. Overall significance designated by: *$p<0.05$, **$p<.0.01$.

The relative rate of recovery of *Pseudomonas* species also differed significantly by environment type (p=0.001; Figure 6B). Drain sites exhibited a different distribution of *Pseudomonas* than most of the other environment types, as drains had a greater proportion of *P. aeruginosa* and other *P. aeruginosa* group members present compared to other environment types. Additionally, the proportion of *P. aeruginosa* group was low in other environment types, consistent with the finding at the level of species group that *P. aeruginosa* group members are primarily found in drains (Figure 4A). The distribution of *Pseudomonas* species from soil sites differ significantly compared to drain sites and miscellaneous moist sites (i.e., dish scrubbing tools, bath toys, etc.). Soils did not differ from the rest of the environment types (with the exception of trash/compost sites), but are dominated by *P. putida* group members. Approximately 75% of *Pseudomonas* recovered from soils fall within the *P. putida* group.

Soil and drain sites had the highest recovery of *Pseudomonas* species; it was for this reason they were sub-categorized for further examination. Drains were sub-divided into kitchen sink drains, bathroom sink drains, and tub/shower drains, while soils were sub-divided into indoor soils (houseplants) and outdoor soils. I compared recovery from the drain subtypes at the level of species group, and found no significant differences for any of the three species groups (data not shown). The relative rates of recovery for all *Pseudomonas* species was also compared for drain sub-types and between seasons for drain recovery, and there were no significant differences between subtypes or season (data not shown). Indoor soils were compared to outdoor soils at the level of species group. No differences were found between indoor and outdoor soils for *P. aeruginosa* group (data not shown), perhaps because of small sample size (only 9 *P. aeruginosa*

group isolates total were collected from soils). There were significant differences in recovery between indoor and outdoor soils for both *P. fluorescens* group and *P. putida* group (p=0.009 and p=0.011, respectively; Figure 7A-B). Both *P. fluorescens* group and *P. putida* group had higher recovery from outdoor soils compared to indoor soils.

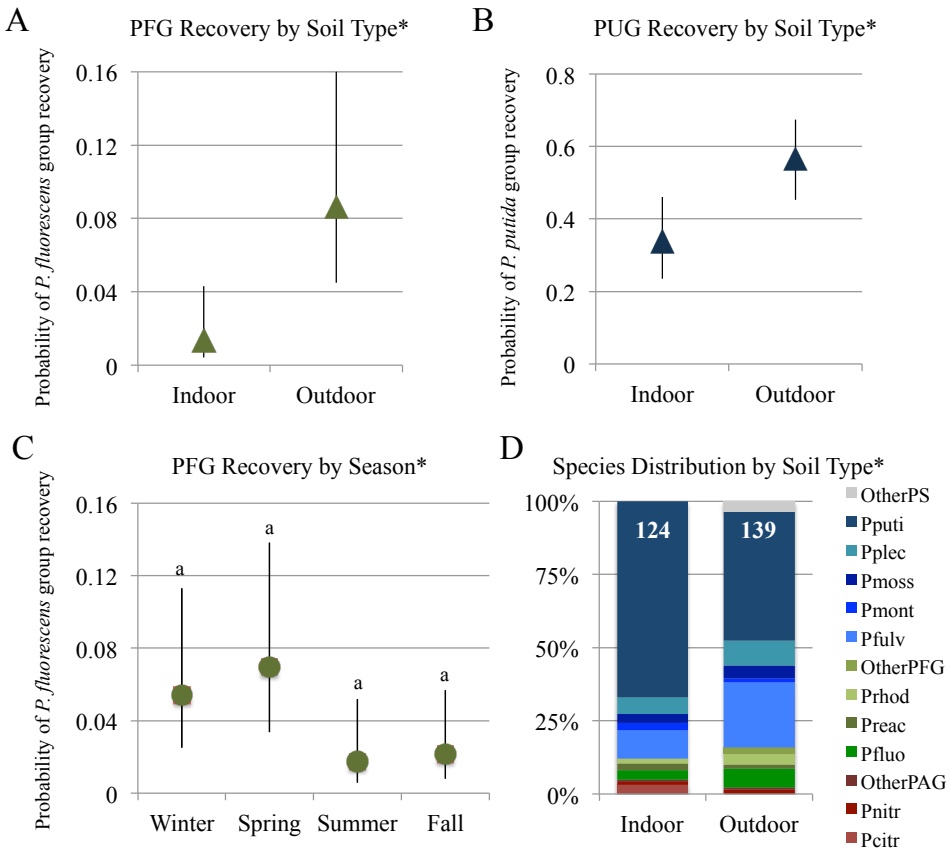

Figure 7. Recovery of *Pseudomonas* from soils. Comparisons of indoor soils to outdoor soils at the level of species group: A) *P. fluorescens* group B) *P. putida* group. C) Seasonal variation in probability of recovery of *P. fluorescens* group isolated from soils. For panels A-C: predicted probability of recovery as calculated from mixed general linear model with *P. fluorescens* group or *P. putida* group recovery as the response variable, soil type and season as predictor variables, house and the interaction of house and environment type as random factors, and sampling as a repeated measure nested within season of recovery. 95% confidence intervals of the probability are shown. The letters over data-points indicate the results of differences of least square means comparisons. Each letter that appears multiple times designates pairs of seasons did not differ significantly. Contrasts were adjusted for multiple comparisons using a Bonferroni correction. D) Distribution of *Pseudomonas* species from indoor and outdoor soils, numbers on the tops of bars indicate sample size (# *Pseudomonas* collected) for each category. Overall significance designated by: *p<0.05.

For each species group, seasonal variability in recovery from soils was examined. There was not any significant seasonal variability for *P. aeruginosa* group or *P. putida* group soils (data not shown). *P. fluorescens* group was found to have significant seasonal variability ($p=0.023$; Figure 7C). This difference was driven by slightly higher *P. fluorescens* group recovery in winter and spring, but pairwise comparisons were not significant after adjustment for multiple comparisons. The relative rates of recovery of all *Pseudomonas* species recovered was compared between indoor and outdoor soils; additionally, the distribution for soil sites was examined for seasonal trends in recovery. The relative rates of recovery for *Pseudomonas* species from soils differed marginally significantly by season ($p=0.06$; data not shown), but were found to differ significantly by soil type ($p=0.05$; Figure 7D). However, *P. putida* group dominates both indoor and outdoor soils.

Discussion

While *Pseudomonas* recovery from the home is not common (9.8%) of samples taken), species were recovered from every season, every house sampled, and every environment type. Patterns of recovery indicate that recovery from soils and drains are significantly more likely. Both at the level of genus, and within each species group, recovery from soil sites and drain sites were high, indicating their importance for household *Pseudomonas*. Classical accounts of *Pseudomonas* species describe them as being common in soil and aquatic sites, and these results are consistent with those observations (Ringen and Drake 1952). Additionally, patterns of recovery vary between species groups, with patterns of recovery from *P. aeruginosa* group differing from those

of *P. fluorescens* group and *P. putida* group. The patterns of recovery for *Pseudomonas* as a genus are very similar to those of the *P. putida* group. This is driven by the numerical dominance of *P. putida* group (60.8%) and species group was not corrected for when analyzing recovery at the genus level. Season also influenced recovery for all taxonomic levels and is important in determining the distribution of *Pseudomonas* in the home. The finding of differences among environment types and in the relative recovery of *Pseudomonas* species provides evidence for niche partitioning within the genus.

Fundamental vs. Realized Niche

Generally, the fundamental niche consists of everywhere the environmental conditions are suitable for an organism to grow and reproduce, and the realized niche can be defined as where, or under what conditions, an organism is actually found. We expected that the fundamental niche be broad and larger than the realized niche for *Pseudomonas*, as *Pseudomonas* have diverse metabolic capabilities and have previously been isolated in numerous varying environments. Consistent with that, *Pseudomonas* was recovered from every season, home, and environment type.

The realized niche is usually smaller than the fundamental niche because of interactions with other species, such as competition, predation, or disease. We found there is also evidence to support that the realized niche could be smaller than the fundamental niche for household *Pseudomonas*. Significant variation in recovery patterns by environment type was found, even at the level of genus. Recovery at the level of genus was highest for soils and followed by drains, while other environment types had much lower recovery. When using finer taxonomic resolutions, and examining recovery

at the level of species group and species, variability between environment types persist. Furthermore, patterns of recovery differ between species groups. It is clear that members of the *P. aeruginosa* group do not necessarily occupy the same types of sites as other *Pseudomonas* species groups (i.e., soils), or at least are not found nearly as frequently in these sites. It is important to note that absence of recovery does not preclude these species being present, and sampling techniques could have biased recovery from sites. However, these data do suggest that while *Pseudomonas* species have a broad fundamental niche, *P. fluorescens* group and *P. putida* group use multiple types of sites, but only a few really well. *P. aeruginosa* group is found at least occasionally in most types of sites, but frequently only in drains. This indicates that all species groups within the genus may have smaller realized niches, which is consistent with other reports of the genus *Pseudomonas* (Remold et al. 2011).

It is unclear from this study what specific factors are influencing the size of the realized niche; however, it is quite possible that biotic factors, such as competition at the local environment, or disease in the form of phage could be decreasing the size of the realized niche. All three *Pseudomonas* species groups discussed in this paper have been shown to be vulnerable to phage (Sillankora et al. 2008; Shaburova et al. 2009; Knezevic et al. 2011). If these phage are present in the home environment, this could contribute to the distributions found. Depending on the way the realized niche is defined, abiotic factors could also influences its size (see Chapter 1; Colwell and Rangel 2009). If one uses this definition for the realized niche it is possible that abiotic environmental conditions such as temperature, pH, humidity, and so on could also be contributing to varied distributions found. Lastly, it is also possible that the realized niche of household

Pseudomonas is constrained by the dispersal limitations or abilities of *Pseudomonas spp* (which are discussed below).

Seasonal Variability

The results of this study demonstrate that season is important for the recovery patterns and distribution of *Pseudomonas* species at all taxonomic levels examined. At the level of genus, recovery is found to be the lowest during the winter months and nearly equal for the other three seasons. At the level of species group, *P. putida* group recovery was higher in the fall and summer and nearly equal for winter and spring. *P. fluorescens* group had a different pattern, where recovery was highest during the spring. The average high temperature in the spring in Louisville is 67.9°F, compared to 45.5°F for winter, 87.5°F for summer, and 69.7°F for fall; while, the average low temperature in the spring in Louisville is 47.4°F, compared to 28.9°F for winter, 68.1°F for summer, and 49.6°F for fall (Climatology – Louisville). *P. fluorescens* group and *P. putida* group differ particularly with respect to recovery in the spring and fall (see Figure 5), where the temperature differential is minimal. This indicates the seasonal differences are driven by something other than temperature.

Season is a broad term that encompasses a variety of environmental and climactic changes including temperature, humidity, precipitation, etc. Therefore it is possible changes in another environmental factor associated with season are driving the difference seen between *P. fluorescens* group recovery and *P. putida* group recovery. Additionally, most of the samples collected in this study were from sites inside the home, where temperature is generally more constant throughout the year. It is also possible that

behavioral patterns of human and animal inhabitants including increased movement from inside to outside, or vice versa, or closed ventilation verses open windows during certain seasons could also potential explain patterns. Further research needs to be done to investigate more precise causes of these differences.

