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Metabolic profiles of household Pseudomonas species groups.

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METABOLIC PROFILES OF HOUSEHOLD *PSEUDOMONAS* SPECIES GROUPS

By

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A Dissertation Approved on

July 28, 2015

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DEDICATION

To my husband, Dr. Sunny Onyiri and my children, Chinunim J. Onyiri and Kelechi C. Onyiri for their unending love, support, and understanding.

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ABSTRACT

METABOLIC PROFILES OF HOUSEHOLD *PSEUDOMONAS* SPECIES GROUPS

Ogochukwu S. Onyiri

July 28, 2015

Resources influence the distribution, behavior, growth and reproduction of bacterial species. Understanding how closely related species utilize resources will shed light on the ecological traits or principles that shape biogeographic patterns and promote coexistence of species. Twelve *Pseudomonas* strains isolated from a single human home and PA01, the standard laboratory strain were evaluated in Luria Bertani media to understand whether their growth traits are influenced by their species group and/or their environment of isolation. Total growth, growth rate, early cumulative area under the growth curve (CUC) over 48 hours and viability (viable bacteria count per ml) at 24 and 48 hours were evaluated for each strain. I found differences in growth strategies that may be driven by species group and/or environment of isolation. These results suggest local adaptation of *Pseudomonas* strains to human home environments.

These strains were also evaluated for their metabolic and oxidative profiles in 31 resources as a way to understand if metabolic and/or oxidative profiles could be predicted by their species group and/or their environment of isolation. Metabolic

profile for each strain was evaluated as the qualitative ability to use a resource coded as 0 or 1, while the oxidative profile in each resource is a quantitative measure that was determined by integrating the CUC for each resource after 48 hours of growth. I found some resources were utilized by all the strains, suggesting similar metabolic adaptations or common ancestry. I also found evidence for resource differentiation that was driven by species group, but very little evidence for environment of isolation or local adaptation. Thus, resource differentiation can reduce competition and promote coexistence of species.

Seventy five strains of *Pseudomonas* species group isolated from multiple human homes were also evaluated in 11 different resources to understand the effect of environment of isolation on their growth profiles. I found extensive variability in their growth profiles and detected no association between environment of isolation and resource use profiles, although there was a significant difference with reference to environments of isolation in the growth rate of the strains in L-glutamic acid resource. Such variations in phenotypic traits can increase fitness, reduce intraspecific competition, and help with the ability to respond to environmental change.

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CHAPTER ONE

GENERAL INTRODUCTION

My research investigated growth traits (total growth, growth rate and CUC at 24 hours and the differences in viability (VBC/ml) at 24 and 48 hours of three major *Pseudomonas* species groups (*P. aeruginosa* group, *P. fluorescens* group and *P. putida* group) (Chapter 2) and the oxidative and metabolic profiles of these strains isolated from a single human home (Chapter 3). In addition, it investigated the diversity of resource use among a larger set of *Pseudomonas aeruginosa* species group isolated from multiple human homes (Chapter 4). Addressing these topics in growth traits, growth profiles, and resource use ecology will provide insights on important traits of these closely related *Pseudomonas* species groups, and the ecology of *Pseudomonas* species in human home environments.

The Human Home as Microbial Habitats

Humans across the globe spend a significant amount of time indoors than outdoors (Flores et al. 2011; Cunningham & Cunningham, 2015), and are in constant interactions with the microbes in their built environments (Rintala et al. 2008). Built environments such as homes, schools, day care facilities and offices offer many habitats that contain numerous microbes (Rintala et al. 2008; Tringe et al. 2008; Amend et al. 2010), yet little

is known about the microbial inhabitants of indoor environments (Flores et al. 2011), and the ecological and evolutionary processes that structure microbial communities in habitats within and around built environments. Microbial niches in the built environment include human skin, upper respiratory tract (Dethlefsen et al. 2007; Remold et al. 2011), soils, live pets, household drains, trash, water, kitchen sponges and surfaces (Remold et al. 2011). It is only recently that researchers have begun to study the microbial composition of indoor and outdoor habitats surrounding built environments.

Several studies have documented the microbial composition of habitats associated with built environments such as: biofilms in domestic drains (McBain et al. 2003), human body sites (Costello et al. 2009), public restroom surfaces (Flores et al. 2011), multiple sites in and around human homes (Ojima et al. 2002; Sinclair and Gerba, 2010; Remold et al. 2011; Purdy-Gibson et al. 2014; Remold et al. 2015), indoor and outdoor air (Kembel et al. 2012) and on surfaces of fresh fruits and vegetables (Leff and Fierer, 2013). Bacterial species that have been isolated from human homes include Coliforms (Ruslin et al. 1998; Ojima et al. 2002), *E. coli* and *Staphylococcus aureus* (Ojima et al. 2002; Medrano-Feliz et al. 2010), and *P. aeruginosa* (Ojima et al. 2002; McBain et al. 2003; Remold et al. 2011; Purdy-Gibson et al. 2014). Remold et al. (2011), in addition to *P. aeruginosa,* isolated other species groups of *Pseudomonas* such as *P. putida* and *P. fluorescens*. Medrano-Feliz et al. (2010) also isolated *Salmonella* and Hepatitis A virus from household environments.

All of the above studies are insightful, contributing to knowledge on the biogeography of human associated bacteria, their composition and diversity. Some studies provide broad coverage on the composition of bacterial species in each of these

environments because they used molecular techniques (culture independent methods) such as 16S rDNA gene amplification, subsequent high throughput sequencing of PCR products and BLAST searches. Culture independent community work needs to be balanced by culture-dependent work on individual isolates, because the latter allows one to get at ecological mechanisms driving biogeographical patterns.

Culture dependent techniques which, although limited in the coverage of bacterial diversity, offer the advantage of obtaining the bacteria in pure culture and freezing at -80^{o}C, so that bacterial strains can be revived at any time in the future for ecological, evolutionary and genomic studies. Most of the above studies were mainly focused on addressing detection, quantification, relative abundance, spatial, and temporal structure of the bacterial diversity in human home environments. However, there is still a knowledge gap in the understanding of the factors that contribute to the structuring of bacterial communities in human home environments. In this research, I used culture dependent methods to investigate the effects of environment or species group on growth traits, growth profiles, and resource utilization profiles of strains from 3 *Pseudomonas* species groups.

The Ecological Niche Theory

The ecological niche is one of the important theories of ecology and evolutionary biology (Lennon et al. 2012). The niche is described as "n-dimensional hypervolume" of environmental conditions (abiotic and biotic factors) that allows a species to exist for an indefinite period of time (Hutchinson, 1957). According to Hutchinson (1957) and Kearney and Porter (2004), the fundamental niche is the set of environmental conditions

and resources in which a species can experience positive population growth in the absence of other species (pre-interaction with other species) (Fig 1.1). Hutchinson (1957) defined the realized niche as the range of environmental conditions and resources that a species actually use in the presence of other species (post interaction with other species) (Fig 1.1). The realized niche is usually more restricted than the fundamental niche (Fig 1.1) (Kearney and Porter, 2004).

The ecological niche theory can be used to estimate the biogeography of species distribution and abundance (Kearney and Porter, 2004). Niche based processes have been shown to contribute to the spatial and temporal patterns of microbial communities (Lennon et al. 2012). Opposing niche based processes are stochastic processes which focuses on stochastic events as being responsible for structuring microbial communities (Dumbrell et al. 2010). Although the niche concept has been debated by some ecologists, it has been used to understand the distribution and abundance of macro-organisms, and may also be useful in understanding microbial diversity (Lennon et al. 2012) and biogeography. This research accessed the role of niche based processes such as growth traits, growth profiles, and resource utilization to test for the role of local adaptation in structuring *Pseudomonas* species in human home environments. An understanding of the extent to which closely related microorganisms living in home environments share important ecological characteristics will shed light on ecological processes structuring microbial communities.

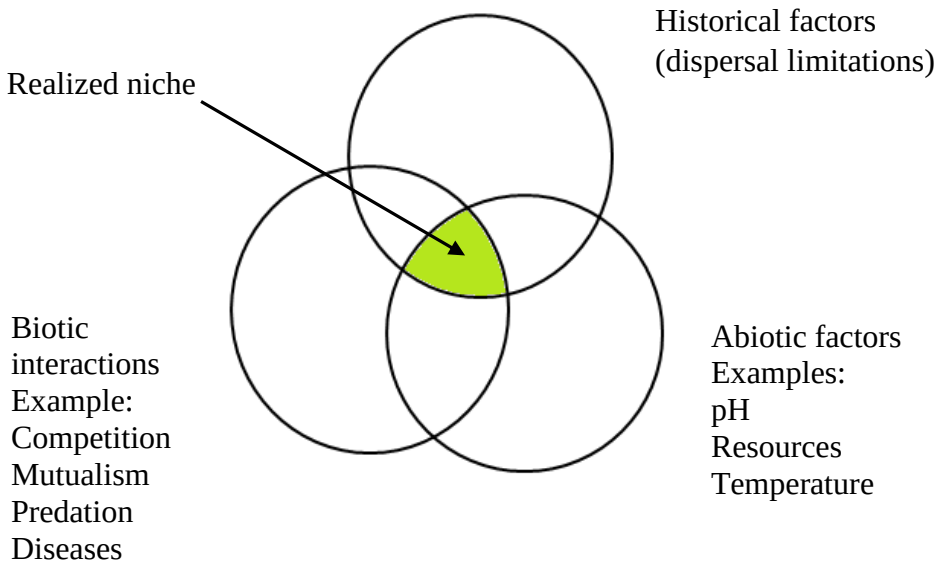


Figure 1.1. The realized niche of a species post interaction with other species (Hutchinson, 1957; Soberon and Peterson, 2005).

Microbial Biogeography

Biogeography is a field that is focused on the distribution of organisms across space and time (Martiny et al. 2006; Fierer, 2008). It was traditionally applied to the distribution of macro-organisms, but is now also applied to microbial ecology to describe patterns of microbial diversity (Martiny et al. 2006; Fierer, 2008). Microbial biogeography is still in its early stage of development as a research field in microbial ecology (Fierer, 2008), and it is receiving increased research attention. As reviewed by Martiny et al., (2006), four alternate hypotheses exist when the biogeography of microbes comes into discussion. The first (null) hypothesis posits that microbes do not exhibit any form of biogeography, that they have a random distribution in space. The second

alternative is that "everything is everywhere, but, the environment selects," (Bass-Becking) which means that microbes are not dispersal limited. The third alternative is that spatial variation results from historical events such as dispersal limitation and past environmental conditions, both of which can lead to genetic divergence of microbial assemblages. The last alternative is that the biogeography of microbes, like that of macro-organisms, is influenced by past events and present environmental conditions. Microbial dispersal, colonization, extinction, environmental heterogeneity, dispersal limitation, and past environmental conditions are key processes determining microbial biogeography (Martiny et al. 2006; Fierer, 2008).

Several studies have demonstrated that microbes exhibit some form of biogeographic patterns in various ecosystems. Salinity, pH, moisture levels, resource availability, redox potentials and light intensity have all been shown to influence biogeographic patterns of microbes to some extent. Belotte et al., (2003) provide evidence of local adaptation of soil bacterial isolates to their home site, as growth performance was better in their home site compared to other sites. Dumbrell et al., (2010) found members of the arbuscular mycorhizal (AM) fungal communities differentiated their niche primarily based on pH (a niche-based process), although there was evidence of dispersal limitation (a stochastic process). Lennon et al., (2012) showed that soil moisture availability influenced the activity and distribution of microbes, suggesting that some microbes may be able to coexist by partitioning their moisture niche axis using contrasting ecological strategies such as dry-adapted generalists or wet-adapted specialists. Elliot et al., (2014) also showed that biological crust bacterial communities

were determined by vegetation type and soil depth and that bacterial communities differed with soil carbon, nitrogen and surface temperature.

According to Fierer, (2008) microbes live in physical environments that are highly variable. It may be difficult to determine the specific environmental factors that are responsible for observed biogeographic patterns. Also, dispersal limitations may influence biogeographic patterns, suggesting separating dispersal limitations from environmental conditions can be difficult (Fierer, 2008). Regardless of whether niche based or stochastic processes, or a combination of these two, explain microbial community structures, it is clear that microbes in various habitats exhibit some spatial and temporal patterns and that further research will provide insights into the processes that structure microbial communities. In this research, I investigated the hypotheses that growth traits, growth profiles, and resource use profiles of *Pseudomonas* species groups are influenced by environments of isolation (selection within particular classes of sites) or species groups (shared traits due to evolutionary history).

Resource Use Characterization and Microbial Growth

Resource availability is an important factor in the regulation of the distribution, behavior, growth, and reproduction of bacterial species. In the presence of resources and favorable environmental factors microbes are able to initiate growth and reproduction. Microbial growth kinetics are relevant in many areas of science. In food microbiology, lag times and growth rates are used to predict food safety or shelf life of products, and identify critical points in the food production process (Zwietering et al. 1991; Cao et al. 2010). Microbial growth in a batch culture occurs in four distinct phases: lag,

exponential, stationary and death phases (Monod, 1949; White, 2007; Madigan et al. 2009).

Phases of Population Growth

Lag Phase

Lag is the interval before growth begins after a bacterial population is introduced into an environment containing new resources (Fig 1.2) (Madigan et al. 2009). It is the time needed for cells to synthesize essential enzymes to catalyze biochemical reactions (White, 2007; Madigan et al. 2009). The duration of the lag phase depends on a variety of factors such as the age of the culture, metabolic activity of the cells, and the type of medium used for the growth (White, 2007; Madigan et al. 2009). For example, if a strain is transferred to a nutrient rich medium, lag may be absent, but present when a strain experiences a nutrient poor medium.

Exponential Phase

During the exponential phase the cell mass or density of the bacterial population increases exponentially with time (Fig 1.2) (White, 2007), and is influenced by the media type, environmental factors, and the genetics of the particular organism (Madigan et al. 2009). The growth rate is often used as a fitness measure in experimental evolution (MacLean and Buckling, 2009) or used to determine fitness in a particular resource environment or under conditions defined by the researcher. The growth rate of a microbe is usually higher in a nutrient rich medium compared to a nutrient poor medium.

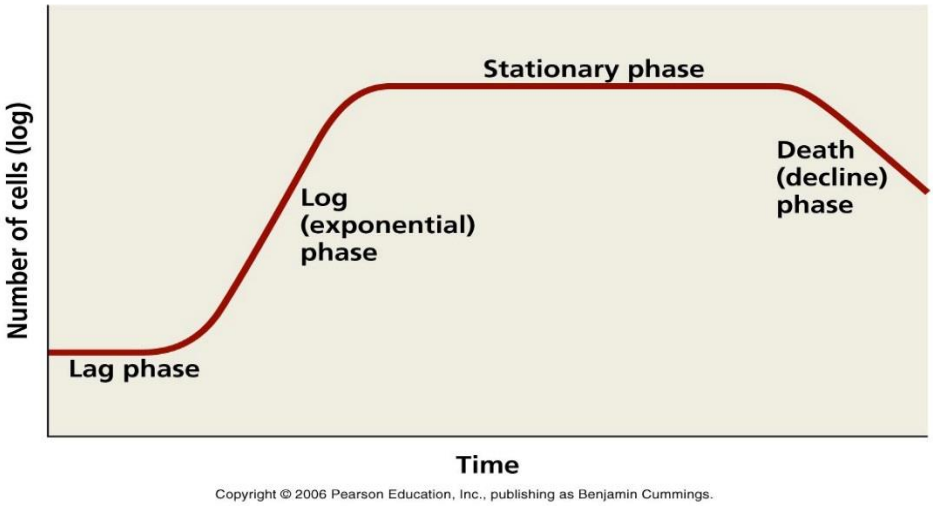


Figure 2: A typical microbial growth curve (http://www.livebinders.com/play/play?id=273899, 2015). Fig 2 was used with permission from Pearson Education, Inc., reference number is 150324-001469.

Stationary Phase

In the stationary phase the growth rate is zero (Fig 1.2) (Monod, 1949; White, 2007; Madigan et al. 2009), as a result of the depletion of an essential nutrient or the buildup of waste materials which exerts a negative feedback on the growth of the population (Madigan et al. 2009). The population then maintains a stationary phase density for a period of time with no net increase in cell number, after which the population enters the death phase, in which lysis of cells may occur. The death phase is also an exponential process, although slower than the exponential growth rate (Madigan et al. 2009).

Estimation of the Growth Rate

Estimation of growth rate is an important measure of performance under various resource and environmental conditions. The growth rate can be fitted by traditional

manual methods (Monod, 1948; Kim et al. 2005; Hall et al. 2014), but traditional manual methods can be time consuming and labor intensive, depending on the sample size. There are now programs that determine the exponential portion of growth curves and estimate the growth rate, but there are challenges and limitations associated with these methods (Hall et al. 2014). According to Hall et al., (2014), researchers are unsure how to estimate growth rates and what time points to include. Technical issues to be addressed include calibration of the plate reader, volume of cell culture to use in the wells of the microtiter plate, and how to precondition the culture for the growth experiment. Additional issues are adjusting to a fixed OD (Riley et al. 2001), diluting the cultures in the growth experiment medium or in a buffer, or diluting initial ODs to a calculated range of ODs (Hall et al. 2014). Standardization of growth rate estimation has implications for comparability across experiments and laboratories around the world.

Hall et al., (2014) described four ways that cultures can be grown for use in growth rate experiments with the strengths and weaknesses of each method. The first is the use of nutrient rich medium such as Luria Bertani (LB) or tryptic soy broth (TSB) to grow cultures overnight. This approach can lead to saturation and produce high numbers of inviable and viable cells with consequences of varying lag times during the growth experiment. The second approach is to inoculate cells into 10 ml of LB or TSB in a 15 ml tightly sealed centrifuge tube to create oxygen limitation, and incubate without shaking. In this approach, the cell density is limited by available dissolved oxygen and consequently fewer dead cells in the broth with resultant shorter and more consistent lag times. The third approach is to provide the limiting carbon in excess (ca.0.2% w/v) and grow the culture to saturation overnight. This approach may result in catabolite

repression, and longer and variable lag times. The fourth is also a minimal medium approach, but the limiting carbon is added at about 0.01% w/v and when the carbon source is exhausted, cell density is limited. During the growth experiment, when the carbon source is supplied in excess, cells resume exponential growth leading to shorter and reproducible lag times.

In this research, I used the traditional approach to estimate my growth rates in the experiment described in chapter two, quantifying variability in growth traits of *Pseudomonas* species groups isolated from human home environments. The traditional manual approach offers an advantage of consistency across experiments and laboratories but very labor intensive for large sample sizes. The automated curve fitting approach was used in the study described in chapter four, evaluating the growth profiles of 75 strains of *Pseudomonas aeruginosa* group isolated from multiple human homes. The automated approach allows visualization of growth curves, exclusion of outliers, good for large sample sizes, but growth rates may vary from laboratory to laboratory as different data points may be used. I grew my strains overnight in LB for chapter two and in tryptic soy broth (TSB) or tryptic soy agar (TSA) in chapter three to prepare them for the growth experiment, while in chapter four I grew them to the early log phase (6 hours) in TSB in a shaking incubator (Riley et al. 2001) for the growth experiment. I adjusted my cultures to a fixed OD for chapters two and three; adjusting to a fixed OD allows for standardization of cell density across experiments and laboratories. In chapter four I diluted the cells in a buffer to accommodate for a large sample size. The cultures were shaken every 30 minutes for chapters two and three and every 15 minutes for chapter four before each OD reading to limit biofilm formation.

The Genus *Pseudomonas*

The genus *Pseudomonas* consists of a large and important group of gram negative, r-strategist bacteria (r-strategist bacteria have rapid growth rates and inhabit highly variable environments), which are members of the γ Proteobacteria (Van Elsas et al. 2007). *Pseudomonas* species are widely known for their metabolic diversity and biodegradation potentials. Members of the genus occur in natural environments such as soils, freshwater, and marine setting, and also in association with plants and animals (Spiers et al. 2000), as normal flora or disease causing agents. The diverse adaptive life history strategies of *Pseudomonas* species enable survival in a variety of ecological niches. The ability to occupy a variety of ecological niches reflects the remarkable physiological and genetic plasticity of *Pseudomonas* species groups, which is a function of the diversity within the genus (Spiers et al. 2000). The genus *Pseudomonas* shows tremendous diversity and strains show heterogeneity of physiological traits (Spiers et al. 2000). This diversity is also manifested at the genome sequence level; where studies show that strains for which complete genome sequences are available composed of only 25% to 35% of the core genes shared by all members of the genus (Loper et al. 2012). This reflects the diversity of functions, which can be seen in the ability of some strains to perform ecosystem functions such as nutrient cycling. For example, *P. aeruginosa* is capable of denitrification, while according to Lalucat et al., (2006) *P. stutzeri* strains are associated with nitrogen fixation.