Rintala and colleagues (2008) found that the diversity of microbial communities from indoor air dust in Finland (mostly Gram-positive organisms) was highest in the spring and lowest in the winter. Moschandreas and colleagues (2003) found that recovery of cultivable organisms from Chicago-area homes (focusing on Gram-positive organisms) was highest in the summer, but also resident dependent. At the genus level, the results of this study do not differ from these accounts, where *Pseudomonas* recovery is lowest in the winter. However geographic differences, in addition to technical differences, make comparing these studies difficult. It is established that external inputs to indoor environments, such as open windows, food, and soil can influence the diversity of microbial communities (Kelley et al. 2013), and it is possible these factors are influencing differences in seasonal recovery of *Pseudomonas* species as well. Seasonal variability in *Pseudomonas* recovery indicates that microbial longitudinal studies must be carefully designed and consider climatic or environmental variables for greatest accuracy.

Dispersal vs. Adaption

At all taxonomic levels *Pseudomonas* recovery significantly varied by environment type, with the environment type varying between different species groups. These results indicate that the distribution of *Pseudomonas* species is influenced by their ability to adapt to certain types of environments. However, at both the level of genus and

the level of species group *Pseudomonas* recovery varied significantly among houses, with the exception of *P. aeruginosa* group, which showed no significant house-to-house variability.

Competing hypotheses in microbial biogeography question whether dispersal limitation is what dictates an organism's distribution, ability to adapt to its environment dictates an organism's distribution or if both dictates its distribution (Martiny et al. 2006). Unfortunately these things are difficult to detangle based on frequency data alone. Evidence for dispersal limitation could include absence or by changes in frequency of recovery (e.g., founder effects, legacy effects); however, differences in frequency of recovery could also be due to adaptation to local environment.

Relative Distributions and Patterns of Niche Partitioning

The relative distribution of species within the genus *Pseudomonas* was found to be variable by both environment type and season (Figure 6), which is indicative of niche partitioning within the genus. Interestingly, the two types of environments with the greatest amount of *Pseudomonas* recovery, soils and drains, showed significant differences in patterns of species distribution. Drains were significantly different compared to most other environment types in their pattern of *Pseudomonas* distribution, with the exceptions of trash/compost sites and water sites. Relative recovery rates of all *Pseudomonas* species from indoor soils compared to outdoor soils differed. This difference could be due to the difference in soil nutrient and characteristic from indoor houseplant soil, which is likely potting soil, compared to yard or garden soil, from differences in water sources and regularity of watering, or from differences in exposure to

larger organisms (i.e., earthworms). The increased recovery of *Pseudomonas* from outdoor soils compared to indoor soils is consistent with Remold and colleagues (2011), also consistent with this study in the increased recovery of *P. fulva* from outdoor soils compared to indoor soils (Figure 7D).

Classically niche partitioning is a result of competition over some sort of limiting resource so that competing organisms do not co-exist in the same niche-space. The patterns of niche partitioning examined in this study are relative only to other *Pseudomonas* and could be indicative of species interactions, such as competition, occurring within household environments. However, this study did not assess whether *Pseudomonas* species were competing with each other, or species of other genera, for a limited resource, so it is possible that the patterns of niche partitioning seen could be caused by other biotic factors. Alternatively the distributions seen could be driven by dispersal limitation and driven by founder effects.

Overall, this study demonstrates that while household *Pseudomonas* have broad fundamental niches, indicated by occasional recovery from most environments, at the level of genus, species group, and species there is evidence for a smaller realized niche for household *Pseudomonas* species. Evidence supporting *Pseudomonas* species adaptation to local environments as well as limited ability to disperse between houses are also shown. Seasonal variability in recovery of *Pseudomonas* species, demonstrates that indoor environments and microbial inhabitants are affected by seasonal changes. Seasonal variability also points to the importance of carefully designed longitudinal studies, so as to not miss or mask variability. Patterns of recovery consistent with niche

partitioning indicate that the factors influencing the distribution of the different species groups may differ, including both species interactions and dispersal limitation.

CHAPTER 3

IDENTIFICATION OF SITES HARBORING *PSEUDOMONAS AERUGINOSA* IN HOMES WITH CF PATIENTS AND HOMES WITHOUT CF PATIENTS

Summary

Pseudomonas aeruginosa infection occurs at an early age in many cystic fibrosis (CF) patients. While most colonizing strains are thought to be environmental, little work has been done to identify source environments important in lung colonization. My goals were to identify environments in the human home that harbor *P. aeruginosa* from which infections could be acquired, examine recovery patterns for seasonal trends, and to compare recovery rates from homes with *P. aeruginosa* culture-positive CF patients to those without. I characterized *P. aeruginosa* recovery patterns from human homes with (n=7) and without (n=8) young CF patients. In all, 75-168 sites representing a broad range of types of environments were sampled at each of 3-8 visits to each home. These included both host-associated (e.g. nose, mouth, eyes of humans and pets), and non-host associated sites (e.g. drains, counters, soils). In all, 11,726 samples were taken. Excluding isolates collected from CF patients, isolates from drains represent 67.3% of *P. aeruginosa* collected. Whereas 28.6% of drains yielded *P. aeruginosa,* recovery from all other types of environments except upper respiratory sites of CF patients was low. The

season of isolation significantly influenced the overall recovery of *P. aeruginosa*, but season did not significantly influence recovery from drains. Recovery from bathroom sink drains was significantly higher than recovery from kitchen sink drains or bathtub/shower drains. *P. aeruginosa* recovery did not differ from drains in CF vs non-CF homes, indicating that high recovery from drains is not explained by spread from patients to drains. These data indicate that household *P. aeruginosa* are mainly found in drains, and suggest that if successful cleaning regimes targeting drains could be identified, they could substantially reduce exposure to environmental *P. aeruginosa* in the home.

Introduction

A majority (90%) of cystic fibrosis patients tested positive for whole-cell antibodies to *Pseudomonas aeruginosa* and nearly 80% of patients' oropharynx cultures were positive for *P. aeruginosa* by 3 years of age (Burns et al. 2001). The prevalence of *P. aeruginosa* infections in CF patients increases with age, with approximately 80% of patients being infected by the age of 20 (FitzSimmons 1993; Koch 2002). As infections establish in the lungs they become more difficult to eradicate. It is well documented that *P. aeruginosa* can be transmitted between siblings (Renders et al. 1997; Tubbs et al. 2001), and cross-infection can occur from patient-to-patient in clinical settings (Cheng et al. 1996; Denton et al. 2002; McCallum 2002; Jones et al. 2003; Scott and Pitt 2004). However, initial infections are frequently found to be genetically more similar to isolates collected from environmental sources compared to those from chronically infected CF patients (Burns et al. 2001; Rau et al. 2010; Jelsbak et al. 2007; Workentine and Surette

2011). This indicates that the source of these initial infections is likely to be somewhere in the patients' everyday environments.

P. aeruginosa is often referred to as "global" or "ubiquitous" (Goldberg 2000; Stover et al. 2000). Even though *P. aeruginosa* has been isolated from many highly variable environments, only a few studies have examined it in environments or at a scale relevant to objects that young CF patients might contact on a daily basis (Mortensen et al. 1995; Ojima et al. 2002; Regnath et al. 2004; Schelstraete et al. 2008; Remold et al. 2011). Four of these five studies (Mortensen et al. 1995; Ojima et al. 2002; Regnath et al. 2004; Schelstraete et al. 2008) focused on the interior of the homes (primarily bathrooms/washrooms and kitchens), and all previous studies consisted of a single sampling of each household. Both the number and types of sites sampled varied between the studies. Remold et al. (2011) examined the many sites spanning inside and outside of the home from 20 homes; however, neither this study nor Ojima and colleagues' study (2002), included any CF patients or their homes. None of these studies addressed variation in recovery across seasons.

This study looks extensively at the human home environment (both interior and exterior), paying particular attention to sites that young children might come into contact with regularly, over multiple time-points and seasons. Additionally, I compare recovery of *P. aeruginosa* in homes with a CF and those without a CF patient. Analyzing isolate recovery from 11,726 samples taken in a longitudinal study of 15 households over 4.5 years, I describe the types of household sites harboring *P. aeruginosa* and other *Pseudomonas* species. Sites from which *P. aeruginosa* are most frequently recovered are potential environmental sources of *P. aeruginosa* infection. Identification of these sites

would also identify parts of the home for which effective cleaning strategies should be implemented in order to minimize patient exposure to household *P. aeruginosa.* I examine recovery of *P. aeruginosa* for seasonal trends in recovery, which would indicate cleaning regimes could be further targeted towards seasons where *P. aeruginosa* is most prevalent. Lastly, I compare recovery from homes with and without a *P. aeruginosa* culture-positive CF patient. Whereas increased recovery in homes with a culture-positive CF patient could occur if droplet contamination from coughing, spitting, etc. affected colonization of non-patient sites, similar recovery patterns across homes with and without a CF patient would indicate that patient dispersal of *P. aeruginosa* is not the primary explanation for recovery and that isolates can be considered environmental in origin.

Materials and Methods

Sample Collection

I collected samples from 15 households in the Louisville, KY (USA) metropolitan area; 7 households had a child with cystic fibrosis (CF) ranging in age from 6 months to 14 years old and 8 did not. All households with a CF patient were recruited through the Pediatric CF Center at the University of Louisville, and each had only one CF patient per house. Most patients had positive *P. aeruginosa* cultures from upper respiratory sites during the course of the study (Table 2).

Table 2. *P. aeruginosa* and other pathogen recovery from upper respiratory sites of enrolled CF patients during the course of their participation in the study. Houses 3-5, 7, 8, 10, 11, 13 did not have a CF patient.

House #	*P. aeruginosa* isolated at clinic visit(s)	*P. aeruginosa* isolates from this study	Other pathogens isolated at clinic visit(s)	Age (in years) at start of study
1	X	X		3
2			*Staphylococcus auerus*	6
6	X	X		14
9	X	X	*Staphylococcus aureus*	11
12	X	X	*Staphylococcus aureus, Stenotrophomonas sp.*	8
14			*Aspergillus fumigatus; Cryptococcus sp.*	0.5
15	X	X	*Staphylococcus aureus; Alcaligenes sp.; Candida albicans; Achromobacter xylosoxidans*	10

Each household was sampled between October 2007 and March 2012 at intervals of approximately three months. Houses were each sampled at least 3-8 times, with over half (n=8) being sampled 8 times; the average number of sampling events per household was 6. A total of 11,726 samples were taken. Within each household for each visit, I collected between 75 and 168 samples (depending on the number of bathrooms, people, pets, etc.) from 123 types of sites in and around the home (Appendix I). Choice of sites did not bias sampling toward environments previously reported to harbor *Pseudomonas*; rather I sampled broadly to include those sites with which young children with CF might have contact or exposure. Subjects were instructed not to clean the home the week before the sampling date. To minimize the risk of cross-contamination, no two households were sampled on the same day. Federal and institutional guidelines and policies regarding use

of human and animal subjects were followed, including signed informed consent forms and assent forms, where applicable.

Samples were collected with sterile swabs pre-moistened with phosphate-buffered saline; the subjects or their parents/guardians collected human fecal and genital samples. All drains were swabbed within the first 1-2 inches from the top of the opening. Surfaces were sampled at locations most likely to have frequent contact with human skin (i.e., knobs, buttons, etc.). Soils were sampled by inserting swab 1-2 inches from the surface, collecting soil on the swab. Swabs were streaked onto *Pseudomonas* isolation agar (PIA) at the homes. Plates were transported on ice and incubated for 48 hours at 28ºC immediately upon return to the laboratory. Where growth occurred, a single colony was picked, re-streaked onto PIA, and frozen for further analysis; where multiple colony morphologies were found for a single sample one of each were frozen for further analysis. On eight instances morphologically different isolates from the same sample were identified to be the same species; for these only one was used in the analyses.

Sample Identification

Isolates were first identified as belonging to the genus *Pseudomonas* using previously described primers that selectively amplify members of the genus *Pseudomonas* (Spilker et al. 2004). Further identification of those isolates identified as *Pseudomonas* was performed using >500bp sequences of the isolates 16s rDNA (8f and 1492r) universal bacterial primer pair for PCR and the 1401r primer for sequencing (Weisburg et al. 1991). Assignments were made to the level of species using two separate BLAST databases: Bioinfo 1200 neucleotide and EzTaxon (Chun et al. 2007; Croce et al. 2010). The two databases agreed on the species assignment for all *P. aeruginosa* isolates.

Isolates classified by 16s sequence as *P. aeruginosa* were confirmed using previously described primers designed to selectively amplify *P. aeruginosa* (Spilker et al. 2004).

Statistical Analysis

For analysis, the 123 types of sites were categorized to create a summary variable: "environment type" which describes ecological similarity (Appendix I). I compared overall recovery of *P. aeruginosa* to other *Pseudomonas* species by looking at presence or absence across all samplings for each site sampled using McNemar's Test (PROC FREQ, SAS 9.3). I also compared recovery of *P. aeruginosa* to other *Pseudomonas* species within each environment type (PROC FREQ, SAS 9.3).

I tested hypotheses regarding seasonal variability in *P. aeruginosa* recovery for overall recovery and for recovery from sites inside the home, humans, and bathroom/kitchen drains (the environment with the highest *P. aeruginosa* recovery) using mixed linear models with presence/absence of *P. aeruginosa* as the response variable, season and house type (whether a CF patient resides in the home or not) as the predictor variables, and sampling as repeated measure nested with season of recovery (PROC GLIMMIX, SAS 9.3). For this and all other analyses odds ratios and least square means were used to generate probability of recovery for each season with 95% confidence intervals (PROC GLIMMIX, SAS 9.3). This is a maximum likelihood approach to estimate the variance for multiple variables (i.e., environment type and house) simultaneously, and is used because of the intrinsic imbalance and multivariate nature of the dataset. For example, raw probabilities would not be able to account for factors such as having three times as many samples collected during a certain season compared to other seasons, while this approach accounts for such variation. Least square means are

generated from parameter estimates of the model and are estimates of fixed effects. Additionally, differences in least squares means were calculated, and adjusted for multiple comparisons using Bonferroni corrections (PROC GLIMMIX, SAS 9.3). *P. aeruginosa* isolated from sites outside of the home were unable to be tested for seasonal trends due to low recovery. Season of recovery was determined based on the meteorological seasons rather than astronomical seasons. The meteorological season considers factors such as average high and low temperatures for the area (Climatology – Louisville).

Then I further examined recovery from the environment with the highest recovery (bathroom/kitchen drains). First, I examined whether drains in homes with a culture-positive CF patient (n=5) were different from drains in homes without a CF patient (n=8). Culture positivity was defined as collection of *P. aeruginosa* in an upper respiratory (sputum, oropharynx, etc.) site either through our study or clinical collection during course of the study. If drains in homes with a culture-positive CF patient have higher recovery rates of *P. aeruginosa* a possible explanation of this would be that patients are dispersing *P. aeruginosa* to their local environment. This was examined using a mixed linear model with presence/absence of *P. aeruginosa* as the response variable and house type (CF or non-CF house) as the predictor variable, and sampling as repeated measure nested with season of recovery (PROC GLIMMIX, SAS 9.3). Second, I also compared *P. aeruginosa* recovery from the three types of drains: kitchen sink drains (including garbage disposal), bathroom sink drains, and bathtub or shower drains. This was done using a mixed linear model with presence/absence of *P. aeruginosa* as the response

variable and type of drain as the predictor variable, and sampling as repeated measure nested with season of recovery (PROC GLIMMIX, SAS 9.3).

Results

Although species from the genus *Pseudomonas* were isolated from all 10 environment types sampled (Figure 8, Appendix I), recovery rates varied greatly between *P. aeruginosa* and other *Pseudomonas* species environment types (Figure 8). The overall recovery rates of *P. aeruginosa* and other *Pseudomonas* were significantly different ($p<0.0001$). Soils had the highest proportion (78.0%) of sites with *Pseudomonas sp.* growth, followed by drains (38.5%). *Pseudomonas sp.* recovery was also high in a number of other environment types, such as water sites (33.6%), trash/compost sites (29.6%), miscellaneous moist sites (26.4%), surfaces (21.4%) and human upper respiratory sites (9.5%).

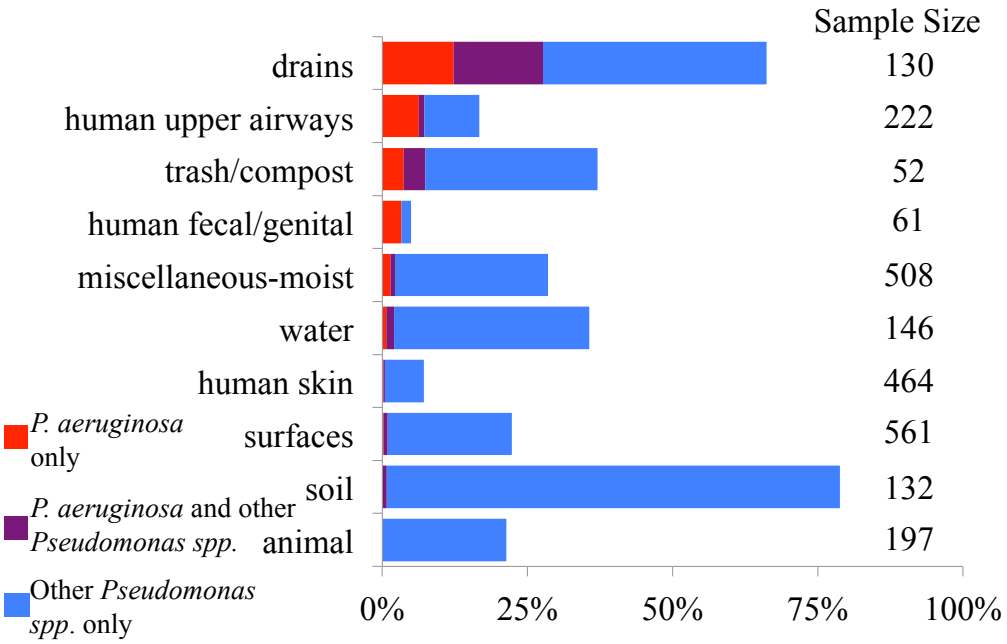

Figure 8. *Pseudomonas* recovery by environment type. Percentage of 2,473 sites from 15 homes ever yielding *Pseudomonas aeruginosa* (red), yielding both *Pseduomonas aeruginosa* and other *Pseuodmonas* species (purple) and yielding only non-*aeruginosa Pseudomonas* (blue). Overall recovery (combination of all environment types) was signigicantly different

between *P. aeruginosa* and other *Pseudomonas* species ($p<0.0001$). Results of tests of differences within each environment are shown (not corrected for multiple comparisons): ***$p<0.0001$; **$p<.001$; ns not significant; ^ unable to be tested because no *P. aeruginosa*.

Drains were the environment type that had the greatest of recovery of *P. aeruginosa* in the home. In fact, over one-half of all *P. aeruginosa* isolates recovered during the study were from drains (51.5%). When host-associated sites of CF patients were excluded, drains represented 67.3% of the *P. aeruginosa* collected. Of the drains sampled, 28.6% harbored *P. aeruginosa* at least once over the course of the study.

As expected, recovery from upper respiratory sites differed between CF patients and non-CF patients; 46.2% of upper respiratory sites of CF patients yielded *P. aeruginosa* over the course of the study, while only 1.55% of upper respiratory sites of non-CF patients yielded *P. aeruginosa* over the course of the study. Interestingly, recovery of non-*aeruginosa Pseudomonas* from upper respiratory sites over the course of the study was approximately equal for CF patients (10.7% of sites over the course of the study) compared to non-CF patients (10.3% of sites over the course of the study), and was substantially higher than *P. aeruginosa* recovery from non-CF patients. Furthermore, *P. aeruginosa* recovery was extremely low for all other types of environments. Extensive sampling of household surfaces (2,424 samples taken) yielded only 5 *P. aeruginosa* isolates from surfaces: a kitchen countertop, a spill on a kitchen counter, 2 bathroom counters, and one from a patient's inhaler (all 5 from different homes). This inhaler was the only sample from patient equipment (PEP masks, compression vests, nebulizers, etc.) to yield *P. aeruginosa*. Even with extensive sampling, only 1 *P. aeruginosa* was recovered from soils (626 samples taken), and no *P. aeruginosa* was recovered from pets (864 samples taken) using our collection method. A full list of all *P. aeruginosa* recovery can be found in Appendix II.

I then examined seasonal variability in recovery of *P. aeruginosa*. Overall, I found that recovery differed significantly across seasons ($p= 0.0077$; Figure 9A). The probability of recovering *P. aeruginosa* was higher in the fall than any of the other seasons. I then examined seasonal variability within sites inside the home, humans (including CF patients), and bathroom and kitchen drains. Season significantly influenced recovery for sites inside the home ($p= 0.0405$). I also found that, while not statistically different, the probability of recovering *P. aeruginosa* in the fall was higher than the other seasons. Recovery from humans and bathroom and kitchen drain environments were not significantly influenced by season (Figure 9C and 9D respectively). Consistent with trends for overall recovery and indoor sites, there appears to be a slight, but not statistically significant, increased probability of recovery of *P. aeruginosa* during the fall for both humans and drains. I did not examine recovery for sites outside of the home for seasonal trends due to low sample size, only 5 *P. aeruginosa* were from outside of the home. Patient status of the home, whether a CF patient resides there or not, did not significantly influence recovery ($p=0.55$).