Pseudomonas aeruginosa is an increasingly prevalent opportunistic human pathogen (Sadikot et al. 2005). *P. aeruginosa* is the third most common cause of nosocomial infections in the human society and is often implicated in chronic and

ultimately fatal lung disease in individuals with cystic fibrosis (CF) (Goldberg et al. 2008). *P. aeruginosa* also causes ventilator associated pneumonia (Yang et al. 2009), urinary tract infections in adults and children (Bitsori et al. 2012), and bacteremia in organ transplant patients (Korvick et al. 1991). Individuals with compromised immunity, and patients with AIDS or malignancies are at increased risk for *P. aeruginosa* infections (Sadikot et al. 2005). *P. aeruginosa* isolates exhibit natural resistance to several antibiotics, and infections caused by this bacterium are increasingly becoming a challenge to eradicate (Lambert, 2002).

Strains of *P. syringae* species stand out as plant pathogen (Patten and Glick, 2002; Goldberg et al. 2008; Loper et al. 2012). Strains of *P. fluorescens* species are also plant associated, but exhibit commensal interaction with plants (Loper et al. 2012). The fluorescent *Pseudomonas* species possess exceptional nutritional capabilities, being able to utilize toxic and unusual chemicals for carbon and energy with applications for biodegradation, bioremediation, and biotransformation research (Goldberg, et al. 2008). *P. fluorescens* strains are also natural biological control agents in plants and help plants resist pathogens (Van loon et al. 1999). This species of *Pseudomonas* also produces the antibiotic 2, 4-diacetylphloroglucinol (2, 4-DAPG) whose presence in soils is associated with natural suppression of diseases caused by soil borne pathogens (Raaijmakers and Weller, 1998).

P. fluorescens and *P. putida* are environmental *Pseudomonas* species groups that are routinely found in bulk soil and plant rhizospheres (Fazli et al. 2014). *P. putida* is a plant growth promoting rhizobacterium that enhances plant root elongation via the production of the auxin indoleacetic acid (IAA), (Patten and Glick, 2002). *P. putida* is

also nutritionally versatile, and is capable of degrading various organic compounds; it is the most frequently encountered bacteria in environments with xenobiotics as a single carbon and energy resource (Timmis, 2002). *P. putida* is used as a laboratory 'workhorse' in many scientific investigations involving bacterial - mediated soil processes because of its rapid growth rate, ease of handling, and amenability to genetic analysis and manipulations (Timmis, 2002). Some *Pseudomonas* species act as biocontrol agents via the production of siderophores which sequester limited iron in the rhizosphere to a form that is unavailable to plant pathogens (Van Elsas et al. 2007). *Pseudomonas* species are therefore involved in ecological interactions that maintain primary productivity in agro-ecosystems and natural environments.

Description of the *Pseudomonas* Strains, Isolation and Identification Method

The *Pseudomonas* strains used in these studies were drawn from the larger *Pseudomonas* study strain collection (Purdy-Gibson et al. 2014; Remold et al. 2015). Strains were isolated from biotic and abiotic environments within and around human homes in the Louisville metropolitan area, Kentucky, USA. Strains were isolated between 2007 and 2012. Strains were isolated from several human associated environments such as: soils, household drains, surfaces, water, humans and an animal (one animal isolate) (H&A), kitchen sponges, and trash (Purdy-Gibson et al. 2014; Remold et al. 2015). The sampling and identification is described in detail in Purdy- Gibson et al., (2014) and Remold et al., (2015). Briefly, strains were isolated by swab-sampling areas in the human home, streaking for isolation on *Pseudomonas* isolation agar (PIA), re-streaking for isolation of a single colony of each distinct colony morphology from each sampled

site, and freezing in 12-15% glycerol in LB medium. Strains were then identified by sequencing at least 500 base pairs of the 16S ribosomal DNA, and comparing this sequence with the Bioinfo 1200 database (Purdy-Gibson et al. 2014; Remold et al. 2015). Strains were then assigned to species groups as defined in Anzai et al (2000). In chapters two and three I described the characterization of 12 strains from three *Pseudomonas* species groups (Table S1). The strains were 4 *P. aeruginosa*, 1 *P. nitroreducens* (*P. aeruginosa* group); 2 *P. fluorescens,* 2 *P. reactions* (*P. fluorescens* group), 2 *P. putida,* and 1 *P. monteilii (P. putida* group*).* Seventy-five *Pseudomonas* strains, all *P. aeruginosa* group were investigated in chapter four, which were comprised of 52 *P. aeruginosa*, 18 *P. nitroreducens*, 3 *P. otitidis*, and 2 *P. citronellolis* (Appendix).

Pseudomonas aeruginosa, PA01 was used as a reference or control strain in the studies described in this dissertation. PA01 is a derivative of the original Australian PAO that was isolated from wound (formerly called *Pseudomonas aeruginosa* strain 1) (Holloway, 1955; Klockgether et al. 2010). PA01 is a well characterized laboratory strain, clones of PA01 have been sequenced multiple times, and PA01 has been used in several studies (Stover et al. 2000; Frimmersdorf et al. 2010; Workentine et al. 2013). PA01 is the most common reference strain of *P. aeruginosa* used in most research laboratories around the world (Klockgether et al. 2010).

Significance and Scope of Research

Household *Pseudomonas* species groups have been shown to vary in recovery by environment types (Remold et al. 2011; Purdy-Gibson et al. 2015; Remold et al. 2015). *P. aeruginosa* species were recovered the most from household drains (Remold et al.

2011; Purdy –Gibson et al. 2014); *P. putida* species and *P. fluorescens* were recovered the highest from soils (Remold et al. 2015). There is limited understanding of the factors structuring the biogeographic patterns of household *Pseudomonas* species groups in human home environments. Differences in resource utilization could explain the differential recovery of *Pseudomonas* species groups in human home environments. The broad research objective in the studies presented here is to test hypotheses on the role of resources in the biogeography of *Pseudomonas* species groups in human home environments.

In this research, I investigated the growth traits (total growth, growth rate, and CUC quantified at 24 hours of growth of strains of three major *Pseudomonas* groups (*P. aeruginosa*, *P. fluorescens* and *P. putida*) isolated from a single human home and differences in viability (viable bacteria count per ml) at 24 and 48 hours in LB medium (Chapter 2).

The total growth is defined as the difference between the initial OD at the beginning of the growth experiment and the maximum OD achieved at the end of the growth experiment (Monod, 1949). The total growth provides an estimate of the bacterial density/carrying capacity at stationary phase or the end of the growth experiment. The OD measures turbidity of the culture and is routinely used to quantify bacterial growth because of the simplicity of the procedure (White, 2007) and can be used as surrogate for bacterial cell density (Francisco Moore, personal communication, July 28, 2015). According to Beer-Lambert's law the OD is directly proportional to the cell density, if the ratio of the mass to cell remains constant; but at higher cell densities this relationship deviates from linearity (White, 2007).

The growth rate is the change in cell density per unit time (Monod, 1949; White, 2007); the CUC integrates the lag, exponential, and stationary phases (Gucket et al. 1996). Luria Bertani (LB) and tryptic soy media were used because they are complex and nutrient rich media that are non-selective and non-inhibitory for the cultivation of bacteria.

The viable bacteria count is a process in which bacterial suspension is serially diluted and the inoculum is deposited and spread on a petri dish of solid growth medium (White, 2007); the petri dish is incubated for 24 to 48 hours. Following incubation viable cells develop into colonies and are counted (White, 2007).

I continued to explore the niche hypotheses that I started earlier in chapter 2 with the same strains by investigating their metabolic and oxidative profiles in 31 resources in the Biolog EcoPlate™ (Biolog Inc., Hayward, CA) (Chapter 3). The Biolog EcoPlate™ is a resource plate that contains carbohydrates, carboxylic acids, amino acids, polymers, phosphorylated compounds, and amines as single carbon resources. The metabolic profile is a qualitative measure of the ability to use a particular resource. It is coded as (1) for the presence of a specific metabolic activity or (0) for the absence of a specific metabolic activity.

The oxidative profile is a quantitative measure, which was evaluated using the cumulative area under the growth curve (CUC), obtained through optical density (OD) readings of cell density over 48 hours. The qualitative is a discrete variable and does not account for differences in rates of utilization over time, while the quantitative is a continuous variable and accounts for differences in the rates of utilization over time

among strains. I used qualitative and quantitative measures to understand if the two variables will support the hypotheses tested.

The diversity of resource use and growth profiles among a larger set of strains of *Pseudomonas aeruginosa* species group isolated from multiple human homes are investigated in chapter four. Addressing these topics in growth traits, growth profiles, and resource use ecology will provide insights into the ecological traits that are unique to these closely related *Pseudomonas* groups, and the ecology of *Pseudomonas* species in human home habitats.

Research Objectives

1. I investigated the growth traits amongst strains of *P. aeruginosa*, *P. fluorescens* and *P. putida* groups isolated from a single human home to understand whether those traits were more influenced by species group and/or environment of isolation. I compared the growth traits of each of these strains to PA01 (a common reference or control strain) to understand if they shared growth traits. I also tested for differential viability amongst species groups at 24 and 48 hours in LB (Chapter 2).
2. I examined whether the metabolic and oxidative profiles of *P. aeruginosa*, *P. fluorescens* and *P. putida* groups isolated from a single human home were influenced by species group and/or environment of isolation. I also investigated the metabolic and oxidative profiles of the common reference strain, PA01 to understand how those compared to household *Pseudomonas* strains (Chapter 3).

3. I also studied resource use in strains of *Pseudomonas aeruginosa* group that were isolated from multiple human homes over spatial and temporal scales to test for environment effects and evidence of local adaptation and in addition, studied resource use in PA01 as a reference or control strain (Chapter 4).

CHAPTER TWO

GROWTH AND SURVIVAL IN LURIA BERTANI (LB) OF *PSEUDOMONAS* SPECIES GROUPS ISOLATED FROM A SINGLE HUMAN HOME.

Summary- Microbial biogeography is influenced by dispersal limitation, abiotic and biotic factors, and local adaptation. The relative role of each of these factors is not clearly understood. Differences among genotypes in the kinetics of population growth may play a role in shaping microbial biogeographic patterns. Understanding how closely related species utilize the same resource can shed light on ecological traits, or principles that shape biogeographic patterns and promote coexistence. Twelve *Pseudomonas* strains isolated from a single human home and a reference strain of *P. aeruginosa*, PA01, were studied for their growth traits. I evaluated the total growth, growth rate, and cumulative area under the growth curve (CUC) over 48 hours for each of these strains when grown in a nutrient rich medium (Luria Bertani) (LB) broth. Differences in viability in LB broth at 24 and 48 hours were also investigated using viable bacteria count (VBC) per ml. Results showed differences in growth strategies that may be driven by environments of isolation and/or *Pseudomonas* species groups. These results suggest local adaptation of *Pseudomonas* species groups in human home environments. Differences in growth strategies may reduce intraspecific competition and promote coexistence in human home environments.

INTRODUCTION

In the presence of resources and favorable environment microbes are able to initiate growth and reproduction. Microbial growth kinetic is a fundamental aspect of all fields of microbiology including physiology, genetics, ecology, and biotechnology (Monod, 1949; Kovarova-Kovar and Egli, 1998). Microbial growth is defined as an increase in the number of cells in a population as a function of time (White, 2007). In bacteria, population growth occurs by binary fission and depends on a variety of chemical reactions such as: synthesis of small molecules; building of macromolecules; energy generation; and reactions that provide cofactors and coenzymes that participate in enzymatic reactions (Madigan et al. 2009). Microbial growth is also influenced by environmental factors such as temperature, pH, salinity, oxygen, and resource availability (Madigan et al. 2009).

Phases of Population Growth

When a bacterial population gains access to a fresh resource growth occurs in four distinct phases: lag, exponential, stationary, and death phases (Monod, 1949; White, 2007; Madigan et al. 2009). The four distinct phases are shown in (Fig 2.1A). These phases can be approximately modeled with an exponential growth model. The rate of growth of populations experiencing exponential growth at time t can be estimated by

$$\frac{dN}{dt} = rN, \quad \text{(dN/dt means change in number as a function of time),}$$

where N is the population density, t is the time and r is the intrinsic rate of natural increase (Cunningham and Cunningham, 2015). The intrinsic rate of increase (r) is not a constant, it is a function of the population density (N), and it is linear on a

log scale. Therefore, when population densities increase, r tends to decrease. As population density increases density dependent factors (intraspecific competition, nutrient exhaustion, and waste accumulation) are imposed on the population, because the environment has limited capacity to provide resources for all members of the population (Cunningham and Cunningham, 2015).

The carrying capacity (K) is the number of individuals of a species or genotype an environment can support with the available resources (Cunningham and Cunningham, 2015). The rate of growth (dN/dt) depends on how close the population size is to the carrying capacity (Cunningham and Cunningham, 2015). Populations that approach the carrying capacity of their environment show logistic growth, which is represented by

$$\frac{\mathrm{dN}}{\mathrm{dt}} = \mathrm{rN}\left(\frac{K-N}{\mathrm{K}}\right)$$ (Cunningham and Cunningham, 2015).

The exponential and logistic growth equations describe phases of a bacterial growth cycle that occur when a small founding population is given access to a resource, for example a fresh container of LB medium in a laboratory setting. In this research, I quantified traits that relate directly to the biological concepts of the growth curve. The specific growth rate is r, maximum optical density (OD) (total growth) is dependent on K; cumulative area under the growth curve (CUC) integrates all of the phases of the growth curve (lag, exponential and stationary phases) and the viable bacterial counts (VBC) quantified the living cell population density at 24 and 48 hours of the growth cycle (Fig 2.1B).

Microbes show different growth strategies based on resource levels, quality, and types (Velicer and Lenski, 1999). For example growth in a single substrate medium may be different from a mixed substrate medium. *Pseudomonas* species are described as r-

strategists, exhibit high growth rate, and are adapted to high resource environments (Van Elsas et al. 2007). The use of different growth strategies by microbes can maximize fitness under different resource environments and can promote niche differentiation and coexistence of species.

Luria Bertani (LB) medium was used in this study to evaluate growth traits of *Pseudomonas* groups. LB is a standard microbiological medium and will allow comparison across studies. It contains yeast extract and tryptone (Atlas, 2010). Yeast extract is a mixture of peptides, eighteen different free amino acids, purines, pyrimidines, water soluble B group vitamins; tryptone also contains peptides and some amino acids (www.organotechnie.com). These resources make LB a very good medium for understanding growth traits of species such as *Pseudomonas* species groups in complex environments. In the natural environments of microbial species, resources are available as mixed substrates rather than a single substrate. For example root exudates are rich in amino acids and sugars (Rovira, 1965). LB therefore, model resources in the natural environments of microbes. The presence of a variety of amino acids and carbon resources provide opportunities for many pathways, sequential utilization of resources, and different strains within the same species or different species can use LB in different ways.

In this study, I investigated the growth traits of three important species groups of *Pseudomonas* (*P. aeruginosa* (PAG), *P. fluorescens* (PFG) and *P. putida* (PUG) groups (Anzai et al., 2000) using LB medium. Twelve strains were isolated from a single home to control for genetic divergence by distance that could result from house to house variability. I tested the hypotheses that the total growth, growth rate and cumulative area under the growth curve (CUC) at 24 hours of growth in LB medium of *P. aeruginosa*, *P.*

fluorescens and *P. putida* group strains isolated from environments in a single human home are influenced by their environments of isolation (human, soils or drains) and/or species group of the strains. I also compared the growth rate, total growth and cumulative area under the curve of each of the *Pseudomonas* strains to a reference strain of *Pseudomonas aeruginosa*, PA01, to understand if they shared phenotypic properties. PA01 was used as a control in this study; it is a well characterized strain and has been used in several studies (Stover et al. 2000; Frimmersdorf et al. 2010; Workentine et al. 2013). PA01 is a derivative of the original Australian PAO that was isolated from wound (formerly called *Pseudomonas aeruginosa* strain 1) (Holloway, 1955; Klockgether et al. 2010).

The viable bacteria counts (VBC) were also quantified at 24 and 48 hours to understand if there were differences driven by species groups or environments of isolation in viability. These research questions will provide insight on the growth traits of *P. aeruginosa*, *P. fluorescens* and *P. putida* subgroups, provide a better understanding of phenotypic differences amongst the strains, and shed light on the role of resources in influencing the biogeographic range of *Pseudomonas* species groups in human home environments.

CHAPTER TWO MATERIALS AND METHODS

A total of 12 *Pseudomonas* strains isolated from human home environments (a human, soils, drains and a kitchen sponge). These strains were comprised of 5 of *P. aeruginosa* group (2 isolates from upper respiratory tract, 1 from human skin, 1 from soil, and 1 from the drain), 4 of *P. fluorescens* group (1 isolate from upper respiratory tract, 2

from soils, and 1 from drain), and 3 of *P. putida* group (1 isolate from the soil, 1 from the drain, and 1 from a kitchen sponge) (Table S1). The recovery rate for *P. fluorescens* and *P. putida* groups for humans is very low, (Remold et al. 2015), no isolate of *P. putida* group was recovered from human in this study. A strain of *Pseudomonas aeruginosa*, PA01 was included as a control in this study. PA01 is a derivative of the original Australian PAO that was isolated from wound (formerly called *Pseudomonas aeruginosa* strain 1) (Holloway, 1955; Klockgether et al. 2010). PA01 is a well characterized laboratory strain, clones of PA01 have been sequenced multiple times, and PA01 has been used in several studies (Stover et al. 2000; Frimmersdorf et al. 2010; Workentine et al. 2013).

Briefly, strains were isolated by swab-sampling areas in the human home, streaking for isolation on *Pseudomonas* isolation agar (PIA), re-streaking for isolation of a single colony of each distinct colony morphology from each sampled site, and freezing in 12-15% glycerol in LB medium. Strains were then identified by sequencing at least 500 base pairs of the 16S ribosomal DNA, and comparing this sequence with the Bioinfo 1200 database (Purdy-Gibson et al. 2014; Remold et al. 2015). Strains were then assigned to species groups as defined in Anzai et al (2000). These strains were studied for their growth traits in LB medium over 48 hours. Pure cultures frozen at -80^{o}C in LB medium with 12-15% glycerol were preconditioned on LB agar for 24 hour and sub-cultured onto fresh LB agar for another 24 hour.

The cells were swabbed off the LB plate and suspended in LB broth and adjusted to 1.0 OD at 600nm with a classical spectrophotometer with a fixed light path of 1.0 cm (Hall et al. 2014). When OD is measured in a 96 well plate using a plate reader, the

length of the light path is determined by the volume in each well (Hall et al. 2014). A culture that gives an OD of 0.2 for a 150 µl sample would give an OD of 0.4 for a 300 µl sample (Hall et al. 2014). Hall et al., (2014) suggests volumes between 100 and 300 µl for growth experiments conducted in a 96 well plate.

One hundred and fifty microliters of the adjusted bacterial suspension was dispensed into each of 96 well micro-plates in triplicate and incubated at 28ºC in a Tecan Infiniti plate reader (Model F200, Tecan, USA) and changes in OD_{595}nm tracked every 30 minutes for 48 hour; starting ODs were in the range 0.25-0.35 for the growth experiment. Forty eight hour incubation was used because a preliminary experiment revealed that some of the strains were still in the exponential growth phase following 24 hour incubation. At the end of the experiment each experimental reading was blanked against an un-inoculated LB control well to correct for background absorbance.

Another micro-plate was inoculated with 150 µl of bacterial suspension (1.0 OD_{600}nm) in duplicate and incubated at 28ºC. Cells were harvested at 24 and 48 hours for VBC/ml estimation. To quantify the VBC for each strain, 100 µl of bacterial suspension of each strain was harvested from a well and diluted in 9.9 ml of 1x phosphate buffered saline (PBS) to obtain 10^{-2} and serially diluted up to 10^{-9}. Dilutions of 10^{-4} to 10^{-9} were plated on LB agar in triplicate and incubated at 28ºC for 48 hours, after which VBC/ml were counted.

Growth rate, total growth, and CUC at 24 hours in LB broth were quantified.