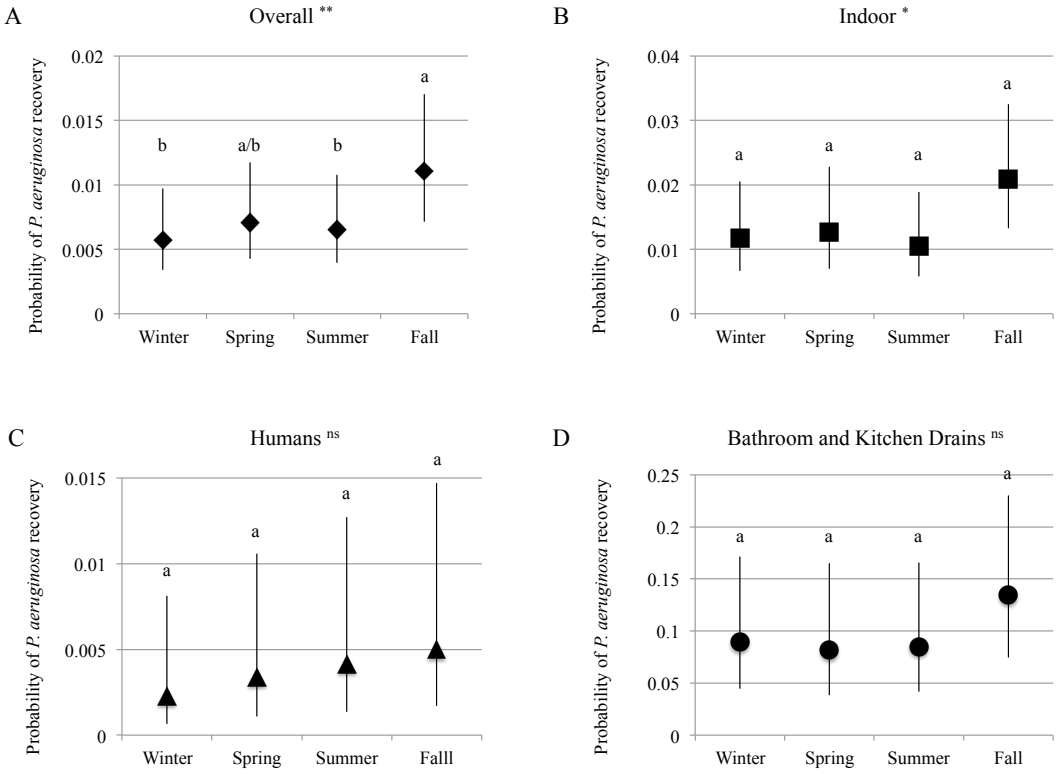

Figure 9. Probability of *P. aeruginosa* recovery by season as calculated from mixed general linear model with *P.aeruginosa* recovery as the response variable, season and house type as predictor variables, and sampling as a repeated measure nested within season of recovery: A) overall B) indoor sites C) humans D) bathroom and kitchen drains. 95% confidence intervals of the probability are shown. The letters over data-points indicate the results of differences of least square means comparisons of seasons. Each letter that appears multiple times designates pairs of seasons did not differ significantly; seasons that differed marginally significantly ($p<0.10$) do not share letters. Contrasts in panels A and B were adjusted for multiple comparisons using a Bonferroni correction. Overall significance designated by: **$p<0.01$; *$p<0.05$.

I then examined patterns of recovery from drains in greater detail. I compared recovery from bathroom and kitchen drains of homes with a *P. aeruginosa* culture-positive CF patient (n=5; see Table 1) to homes without a CF patient (n=8). I found that the probability of recovery from drains of homes without a CF patient was higher than from a home with a culture positive CF patient (0.1146 and 0.07 respectively), but not significantly so (Figure 10A). I compared recovery of kitchen sink drains (including

garbage disposals), bathroom sink drains, and bathtub/shower drains, combining data from all houses in light of the absence of a significant difference in drain recovery between houses with and without a CF patient. I found that the probability of *P. aeruginosa* recovery differed significantly overall, and was driven by higher recovery from bathroom sink drains (Figure 10B). The difference between bathroom sinks and kitchen sinks and the difference between bathroom sinks and bathtubs or showers are only marginally significant after adjusting for multiple comparisons (Figure 10B).

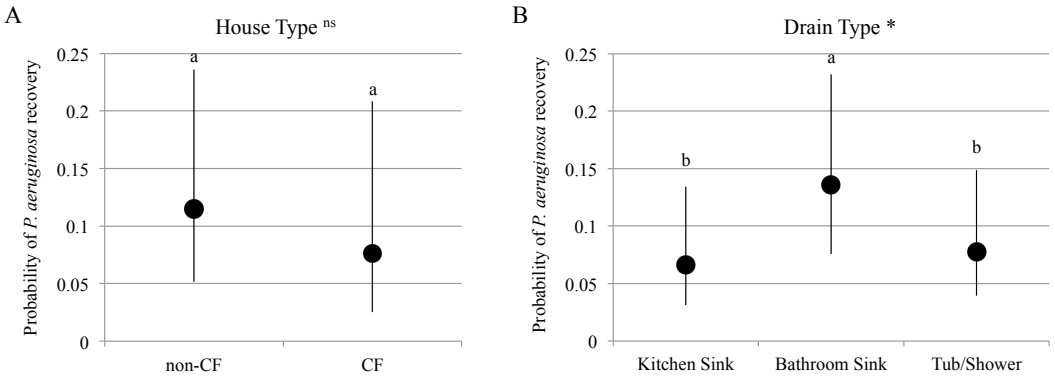

Figure 10. Probability of *P. aeruginosa* recovery in bathroom and kitchen drains as calculated from mixed general linear model with *P.aeruginosa* recovery as the response variable, drain type and house type as predictor variables, and sampling as a repeated measure nested within season of recovery by: A) house type (CF houses with a *P. aeruginosa* culture-positive patient (n=5) compared to non-CF houses) B) drain types (bathroom sink, kitchen sink, and tub/shower). 95% confidence intervals of the probability are shown. The letters over data-points indicate the results of differences of least square means comparisons. Each letter that appears multiple times designates pairs of seasons did not differ significantly; seasons that differed marginally significantly ($p<0.10$) do not share letters. Contrasts were adjusted for multiple comparisons using a Bonferroni correction. *$p<0.05$

Discussion

Species from the genus *Pseudomonas* were collected from every home sampled, and from locations and environments both inside and outside of the homes. My data suggest that, as a genus, *Pseudomonas* is very versatile. This is consistent with other studies (Stainer et al. 1966; Nelson et al. 2002; Özen et al. 2011). In contrast, although *P.*

aeruginosa was recovered from every home sampled (regardless of patient status), the patterns of recovery from these homes were not consistent with this species being ubiquitous in nature with respect to environment type. Rather, *P. aeruginosa* was primarily found in drains, consistent with Ojima, et al. 2002, Regnath et al. 2004, and Remold et al. 2011.

Potential sources of *P. aeruginosa* to which exposure could be controlled are of particular interest. While I recovered some *Pseudomonas* species from household pets (primarily cats and dogs were sampled), I did not recover any *P. aeruginosa*. This suggests that pets do not commonly act as a reservoir for *P. aeruginosa*, and that their presence is likely not a primary source of risk to CF patients regarding acquisition of *P. aeruginosa*. Similarly, I only recovered a single *P. aeruginosa* isolate from all 626 yard, garden, or houseplant soil samples taken; this isolate was from an indoor houseplant. *P. aeruginosa* is often reported as a soil organism, and other studies have isolated *P. aeruginosa* from the soil (Green et al. 1974; Mukherjee et al. 2011). These studies collected much larger soil samples and suspended soil samples in water before inoculating media, so it is possible that differences in sampling methods contributed to the differences in observed recovery. The low recovery of *P. aeruginosa* from soils in this study is consistent with the results reported by Remold and colleagues (2011), which sampled more houses than this study, but used a similar technique. Failure to detect *P. aeruginosa* in these studies is not due to a failure to detect *Pseudomonas* (Figure 8); however, it is possible different *Pseudomonas* species could use different components of soil and may then vary in their ability to be recovered. Food sources, particularly fresh produce, have been cited as potential sources for *P. aeruginosa*. Studies have isolated *P.*

aeruginosa at varying levels on supermarket fruits and vegetables (Schwaiger et al. 2011; Allydice-Francis and Brown 2012). When present, I sampled vegetables grown in the ground (potatoes, carrots, etc) and sampled the vegetable drawer of the refrigerator but I recovered no *P. aeruginosa* from these sites. However, my sample sizes of 67 vegetables and 92 vegetable drawers (totals reflect multiple sampling events) are small compared to other studies.

Recovery from neither humans nor drains differed significantly across seasons, but the trends are consistent with pattern of recovery across all sites, in that the probability of *P. aeruginosa* is higher in the fall for both. The increased recovery in fall could be due to the change in weather, or due to the change in behavioral patterns, such as going back to school or increased movement in and out of the home, that are associated with the fall. The average high temperature for fall in the Louisville area is 69.8°F, the average low temperature for the fall in the Louisville area is 49.6°F, and the average precipitation in the fall for the Louisville area is 9.86" (Climatology – Louisville).

Johansen and colleagues' (1992) examination of seasonal variability of CF patients' initial acquisition of *P. aeruginosa* found that there is an increase in the winter months. This study was conducted in Denmark and defined winter as October through March. Our study demonstrates that there is seasonal variability in recovery from the home environment and that patients could possibly have an increased risk of *P. aeruginosa* exposure from the home during certain times of the year (in this case fall). However, the increase in recovery for fall demonstrated in this study is indicative only of the Ohio River Valley and may not be able to be extrapolated to other climates and geographies.

If drains in homes with a culture-positive CF patient had higher recovery rates of *P. aeruginosa* a possible explanation of this would be that culture-positive CF patients were disseminating *P. aeruginosa* to the drains in their homes. The probability of *P. aeruginosa* was not significantly different for drains in homes without a CF patient, indicating that *P. aeruginosa* is not being distributed to drains from *P. aeruginosa* positive patients at a rate significantly higher than the dispersal rates *P. aeruginosa* in homes without a patient. This result is consistent with those of Panagea and colleagues (2005), who report colonized patients disseminating *P. aeruginosa* to their surroundings at detectable levels and indicate this dissemination is only to areas in the patients' direct vicinity (i.e., clothing) and contamination appeared to be transient. I also looked at differences in types of drains: kitchen sink, bathroom sink, and bathtub or shower drains. There is some evidence to suggest that recovery of *P. aeruginosa* is higher for some types of drains (bathroom sinks), and the higher recovery from bathroom sink drains is consistent with other comparisons of *P. aeruginosa* in household drains (Remold et al. 2011). However, most important is that *P. aeruginosa* recovery is highest for household drains. While the outcome of the statistical analysis for drain type is different here than in Chapter 2, the trends are consistent. This difference is likely due to analysis at the level of species group (*P. aeruginosa* group) in Chapter 2 compared to analysis of just *P. aeruginosa* proper here, and slight differences in the model. While the exact source of the *P. aeruginosa* in household drains is unknown, it is possible that tap water, a regular input to both bathroom and kitchen drains could be a potential source. *P. aeruginosa* has been recovered from tap water in both home and hospital environments (Anaissie et al.

2002; von Baum et al. 2010). One study of household tap water in Germany found *P. aeruginosa* in the tap water of nearly 11% households sampled (von Baum et al. 2010).

Biofilms, found both in the drain environment and the lung environment, may help explain the increased recovery of *P. aeruginosa* from these sites. Biofilms are an aggregate of microbial organisms, usually consisting of multiple species, contained within a sticky, extracellular matrix of polysaccharides, proteins, and DNA, facilitating adherence to surfaces (Høiby et al 2010). *P. aeruginosa* has a number of characteristics that cause them to be successful in biofilm environments. First, they can be facultative anaerobes allowing them to continue to grow and reproduce in anaerobic regions of biofilms which other organisms may not be able to survive (Wagner and Iglewski). Second, *P. aeruginosa* species in biofilms are capable of adhering to surfaces (i.e., piping) and this could give them an advantage in biofilms that are subject to shear force, as in drains. Third, in biofilms *P. aeruginosa* species are in close contact with neighboring organisms, and could be at an advantage due to their diverse metabolic capabilities. Last, biofilm formation has been shown to greatly increase antibiotic resistance of *P. aeruginosa*, increasing its ability to withstand the disturbance event of antibiotic exposure (Wagner and Iglewski 2008).