I calculated total growth as the difference between initial absorbance and maximum absorbance achieved (Monod, 1949).

$$\text{Total growth} = (X_{max} - X_{min}) \text{ (Fig 2.1B)}$$

I estimated the growth rate using the following equation:

Specific growth rate = ln (X_2/X_1) / (T_2-T_1) (Kim et al., 2005),

where X_2 = absorbance readings at 75%, X_1 = 25% of maximum absorbance, T_2 = time at X_2 and T_1 = the time at X_1 (Thomas Hundley, personal communication, 2011) (Fig 2.1B).

I calculated the cumulative area under the curve (CUC) using the trapezoid method as described by Gucket et al., (1996) using the following equation:

$$CUC = \sum \frac{X2+X1}{2} * (T2 - T1) + \frac{X3+X2}{2} * (T3 - T2) + .. + \frac{Xn+Xn-1}{2} * (Tn - (Tn - 1)).$$

Where $X1$, $X2$, $X3$ and Xn are the absorbance values, while T1 = time 0 and Tn = 24 hours (Fig 2.1B).

Statistical Analysis

The total growth measurements were log(X+1) transformed, the CUC at 24 hours, and the VBC/ml were log(X) transformed to improve normality and homogeneity of variance before statistical analyses (Zar, 2010). The growth rate was estimated on a log scale and did not require transformation. Mixed linear models examining the effect of the fixed effects species groups, environment (predictor variables), and the random effect of strains nested within species groups on total growth, growth rate, and CUC at 24 hours (response variables) were performed (PROC MIXED, SAS 9.4). In these models the nested random factor was fitted with and without the assumption of unequal variances among levels of the grouping variable to test for differences in variance among species groups. These analyses required the likelihood ratio test done using sub-models with the parameter of interest removed from the models. Strain 1710, an isolate from a kitchen

sponge was excluded from the analyses because of the lack of biological replication for the environment of isolation. Significant effects were explored with the adjusted Tukey-Kramer tests to correct for multiple comparisons using the Least Squares Means (LSMeans) statement (PROC MIXED, SAS 9.4).

Pearson pairwise correlation analyses, among the variables were calculated for growth rate, total growth, and CUC at 24 hours (PROC CORR, SAS 9.4). Two sample *t* tests (two tailed) were performed to compare the total growth, growth rate, and the CUC of each of the isolates to the reference strain PA01. Bonferroni correction was used to control for multiple comparisons. Each individual hypothesis was tested at $\alpha = 0.05/12 = 0.0042$.

The differences in viability at 24 and 48 hours among species groups or environment types were modeled using the Mixed Linear Models (PROC MIXED, SAS 9.4) with species group, environment, time-point (24 and 48 hours), and species group by environment by time-point interactions as predictor variables and the population density (VBC/ml) as the response variable. Adjusted Tukey-Kramer test was performed using the LSMeans statement to explore significant effects (PROC MIXED, SAS 9.4).

RESULTS

Growth Traits of *Pseudomonas* Species in LB after 48 Hours

Using changes in optical density, I quantified three characteristics of bacterial growth kinetics; total growth, growth rate, and CUC at 24 hours. Total growth ranged from log OD 0.31 – 0.322 (mean = 0.32 ± 0.012) for the human strains, 0.167 – 0.344 for the drain strains (mean = 0.26 ± 0.22), and 0.18 – 0.324 for the soil strains (mean = 0.26

± 0.093). The mean total growth of the human strains is 18.75% more than the mean total growth of soil and drain strains. The error associated with each mean is the 95% confidence interval and this also applies to the means of the growth rate and the CUC at 24 hours for the different environments.

The log growth rate hr^{-1} ranged from 0.1 – 0.113 (mean = 0.11 ± 0.112) for the human strains, 0.08 – 0.19 for the drain strains (mean = 0.133 ± 0.137), and 0.08 – 0.15 (mean = 0.11 ± 0.05) for the soil strains. There is a 17.3% fold increase in the mean growth rate of drain strains compared to the means of human and soil strains. The log CUC at 24 hours ranged from 1.26 – 1.36 (mean = 1.322 ± 0.12) for the human strains, 1.12 -1.30 for the drain strains (mean = 1.22 ± 0.15), and 1.12 – 1.35 (mean = 1.26 ± 0.22) for the soil strains. The mean CUC at 24 hours for the human strains is 7.72% higher than the mean for the drain strains and 4.7% higher than the mean for the soil strains, while the mean CUC at 24 hours for the soil strains is 3.2% higher than the mean for the drain strains.

I investigated the relationships among these growth traits using Pearson pairwise correlation analyses. I found significant associations between total growth and CUC at 24 hours (r = 0.77828, p <.0001), growth rate and CUC at 24 hours (r = 0.44 p = 0.01), and total growth and viability at 48 hours (r=0.44; p = 0.01). The correlations were not significant for growth rate and total growth (r = 0.102, p = 0.55) and total growth and 24 hour viability (r=0.053, p = 0.76), negative associations were detected for growth rate and viability at 48 hours (r = - 0.18, p = 0.28) and viability at 24 and 48 hours (r= -0.21, p = 0.22). These results indicate total growth and CUC at 24 hours; growth rate and CUC at 24 hours, and total growth and viability at 48 hours may be biologically related, while

growth rate and total growth relate to different aspects of the biology of the growth curve (Fig 2.1B). The significant correlations between total growth and CUC at 24 hours, growth rate and CUC at 24 hours could be explained by the fact that the CUC at 24 hours integrates all phases of the growth curve. The negative association between growth rate and viability at 48 hours indicate that the trend in the growth rate is to decrease with increasing population density. Also negative correlation between viability at 24 hours and 48 hours indicate the trend is for population density to decrease with time.

I then explored differences among household *Pseudomonas* strains in three characteristics of growth dynamics; total growth, growth rate, and CUC at 24 hours, testing the hypothesis that each of these differed with respect to a strain's environment of isolation and/or species group. The human associated strains showed the least variability in their total growth, growth rate and CUC at 24 hours (Figs 2.2A, 2.2B & 2.2C); while the drain, soil and sponge isolated strains were more variable in their growth traits (Figs 2.2A, 2.2B & 2.2C). I performed 3 separate analyses to test the fixed effect of environment of isolation and/or species group on the total growth, growth rate, and CUC at 24 hours of *Pseudomonas* isolates in LB (PROC MIXED, SAS, 9.4) (Table 2.1). The statistical analyses showed a marginally significant effect of environment of isolation on total growth, and significant effects on growth rate, and CUC at 24 hours of *Pseudomonas* strains (p = 0.08, <.0001, 0.0042 respectively) (Table 2.1).

The adjusted Tukey-Kramer tests showed that, strains isolated from drains had significantly higher mean growth rate than those from soils (p = 0.01) and those from a human (p <.0001), but the mean growth rate for soil-isolated strains did not differ from the mean for human isolates (p= 0.15) (Fig 2.2B). For the CUC at 24 hours, human

isolates had significantly higher mean from the soil- isolated strains (p = 0.01) and from drain -isolated strains (p= 0.005), but soil- isolated strains did not differ from drain isolates (p= 0.14) (Fig 2.2C).

These analyses also detected no fixed effects of species group on total growth, growth rate, or CUC at 24 hours (Table 2.1). The likelihood ratio test was performed on the full model (with unequal variances) and the reduced model (in which a single variance among all strains was fit) to test the hypothesis that species groups differ in among-strain variance in total growth, growth rate, and CUC at 24 hours (Table 2.1). In contrast to the main effect of species groups, I detected significant differences in among strain variances among species groups in total growth, CUC at 24 hours, and marginally significant difference in among strain variance among species groups in growth rate of these strains (Table 2.1).

Two sample *t* tests (two tailed) were used to compare the growth traits of each strain to PA01 (Figs 2.2A, 2.2B & 2.2C). Some strains performed similarly to PA01 in their growth properties; while others differed in their performance in total growth, growth rate, and CUC at 24 hours (Figs 2.2A, 2.2B & 2.2C). Comparison of the species group pattern in total growth, growth rate and CUC at 24 hours suggests *P. aeruginosa* strains were more similar to PA01 than strains from the other two species groups. For all three growth traits at least one strain differs from PA01 (Figs 2.2A, 2.2B & 2.2C).

Viability of *Pseudomonas* Species Groups in LB at 24 and 48 Hours

To test for differences in viability after 24 hours, I quantified VBC/ml of *Pseudomonas* species groups at 24 and 48 hours (Figs 2.3A, 2.3B & 2.3C). Viability

ranged from 9.03 x 10^8CFU/ml. to 1.10 x 10^{12}CFU/mL at 24 hours and from 3.93 x 10^8 CFU/mL to 4.33 x 10^{10}CFU/ml at 48 hours of growth in LB medium. The statistical analyses (PROC MIXED, SAS 9.4) showed that there were no main effects of species groups or environment, nor was there an overall effect of species group by environment interaction (Table 2.2). The model indicated significant effects of time-point, species by time-point interaction, environment by time-point interaction, and species group by environment by time-point interactions (Table 2.2). These significant interactions indicate change in population density of the strains over time that are driven by species groups and/or environment, within species group differences in different environments, and between species group differences in the same environments (Figs 2.3D, 2.3E, 2.3F, 2.3G & 2.3H). Species group, time point, and environment of isolation all influenced the VBC/ml to some extent.

One hundred and twenty multiple comparisons were performed using LSMeans and adjusted Tukey-Kramer tests for all possible combinations of species by environment by time-point interactions (PROC MIXED, SAS 9.4). These analyses revealed 35.8% of the 120 comparisons were significantly different, 3.3% were marginally significant, and 60.8% were not different (data not shown). In these three level comparison analyses (species group x environment x time –point interactions), to avoid confounding factors such as environment of isolation and species group, I considered only those comparisons where two factors were held constant, while one factor varied. For example, species group and time (24 or 48 hours) held constant while the environment of isolation varied.

I detected differences within species groups ($p \leq 0.003$) in different environments. For the *P. aeruginosa* group, human isolated strains differed from soil and drain strains at

24 hours (Fig 2.3D), while for the *P. putida* group, drain- and soil-isolated strains differed at 24 and 48 hours (Fig 2.3E) (Tables S2). Only the *P. fluorescens* group strains did not differ by environment type at 24 or 48 hours. Isolates of the three species groups from the soil and drain environments (p <0.0001) significantly changed their population density (VBC/ml) over time (Figs 2.3F, 2.3G & 2.3H) (Table S2). Between species group differences were also detected in the same environment at the same time point (p< 0.03) (Table S2). Drain and soil strains differed for *P. aeruginosa* group and *P. putida* group at 24 hours, for the *P. fluorescens* group, soil and drain isolates differed from *P putida* group at 24 and 48 hours ($p\leq 0.003$); and drain isolate marginally differed for *P. aeruginosa* group and *P. fluorescens* group (p = 0.06) at 48 hours (Table S2). These differences may contribute to the differences in their distribution in human home environments.

DISCUSSION

Household *Pseudomonas* Strains Vary with Respect to Many Aspects of their Growth Dynamics

In microbial biogeography niche based (local adaptation) and stochastic (dispersal limitation) processes are often tested to understand their relative roles in microbial community structure (Martiny et al. 2006; Drumbell et al. 2010). In this study, the growth traits of strains of *Pseudomonas* species groups isolated from human home environments were investigated to understand whether species group or environment of isolation influenced their growth traits in LB over 48 hours. Results showed that the total growth

was marginally influenced, while the growth rate and CUC at 24 hours were significantly influenced by environments of isolation (Figs 2.2A, 2.2B & 2.2C) (Table 2.1).

However, because of strong correlations between environment of isolation and species group, the interaction between species and environment could not be evaluated for the growth traits in LB medium. The adjusted Tukey-Kramer tests detected that strains isolated from one or more environment types were different for growth rate and CUC at 24 hours, except for the growth rate (soil - isolated strains were not different from human – isolated strains), and for the CUC at 24 hours (soil – isolated strains were not different from drain isolates) (Figs 2.2B & 2.2C).

Pearson correlation analyses confirmed that total growth and growth rate are not significantly associated ($r = 0.102$, $p = 0.55$) and total growth and viability at 24 hours ($r = 0.053$, $p = 0.76$). This indicates the absence of a tradeoff between these traits. Tradeoff occurs when two traits are negatively associated (Pianka, 1970). Tradeoffs may be small but adequate to influence the coexistence of organisms (Bohannan et al. 2002). Organisms show tradeoffs in their abilities to respond to factors that limit their fitness and abundance (Tilman, 2000). The outcomes of tradeoffs are organisms that have different growth requirements and different environmental impact with consequences for reduced competition and coexistence of species (Dykhiuzen, 1998; Bohannan et al. 2002). Growth rate and CUC at 24 hours and total growth and CUC at 24 hours may be biologically related, because the CUC at 24 hours integrate the lag, exponential and stationary phases of the growth curve. When each strain was compared to PA01 in their total growth, growth rate and CUC, some strains were similar, while others were significantly different (Figs 2.2A, 2.2B & 2.2C).

The viability of *Pseudomonas* species after 24 and 48 hours in LB shows that the population density (VBC/ml) (Figs 2.3A, 2.3B & 2.3C) of *Pseudomonas* groups differed by environment types for the same species group (Figs 2.3D & 2.3E), differed between different species groups in the same environment, and also changed over time points (Figs 2.3F, 2.3G & 2.3H) (Table S2). *P. fluorescens* group did not differ by environment type, strains maintained similar VBC/ml regardless of environment of isolation (Fig 2.3B). These results suggest that time is important and strains vary by species groups, and environment of isolation with time suggesting effect of environment and past historical events on the distribution of household *Pseudomonas* strains. This finding of no differences in growth traits of *P. fluorescens* species group between environments contrast the findings of Loper et al., (2012), who documented the variability of strains of *P. fluorescens* group isolated from bulk soil, rhizosphere, and plant surfaces. This could be as a result of the small sample size used in this study.

Although the finding of a significant effect of environment of isolation is consistent with local adaptation, significant interaction between species and environment is a stronger indication (Kraemer and Kassen, 2015). Even stronger support would be provided by a comparison of growth performance (fitness) between home (environment of isolation) and away (transplant) sites to demonstrate tradeoff (Belotte et al. 2003). However, Velicer and Lenski (1999) showed that bacteria do not necessarily have to show tradeoffs under different resource conditions. Instead they are able to adapt via multiple evolutionary pathways. In this study a transplant experiment was not conducted to measure tradeoffs in growth traits, but a significant environment effect on growth traits could provide evidence for local adaptation of *Pseudomonas* species in human home

environments. For the growth rate human isolates differed from the drain and soil isolates; while for the CUC at 24 hours drain isolates had higher mean compared to human and soil isolates suggesting strains are adapting their growth strategies to resources in human home environments.

Differences in growth traits based on environment of isolation have previously been documented by Belotte et al., (2003). Differences in bacteria growth strategies have implications for growth rate and carrying capacity (Pianka, 1970; Van Elsas et al. 2007). In addition, differences in growth traits could mean that the strains are using the resources differently, which is consistent with niche partitioning.

The analyses did not detect the main effect of species group but the log likelihood ratio tests detected significant differences in variance in total growth, growth rate, and CUC at 24 hours among species groups (Table 2.1). Variability in total growth, growth rate, and CUC at 24 hours of *Pseudomonas* strains could increase fitness in human home environments. According to Zhang and Hill, (2005), phenotypic homogeneity in traits may have an associated fitness cost due to ecological and biochemical constraints. Strains show genetic, phenotypic, and environmental variance in quantitative traits (Zhang and Hill, 2005) and each strain may have an "optimal phenotype" that depends on growth strategies and niche partitioning abilities.

For example, *Esherichia coli* have been shown to evolve to partition one resource into three different niches: some strains evolved to use glucose, some evolved to use glycerol (a metabolite of glucose), and some evolved to use acetate (also a metabolite of glucose), which reduced intraspecific competition for glucose and promoted coexistence of species (Helling, Vargas and Adam, 1987). The *E. coli* strains that evolved this

partitioning also showed differences in growth rate, transport of glucose, and excretion of metabolites (Helling, Vargas and Adam, 1987). Also, according to Friesen et al., (2004), experimentally evolved *E. coli* populations showed a tradeoff of fast versus slow growth rate in the different phases of their growth cycle which allowed for coexistence. The differences in growth traits showed by *Pseudomonas* groups isolated from a single human home could reflect adaptive traits, which could promote coexistence in human home environments.

It should be noted that there is substantial imbalance in the collection of the isolates used in this study, such that most human associated isolates were of *P. aeruginosa* group, and most environmental isolates were of *P. fluorescens* and *P. putida* groups (Table S1, Figs 2.2A, 2.2B & 2.2C). The effects of environment of isolation and species group are therefore not fully separable, and the results presented here should be interpreted with caution.

In studying the growth traits of *P. aeruginosa*, *P. fluorescens* and *P. putida* groups isolated from a single human home, findings suggest marginal effect of environment of isolation and on total growth, but significant effects on growth rate, CUC at 24 hours and effects of environment and species group on VBC/ml at 24 and 48 hours providing evidence for the influence of contemporary environment and phylogenetic history on the distribution of household *Pseudomonas* species groups in human home environments. These results also highlight the phenotypic variability in total growth, growth rate and CUC at 24 hours of household strains of *Pseudomonas* groups. Phenotypic variability in general has the potential to increase future niche differentiation, coexistence, and ability of strains to respond to environmental change.

Tables

Table 2.1. Mixed linear models examining the effect of the fixed effects species groups, environment (predictor variables), and the random effect of strains nested within species groups on total growth, growth rate, and CUC at 24 hours (response variables) were performed (PROC MIXED, SAS 9.4).

Source	DF	Test Statistic
Total growth		
Environment (fixed, tested with F)	2, 2.01	11.76^{+}
Species group (fixed, tested with F)	2, 1.2	1.67 NS
Strains (species group) (variances) (Random, tested with LR Test)	2	**11.1****
Strains (species group) (means) (Random, tested with LR Test)	1	**94.1*** **
Growth rate		
Environment (fixed, tested with F)	2, 24.9	**19.23*** **
Species group (fixed, tested with F)	2, 1.21	2.86 ^{NS}
Strains (species group) (variances) (Random, tested with LR Test)	2	5.7+
Strains (species group) (means) (Random, tested with LR Test)	1	**30.2*** **
CUC of species at 24 hours		
Environment (fixed, tested with F)	2, 2.19	**163.11****
Species group (fixed, tested with F)	2, 1.2	4.22 ^{NS}
Strains (species group) (variances) (Random, tested with LR Test)	2	**10.8****
Strains (species group) (means) (Random, tested with LR Test)	1	**77.91*****

DF indicates degrees of freedom, denominator DF for F test is estimated using the Kenward-Roger approximation. DF for likelihood ratio (LR) tests are equal to the difference in the number of parameters in the full and reduced models. The fixed effect is tested with an appropriate F test. Random effects are tested with the (LR) tests; the LR test is -2x (maximum likelihood for the test's full model - maximum likelihood for the reduced model), from which the variance component for the reduced model has been reduced, and is chi square distributed. In the tests of variance effects, variances are constrained to be equal in the reduced model.
^{NS} p>0.1; +0.05<p<0.10; **0.001<p<0.005; ***0.00001<p<0.0005.

Table 2.2. Mixed general linear models of the viability of *Pseudomonas* species groups measured as VBC/ml at 24 hours and 48 hours in LB medium.

Source	DF	Type 111 F	P>F
Fixed effects			
Species group	2, 1.34	15.85	0.1162
Environment	2, 1.17	0.63	0.6527
Time point	1, 47	603.6	<.0001
Species group x Environment	3, 1	4.75	0.3223
Species group x Time point	2, 47	215.83	<.0001
Environment x Time point	2, 47	128.65	<.0001
Species group x Time point x Environment	3, 47	238.77	<.0001

Figure legends

Figure 2.1A: Typical growth curve (http://www.livebinders.com/play/play?id=273899, 2015)**.** Fig 1A was used with permission from Pearson Education, Inc., reference number is 150324-001469.

Figure 2.1B: Growth cycle information used for the calculation of the total growth, growth rate and cumulative area under the curve for each of the 13 isolates.

Figure 2.2: (A) Total growth ± 95% confidence interval (CI) in LB for 48 hours. (B) Growth rate ± 95% CI. (C) CUC at 24 hours ± 95% CI. Each data point represent mean of 3 replicates. Significance indicates 2 sample *t* test comparisons of each strain to PA01. *0.0042 $p < 0.005$; ** 0.0001 $p < 0.0005$; *** 0.00001 $p < 0.000005$. Each letter that appears multiple times designates pairs of environments that did not differ significantly; environments that differed significantly ($p<0.05$) do not share letters. Contrasts were adjusted for multiple comparisons using a Bonferroni correction.

Figure 2.3: (A) The VBC/ml in LB medium at 24 and 48 hours of strains of *Pseudomonas aeruginosa* group, (B) *Pseudomonas fluorescens* group, (C) *Pseudomonas putida* group, (D) differences within PAG by environment, (E) differences with PUG by environment, (F) change over time in viability in PAG, (G) change over time in viability in PFG, and (H) change over time in viability in PUG. Error bars represent ± 95% CI of mean of 3 repeated replicates. Each letter that appears multiple times designates pairs of isolates or time point that did not differ significantly; isolates or time point that differed

significantly ($p<0.05$) do not share letters. Contrasts were adjusted for multiple comparisons using a Bonferroni correction

Figures

Figure 2.1A

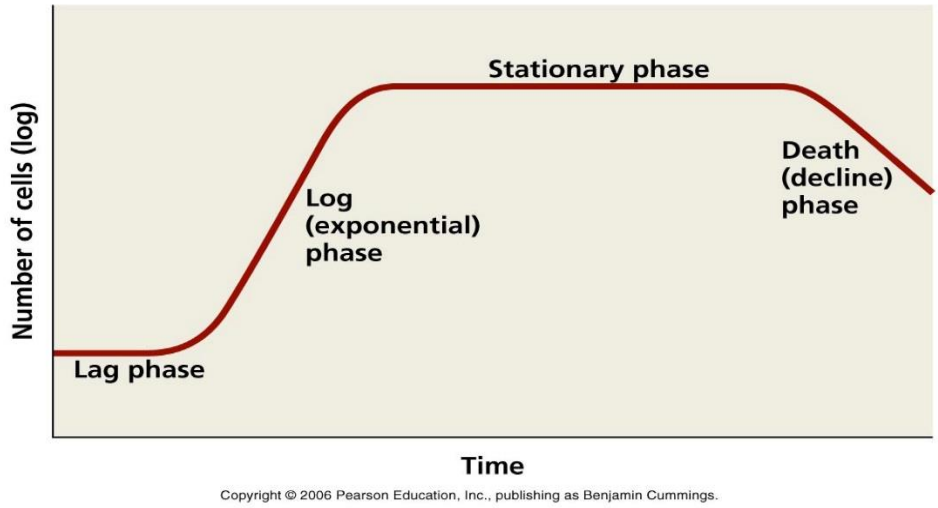


Figure 2.1B

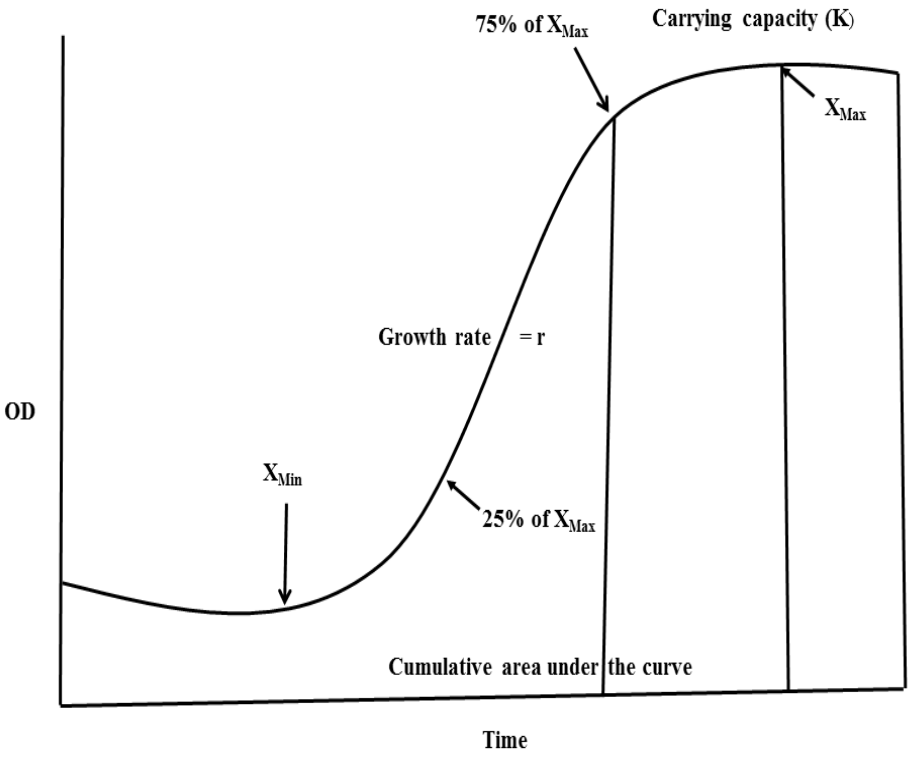


Figure 2.2

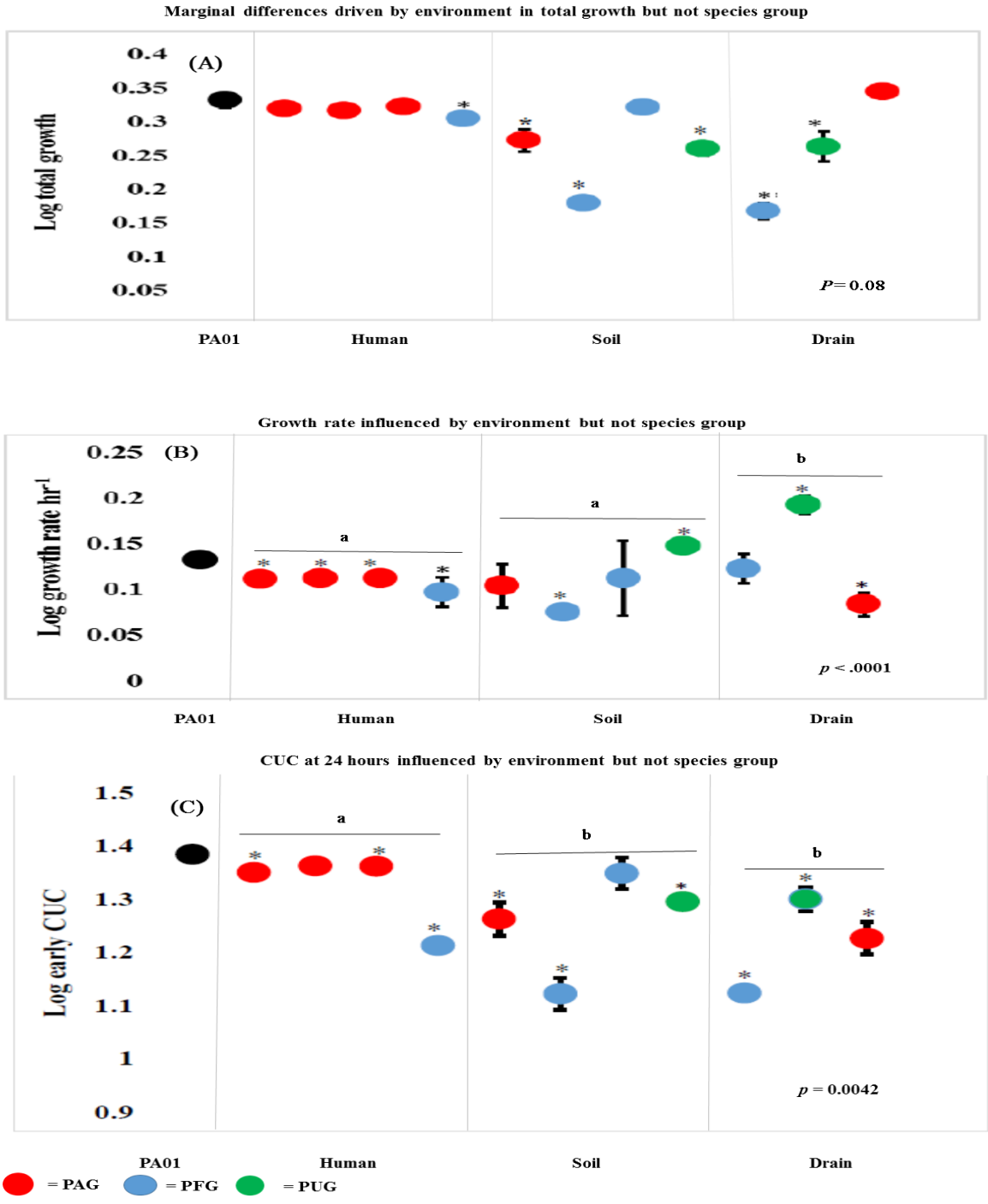
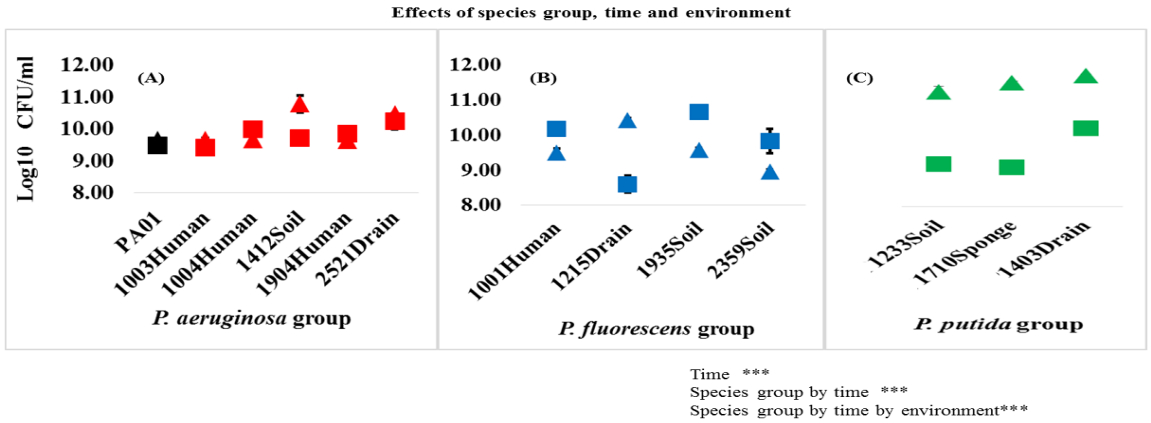
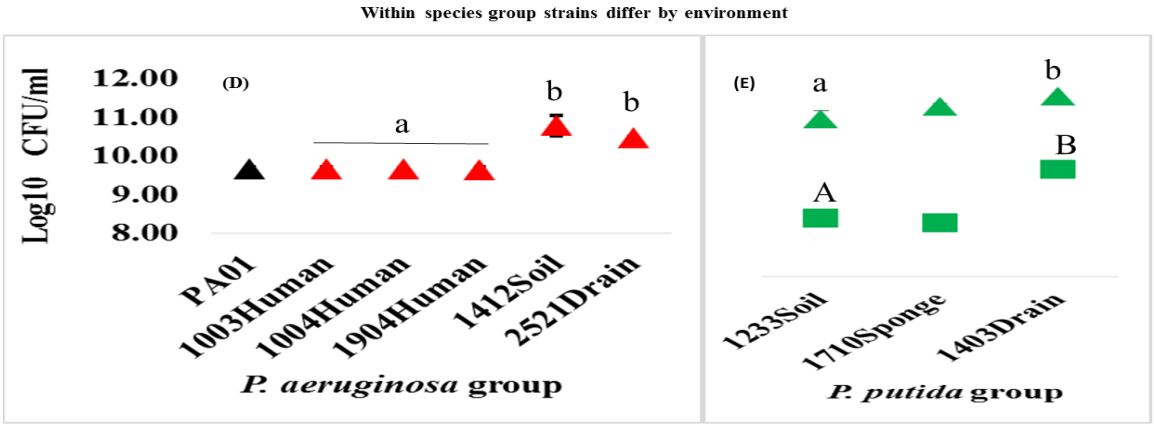
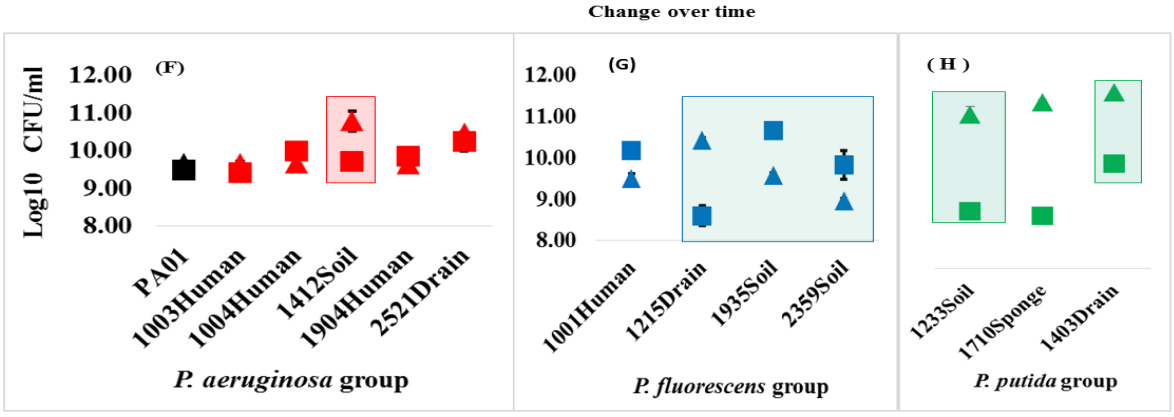

Marginal differences driven by environment in total growth but not species group
(A)
Log total growth
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
P= 0.08
PA01
Human
Soil
Drain
Growth rate influenced by environment but not species group
(B)
Log growth rate hr-1
0.25
0.2
0.15
0.1
0.05
0
a
a
b
p < .0001
PA01
Human
Soil
Drain
CUC at 24 hours influenced by environment but not species group
(C)
Log early CUC
1.5
1.4
1.3
1.2
1.1
1
0.9
a
b
b
p = 0.0042
PA01
Human
Soil
Drain
= PAG
= PFG
= PUG

Figure 2.3


Effects of species group, time and environment
Log10 CFU/ml
(A)
12.00
11.00
10.00
9.00
8.00
PA01
1003Human
1004Human
1412Soil
1904Human
2521Drain
P. aeruginosa group
(B)
1001Human
1215Drain
1935Soil
2359Soil
P. fluorescens group
(C)
1233Soil
1710Sponge
1403Drain
P. putida group
Time ***
Species group by time ***
Species group by time by environment***


Within species group strains differ by environment
Log10 CFU/ml
(D)
a
b
b
12.00
11.00
10.00
9.00
8.00
PA01
1003Human
1004Human
1904Human
1412Soil
2521Drain
P. aeruginosa group
(E)
a
b
A
B
1233Soil
1710Sponge
1403Drain
P. putida group


Change over time
Log10 CFU/ml
(F)
12.00
11.00
10.00
9.00
8.00
PA01
1003Human
1004Human
1412Soil
1904Human
2521Drain
P. aeruginosa group
(G)
1001Human
1215Drain
1935Soil
2359Soil
P. fluorescens group
(H)
1233Soil
1710Sponge
1403Drain
P. putida group
24 HR =
48 HR =

Supplementary Information

Table S1: Strain description showing environment of isolation, species, species group, and house**.**

Genotype		Niche	Species	Group code
SRP	**2521**	Drain	*P. aeruginosa*	PAG =*P. aeruginosa* group
SRP	**1003**	Human Mucus Membrane	*P. aeruginosa*	PAG
SRP	**1004**	Human Exterior	*P. aeruginosa*	PAG
SRP	**1904**	Human Mucus Membrane	*P. aeruginosa*	PAG
SRP	**1412**	soil	*P. nitroreducens*	PAG
SRP	**1001**	Human Mucus Membrane	*P. reactans*	PFG = *P. flourescens* group
SRP	**1215**	Drain	*P. fluorescens*	PFG
SRP	**2359**	soil	*P. fluorescens*	PFG
SRP	**1935**	soil	*P. reactans*	PFG
SRP	**1403**	Drain	*P. putida*	PUG= *P. putida* group
SRP	**1233**	Soil	*P. Monteilii*	PUG
SRP	**1710**	Sponge	*P. putida*	PUG

Table S2. LSMeans comparisons of species by environment by time point interactions of the VBC/ml of strains of *Pseudomonas* species groups isolated from human home environments.

Effect	Species	Environment	Timepoint	Species	Environment	Timepoint	DF	t Value	Adj P
Within species differences in different environments at the same time-point									
Specie*Environment*Timepoint	PAG	Drain	24	PAG	Human	24	2.34	4.6	0.003
Specie*Environment*Timepoint	PAG	Human	24	PAG	Soil	24	2.34	-6.41	<.0001
Specie*Environment*Timepoint	PUG	Drain	24	PUG	Soil	24	47	6.19	<.0001
Specie*Environment*Timepoint	PUG	Drain	48	PUG	Soil	48	47	13.17	<.0001
Change over time-points within species									
Specie*Environment*Timepoint	PAG	Soil	24	PAG	Soil	48	47	12.91	<.0001
Specie*Environment*Timepoint	PFG	Drain	24	PFG	Drain	48	47	21.99	<.0001
Specie*Environment*Timepoint	PFG	Soil	24	PFG	Soil	48	47	-16.51	<.0001
Specie*Environment*Timepoint	PUG	Drain	24	PUG	Drain	48	47	19.59	<.0001
Specie*Environment*Timepoint	PUG	Soil	24	PUG	Soil	48	47	26.57	<.0001
Between species differences in the same environment at the same time-point									
Specie*Environment*Timepoint	PAG	Drain	24	PUG	Drain	24	3.09	-9.71	<.0001
Specie*Environment*Timepoint	PAG	Soil	24	PUG	Soil	24	3.09	-4.6	0.003
Specie*Environment*Timepoint	PFG	Soil	24	PUG	Soil	24	1.07	-6.17	<.0001
Specie*Environment*Timepoint	PAG	Soil	24	PFG	Soil	24	1.38	3.86	0.0264
Specie*Environment*Timepoint	PFG	Drain	48	PUG	Drain	48	1.04	-3.54	0.0609

CHAPTER THREE

METABOLIC PROFILING OF *PSEUDOMONAS* SPECIES GROUPS ISOLATED FROM A SINGLE HUMAN HOME

Summary- In natural environments resources help to determine where organisms survive and reproduce. I tested the hypotheses that carbon utilization and oxidative profiles of *Pseudomonas* groups could be predicted based on the type of environment from which a strain was isolated and/or the species group to which it belongs. The *Pseudomonas* strains used in this study belong to *P. aeruginosa*, *P. fluorescens* and *P. putida* species groups and were isolated from drains, human upper respiratory tract, human skin, soils, and a kitchen sponge. The ability to utilize each of the 31 carbon sources in the Biolog EcoPlate™ was scored as 0 or 1 and analyzed using logistic regression to determine differences in resource utilization driven by species group, resource guild (the chemical group of the resource for example, carbohydrate resource guild), environment from which they were isolated and their interactions. Significant effects of environment, species group by resource guild, and environment by resource guild interactions (p = 0.0413, <.0001, and 0.0017, respectively) were found. Resources in the amino acid guild were utilized by the 3 species groups suggesting similar metabolic adaptations or common ancestry, resources in the phosphorylated guild were utilized differently amongst the 3 species groups. The oxidative profiles of household *Pseudomona*s strains in 30 resources were quantified as

the CUC and subjected to a principal component analysis (PCA). The principal component scores were analyzed with SAS mixed linear models. Mixed linear models of the first three principal component scores (PCS) of the cumulative area under the growth curves (CUCs) of growth in 30 resources indicated significant effect of species group in principal components (PCs) 1 and 2 and significant effect of species group by environment interaction in principal component 2 (p = 0.0143, 0.023 and 0.0314, respectively). There was no significant difference indicated for the principal component 3 scores for any of the three predictors included in the model. Significant species group-specific patterns of resource use profiles with respect to the presence or absence of a specific metabolic activity and the first two PCs of CUCs support resource differentiation by *Pseudomonas* species groups. The inability of the models to detect any significant effect of environment indicates there is little local adaptation with respect to resource-use diversity of these strains to human home environments.

INTRODUCTION

Engineered environments such as human homes are heterogeneous ecosystems that contain numerous niches that harbor different microbial species (Rintala et al. 2008; Tringe et al. 2008; Amend et al., 2010). *Pseudomonas* species are found in many environments in human homes (Ojima et al. 2002, McBain et al. 2003, Remold et al. 2011; Purdy-Gibson et al 2014; Remold et al. 2015), but have been shown to differ in their rates of recovery from different environment types (Remold et al. 2011; Purdy-Gibson et al. 2014; Remold et al. 2015). Variability in resource utilization may contribute to differences among species in the use of environments in the human home.