My data show that within the human home environment *P. aeruginosa* is primarily found in bathroom and kitchen drains and that *P. aeruginosa* is primarily a drain specialist regardless of patient status of the home. It is known that *P. aeruginosa* isolated from initial infections of CF patients are more genetically similar to isolates collected from the environment than those isolates collected from chronically infected patients (Burns et al. 2001; Jelsbak et al. 2007; Rau et al. 2010; Workentine and Surette

2011). It is possible the environmental source(s) of initial infections are drains from patients' homes; this is consistent with the findings of Schelstraete, et al. 2008, where nearly 20% of isolates from newly infected CF patients where identical to isolates from patients' homes.

One should be cautious about interpreting presence/absence data for particular sites or environments because detection of *P. aeruginosa* does not address the relative ability of strains to establish infection. Nevertheless, my results suggest that identifying and implementing effective cleaning strategies for drains in patients' homes could be effective in minimizing exposure to *P. aeruginosa*. Identifying the most effective cleaning strategies is key, as household drains are likely to contain difficult to eradicate biofilm communities. Future research focusing on correlations between cleaning regimes, cleaning agent, drain materials and *P. aeruginosa* recovery and infection rates would be a beneficial addition to the body of knowledge about *P. aeruginosa*.

CHAPTER 4

RECOVERY OF *BURKHOLDERIA* SPECIES FROM THE HUMAN HOME ENVIRONMENT

Summary

Burkholderia cepacia complex species can cause life-threatening illness in cystic fibrosis patients. While clinical efforts to minimize transmission (particularly patient-to-patient) of *B. cepacia* complex species have been very successful, new cases still arise with unknown sources. I investigated the human home as a potential source of *Burkholderia sp.* by looking at the recovery rate of various types of environments. Overall, the recovery rate of *Burkholderia sp.* was very low (0.22%) with only 14 isolates being recovered out of 6,495 samples taken. These 14 isolates were recovered primarily from soil and soil-like environments (62.3%) and drains (28.6%). Isolates evenly clustered within two phylogenetic clades: a plant-associated beneficial environmental group described by Suárez-Moreno et al. (2012) and *B. cepacia* complex. Because of the culture-based nature of the study, the data represent a conservative estimate of *Burkholderia* in the household environment; however, even with low recovery, sites within the house cannot be eliminated as potential sources of new infection.

Introduction

Burkholderia cepacia complex members are considered opportunistic pathogens and are of particular interest to the cystic fibrosis (CF) community. *B. cepacia* complex infections in the CF lungs of some individuals can lead to cepacia syndrome, a rapid decline in lung function, overall prognosis, and ultimately death (Mahenthiralingam et al. 2008). *B. cepacia* complex has been shown to be transmissible from person-to-person, and efforts to minimize this transmission have proven greatly effective (LiPuma 1990, Baldwin et al. 2008). However, these measures have not completely eliminated the occurrence of new infections among patients who have not come into contact with an infected patient. Therefore, it is possible that the source of some patients' infections are non-clinical or environmental (LiPuma 2010).

Other *Burkholderia* species, including some of those in the *B. cepacia* complex, have been found in close association with plant roots and have been found to promote plant growth. Some strains have been reported to be capable of N_2-fixation, making them of particular interest to the agriculture community (Parke and Gurian-Sherman 2001). Much work has been done on the use of *Burkholderia* species for purposes of bioremediation, due to their diverse metabolic capabilities (reviewed in O'Sullivan and Mahenthiralingam 2005). The use of *Burkholderia* species, specifically *B. cepacia,* for biocontrol and bioremediation purposes has been concerning for the CF community because the introduction of these organisms to places where vulnerable people might come into contact with them could result in infections (Chiarini et al. 2006).

It was once believed that the beneficial environmental and clinical strains could be differentiated from each other; however, LiPuma and colleagues (2002) identified an

epidemic clinical isolate of *B. cepacia* complex in soil samples. Additionally, another study (Baldwin et al. 2007) found that over 20% of tested clinical isolates could not be distinguished from isolates from the environment. This points to the environment as a potentially important reservoir for infectious strains. A study by Fisher and colleagues (1993) examined a small number of sites in patient homes and control homes (refrigerator bins, refrigerator drain pans, sink drains, and soil samples), salad bars, and food stores for *B. cepacia.* Their recovery rate for *B. cepacia* in homes was approximately 1% with isolates being recovered from a refrigerator bin, two refrigerator drain pans, a bathroom sink drain and a kitchen sink drain. Recovery from food stores and salad bars was approximately 4.5%. We now know that in addition to *B. cepacia* many additional members of the *B. cepacia* complex can cause infections in CF patients. In this study we aim to examine many more sites within each home (75-168 per home) as well as sample each home repeatedly (3-8 times each) for all members of the genus *Burkholderia*.

Materials and Methods

Sample collection

Samples were collected from 15 households in the Louisville, KY (USA) metropolitan area. Each household was sampled between October 2007 and March 2012 at intervals of approximately three months. Houses were each sampled between 3 and 8 times, with the average household being sampled 6.5 times. Within each household, we collected between 53 and 85 samples (depending on the number of bathrooms, toys etc.) from 97 types of sites in and around the home (Appendix I). A total of 6,495 samples were taken. Subjects were instructed not to clean the home the week before the sampling

date. To minimize the risk of cross-contamination of households, no two households were sampled on the same day. Federal and institutional guidelines and policies regarding use of human and animal subjects were followed, including signed informed consent forms (and assent forms, where applicable).

Samples were collected with sterile swabs pre-moistened with phosphate-buffered saline. All drains were swabbed within the first 1-2 inches from the top of the opening. Surfaces were sampled at locations most likely to have frequent contact with human skin (i.e., knobs, buttons, etc.). Soils were sampled by inserting swab 1-2 inches from the surface, collecting soil on swab. Swabs were streaked onto *Pseudomonas* isolation agar (PIA) at the homes. While selective media has been developed specifically for *Burkholderia*, PIA does not inhibit the growth of *Burkholderia*, and is used for growth of *Burkholderia* species in other studies (Hardy Diagnostics: PIA 1996; Sokol et al. 1999; Lagatolla et al. 2002; Ferreira et al. 2007). Plates were transported on ice and incubated for 48 hours at 28ºC immediately upon return to the laboratory. Where growth occurred, a single colony was picked, restreaked onto PIA, and frozen for further analysis; where multiple colony morphologies were found for a single sample one of each were frozen for further analysis.

Sample Identification

763 isolates not previously identified (as described in Chapters 2 and 3) were extracted using a technique described by Spilker and colleagues (2009). Successful DNA extracts were confirmed using 16s rDNA universal bacterial primers 8f and 1492 (Weisburg et al. 1991) and amplified with the *RecA* primers designed as part of a multi-locus sequencing type (MLST) set for *Burkholeria* species (Spilker et al. 2009). Of the

814 isolates 5 were unable to be examined further. Because at least three extraction and amplifications with the universal bacterial primers had failed, these were concluded to be non-bacterial. Potential *Burkholderia* isolates (samples amplified by *Burkholderia RecA* primers) were identified using >500bp sequences of the isolates 16s rDNA (1401r primer for sequencing) (Weisburg et al. 1991), as well *RecA* as using sequences (Spilker et al. 2009). I assigned each isolate to the level of species based on 16s sequences, using three BLAST databases (EzTaxon, Bioinfo 1200 nucleotide, Bioinfo 2 sequences) (Chun et al. 2007; Croce et al. 2010). Where the databases disagreed a species assignment was made using the designation from the Bioinfo 1200 nucleotide database (Croce et al. 2010). The *Burkholderia RecA* primers were designed as part of an MLST set, and the amplicon for these primers was 704 base pairs (Spilker et al. 2009). Due to this, *RecA* sequences were used to confirm the assignment to the genus *Burkholderia*.

Data Analysis

16s rDNA sequences of the 14 collected isolates plus 7 *Burkholderia* type strains were used to construct a maximum likelihood tree. In order to contrast the tree, sequences were aligned using MUSCLE (v3.7; Edgar 2004). Ambiguous regions of sequences after alignment were removed using Gblocks (v0.91b; Castresana 2000). The maximum likelihood phylogenetic tree was constructed and bootstrap values calculated (100 replicates) in the PhyML program (v3.0; Anisiova and Gascuel 2006; Dereeper et al. 2008; Guindon et al. 2010). The tree was then graphically represented using TreeDyn (v198.3; Chevenet et al. 2006).

Results

Burkholderia species were very rarely found in the human home, with only 14 isolates being recovered (Table 3). The overall recovery was 0.22%. The *Burkholderia* isolates recovered came almost exclusively from two types of environments: soil samples or soil-like environments (9/14) and drain samples (4/14). On two occasions *Burkholderia* isolates were recovered from the same physical site at different sampling events (Table 3), and in neither case were they consecutive sampling events. Interestingly, for both sites it was the same species that was recovered twice. Both of these cases indicate that the reported recovery rate should be considered a lower limit, as there is a potential for a high false negative rate.

Table 3. Recovery of *Burkholderia sp.* from 15 households. ^ indicates recovered from the same floor drain at different sampling events; * indicates recovered from the same houseplant at different sampling events.

Site	16S Identification	House	Sampling	Sample ID#
Floor Drain^	*B. cepacia*	1	3	1281
Floor Drain^	*B. cepacia*	1	5	1625
Sandbox	*B. cepacia*	14	5	2129
Stovetop	*B. cepacia*	4	8	2518
Floor Drain	*B. fungorum*	10	5	3176
Houseplant	*B. mimosarum*	4	7	2350
Houseplant	*B. multivorans*	1	5	1627
Houseplant	*B. multivorans*	7	3	1547
Houseplant	*B. tropica*	5	4	3080
Houseplant*	*B. tuberum*	4	5	1926
Houseplant*	*B. tuberum*	4	7	2349
Garbage Disposal	*B. vietnamiensis*	10	2	2889
Yard Soil	*B. vietnamiensis*	15	7	2610
Houseplant	*B. xenovorans*	4	7	2355

I was interested in examining the distribution of the isolates collected across the 15 houses sampled. Of the 15 houses sampled, 7 harbored *Burkholderia*. Recovery from

each home varied from 0 to 5 isolates per house. Since most of the *Burkholderia* recovered was isolated from soil environments, it is possible that the variability in recovery from house to house could be explained by variability in the number of houseplants in each house. The number of houseplants sampled (pooled across all samplings per house) varied from 0 to 131 per house. Over one-third of the *Burkholderia* isolates came from the house that had the greatest number of houseplants (house 4). A total of 275 strains were collected from houseplants with 7 of those isolates identified as *Burkholderia* species (2.5%).