In this study, I focused on 12 strains within three *Pseudomonas* species groups (*P. aeruginosa*, *P. fluorescens* and *P. putida*) (Purdy-Gibson et al. 2014; Remold et al. 2015) isolated from a single human home, plus a reference strain of *Pseudomonas aeruginosa*, PA01 (i.e., n =13). Strains were chosen such that members of different species groups shared environment types of isolation (e.g. soils, drains, human). I assessed the degree to which members of these three groups differ in their use of the 31 carbon resources found in the Biolog EcoPlate™ (Biolog Inc., Hayward, CA), to understand whether differences in resource use profiles could contribute to differences in their distributions around the home. I tested the hypotheses, that environments of isolation and/or species groups predicts resource use patterns. Finding that environments of isolation rather than species group determines resource use patterns would suggest that local adaptation to a currently-occupied environment can supersede differences among species groups. A stronger support for local adaptation is a significant interaction between species group and environment (Kraemer and Kassen, 2015). Alternatively, finding that species group determines resource use indicates that resource use in these strains is ancient and not as a result of local resource adaptation to human home environments.

CHAPTER THREE MATERIALS AND METHODS

Description of Strains

Twelve *Pseudomonas* strains isolated from a single human home (Table S1, Chapter One) and a reference strain of *P. aeruginosa*, PA01, were studied. The 12 strains were isolated from a single house to control for genetic divergence by distance that could result from house to house variability. Strains were isolated from drains, soils, a kitchen

sponge, and sites on a human body (Purdy-Gibson et al., 2014; Remold et al. 2015). These strains comprised 5 isolates of *P. aeruginosa* group (PAG) (2 isolates from human upper respiratory tract, 1 from human skin, and 1 each from soil and drain), 4 from the *P. fluorescens* group (PFG) (1 isolate from human upper respiratory tract, 2 from soils, and 1 from drain), and 3 strains from the *P. putida* group (PUG) (1 isolate each from the drain, kitchen sponge, and soil). Briefly, strains were isolated by swab-sampling areas in the human home, streaking for isolation on *Pseudomonas* isolation agar (PIA), re-streaking for isolation of a single colony of each distinct colony morphology from each sampled site, and freezing in 12-15% glycerol in LB medium. Strains were then identified by sequencing at least 500 base pairs of the 16S ribosomal DNA, and comparing this sequence with the Bioinfo 1200 database (Purdy-Gibson et al. 2014; Remold et al. 2015). Strains were then assigned to species groups as defined in Anzai et al (2000).

PA01 was used as a control strain and it is a derivative of the original Australian PAO that was isolated from wound (formerly called *Pseudomonas aeruginosa* strain 1) (Holloway, 1955; Klockgether et al. 2010). PA01 is a well characterized laboratory strain, clones of PA01 have been sequenced multiple times, and PA01 has been used in several studies (Stover et al. 2000; Frimmersdorf et al. 2010; Workentine et al. 2013).

Biolog EcoPlate Assays

The qualitative resource utilization profiles measured as the presence/absence of the ability to use a particular resource (0 or 1) and the quantitative oxidative profiles measured as the growth of *Pseudomonas* strains were determined using the Biolog

EcoPlate™ (Biolog Inc., Hayward, CA). The Biolog EcoPlate™ contains a triplicate of 31 different resources, plus one control well, without a carbon resource (Fig 3.1). The Biolog EcoPlate™ contains a broad range of resources that Zak et al., (1994) classified into resource guilds such as carbohydrates, carboxylic acids, amino acids, polymers, phosphorylated compounds, and amines based on chemical similarities (Fig. 3.1).

In a Biolog EcoPlate™, as cells respire, the tetrazolium violet dye present in the well is reduced and irreversibly converted from colorless to purple (Bochner et al. 2001). Ability to use a resource is indicated by a purple color change and was scored on a minimum OD of 0.28 after blanking with the water control well. This value is very similar to the > 0.25 OD that was used as the threshold for positive wells in the Gucket et al., (1996) study that also utilized Biolog EcoPlate™. Metabolic capabilities was estimated as the (# of resources utilized by each strain ÷ by the total number of resources x 100) (Meyer et al. 2004). Oxidative profiles are assayed by quantifying amount and rate of color change over time.

To precondition cells, cultures were grown in 10 mL tryptic soy broth (TSB) or on tryptic soy agar (TSA) without glucose for 24 hrs. Media contained 15 g tryptone, 5 g soytone, 5 g NaCl (TSB), and 15 g agar for the (TSA) (Atlas, 2010). Cells were harvested by centrifugation, rinsed 3x in 1x phosphate buffered saline (PBS) and re-suspended in 1x PBS, after which cells were adjusted to an OD_{600nm} of 1.0 (Riley et al. 2001). Rinsing of cells ensured no carbon resource was carried over to the EcoPlate assays. One hundred and thirty microliters of bacterial suspension was inoculated into each well in the Biolog EcoPlate™ and incubated at 28ºC in a Tecan Infiniti plate reader (Tecan, USA), and changes in absorbance at OD_{595nm} tracked every 30 minutes for 48hr. During the

experiment, plates were shaken before each OD reading; shaking ensured cells were uniformly suspended and well aerated. Each strain was assayed three times in an incomplete block design in which strains were assessed in different combinations in each block. One isolate (1935) had unusually high ODs in 2 out of the 3 replicates in the lactose resource (OD > 0.28) and was considered an outlier as these *Pseudomonas* species groups do not normally use lactose (Fig 3.1) (Stanier et al. 1966). Therefore, lactose was excluded from all the statistical analyses in this study.

Statistical Analyses

At the end of the experiments the OD values obtained for each strain for the 31 resources were blanked by subtraction of the OD values obtained in a substrate-free negative control well. I evaluated the qualitative carbon utilization profiles, which reflect the presence or absence of the ability to use a particular resource (coded as 1 for utilization, or 0 for lack of utilization) for each of the 31 resources. The quantitative oxidative profiles were evaluated as the cumulative area under each growth curve (CUC) of OD over time in a given resource environment. The CUC integrates the lag, exponential, and stationary phases into one variable (Gucket et al. 1996). The CUC could provide insight into the relationships between resource use profiles and species groups and/or environments of isolation.

In the analyses presented in this study, the strains were classified into two environment types (human-associated or environmental) to increase statistical power. Strains isolated from sites on a human body were classified as human-associated, while the soil, drain, and sponge strains were classified as environmental.

There is an imbalance in the data, there is/are no human-associated *P. putida* group strain(s); I performed these analyses with or without the *P. putida* group strains and there are no qualitative differences. Therefore, the results were presented with the *P. putida* group strains included.

The results of the 0 or 1 (response variable) were analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS 9.4) with species group, resource guild, environment, species group by resource guild, and environment by resource guild interactions as predictor variables, and resources nested within guild and genotype nested within species group as random factors. Significant effects were explored using the adjusted Tukey-Kramer test to correct for multiple comparisons (PROC GLIMMIX, SAS 9.4). To achieve balance in the model, the phosphorylated compounds were excluded from the model (but not the analysis of CUC, see below); because only strains from the *P. fluorescens* group utilized them, their inclusion resulted in the failure of the model.

The cumulative areas under the growth curves (CUCs) for the 31 substrates for the 3 replicates were also computed using the trapezoid method (Gucket et al. 1996), after which the 3 replicate CUCs for each resource were averaged (mean centered) for the 13 isolates. The CUCs provide information on the overall performance of each strain in a given resource environment and is not dependent on a range of data points chosen by the researcher (Gucket et al. 1996). Lactose was excluded from the PCA because *Pseudomonas* strains rarely utilize it for growth and energy (Stanier et al. 1966).

The mean of each of the CUCs for the thirty resources for the 13 isolates were subjected to a PCA using XLSTAT 2014.4.01 (http://xlstat-win.soft112.com), to identify common carbon utilization patterns that may be influenced by species groups and/or

environments of isolation. The relationships between the scores of the first three principal components (PCs) (response variable) and species group, environment type, and species group by environment type interaction (predictor variables) were analyzed using three parallel mixed linear models (PROC MIXED, SAS 9.4). For the PC1 the (not statistically significant) interaction term was dropped so that replication at the lowest level of the model, the main effect, would allow comparisons among species groups. Thereafter, the significant effect of species group for PC1 was explored via least squares means using the adjusted Tukey -Kramer tests (PROC MIXED, SAS 9.4) to correct for multiple comparisons.

RESULTS

Carbon Utilization Profiles Evaluated as the Ability to Use a Particular Resource

The presence or absence of utilization of a resource was evaluated to understand whether there are relationships between species groups and/or environments of isolation, and resource utilization profiles of *Pseudomonas* species groups. Some substrates were consistently utilized and some showed variable use across the three technical replicates. The amino acids were utilized by most of the strains regardless of species groups (Fig 3.1). D-galacturonic acid and D-galactonic acid $^{\gamma}$ -lactone were not utilized by any of the *P. aeruginosa* group strains (Fig 3.1). D- mannitol and N-acetyl glucosamine were not utilized by any of the *P. putida* group strains (Fig 3.1). β- methyl D-glucoside, i-erythritol, D-cellobiose, α-D- lactose, D-glucosaminic acid, α-cyclodextrin, and the phosphorylated compounds were not utilized by any of the strains of *P. aeruginosa* and *P. putida* groups (Fig 3.1). Some strains of the *P. fluorescens* group utilized resources in all of the resource guilds. None of the strains utilized 2-hydroxy benzoic acid.

Metabolic capabilities ranged from 45.2% to 54.84% for the *P. aeruginosa* group, 58.06% to 90.32% for the *P. fluorescens* group and 58.07% to 61.29% for *P. putida* group (Fig 3.1). Logistic regression of the ability to use a particular resource response, scored as yes or no, showed significant effects of environment, species group by resource guild, and environment and resource guild interactions (Table 3.1). This model could not be performed with the inclusion of the phosphorylated compounds (that were only utilized by strains of *P. fluorescens* group), because inclusion resulted in failure of the model. This indicates a strong resource guild by species group effect that could not be modeled.

Mean comparisons of species group by resource guild interaction using the adjusted Tukey-Kramer test (PROC MIXED, SAS 9.4) revealed that strains of *P. fluorescens* group marginally utilized a higher number of resources in the carbohydrate guild from strains of *P. aeruginosa* group and significantly higher number than strains from *P. putida* group ($p = 0.0521$, 0.007, respectively), *P. fluorescens* group marginally utilized lower number of resources in the amine guild from the strains of *P. putida* group ($p = 0.092$). These results suggest that there may be differences in how these resources were utilized by *Pseudomonas* groups that are driven by species group, resource guild and environment of isolation.

Oxidative Profiles of *Pseudomonas* Species Groups in Biolog EcoPlate

To understand if the quantitative oxidative profiles varied by species group and/or environment of isolation, the CUCs of growth curves in 30 resources were subjected to a PCA. The first three components explained 74.39% of the variability in the data.

Loadings on the first three PCs were not broken down by resource guild (Table 3.2). Some carbohydrates, carboxylic acids, phosphorylated compounds, an amine, and a polymer were most strongly loaded on PC1, different carbohydrates, a carboxylic acid, an amino acid, and polymers on PC2 and different carbohydrates, carboxylic acids, an amino acid, an amine, and a polymer for PC3 (Table 3.2).

To evaluate the hypotheses that oxidative profiles in the Biolog Ecoplate were influenced by *Pseudomonas* species group and/ or the environment of isolation, I constructed SAS general linear mixed models (PROC MIXED, SAS 9.4) with species group, environment type of isolation and the interaction between environment type of isolation and species as predictors on PCs 2 and 3 scores from the PCA. A similar model was used to analyze the PC1 scores with the interaction term dropped to enable LSMEANS contrasts. I detected a significant difference among PC1 scores with respect to species group but not environment type of isolation (p = 0.0143, and 0.36, respectively; general linear mixed model with PC1 scores as response variable and species group and environment type as predictors).

The adjusted Tukey test showed that the mean PC1 score for *P. fluorescens* group isolates was significantly higher than the mean PC1 score for *P. aeruginosa* group isolates (p = 0.02) and marginally significantly higher than that of *P. putida* group (0.073), but the mean PC1 scores for *P. aeruginosa* group and *P. putida* group were not different (p = 0.79) (Figs 3.2A & S1). An analogous model of PC2 scores detected significant effects of species group and species group by environment type interaction (p = 0.023, and 0.0314, respectively), but no significant environment type effect (p = 0.33) (Figs 3.2B & S2). Adjusted Tukey-Kramer detected that human-associated strains of *P.*

aeruginosa group differed (higher mean score) from environmental strains of *P. putida* group ($p = 0.004$) and environmental strains of *P. fluorescens* group (higher mean score) differed from environmental strains of *P. putida* group ($p = 0.033$), but human *P. aeruginosa* group strains do not differ from environmental *P. fluorescens* group strains. No effects of species group, environment type, and environment type by species group interaction were detected for PC3 scores (p =0.1022, 0.5231, and 0.1754 respectively) (Fig not shown). To determine whether the outlier strain 1935 (Fig 3.1), which utilized the highest number of resources in the Biolog EcoPlate™ was strongly influencing the results of the statistical analyses, especially tests for differences among species groups, models were run a second time excluding this strain. I found no qualitative differences between the results of these models. Therefore, the results were presented with the outlier included.

On average the *P. fluorescens* group isolates presented higher CUC values than strains of *P. aeruginosa* and *P. putida* groups in 9 out of 11 substrates associated with high loadings in the PC1 (Table S1). *P. aeruginosa* group and *P. putida* had higher average CUC in each of the two other resources in PC1. An evaluation of the CUCs for the resources with high loadings in the PC2 (Table 3.2) shows that the *P. aeruginosa* isolates have higher average CUC values in N-acetyl –D- glucosamine, itaconic acid, tween 40, and tween 80, while *P. fluorescens* isolates have higher average CUC in D-mannitol and *P. putida* higher average CUCs in glutamic acid and phenylethylamine (Table S2). *P. putida* isolates have higher average CUCs for resources with high loadings in the PC3, except for the pyruvic acid methyl ester (Table S3). These

differences in average CUCs may contribute to the scores of each of the species groups in the top three principal components.

DISCUSSION

Carbon Utilization Profiles of strains of *Pseudomonas* Species Group

Pseudomonas species groups showed carbon utilization patterns that suggests species group differences (Fig 3.1), but statistical analysis did not detect main effects of species group and resource guild (Table 3.1), rather there were significant effects of environment, species group by resource guild, and environment by resource guild interactions (Table 3.1). The significant interaction between species group and resource guild (even though the resources in the phosphorylated guild were dropped from the model) indicates species group differ but in resource guild specific ways (Fig 3.1). The significant environment by resource guild interaction also suggest differences driven by environment of isolation with respect to the resource guilds. Mean comparisons revealed differences in the utilization of carbohydrate resources for the 3 species groups and in the utilization of the amine resources for *P. fluorescens* group and *P. putida* group.

All of the strains were able to utilize most of the amino acid resources. Meyers et al., (2004) also found that growth was most supported for cold-adapted fluorescent pseudomonads isolated from alpine soils by the amino acid guild. In a metabolomics study, Behrends et al., (2013), found that clinical *Pseudomonas aeruginosa* strains showed increased capabilities for amino acid utilization. The utilization of amino acids by *Pseudomonas* species groups isolated from a variety of environments may suggest similar genetic adaptations for the pathways for amino acid metabolism. Amino acids are

found in a variety of environments such as cystic fibrosis lungs (Behrends et al. 2013), root exudates in soils (Rovira, 1965) and secretions from human skin glands (Madigan et al. 2009).

Pseudomonas species are able to colonize a variety of environments that vary in levels of different resources. The ability to utilize a variety of amino acids may be a trait that increases fitness in multiple environments and indicates similar genetic adaptations, or common ancestry by the *Pseudomonas* species groups. Overall, these *Pseudomonas* strains showed remarkable resource use diversity and this is consistent with the metabolic plasticity of the genus *Pseudomonas*. These findings of metabolic diversity in *Pseudomonas* species are consistent with studies of Stanier et al., (1966), Clarke, (1982), and Meyer et al., (2004). Metabolic diversity has the potential to promote adaptation to new resource environments and niche differentiation with consequences for mediating intraspecific competition and co-existence of species.

There were resources in which the strains showed resource use that varied between replicates (Fig 3.1). Variable response in resource use patterns between replicates has also been reported by Wiedmann et al., (2000) for *Pseudomonas* species and by Riley et al., (2001) for experimentally evolved populations of *Ralstonia* strain TFD41; both studies also used the Biolog resource plate technology. It is unclear why bacterial clones of the same genotype will show variable response in resource utilization across technical replicates performed on separate days.

Oxidative Profiles of *Pseudomonas* Strains Species Groups

I tested the hypotheses that local adaptation results in parallel patterns of resource use in species that share environmental distributions or whether closely related species differ in resource use even if they use the same environments. The clear separation based on species groups in PC1 and PC2 (Figs S1 & S2) suggests biological differences in the oxidative abilities of the *Pseudomonas* species groups used in this study. The significant effects of species groups in PC1 and PC2 (Figs 3.2A, 3.2B, S1 & S2) provide evidence for resource differentiation by strains of *P. aeruginosa*, *P. fluorescens* and *P. putida* groups isolated from a single human home, while the significant species groups by environment type interaction in the PC2 suggest that the species groups and environment of isolation both influenced the CUCs of resources associated with PC2. Species groups use different ecological strategies in dealing with the same ecological conditions.

There was no main effect of environment for the PC1, PC2, and PC3 scores. However, there was significant species group by environment interaction for the PC2. The *P. putida* group strains, all isolated from the environment, were lower in their PC2 scores than *P. aeruginosa* group isolates from human ($p = 0.004$) but not from the *P. aeruginosa* group isolates from the environment, and they were also lower than *P. fluorescens* group isolates from the environment, ($p = 0.033$), but not the *P. fluorescens* strain from human (Fig 3.2B). A significant interaction between environment and species indicates local adaptation (Kraemer and Kassen, 2015). In this study this evidence of local adaptation should be interpreted carefully; because the recovery of *P. fluorescens* and *P. putida* groups from humans is very low (Remold et al. 2015) and in this study there is no *P. putida* strain isolated from a human.

There is little support for the alternate hypothesis that local adaptation results in parallel patterns of resource utilization in species that share similar environmental distributions. There is a stronger support for resource differentiation amongst *Pseudomonas* species groups. Resource differentiation can reduce interspecific competition with consequences for promotion of coexistence of species (Kneitel and Chase, 2004). This is consistent with niche differentiation (Cunningham and Cunningham, 2015). Niche differentiation by microbes has been documented by (Dumbrell et al. 2010; Remold et al. 2011; Lennon et al. 2012; Elliot et al. 2014). The resources that our strains utilized (Fig 3.1) are similar to the resources that were utilized by the *Pseudomonas* strain used by Paixoa et al., (2003), and the *Pseudomonas* strains in the Aagot et al., (2001) studies.

Overall, the findings support the hypothesis that closely related strains of *Pseudomonas* species groups differ in their metabolic and oxidative profiles even though they use the same environment. Although, there is slight evidence for local adaptation, these results should be interpreted with caution; because the recovery rates for *P. fluorescens* and *P. putida* groups from humans is very low (Remold et al. 2015), and as such there is no human representative strain for *P. putida* group in this study. Findings indicate that resource utilization in *Pseudomonas* species groups is ancient and is not as a result of local resource adaptation in these species.

Tables

Table 3. 1: Generalized linear mixed model (PROC GLIMMIX) analysis of the presence or absence of a specific metabolic activity of *Pseudomonas* species groups after 48 hours of growth in Biolog Ecoplate ™ resources with species group, resource guild, environment, and their interactions and resources nested within resource guild and genotype nested within species group as random factors.

Effect	DF	F Value	Pr > F
Species group	2, 10.91	1.61	0.2442
Resource guild	4, 3.578	0.91	0.5423
Environment	1, 991	4.18	0.0413
Species group*Resource guild	8, 991	5.62	<.0001
Resource guild*Environment	4, 991	4.36	0.0017

Table 3.2. The loadings for the first - three principal components of the mean CUCs of each of the 30 resources for strains of *Pseudomonas* species groups and PA01. Values in bold are resources that have ± 0.6 and above in the principal component loadings.

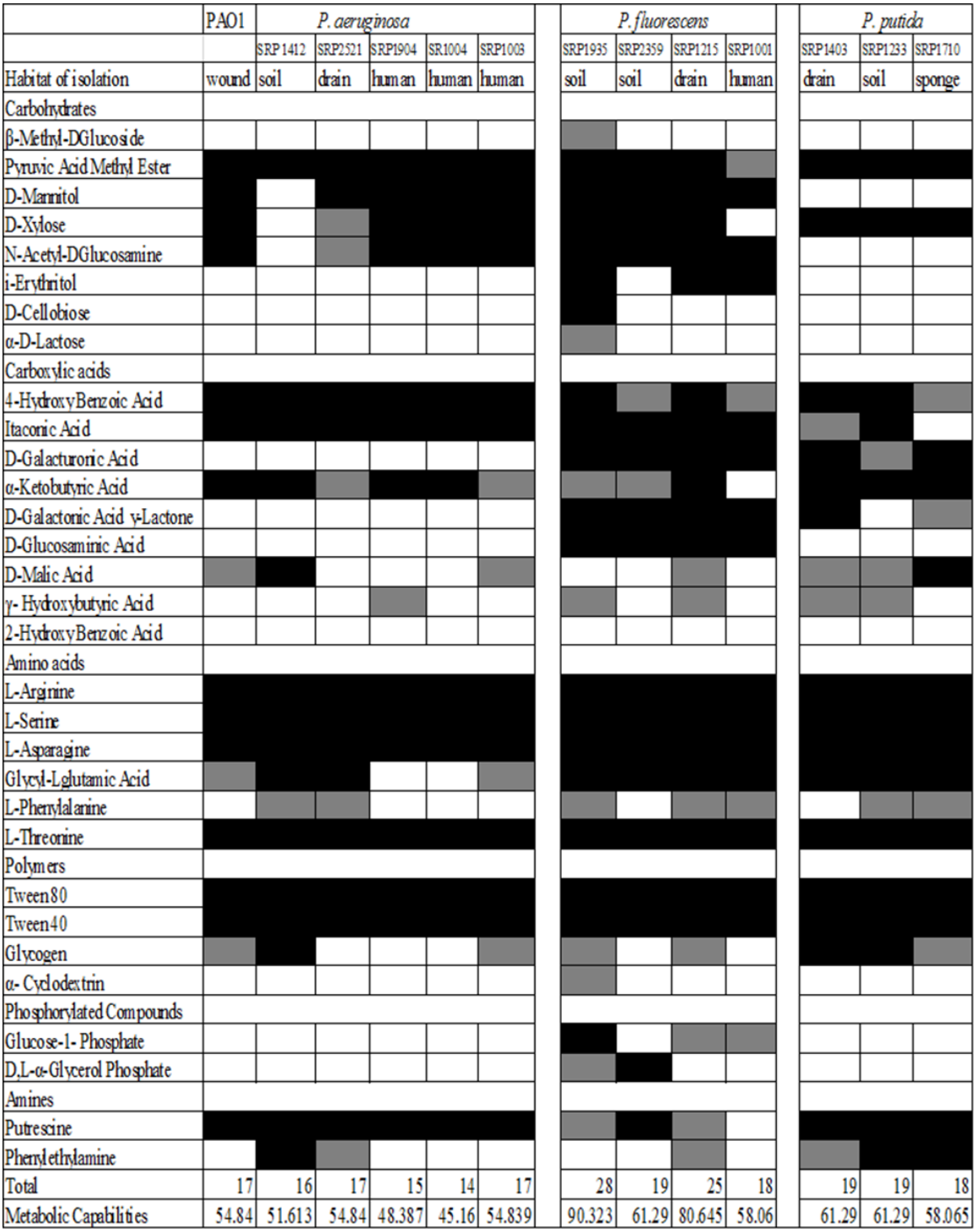
Resources	PC1	PC2	PC3
CARBOHYDRATES			
β-methyl-D-glucoside	**0.840**	0.263	0.388
pyruvic acid methyl ester	-0.460	0.076	**0.857**
D-xylose	0.450	0.072	**0.677**
I-erythritol	**0.881**	0.147	0.004
N-acetyl-D-glucosamine	0.192	**0.871**	0.173
D-galactonic acid ɣ-lactone	**0.778**	-0.045	-0.021
D-mannitol	0.171	**0.906**	-0.168
D-cellobiose	**0.762**	0.188	0.512
CARBOXYLIC ACIDS			
D-galacturonic acid	**0.732**	-0.343	-0.093
D-glucosaminic acid	**0.807**	0.167	-0.346
itaconic acid	-0.370	**0.774**	0.186
α-ketobutyric acid	-0.144	-0.148	**0.845**
γ-hydroxybutyric acid	0.451	-0.160	**0.707**
4-hydroxy-benzoic	**-0.631**	0.444	0.538
AMINO ACIDS			
L-Asparagine	-0.541	-0.169	**0.699**
Glutamic acid	0.555	**-0.704**	0.242
PHOSPHORYLATD COMPOUNDS			
Glucose-1-phosphate	**0.923**	0.224	0.164
D,L-α-glycerol phosphate	**0.754**	0.413	0.308
POLYMERS			
tween 40	-0.427	**0.664**	0.270
tween 80	-0.356	**0.618**	0.527
Glycogen	0.525	-0.212	**0.693**
α-cyclodextrin	**0.678**	0.427	0.525
AMINE			
Putrescine	**-0.620**	-0.191	**0.698**
Phenylethylamine	-0.160	**-0.809**	0.277

Figures Legends

Figure 3.1: Carbon utilization patterns (the presence or absence of the ability to use a particular resource) of *Pseudomonas* species groups. Black cells indicate carbon resources that were consistently utilized (three times out of the three independent replicates). Gray cells indicate carbon resources where the strains showed a variable response (utilized once or twice out of the three independent replicates). The white cells indicate carbon resources that were not utilized by any of the strains.

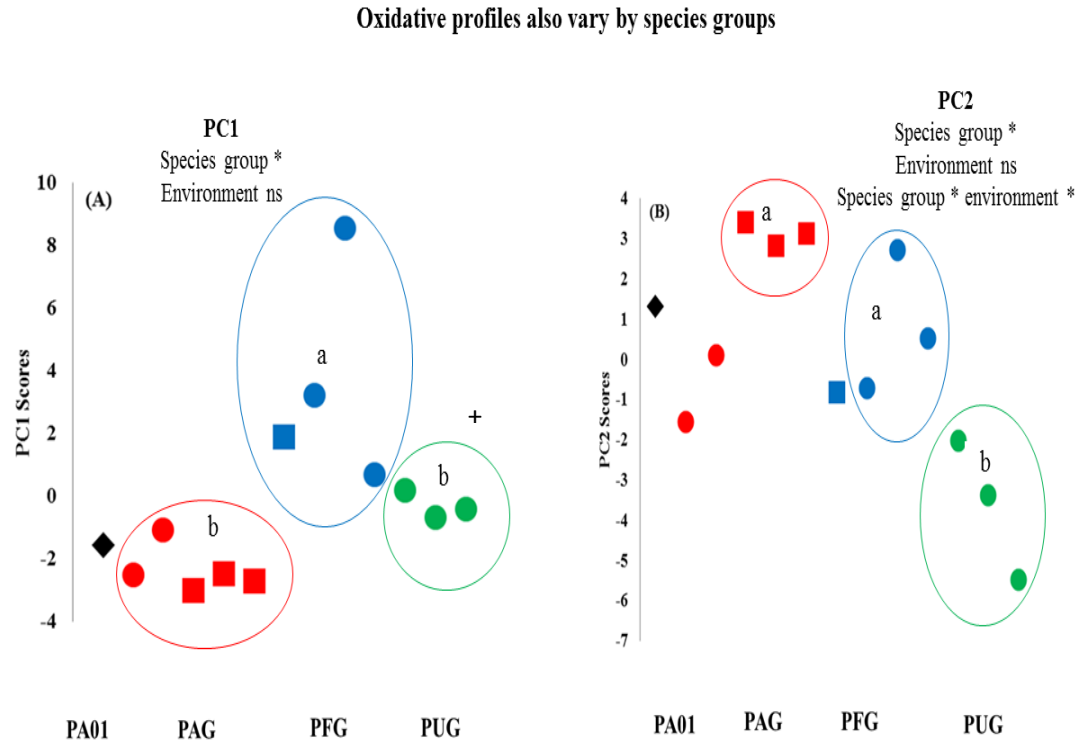
Figure 3.2: Principal component 1 (A) and Principal component 2 (B) scores of *Pseudomonas* species groups isolated from a single human home plus a reference strain of PA01. PAG = *P. aeruginosa*, PFG = *P. fluorescens* group, and PUG = *P. putida* group. Each letter that appears multiple times designates pairs of isolates or time point that did not differ significantly; isolates or time point that differed significantly ($p<0.05$) do not share letters. Contrasts were adjusted for multiple comparisons using a Bonferroni correction. The + sign on PC1 figure indicate marginal significant contrast between *P. fluorescens* and *P. putida* groups.

Figure 3.1



	PAO1	*P. aeruginosa*					*P. fluorescens*				*P. putida*		
		SRP1412	SRP2521	SRP1904	SR1004	SRP1003	SRP1935	SRP2359	SRP1215	SRP1001	SRP1403	SRP1233	SRP1710
Habitat of isolation	wound	soil	drain	human	human	human	soil	soil	drain	human	drain	soil	sponge
Carbohydrates													
β-Methyl-DGlucoside													
Pyruvic Acid Methyl Ester													
D-Mannitol													
D-Xylose													
N-Acetyl-DGlucosamine													
i-Erythritol													
D-Cellobiose													
α-D-Lactose													
Carboxylic acids													
4-Hydroxy Benzoic Acid													
Itaconic Acid													
D-Galacturonic Acid													
α-Ketobutyric Acid													
D-Galactonic Acid γ-Lactone													
D-Glucosaminic Acid													
D-Malic Acid													
γ- Hydroxybutyric Acid													
2-Hydroxy Benzoic Acid													
Amino acids													
L-Arginine													
L-Serine													
L-Asparagine													
Glycyl-Lglutamic Acid													
L-Phenylalanine													
L-Threonine													
Polymers													
Tween80													
Tween40													
Glycogen													
α- Cyclodextrin													
Phosphorylated Compounds													
Glucose-1- Phosphate													
D,L-α-Glycerol Phosphate													
Amines													
Putrescine													
Phenylethylamine													
Total	17	16	17	15	14	17	28	19	25	18	19	19	18
Metabolic Capabilities	54.84	51.613	54.84	48.387	45.16	54.839	90.323	61.29	80.645	58.06	61.29	61.29	58.065

Figure 3.2



Human associated strains = squares
Environmental strains = circles

Supplementary Information

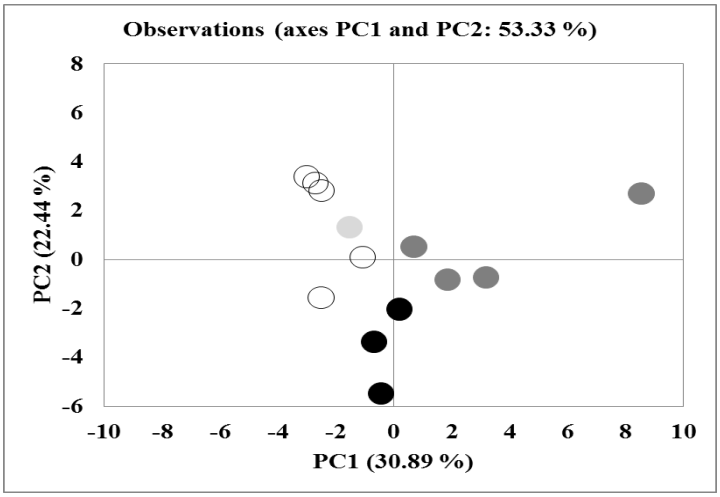


Fig. S1: First and second principal component ordination diagram of the oxidative profiles obtained from the Biolog Ecoplate for the 13 *Pseudomonas* strains used in this study. Black circles represent the *P. putida* species group, open circles represent the *P. aeruginosa* species group, light gray circle represent PA01 (the reference strain) and dark gray circles represent the *P. fluorescens* species group.

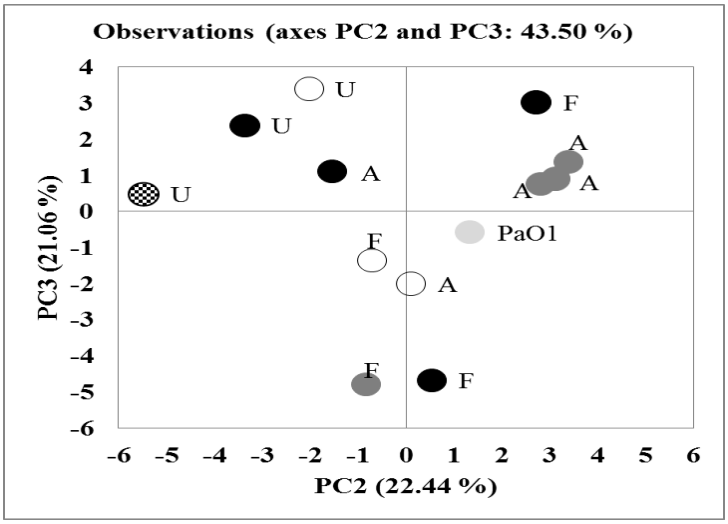


Fig. S2: Second and third principal component ordination diagram color coded to show the environments of isolation of each *Pseudomonas* strain used in this study. Dark gray circles represent human body sites, black circles represent soils, open circles represent drains, patterned circle represent kitchen sponge and light gray circle represent PA01 isolated from burn wound. Letters stand for species group, A = *P. aeruginosa* strains, F = *P. fluorescens* strains and U = *P. putida* strains.

Supplementary Information *Continued*

Table S1: Shows that *P. fluorescens* isolates are associated with higher mean CUCs for most of the carbon resources associated with high loadings in PC1.											
P. aeruginosa isolates	β-methyl-D-glucoside	i-erythritol	D-galactonic acid γ-lactone	D-cellobiose	D-galacturonic acid	D-glucosaminic acid	glucose-1-phosphate	D,L-α-glycerol phosphate	α-cyclodextrin	Putrecine	4-Hydroxy benzoic acid
PAO1	0.21695	0.198566667	0.06585	2.326941667	0.11605	0.371833333	1.874183333	2.265816667	1.874183333	26.1235	35.65375
SRP1412	0.426891667	0.227175	0.04305	2.91800833	0.492425	0.585933333	0.8525	0.792083333	0.8525	34.03397	39.53275833
SRP2521	0.082666667	0.461525	0.247125	0.964308333	0.212708333	0.631408333	0.874033333	3.0242	0.874033333	15.76856	31.02905
SRP1904	1.00395	0.857292	0.991217	1.734308333	1.15785	1.586983	2.71905	4.580216667	2.71905	36.33286	51.495075
SRP1004	0.207566667	0.477858	0.1482	0.73406667	0.5075	1.974425	2.22405	3.857325	2.22405	36.23757	46.84349167
SRP1003	0.963117	0.67901667	1.415741667	1.294925	1.33025	1.79184167	1.79295	3.627325	1.79295	35.17245833	47.89229167
Mean	**0.536384**	**0.540573334**	**0.569066733**	**1.529123333**	**0.740146667**	**1.314118267**	**1.692516667**	**3.17623**	**1.692516667**	**31.50908367**	**43.35853333**
P. fluorescens isolates											
SRP1001	0.664633333	10.56820833	16.774475	0.960708333	27.757525	13.926525	10.62955	1.194925	0.44361667	0.2799	2.410866667
SRP1215	2.224983333	23.91951667	28.44845	1.04343333	46.01113333	25.73688333	8.75635	1.828833333	0.886741667	1.792275	20.35031667
SRP1935	20.7748	26.27788333	33.28361	16.892925	36.82629167	31.01854167	32.15773333	23.32571667	10.72258333	8.422408	12.651225
SRP2359	0.32705	0.008691667	24.60339	0.7448	5.7056	31.16155	1.446758333	7.544525	0.154625	9.788542	7.5724
Mean	**5.997866667**	**15.193575**	**25.77748125**	**4.910466666**	**29.0751375**	**25.460875**	**13.24759792**	**8.473500001**	**3.051891667**	**5.07078125**	**10.74620208**
P. putida isolates											
SRP1403	2.2859	0.448583333	35.13621	4.523975	7.914333	2.509066667	2.566117	4.926858	2.327517	51.1646	45.18968333
SRP1233	0.800383333	0.709091667	0.093375	3.311233333	4.044933333	3.053241667	1.689308333	2.852983333	1.292433333	**38.7246833**	**27.88168333**
SRP1710	1.1201	0.697308	4.716075	1.57225	40.306525	0.881808333	2.028341667	1.087367	0.686808333	38.67493	5.179541667
Mean	**1.402127778**	**0.618327667**	**13.31522**	**3.135819444**	**17.42193044**	**2.148038889**	**2.094589**	**2.955736111**	**1.435586221**	**42.85473777**	**26.08363611**

Supplementary Information *Continued*.

Table S2: Shows that *P. aeruginosa* isolates are associated with higher mean CUCs some of the carbon resources associated with high loadings in PC2.

***P. aeruginosa* isolates**	**N-acetyl-D-glucosamine**	**D-mannitol**	**itaconic Acid**	**tween 40**	**tween 80**	**glutamic**	**phenylethylamine**
PA01	24.06603333	31.622225	39.134625	36.50552	18.7433	9.296225	1.234725
SRP1412	1.171425	0.251817	31.2058083	39.413642	16.14521	26.8929	18.35765833
SRP2521	4.409741667	25.4777	38.8276583	40.38221	12.41106	13.20273	10.51090833
SRP1904	33.03354167	42.25615	53.4209	51.11005	38.23404	5.283475	5.181208333
SRP1004	33.95456667	45.44442	52.571275	49.224233	36.38368	6.8535	4.659766667
SRP1003	35.18605833	43.8853917	53.9578333	49.422483	37.37002	3.587683	3.358358333
Mean	**21.55106667**	**31.4630957**	**45.996695**	**45.910524**	**28.1088**	**11.1641**	**8.413579999**
***P. fluorescens* isolates**							
SRP1001	14.548275	19.14283	4.24133333	20.79568	14.09654	25.23793	2.660391667
SRP1215	18.00508333	34.7571667	31.2886667	27.163225	12.17194	23.28861	6.975625
SRP1935	40.27075	45.2857333	30.315475	34.12362	23.62426	30.84636	2.711425
SRP2359	8.336366667	35.189442	22.4102667	28.4066	13.76718	12.64952	0.247308333
Mean	**20.29011875**	**33.593793**	**22.0639354**	**27.622281**	**15.91498**	**23.0056**	**3.1486875**
***P. putida* isolates**							
SRP1403	2.193833	1.364467	4.79810833	37.91776	24.32423	26.82284	7.076817
SRP1233	1.547758333	0.709225	29.6669667	11.85137	21.69236	34.35255	34.06656667
SRP1710	0.933816667	0.529983	0.81144167	29.537108	15.30253	26.4837	48.48265
Mean	**1.558469333**	**0.86789167**	**11.7588389**	**26.435413**	**20.43971**	**29.2197**	**29.87534456**

Table S3: Shows that P. putida isolates are associated with higher mean CUCs for the carbon resources associated with high loadings in PC3 except for pyruvic acid methyl ester.