A maximum likelihood tree was constructed from 16s rDNA sequences to examine relatedness of samples collected from households to recognized *Burkholderia* type strains (Figure 11). The type strains clustered into two distinct groups previously described by Suárez-Moreno and collaborators (2012). Suárez-Moreno et al. (2012) characterize these clades as comprising plant-associated beneficial and environmental group (PBE) species, and the *Burkholderia cepacia* complex (BCC group) species. Of the isolates collected from household environments, one half (7/14) cluster within the PBE group, while the other half (7/14) cluster within the BCC group.

I did not see strong habitat of isolation differences when examining in which clade an isolate. Isolates collected from drain sites mainly cluster within the BCC group (3/4 of drain isolates), while soil and soil-like isolates are evenly split between the two groups (PBE: 5/9; BCC group: 4/9). Of the isolates collected from houseplants specifically most (5/7) cluster in the PBE clade; however, most of those (4/5) were isolated from a single house (house 4). Interestingly, there is clade-specificity in the location of isolation at the level of house. All 5 *Burkholderia* isolates from house 4

cluster within the PBE clade of the tree. All 3 *Burkholderia* isolates from house 1 cluster within the BCC clade of the tree, including the isolate collected from a houseplant.

When I compare the species assignments of our isolates to the location of those species in the clades of the Suárez-Moreno et al. (2012) tree, I see concordance, except in one case. The isolate collected from the stovetop of house 4 (ID# 2518) was classified as a *Burkholderia cepacia*; however, the 16s rDNA tree constructed (Figure 11) shows that this isolate does not cluster with the rest of the *B. cepacia* complex, but rather clusters within the PBE. The three databases used for species assignment to species disagreed regarding this isolate. The Bioinfo 1200 nucleotide database was used for identification (Croce et al. 2010) and identified this isolate as *B. cepacia.* Interestingly, the other two databases used classified this isolate as *B. phenoliruptrix*, which is more consistent with the tree constructed from sequence data.

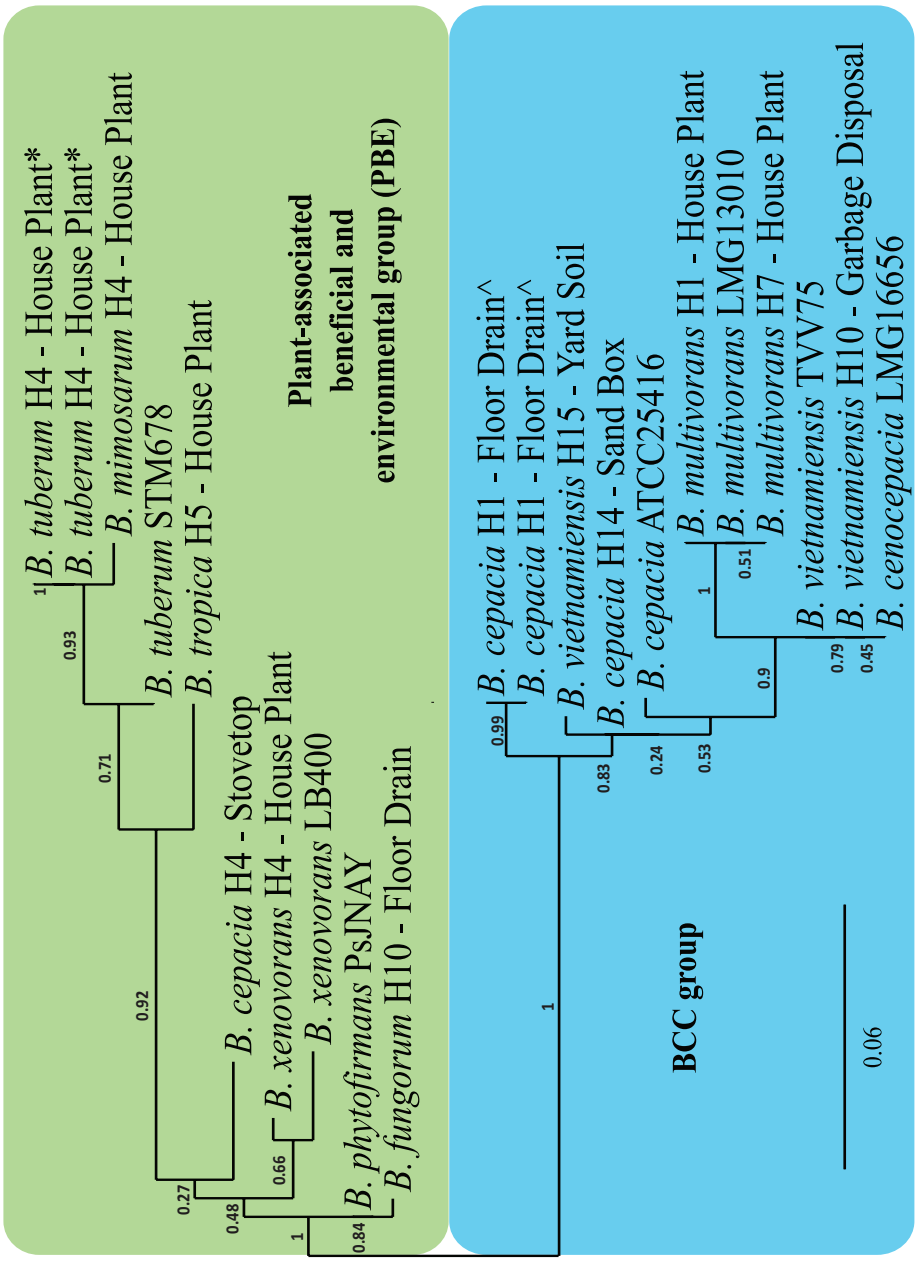

Figure 11. Maximum likelihood tree of isolates and selected *Burkholderia* type strains. House number are represented by "H#" and are followed by the location of isolation. The * indicates isolates recovered from the same houseplant at different sampling events; ^ indicates isolates recovered from the same floor drain at different sampling events. The branch lengths (bar=0.06) are proportional to the number of nucleotide substitutions per site (Dereeper et al 2008). Bootstrap values are giving for branch nodes. Green shading – plant-associated beneficial and environmental group (PBE); blue shading – *Burkholderia cepacia* complex group (Suárez-Moreno et al. 2012).

Discussion

Overall I found very little *Burkholderia* in the human home environment, a finding consistent with Fisher and colleagues' (1993) examination of home environments. Consistent with this study Fisher and colleagues recovered *Burkholderia cepacia* from household drains (refrigerator, bathroom, and kitchen), but interestingly only recovered one isolate from the soil. The majority of their isolates were collected from food sources, and ground vegetables were the only food items sampled in our study (Appendix I). Even though recovery was low, caution should be taken in concluding that lack of isolation indicates absence; additionally, for sites where isolation of *Burkholderia* did occur it could be more frequent than our recovery indicate. Culture-based techniques, including this study provide an indication of the organism(s) present, but can miss organisms not present at high frequencies or not culturable. Of the *Burkholderia* isolates recovered approximately one-half fall into the *B. cepacia* complex clade that is typically associated with infections in CF patients.

Nosocomial outbreaks of *Burkholderia cepacia* complex have been linked to mouthwash (Kutty et al. 2007), washcloths (Martin et al. 2011), multi-use solutions (De Smet et al. 2012), and other contaminated products including water (Mann et al. 2010). A 2010 study (Lucero et al.) implicated hospital sinks and drains as the source of an outbreak of *Burkholderia cepacia* complex. This study demonstrated recovery of members of the *B. cepacia* complex from household drains. Without further study it is impossible to say if the household drain strains described here share phenotypic characteristics with clinical isolates or are as virulent as those recovered from hospital drains, but we have shown that they are present in some household drains, in absence of

an infected patient. This indicates that respiratory secretions of infected patients were not the source of these isolates.

I have demonstrated that *Burkholderia* species, including members of the plant-associated beneficial and environmental group (PBE) as described by Suárez-Moreno et al. (2012), as well as the *B. cepacia* complex are recoverable from environments at a scale relevant to patient contact. Soil and soil-like samples are the source of approximately two-thirds of isolates collected in this study, and are split evenly between the two clades of the tree (Figure 11). While recovery rates of *Burkholderia* are very low, we cannot eliminate the possibility that sites in the home (particularly soils and drains) could be an environmental source of infection. In particular, a reevaluation of the risk associated with patient-soil contact could be beneficial.

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APPENDIX I

SITES SAMPLED IN THE HUMAN HOME

* Were eliminated from analyses in Chapter 2.

^ For analyses in Chapter 3 CF Equipment sites were included in surfaces.

[t] Were eliminated from analyses in Chapter 4

Upper Respiratory*[t]	Human Skin[t]	Animal[t]	CF Equipment*^[t]
human mouth	armpits	skin	compression vest
human nose	between toes	eyes	inhaler
human throat	eyes	foot pads	nebulizer
human sputum	navel	outer ear canal	PEP mask
	human outer ear canal	wounds	suction device
	piercings	rectum	
	scalp		
	under fingernails		
	wounds		
	fecal		
	genital		

Drains	Soils	Trash
garbage disposal	house plant soil	kitchen compost
refrigerator drain	garden soil	kitchen trash can
kitchen sink drain	yard soil	pet litter box
bathroom sink drain	sand box	diaper disposal bin
bathtub/shower drain		outside compost bin
floor drain		outside trash can

Miscellaneous Moist	Surfaces	Water
dish drying rack	coffee maker	tap water
dish rag	kitchen counter	toilet
dish scrubber brush	kitchen counter spills	decorative fountain
dish sponge	ground vegetables	dehumidifier
ice/water dispenser	microwave	fish tank
moisture in undersink cabinet	vegetable drawer	flower vase
refrigerator spills	stove top	humidifier
water filter	bathroom counter	pet water bowl
bath toy	tubs cream/lotion	bird bath
loofah	computer keyboard	outdoor bucket
poof	contact lens storage	outdoor fountain
shower head	pet bedding	garden pond
bathroom soap dish	pet toy (non-chew)	pool/hot tub
spigot in tub	baby carrier	standing water
washcloth	baby swing	watering can
denture/retainer storage	car seat	
moist/mildewed areas	changing table	
pet chew toy	crib	
baby bathtub	electronic toy controls	
children's bathing suits	frequently worn clothes	
bottle drying rack	high chair tray	
bottle warmer	stroller	
bottles	toy boxes	

breast pump

food spoons

pacifier

spit-up clothes

teething rings

training toilet

garden hose

outdoor water toys

toys

wipes

A/C unit

outdoor grill

outdoor play area

APPENDIX II

PSEUDOMONAS AERUGINOSA ISOLATE LIST

Houses 1, 2, 6, 9, 12, 14, ad 15 had CF patients residing in them

House	Season	Specific Isolation Site	Environment Type	SRP#
1	Fall	Kitchen Counter	surfaces	1062
1	Fall	Human Throat	upper respiratory	1054
1	Fall	Bath Tub/shower drain	drains	1068
1	Spring	Human Mouth	upper respiratory	1266
1	Spring	Human Throat	upper respiratory	1267
1	Spring	Kitchen Sink drain	drains	1274
1	Spring	Bath Bath toy	miscellaneous-moist	1276
1	Summer	Human Mouth	upper respiratory	1426
1	Summer	Human Sputum	upper respiratory	1431
1	Summer	Human Nose	upper respiratory	1427
1	Summer	Human Throat	upper respiratory	1428
1	Summer	Kitchen Sink drain	drains	1435
1	Summer	Bath Bath toy	miscellaneous-moist	1439
1	Fall	Kitchen Trash container	trash/compost	1618
1	Fall	Inside-other House plants	soils	1632
1	Summer	Kitchen Garbage disposal	drains	1952
2	Fall	Outside Trash cans	trash/compost	2004
3	Fall	Bath Bath toy	miscellaneous-moist	2048
3	Winter	Bath Sink drain	drains	2230
3	Winter	Kitchen Sink drain	drains	2684
3	Winter	Bath Bath toy	miscellaneous-moist	2704
3	Fall	Children's areas Food spoons	miscellaneous-moist	3091
4	Fall	Human Between toes	human skin	1004
4	Fall	Human Sputum	upper respiratory	1005
4	Fall	Bath Tub/shower drain	drains	1027
4	Fall	Bath Sink drain	drains	1031
4	Summer	Outside Garden hose	miscellaneous-moist	1421
4	Spring	Human Sputum	upper respiratory	1906
4	Spring	Human Throat	upper respiratory	1904
4	Spring	Outside Bird bath	water	1943
4	Spring	Outside Bird bath	water	2361
4	Summer	Bath Sink drain	drains	2521
5	Fall	Kitchen Counter spills	surfaces	2620