***P. aeruginosa* isolates**	**Pyruvic acid methyl ester**	**D-Xylose**	**L-Asparagin**	**γ-Hydroxybutyric acid**	**Glycogen**	**α-Ketobutyric acid**	**Putrescine**
PAO1	29.77823333	17.8038	69.7064333	2.729891667	10.6926	11.4688	26.1235
SRP1412	38.384625	5.158517	64.21365	5.605616667	14.4798	23.58015	34.03397
SRP2521	19.38596667	13.58073	54.5967583	6.073575	7.10715	9.933766667	15.76856
SRP1904	35.912525	17.78879	67.05246	5.232441667	6.04977	17.3816	36.33286
SRP1004	35.508225	19.9953	62.6891917	4.177858333	5.49078	15.54551667	36.23757
SRP1003	36.14979167	17.48821	64.801325	4.678533333	5.86379	20.58816667	35.17245833
Mean	**33.06822667**	**14.802309**	**62.670677**	**5.153605**	**7.79826**	**17.40584**	**31.50908367**
***P. fluorescens* isolates**							
SRP1001	8.117533333	4.777267	39.229	4.103866667	5.28703	3.433208333	0.2799
SRP1215	22.16405833	23.05021	55.0696667	6.447125	10.4129	12.862275	1.792275
SRP1935	25.12504167	32.34288	49.9092417	10.86785833	20.4299	19.14271667	8.422408
SRP2359	11.13645	9.693758	51.2203417	1.730891667	4.08364	7.035158333	9.788542
Mean	**16.63577083**	**17.466029**	**48.8570625**	**5.787435416**	**10.0534**	**10.61833958**	**5.07078125**
***P. putida* isolates**							
SRP1403	38.8586	21.72278	78.086825	11.86715	17.1754	22.7531917	51.1646
SRP1233	32.19775833	27.36576	68.0457167	9.484708333	13.728	32.391225	38.7246833
SRP1710	28.3144	19.21705	66.1852833	3.970525	8.48553	13.09291667	38.67493
Mean	**33.12358611**	**22.76853**	**70.7726083**	**8.440794444**	**13.1297**	**22.74577779**	**42.85473777**

CHAPTER FOUR

GROWTH PROFILES OF STRAINS OF *PSEUDOMONAS AERUGINOSA* GROUP ISOLATED FROM MULTIPLE HUMAN HOMES

Summary-The *Pseudomonas aeruginosa* group is able to inhabit variable environment types and utilize a variety of resources for growth and energy. Understanding the role of resources in structuring human associated strains of *P. aeruginosa* group could be of practical importance in understanding its biogeography in human home environments. In this research, I studied the growth of 75 strains of the *P. aeruginosa* group isolated from household drains, surfaces, water, trash, soils, sponges, and from the humans, an animal in the homes, and a reference strain of *Pseudomonas aeruginosa,* PA01. I evaluated their resource utilization capabilities (presence/absence of the ability to use a particular resource) in D-mannose, D-mannitol, N-acetyl glucosamine (NAG), D-galactose, alpha-ketobutyric acid, L-rhamnose and L-glutamic acid. Though the strains showed great variability in growth profiles, I detected no association between environment of isolation and resource use profiles of the strains. Principal component analysis (PCA) of the growth rate profiles revealed random distribution of the strains. Mixed linear models with mean growth rate for each strain as the response variable, and environment of isolation as a fixed predictor and house of isolation and its interaction with environment of isolation as random predictors identified significant differences among strains in growth rate in L-glutamic acid ($p = 0.02$). In addition, PERMANOVA of the growth rate

profiles with environment of isolation as the predictor variable and the growth rate profiles of the strains as the response matrix did not detect any significant effect ($p > 0.05$). The large amount of variance in growth rate profiles for strains within any of the environments for a given resource provide evidence for quantitative differences in the use of these resources. Differences in quantitative abilities could be adaptive and may reduce intraspecific competition with consequences for the coexistence of these species in human home environments.

INTRODUCTION

Pseudomonas aeruginosa group include *P. citronellolis, P. multiresinivorans, P. nitroreducens, P. otitidis, P. alcaligenes,* and *P. aeruginosa* (type species for *P. aeruginosa* group) (Anzai et al. 2000; Gomila et al. 2015). These bacteria are human associated and have been recovered from human home environments (Remold et al. 2011; Purdy-Gibson et al. 2014). *P. citronellolis* has been shown to utilize toxic compounds such as poly aromatic hydrocarbons (Jacques et al. 2005) and methyl *tert*-butyl ether (Bravo et al. 2015), and *P. otitidis* causes acute and chronic ear infections in humans (Clark et al. 2006). *P. alcaligenes,* not included in this study, is a rare opportunistic human pathogen, that is resistant to several antimicrobial agents (Suzuki et al. 2013) and capable of arsenic biotransformation (Zhang et al. 2015).

P. aeruginosa is an opportunistic pathogen that causes acute and chronic infections in humans (Palmer et al. 2005; Khan et al. 2007; Goldberg et al. 2008; Mulcahy et al. 2010). Free living *P. aeruginosa* strains are found in a variety of environments such as soils, aquatic habitats, plants, animals (Kiewitz and Tummler, 2000; Khan et al. 2007; Kida et al. 2010) and human associated ecological niches (Remold et al. 2011;

Workentine et al. 2013; Purdy-Gibson et al. 2014; Remold et al. 2015). *P. aeruginosa* is described as "ubiquitous" but more recently research is revealing that its distribution is in fact restricted.

Remold et al. (2011) found *P. aeruginosa* to be over represented in household drains and Selezska et al. (2012) found it to be predominantly in aquatic habitats. The ability of *P. aeruginosa* to occupy different ecological niches may be a consequence of its large genome and extensive ability to sense, respond and grow in a variety of resource environments (Stover et al. 2000). According to Kiewitz and Tummler (2000), *P. aeruginosa* strains possess high codon adaptation indices (0.55 to 0.85) (adaptation index is a quantitative measure of synonymous codon bias of genes), which facilitates its adaptation to new environments. *P. aeruginosa* can grow in minimal media (Stover et al. 2000) and can also survive in low resource environments (Kida et al. 2010). It has genes necessary for the synthesis of amino acids, nucleic acids and cofactors, it can import a variety of nutrients and other molecules from its environment using cytoplasmic transport systems, and though it has limited ability to grow on sugars it can use other carbon compounds for growth and energy (Stover et al. 2000).

Resources help to regulate the behavior, growth, reproduction, interactions and distribution of microbes. Differences among microbes in the resources used for growth and energy could help explain differential distribution among environment types. Human homes contain sites that differ in resources and environmental conditions and those could lead to local adaptations of human associated *P. aeruginosa* group. Natural selection could be imposed by the nutritional resources available in an organism's environment (Wong et al. 2012), such as those found in human homes and host environments.

There is the need to understand the relationship between human associated *P. aeruginosa* group and their resource environments as such information can be of practical importance in understanding the mechanisms that shape the biogeography of *P. aeruginosa* group in human home environments. D-mannitol, N-acetyl glucosamine, L-glutamic acid, and α-ketobutyric acid are resources used in this chapter that were chosen from the Biolog Ecoplate™ resources tested in a previous study (Chapter Three); these resources reflect those in which the *Pseudomonas* groups showed variability across strains in their utilization. In this chapter, I examined the resource utilization patterns of human associated strains of *P. aeruginosa* group to understand if they are influenced by the environment types of isolation. I also tested the hypothesis that the growth rate profiles of these strains in a variety of resources were influenced by their environments of isolation. Addressing these research questions sheds light on the relationship between human associated *P. aeruginosa* group and their environments of isolation based on resource utilization, and on the role of local resource adaptation in structuring *P. aeruginosa* species group in human associated environments.

CHAPTER FOUR MATERIALS AND METHODS

A total of 75 strains of *P. aeruginosa* group, comprised of 52 *P. aeruginosa*, 18 of *P. nitroreducens*, 3 of *P. otitidis*, and 2 of *P. citronellolis* were investigated in this study (Appendix). These strains were isolated from household drains (n = 29), surfaces (n = 11), water (n = 10), trash (n = 4), soils (n = 6), CF equipment (n = 1), sponges (n = 3), humans, and animal (H&A) (n = 11) (Remold et al. 2011; Purdy-Gibson et al. 2014). Briefly, strains were isolated by swab-sampling areas in the human home, streaking for

isolation on *Pseudomonas* isolation agar (PIA), re-streaking for isolation of a single colony of each distinct colony morphology from each sampled site, and freezing in 12-15% glycerol in LB medium. Strains were then identified by sequencing at least 500 base pairs of the 16S ribosomal DNA, and comparing this sequence with the Bioinfo 1200 database (Purdy-Gibson et al. 2014; Remold et al. 2015). Strains were then assigned to species groups as defined in Anzai et al (2000).

A reference clinical strain of *P. aeruginosa*, PA01was also included. PA01 is a derivative of the original Australian PAO that was isolated from wound (formerly called *Pseudomonas aeruginosa* strain 1) (Holloway, 1955; Klockgether et al. 2010). PA01 is a well characterized laboratory strain, clones of PA01 have been sequenced multiple times, and PA01 has been used in several studies (Stover et al. 2000; Frimmersdorf et al. 2010; Workentine et al. 2013). PA01 is the most common reference or control strain of *P. aeruginosa* used in laboratories around the world (; Klockgether et al. 2010).

These strains were studied for their growth profiles in 11 different minimal media that contained D-mannose, D-mannitol, N-acetyl glucosamine (NAG), D-galactose, alpha-ketobutyric acid (keto), L-rhamnose, L-glutamic acid, D-glucose, DNA, alginate and L- 3, 4-dihydroxyphenyalanine (L-DOPA) as a single carbon resource in each medium. D-mannose, D-mannitol, NAG, D-galactose, keto, L-rhamnose, and L-glutamic acid were included in the PCA and downstream analyses, while D-glucose, DNA, alginate and L-DOPA are discussed separately (see below).

Preparation of the Characterization Medium and the Carbon Resources

Tryptic soy agar (TSA) and broth (TSB) prepared without added glucose (Atlas, 2010) were used as preconditioning media. The characterization medium (CM) was prepared by adding 1.25 g of ammonium phosphate monobasic ($NH_4H_2PO_4$), 0.25 g of potassium chloride (KCl), 0.25 g of magnesium sulfate ($MgSO_4.7H_2O$) to 1 L of reverse osmosis (RO) H_20 (Kennedy, 1994). The pH of the CM was adjusted to 7.0 using 1.0 N NaOH, after which the CM was autoclaved (Kennedy, 1994). After autoclaving, 100 ml of the CM were dispensed into 200 ml sterile glass bottles and with the exception of DNA and alginate, each of the carbon resource was prepared for use at a final concentration of 0.1% (w/v) and filter sterilized with a 0.2 µm pore size filter (Kennedy, 1994). DNA (salmon sperm ultrapure DNA, invitrogen™) for use as a carbon source was prepared at a concentration of 2.5 mg/ml (Mulcahy et al. 2010) in sterile CM. Alginate minimal medium was autoclaved for sterilization, because filter sterilization of alginate proved difficult because it is a large molecule. Tetrazolium blue chloride solution was filter sterilized and added to each CM + carbon resource in the sterile glass bottle at a final concentration of 0.02% (v/v) as an indicator of bacterial respiration; tetrazolium blue chloride turns blue when cells utilize the carbon resource.

Preparation of Bacteria Strains

Strains frozen in 12-15% glycerol at -80^{o}C were preconditioned on (TSA) for 24 hours at 28^{o}C. Cells were then transferred into tryptic soy broth (TSB) and incubated in a shaking incubator (150 rpm) and grown for 6 hours (early log phase based on the growth

curves of some strains in Luria broth, Chapter Two) at 28^{o}C. Cells were then transferred to 15ml centrifuge tubes and harvested by centrifugation at 4000 rpm for 15 minutes and rinsed thrice in 1x phosphate buffered saline (PBS) to remove all exogenous carbon resources. The pellet was re-suspended in 1 ml of 1x PBS, after which cells were diluted 1:20 in 1x PBS. One hundred microliters of the carbon resource media was dispensed into each well in a 96 well microplate and 50 µl of the diluted bacterial suspension was added into the wells.

Microplates were incubated in plate readers (Tecan Infiniti F 200, Tecan Sunrise and Spectramax), and absorbance at $OD595_{nm}$ measured every 15 minutes for 48 hours. The approach used, 1:20 dilution as opposed to adjusting cell density to a fixed OD, does not standardize starting cell density. It does allow density to vary with natural stationary phase density differences between strains (Francisco Moore, personal communication, July 28, 2015). Starting cell density ranged between 0.126 to 0.33 OD for most of the blocks. Three plate readers were used in this study to accommodate for a sample size of 76 with 3 technical replicates performed on 3 separate days and the inability to fit more than 6 strains in one 96 well microplate.

Each strain was tested for its ability to grow in the 11 different carbon resources. All of the resources were tested in each plate (block), only six strains were evaluated/plate such that approximately 14 microplates made up one replicate. Three complete replicates were performed for each strain and strains were tested with unbalanced distribution across plate readers. Each replicate was blanked against the average of the first three readings to accommodate for differences in cell density.

A set of three control plates with 18 replicate wells/plate of PA01 grown in TSB inoculated from a single preconditioning preparation were evaluated on the three plate readers. Although, the Tecan Sunrise and Spectramax did not differ significantly in the growth rates they yielded, the Tecan Infiniti differed from both of the others. The difference in growth rate between the Tecan Infiniti and the other two readers is 5×10^{-6} hr^{-1}, this value lies within the 95% confidence interval (3.5×10^{-2}) (data not shown) for the growth rates in L-glutamic acid for all the strains across environment types. L-glutamic acid is a resource that was utilized by most of the strains. This suggest that the variability contributed by the Tecan Infiniti is less than the variability of the strains across environment types.

Resource Utilization Capabilities and Growth Rate Profiles

The data were exported into the Curve Fitter program (http://www.evolvedmicrobe.com/CurveFitter/Download.html) (Delany, 2011) for scoring of resource utilization and calculation of growth rate. The data were blanked by subtracting the average of the first three-absorbance readings from the OD values generated during the kinetic cycle. The ability to utilize a particular resource was determined based on evidence of growth (positive OD values) after blanking and a maximum OD of 0.1 and above. Resource utilization capability was coded as 0 or 1, where 0 means the growth of the isolate was not supported by the carbon resource and 1 means the growth of the isolated was supported by the resource in the three technical replicates. If a strain utilized a resource one time out of the 3 replicate it was scored 0.33 and 0.66 for 2 out of the 3 technical replicates. The growth rate for each strain in different

resources was fitted with the exponential model using the Curve Fitter program. Growth rates were calculated with data points between the minimum OD to fit and 75% of the maximum absorbance values. Outliers were excluded to improve the R^2 and obtain better fit curves.

Statistical Analyses

The growth rates in D-mannose, D-mannitol, N-acetyl glucosamine (NAG), D-galactose, alpha-ketobutyric acid, L-rhamnose, and L-glutamic acid (7 resources) for isolates of *P. aeruginosa* group isolated from human home habitats were mean centered and analyzed by principal component analysis (PCA) using XLSTAT 2014.4.01 (http://xlstat-win.soft112.com). PERMANOVA was done using Primer statistical analysis software (Clarke et al. 2014) to test for significant effect of environment of isolation (predictor variable) on growth rate profiles (response variable) of human associated isolates of *P. aeruginosa* group.

RESULTS

Resource Utilization Capabilities

I evaluated the resource utilization capabilities of household isolates of *P. aeruginosa* group by testing their abilities to grow in 11 different carbon resources. Seven of those are shown in (Table 4.1): D-mannose, D-mannitol, N-acetyl glucosamine (NAG), D-galactose, alpha-ketobutyric acid, L-rhamnose, and L-glutamic acid. Glucose, L-DOPA, DNA and alginate (4 resources) were excluded from the analyses. Glucose was excluded to eliminate redundancy in the data set, because all of the isolates utilized it. L-

DOPA was excluded because it is capable of oxidation at the slightest exposure to oxygen; separating self -oxidation from real growth was not possible in this assay. DNA and alginate were excluded because isolates showed unusual growth curves and many strains had too many negative OD values after blanking.

Household isolates of *P. aeruginosa* group were variable across replicates in their resource utilization profiles (Table 4.1). There were patterns, where a resource was either utilized once or twice out of the three technical replicates (Table 4.1). D-mannose, L-rhamnose, D-galactose and α-ketobutyric acid showed the most variability, while D-mannitol, NAG and L-glutamic acid showed the least variability in resource use patterns (Table 4.1). The majority of the strains utilized all the resources offered.

Growth Rate Profiles

To test whether the growth rate profiles of strains of *P. aeruginosa* group were influenced by environments of isolation, I also evaluated their growth rate profiles in D-mannose, L-rhamnose, D-galactose, D-mannitol, NAG, L-glutamic acid and α-keto butyric acid. Result of the principal components analysis (PCA) of the growth rates in these seven resources is shown in Figure 4.1. A second PCA was conducted in which growth in glucose was included. Because results of the two analyses did not differ qualitatively, only the seven resources included in the utilization analysis (previous section) is shown.

The first 3 components of the PCA model explained 77% of the variance in the growth rate profiles of strains of *P. aeruginosa* group. D-mannose, D-galactose, L-

rhamnose and D-mannitol (carbohydrates) accounted for majority of the variance in principal component (PC) 1, L-glutamic acid and NAG (amino acid and amino sugar) explained majority of the variance in PC 2, while, α- ketobutyric acid (carboxylic acid) accounted for majority of the variance in PC 3 (Table 4.2). These loadings of variables of the PCAs indicate the variance of the PCA is broken down by resource similarity. Pearson's pairwise correlation coefficients indicate that correlations among growth in different resources are strongest among sugars (mannose, galactose and rhamnose, $r >$ 0.6, $p <.0001$ for all pairwise comparisons) and between NAG and mannitol ($r = 0.66$, $p <.0001$).

There was no association between environment of isolation and growth rate profiles; in addition, no phenotype or dominant ecotype could be linked to any environment. Mixed linear models with mean growth rate for each strain as the response variable, and habitat of isolation as a fixed predictor and house of isolation and its interaction with habitat of isolation as random predictors identified significant differences among strains in growth rate in L-glutamic acid ($p = 0.02$), (Fig 4.2). There were no significant differences among habitat types in any of the other resources.

A permutational ANOVA (PERMANOVA) with the Euclidean distance matrix calculated from the mean growth rate of each strain in the 7 resources, and environment type (fixed) and house of isolation (random) and their interaction as predictor variables did not show any significant effect of any of the three predictors on the overall growth rate profiles of the household *P. aeruginosa* isolates studied ($p > 0.05$). The PERMANOVA was performed with and without glucose and there was no qualitative difference detected. The variability in resource utilization by *P. aeruginosa* strains

isolated from human home environments is shown in (Fig. 4.3), the isolate from the CF equipment was excluded from Fig 4 3 because the CF equipment lacked biological replication. Findings showed large amount of variability within each environment for any given resource (Fig. 4.3). PA01 either showed high or intermediate growth phenotype in all the resources.

These results show quantitative differences in growth rates in different resources. There was no specific pattern associated with any resource or environment in the growth rate profiles (Fig. 4.3). The majority of the strains had lower growth rates in α-ketobutyric acid and the highest growth rates were in mannitol, glutamic acid and NAG (Fig 4.3).

DISCUSSION

Resources generally help to determine where an organism is found. In this study, I considered resources in which 12 household *Pseudomonas* from three species groups and PA01 showed variability in utilization (Chapter Three) and assessed whether they are differentially used by isolates of *P. aeruginosa* group from different environments.

Resource Utilization and Growth Rate Profiles are Highly Variable among Household *P. aeruginosa* Group Isolates

Household strains of *P. aeruginosa* group showed diversity in both resource utilization and growth rate profiles. Previous studies have also reported the metabolic diversity of strains in the genus *Pseudomonas* (Stanier et al. 1966; Wilson and Lindow, 1994; Johnson et al. 1996; Wiedmann et al. 2000; Aagot et al. 2001; Meyer et al. 2004;

Oksinska et al. 2011). Stanier et al., (1966) showed that *P. aeruginosa* strains can utilize a wide range of resources including carbohydrates, organic acids, amino acids and other nitrogenous compounds, alcohols, amines, aromatic compounds and many more groups of resources. Findings from this study are consistent with *P. aeruginosa* being able to utilize a variety of resources.

There was variability among technical replicates not only in growth rates, but even in whether or not any use of a resource was detected (Table 4.1). Wiedmann et al., (2000), Riley et al. (2001), and Aagot et al., (2001) also had similar findings of differences in the ability to utilize a resource between technical replicates. Differences in carbon utilization between replicates may result from stochastic events. Variability can also result from replicate to replicate differences in cell density. In this study, cells where adjusted to 1:20 dilution, rather than adjust to a fixed OD. The starting bacterial density in this study were in the range (0.126 -0.33 OD) and varied between replicates.

To accommodate for differences in starting densities, I blanked readings for each well using the average of the first 3 readings, rather than blanking against a common control well. This strategy controls the location of the baseline measurement for differences in starting density, but it is still possible that starting density variation can have additional effects not controlled for in my experiment. For example, if some strains have a threshold density required for use of a particular resource, it is also possible that some of this among-replicate variability is attributable to an Allee effect (Courchamp, Clutton-Brock and Grenfell, 1999). An Allee effect results in populations exhibiting one phenotype when they occur above the threshold density (in this case, this phenotype

could be utilization of a resource), and a different phenotype (here failure to use a resource) when below the threshold.

Resource Utilization and Growth Rate Profiles also cannot be predicted from Environment of Isolation

Despite substantial variability in resource utilization and growth rate among the resources, among-strain variability was not strongly correlated with environment of isolation (Table 4.1, Figures 4.1 & 4.3). This is consistent with the work of others (Pirnay et al. 2005; Wiehlmann et al. 2007; Tielen et al. 2011; Kidd et al. 2012; Workentine et al. 2013). There is no dominant group or phenotype of *P. aeruginosa* that has been associated with any environment (Wiehlmann et al. 2007).