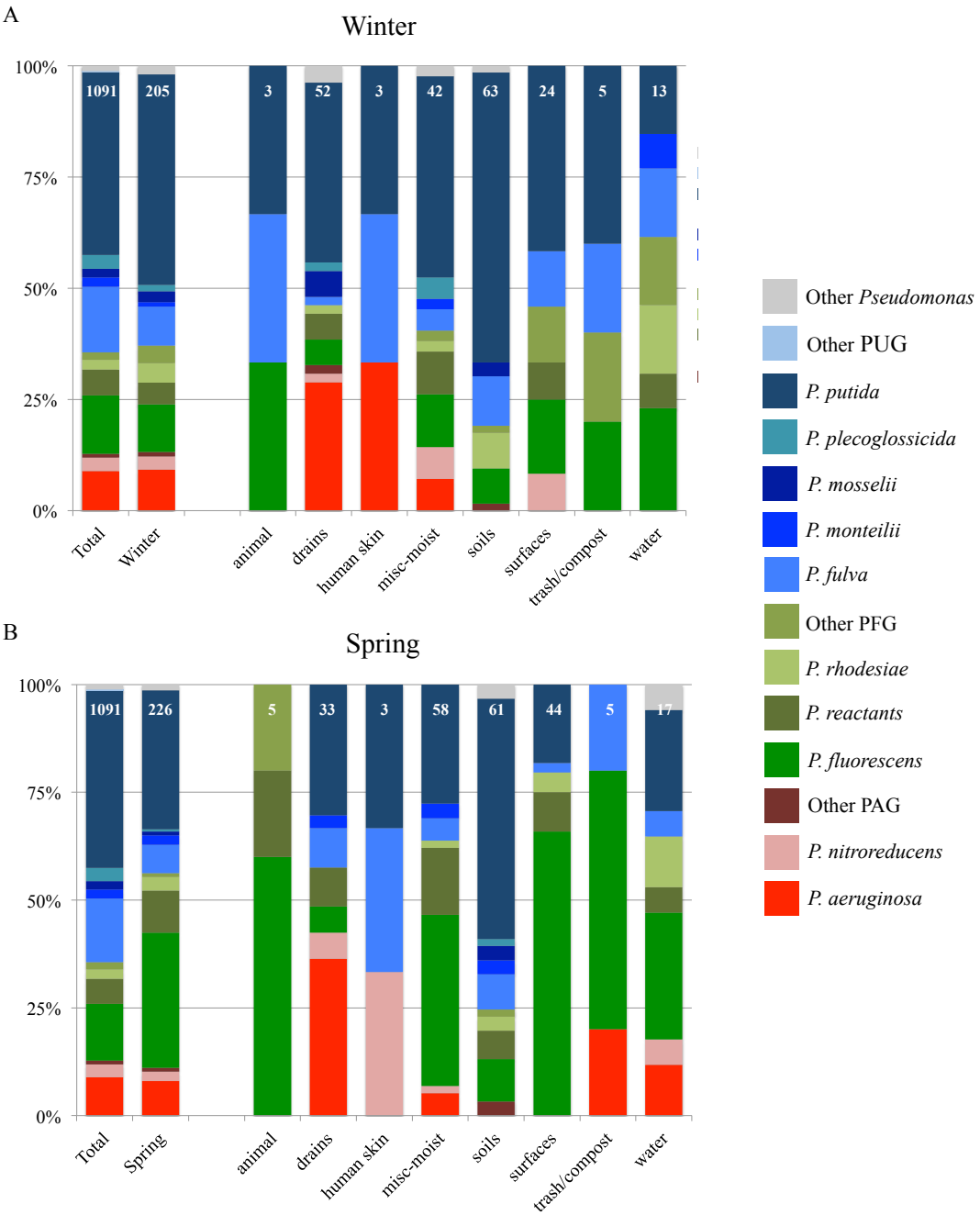
House	Season	Specific Isolation Site	Environment Type	SRP#
5	Fall	Bath Tub/shower drain	drains	2629
5	Fall	Bath Tub/shower drain	drains	2630
5	Fall	Bath Sink drain	drains	2633
5	Spring	Bath Sink drain	drains	2909
5	Summer	Bath Soapdish	miscellaneous-moist	2933
5	Fall	Bath Sink drain	drains	3061
5	Winter	Bath Sink drain	drains	3151
6	Fall	Human Mouth	upper respiratory	1565
6	Fall	Human Sputum	upper respiratory	1571
6	Fall	Human Throat	upper respiratory	1568
6	Fall	Bath Counter	surfaces	1582
6	Spring	Human Mouth	upper respiratory	1810
6	Spring	Human Sputum	upper respiratory	1814
6	Spring	Human Throat	upper respiratory	1811
6	Spring	Bath Moist or mildewed areas	miscellaneous-moist	1831
7	Winter	Bath Sink drain	drains	1162
7	Winter	Bath Sink drain	drains	1150
7	Summer	Bath Sink drain	drains	1320
7	Summer	Bath Sink drain	drains	1321
7	Fall	Bath Sink drain	drains	1535
7	Fall	Bath Sink drain	drains	1537
8	Fall	Kitchen Sink drain	drains	1607
8	Fall	Bath Sink drain	drains	1609
8	Winter	Bath Sink drain	drains	1772
8	Fall	Kitchen Trash container	trash/compost	2146
8	Fall	Bath Sink drain	drains	2151
8	Winter	Bath Sink drain	drains	2253
8	Summer	Bath Tub/shower drain	drains	2544
8	Summer	Bath Sink drain	drains	2545
8	Spring	Bath Sink drain	drains	2836
8	Fall	Bath Sink drain	drains	3132
8	Spring	Bath Sink drain	drains	3209
9	Spring	Human Mouth	upper respiratory	1790
9	Spring	Human Sputum	upper respiratory	1794
9	Spring	Human Throat	upper respiratory	1792
9	Fall	Human Mouth	upper respiratory	2087
9	Fall	CF Inhaler	surfaces	2115
9	Fall	Human Sputum	upper respiratory	2091
9	Fall	Human Throat	upper respiratory	2089
9	Fall	Human Under fingernails	human skin	2088
9	Spring	Human Sputum	upper respiratory	2306
9	Summer	Human Mouth	upper respiratory	2407

House	Season	Specific Isolation Site	Environment Type	SRP#
9	Summer	Human Throat	upper respiratory	2408
9	Summer	Bath Sink drain	drains	2415
9	Winter	Bath Shower head	miscellaneous-moist	2670
9	Winter	Bath Sink drain	drains	2674
9	Spring	Bath Sink drain	drains	2877
10	Fall	Bath Tub/shower drain	drains	2594
10	Fall	Bath Counter	surfaces	2597
10	Fall	Bath Sink drain	drains	2598
10	Fall	Bath Moist or mildewed areas	miscellaneous-moist	2599
10	Spring	Bath Tub/shower drain	drains	2892
10	Spring	Bath Sink drain	drains	2894
10	Summer	Bath Tub/shower drain	drains	2964
10	Summer	Bath Sink drain	drains	2967
10	Fall	Bath Tub/shower drain	drains	3100
10	Fall	Bath Sink drain	drains	3101
10	Winter	Bath Tub/shower drain	drains	3173
11	Fall	Bath Tub/shower drain	drains	2562
11	Fall	Bath Sink drain	drains	2563
11	Winter	Bath Tub/shower drain	drains	2696
12	Fall	Human Sputum	upper respiratory	3110
12	Fall	Bath Sink drain	drains	3122
12	Winter	Bath Sink drain	drains	3199
13	Winter	Human Nose	upper respiratory	1169
13	Winter	Kitchen Dishwash scrubber/brush	miscellaneous-moist	1172
13	Winter	Kitchen Garbage disposal	drains	1173
13	Winter	Kitchen Sink drain	drains	1175
13	Winter	Bath Tub/shower drain	drains	1177
13	Fall	Bath Tub/shower drain	drains	2578
14	Fall	Kitchen Dishwash scrubber/brush	miscellaneous-moist	1078
14	Fall	Kitchen Garbage disposal	drains	1082
14	Fall	Kitchen Sink drain	drains	1086
14	Fall	Bath Tub/shower drain	drains	1091
14	Fall	Outside Standing water	water	1105
14	Summer	Kitchen Garbage disposal	drains	1458
14	Winter	Human Fecal	human skin	1642
14	Spring	Kitchen Garbage disposal	drains	1858
14	Spring	Kitchen Trash container	trash/compost	2299
14	Spring	Children's areas Bottle drying rack	miscellaneous-moist	2304
14	Summer	Human Fecal	human skin	2499

House	Season	Specific Isolation Site	Environment Type	SRP#
14	Summer	Kitchen Garbage disposal	drains	2500
15	Spring	Kitchen Garbage disposal	drains	1256
15	Spring	Kitchen Sink drain	drains	1258
15	Spring	Bath Tub/shower drain	drains	1260
15	Summer	Human Sputum	upper respiratory	1348
15	Fall	Bath Tub/shower drain	drains	1505
15	Fall	Bath Tub/shower drain	drains	1507
15	Spring	Bath Sink drain	drains	1845
15	Summer	Human Sputum	upper respiratory	2433
15	Summer	Human Throat	upper respiratory	2431
15	Summer	Bath Tub/shower drain	drains	2438
15	Fall	Human Sputum	upper respiratory	2554
15	Fall	Human Throat	upper respiratory	2552
15	Fall	Bath Tub/shower drain	drains	2604
15	Fall	Bath Sink drain	drains	2605
15	Winter	Human Sputum	upper respiratory	2769
15	Winter	Human Throat	upper respiratory	2768
15	Winter	Bath Sink drain	drains	2785

Appendix III

Distribution of *Pseudomonas* Species by Season



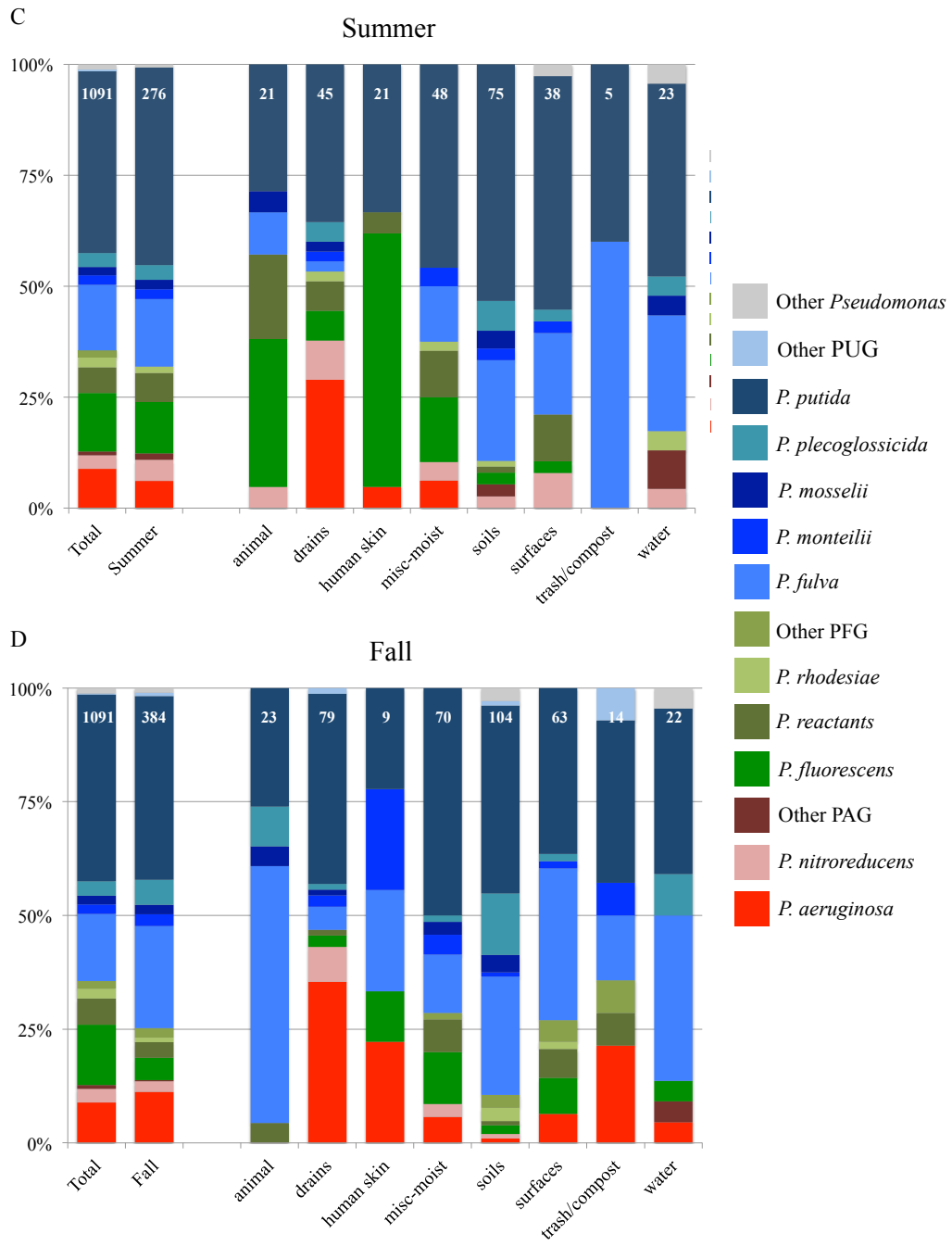


Figure 1. Recovery by species for all *Pseudomonas* by seasons A) winter B) spring C) summer D) fall. Coloring represents species (see legend) and group: reds - *P. aeruginosa* group, greens - *P. fluorescens* group; blues – *P. putida* group. Numebrs on the tops of bars indicate sample size (# *Pseudomonas* collected) for each category.

CURRICULUM VITAE

MEGAN E. PURDY

e-mail: mepurd01@gmail.com

2730 Hollywood Terrace Unit #19, Louisville, KY 40206 phone: (270) 993-3660

EDUCATION

Ph.D. (expected) **Biology** *Ecology, Evolution and Behavior concentration*, University of Louisville, Louisville, KY **2013**
GPA: 3.9

B.A. **Biology**, University of Louisville, Louisville, KY - *magna cum laude* **2008**

TEACHING EXPERIENCE

Head Teaching Assistant
BIOL 358 – Microbiology Laboratory for Biology Majors **Fall 2012**
BIOL 258 – Introduction Microbiology Laboratory for non-Biology Majors **Fall 2011 – Spring 2012**
Survey of microbes including: microscopy, differential media, antimicrobial agents, diversity of lifestyles, habitats used and relevance for human health and ecosystem functions
University of Louisville, Department of Biology

- Prepared media and supplies for up to 150 students.
- Planned and ran laboratory activities for twice weekly class meetings.
- Wrote and graded handouts, quizzes, and exams.
- Provided guidance to students on culminating unknown organism project and graded culminating papers.
- Assisted students one-on-one as needed.

Teaching Assistant **Summer 2009 – Fall 2009; Summer 2010**
BIOL 258 – Introduction Microbiology Laboratory for non-Biology Majors
Survey of microbes including: microscopy, differential media, antimicrobial agents, diversity of lifestyles, habitats used and relevance for human health and ecosystem functions.
University of Louisville, Department of Biology

- Ran laboratory activities for twice weekly class meetings for approximately 40 students
- Graded handouts, quizzes, and exams.
- Provided guidance to students on culminating unknown organism project and graded culminating papers.
- Assisted students one-on-one as needed.

Teaching Assistant **Spring 2010**
BIOL 104 – Laboratory for Introduction to Biological Systems
Survey of biology including an introduction to biological molecules, ecology, and genetics.
University of Louisville, Department of Biology

- Ran laboratory activities for weekly class meetings for approximately 130 students.
- Wrote and graded handouts, quizzes, and exams
- Graded weekly lab reports

- Assisted students one-on-one as needed.

ADDITIONAL TEACHING ACTIVITIES

Guest Lecturer **Spring 2012**
BIOL 671 – Research Design and Analysis
University of Louisville, Department of Biology
- Guest lectured for two weeks
- Topics of dataset formatting, visual display of data, and use of Proc Glimmix in SAS.

Guest Speaker **Fall 2009 – Spring 2012**
Microbiology Citizen Science Program
Seneca High School; Fairdale High School, Louisville, KY
- Provided a hands-on introduction to microbiology to high school students.
- Aided in high school student involvement in a NSF funded citizen science project.
- Worked with teachers to develop an assessment examining attitudes towards and understanding of microbiology.

Guest Speaker
Introduction to the World of Microbes **Spring 2012**
Born to Do Science, New Albany Public Library, New Albany IN
- Provided a hands on introduction to microbiology to children ages 7-10.

Guest Lecturer **Fall 2011**
BIOL 409 – Evolution
University of Louisville, Department of Biology
- Topics: phylogenetic tree building, parsimony.

Guest Lecturer **Fall 2008**
BIOL 391 – Special Topics: Microbes in Ecology and Evolution
University of Louisville, Department of Biology
- Topic: Hardy-Weinberg equilibrium.

Graduate Assistant **Aug. 2008 – May 2009**
REACH, Department of Undergraduate Affairs, University of Louisville
Louisville, KY
* Worked as graduate student manager of university tutoring center.
* Used TutorTrac software to managing scheduling of tutors and tutees.
* Trained and was responsible for scheduling of federal work study student workers.
* Aided in processing of statistical data for REACH.
* Participated in CRLA certified tutor training program.
* Tutored students in the following courses:
    - BIOL 102 – Introductory Biology for Non-Majors
    - BIOL 240 – Unity of Life
    - BIOL 357 – Evolutionary Ecology of
    - BIOL 358 – Disease Microbiology for Majors
    - MATH 109 – Introductory Statistics

PROFESSIONAL EXPERIENCE

Research Assistant **Aug. 2010 – May 2011; Aug. 2012 – Aug. 2013**
University of Louisville, Department of Biology
Louisville, KY
*Coordinated environmental sampling of volunteer households as part of investigation of pathogens affecting children with cystic fibrosis.
* Mentored and trained undergraduate students in microbiological and molecular processing techniques.

* Supervised lab members in microbiological processing of more than 3,000 bacterial samples collected for the study, which included collecting and tagging samples in the field, processing samples in the lab according to established protocols, and documenting and tracking isolates.
* Managed study data set of genotypic and phenotypic information for ≥11,000 human, pet, and environmental samples collected from up to 150 different niches/sites in 15 homes, and oversaw all data entry into data set.
* Employed molecular and microbiological techniques addressing questions about biogeography of *Pseudomonas* and *Burkholderia* species in the human home.
* Built statistical models to explain relationships between recovery rates and ecological and geographical information.
* Utilized Microsoft Excel, SAS and Adobe Illustrator to conduct analyses and creates, charts, graphs and other figures.
* Maintained data sets for various projects, including HIPAA sensitive information.
* Developed and presented presentations from data and analyses and prepared manuscripts for submission to peer-reviewed journals.

RELEVANT COURSEWORK

Advanced Biostatistics
Chronic Disease Biology†
Ecosystem Ecology
Evolution
Evolution of Disease Seminar
Evolutionary Medicine†
Gene Structure and Function
Human Virus and Disease†
Immunobiology
Microbial Community Assembly*
Microbes in Ecology and Evolution†
Population and Community Ecology
Research Design and Analysis

†*Course taken as an undergraduate student.*
**Course taken as independent study.*

PRESENTATIONS

Purdy ME, France M, Hundley T, Eid N, Remold S. Proceedings from the 2011 North American Cystic Fibrosis Conference: *Identification of sites harboring Pseudomonas aeruginosa in CF vs. non-CF homes.* Anaheim, CA

Purdy ME, France M, Hundley T, Eid N, Remold S. Proceedings from the 2011 Gordon Research Conference: Microbial Population Biology: *Biogeography of Pseudomonas species at multiple spatial and temporal scales.* Andover, NH.

Purdy ME, France M, Hundley T, Eid N, Remold S. Proceedings from the 2011 University of Louisville Graduate Research Symposium: *Pseudomonas biogeography in the human home.* Louisville, KY

Purdy ME, Perpich J, Hundley T, Brown CK, Farris J, Carrier A, Remold S. Proceedings from the 2009 North American Cystic Fibrosis Conference: *Where to Pseudomonas genotypes that successfully colonize the CF lung come from?* Minneapolis, MN

Purdy, ME, Perpich J, Hundley T, Brown CK, Farris J, Carrier A, Remold S. Proceedings from the 2009 Gordon Research Conference: Microbial Population Biology: *Pseudomonas biogeography in the human home at multiple spatial scales.* Andover, NH

PUBLICATIONS

Purdy ME, France M, Hundley T, Eid N, Remold S. (2013). Identification of sites that harbor *Pseudomonas aeruginosa* in CF and non-CF homes. In Prep.

Remold SK, Brown CK, Farris JE, Hundley TC, Perpich JA, **Purdy ME** (2011). Differential habitat use and niche partitioning by Pseudomonas species in human homes. *Microb. Ecol., 62(3)*, 505-17.