However, there are studies that have found associations between *P. aeruginosa* strains and their environments of isolation. Selezska et al., (2012), showed that *P. aeruginosa* population structure in a river in northern Germany may be influenced by water quality (different levels of pollution). Khan et al. (2007) also found that there may be *P. aeruginosa* clones unique to the open ocean, but that there was evidence of phenotypic heterogeneity. While the freshwater and animal strains showed more diversity, there was no genetic difference between environmental, and clinical strains (Khan et al., 2007). This is consistent with the finding of environment influencing the distribution of household *Pseudomonas* strains in chapter two. Although, *P. aeruginosa* is notorious as an opportunistic human pathogen (Palmer et al. 2005; Khan et al. 2007; Goldberg et al. 2008; Mulcahy et al. 2010), it is a bacterium that is routinely isolated from environments contaminated with organic compounds such as crude oil (Norman et al. 2002; Zhang et al. 2012).

Variability in Growth Rate Profiles May Have Ecological Relevance

According to Fig 4.3, strains with a range of growth rate profiles can co-exist in human home environments. The variability in growth rates could reflect quantitative differences in the rate of conversion of a resource into cells. This provides evidence of divergence in their growth rate profiles. Organisms show phenotypic heterogeneity in fitness traits to overcome biochemical and ecological constraints (Zhang and Hill, 2005). Phenotypic variance can promote niche colonization and potential for future niche differentiation. MacLean et al., (2005) showed that evolved *P. fluorescens* populations showed differentiation in their ability to utilize different resources. Experimental evolution has shown that *E. coli* strains can diversify into two ecotypes identifiable by their different 24 hour growth profiles; one ecotype evolved to become slow switchers, and the other ecotype evolved to become fast switchers between diauxic growth phases over 1000 generations (Tyerman et al., 2008).

Pseudomonas strains are known to undergo high rate of recombination (Wiehlmann et al. 2007; Kidd et al. 2012); in the absence of a particular carbon resource the genes necessary for the metabolism of that resource may be lost by mutation (Meyer et al. 2004). These results support previous research that *Pseudomonas* strains can use a broad range of resources, exhibit tremendous diversity in various traits, and no pattern can be discerned based on environment of isolation. Intraspecific variability in resource utilization may contribute to the overall fitness of *P. aeruginosa* group in multiple environments with multiple resources. Variability in traits could reduce intraspecific competition, promote co-existence and influence distribution patterns of microbes.

Tables

Table 4.1. Resource utilization patterns of household strains of *P. aeruginosa* group. Black cells indicate the carbon resources that were consistently utilized in the three replicates, dark gray cells indicate utilization was detected in 2 of the 3 replicates, light gray cells indicate utilization was detected in one replicate and white cells indicate utilization was never detected.

Environment	Mannose	Galactose	Rhamnose	Mannitol	Glutamic	NAG	Keto
PA01							
Drain							
Drain							
Drain							
Drain							
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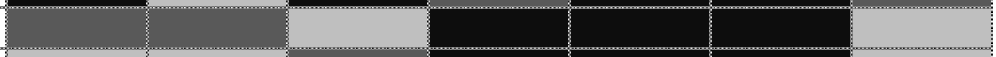
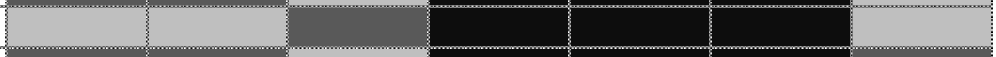
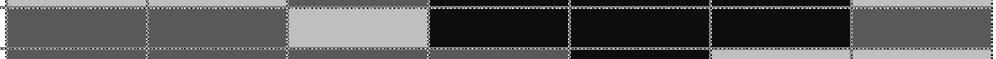
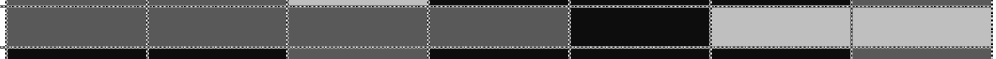
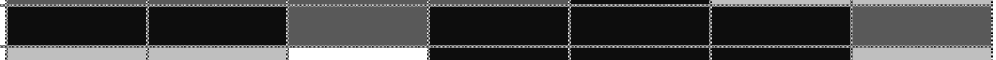
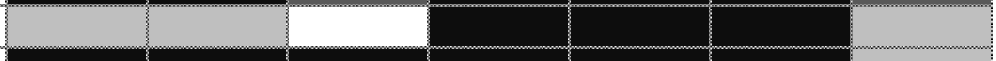
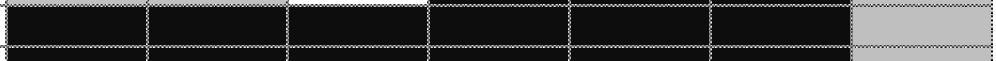
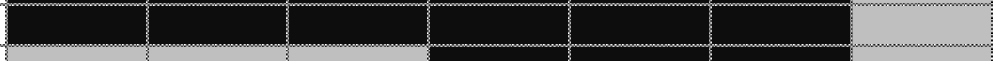
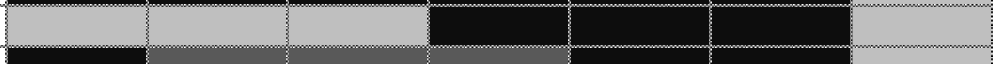
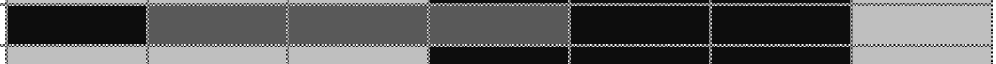
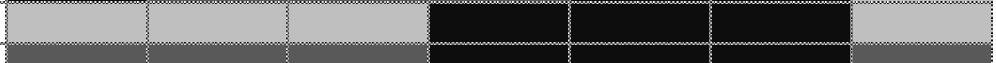
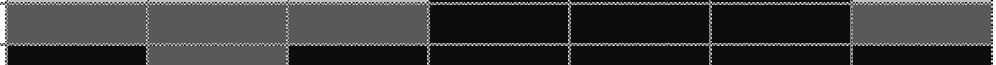
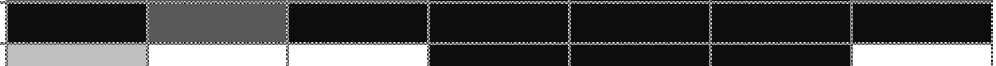
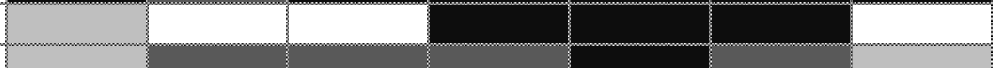
Table 4.1 ***continued***

Drain	
Drain	
Drain	
Drain	
Drain	
Drain	
Soil	
Soil	
Soil	
Soil	
Soil	
Soil	
Surface	
Surface	
Surface	
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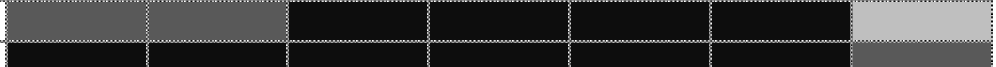
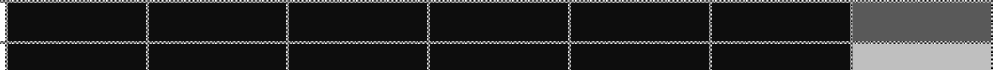
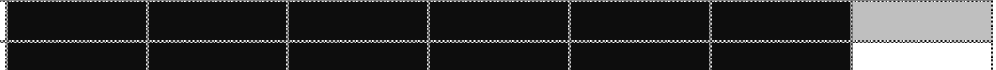
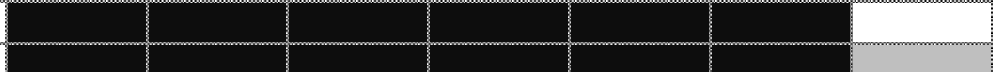
Surface	
Surface	
Surface	
Surface	
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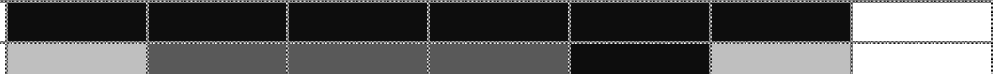
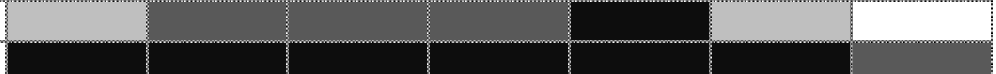
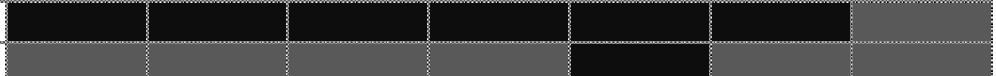
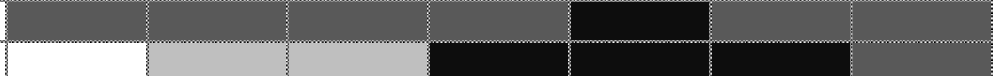
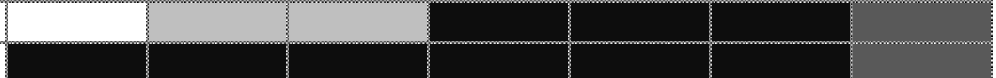
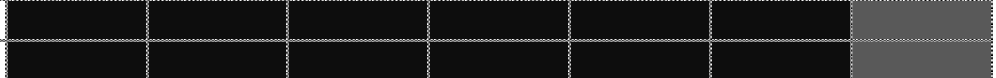
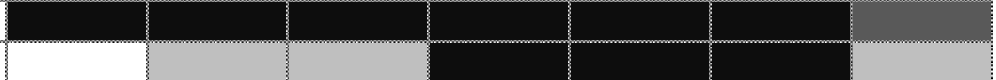
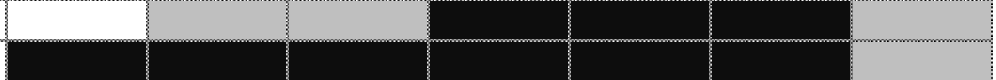
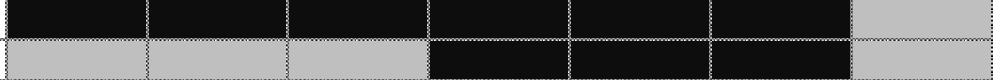
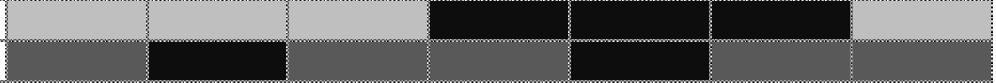
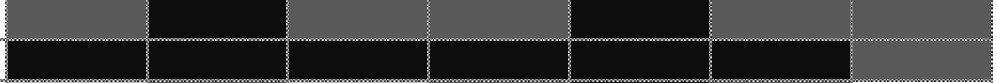
Surface	
Surface	
H&A	
H&A	
H&A	
H&A	
H&A	
H&A	
H&A	
H&A	
H&A	
H&A	
H&A	

Table 4.1 ***continued***.

Water							
Water							
Water							
Water							
Water							
Water							
Water							
Water							
Water							
Water							
Trash							
Trash							
Trash							
Trash							
Sponge							
Sponge							
SP							
CF							

Table 4.2. The contribution of (loading) each of the seven resources included in a principle components analysis of growth rates in a range of resources of PA01 and 75 isolates belonging to *Pseudomonas* species group isolated from human home environments. Values in bold represent loadings higher than 0.6.

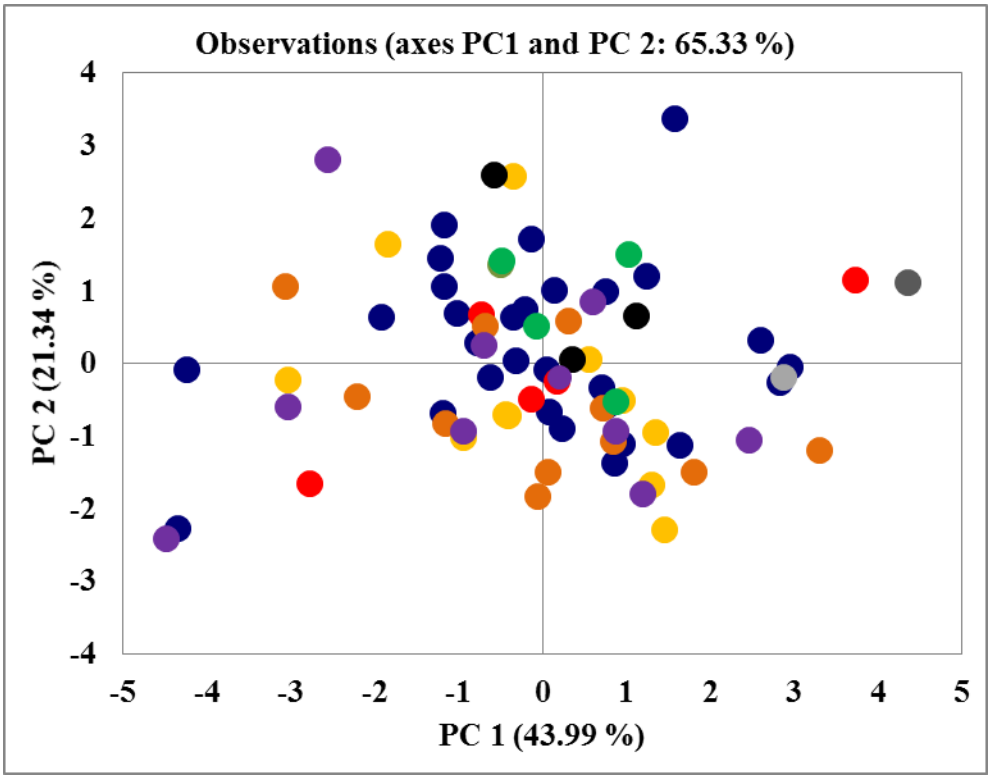
	PC1	PC2	PC3
Mannose	**0.78647**	-0.3382	-0.0853
Galactose	**0.81527**	-0.3359	-0.1792
Rhamnose	**0.76312**	-0.3579	-0.2148
Mannitol	**0.74363**	0.42633	-0.0432
Glutamic	0.33675	**0.68859**	0.12518
NAG	0.57435	**0.65261**	-0.0497
Keto	0.46671	-0.2374	**0.84756**

Figure 4.1: Relationship between the first two principal components from the PCA of the growth rates in different resources for strains of *P. aeruginosa* group isolated from human home environments.

Figure 4.2: Growth rate of strains of *Pseudomonas aeruginosa* group in L-glutamic acid ± 95% confidence interval of the mean.

Figure 4.3. Growth rate profiles of household isolates of *Pseudomonas aeruginosa* group in different media types.

Figure 4.1.



PA01 = H&A = Sponge =

Trash = Surfaces= Water =

Soils = Drains = CF equipment =

Figure 4.2

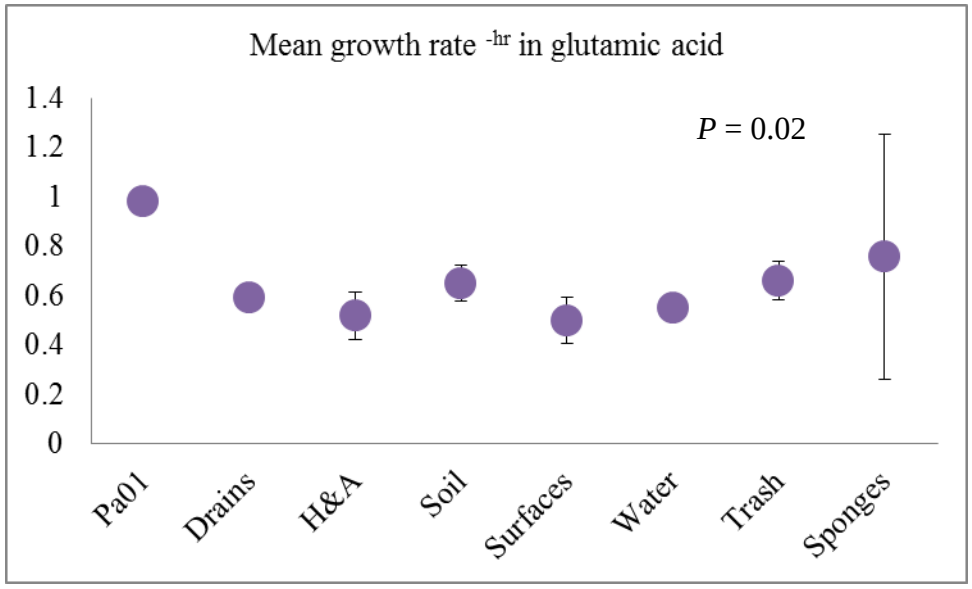
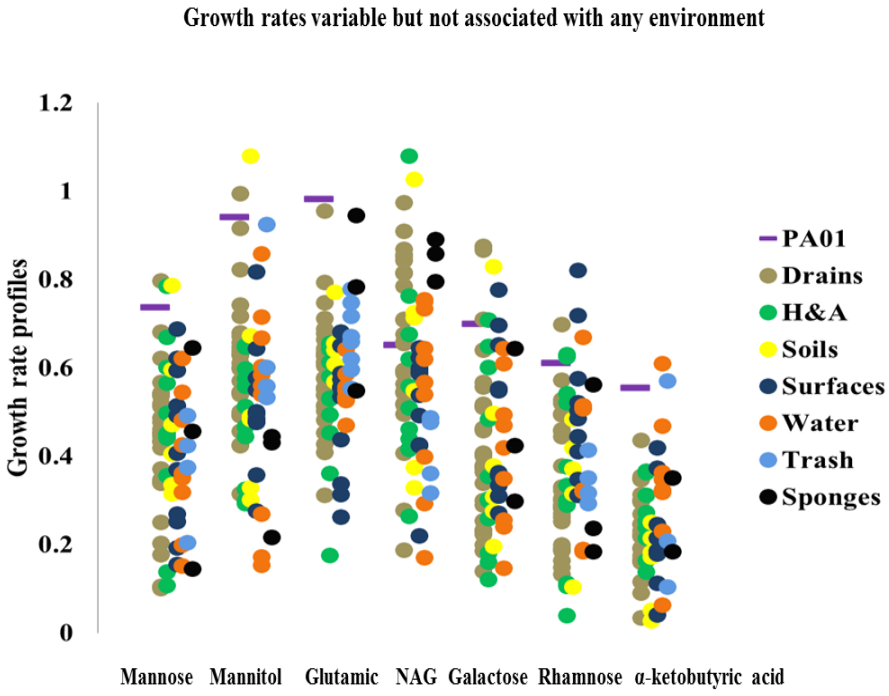

Mean growth rate -hr in glutamic acid
P = 0.02
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Pa01
Drains
H&A
Soil
Surfaces
Water
Trash
Sponges

Figure 4.3.


Growth rates variable but not associated with any environment
Growth rate profiles
1.2
1
0.8
0.6
0.4
0.2
0
Mannose
Mannitol
Glutamic
NAG
Galactose
Rhamnose
α-ketobutyric acid
PA01
Drains
H&A
Soils
Surfaces
Water
Trash
Sponges

CHAPTER FIVE

CONCLUSION

This research investigated the relative roles of species groups and/or environments of isolation on the biogeographic patterns of household *Pseudomonas* species groups. . Resources help to determine where organisms are found and can impact survival and reproduction. The broad objective of this research was to test the role of resources in the distribution patterns of household strains of *Pseudomonas* species groups. A framework developed by Martiny et al., (2006) was modified and used to test the contributions of environments of isolation and phylogenetic history (species groups) on the distribution of household *Pseudomonas* strains (Fig 5.1) with respect to resource utilization. These studies determined that species groups and/or environments of isolation based on resource utilization influenced the distribution of household *Pseudomonas* species groups.

According to Fig 5.1(quadrant A), if there are non-significant effects of environment and species group, this indicates resources in the environment and species group (deeper history of the strains) does not influence biogeographic pattern of household *Pseudomonas* strains. If environment is significant and species group is non significant, this suggest local resource adaptation influence biogeographic patterns and

-

that *Pseudomonas* species groups could be adapting to human home environments (Fig 5.1, quadrant B). When environment is non-significant, but species group is significant, this suggest that resource use is influenced by phylogenetic history and not the environment (Fig 5.1, quadrant C) and if both environment and species group influenced resource utilization, then current environment and deeper history influences the biogeographic pattern of household *Pseudomonas* species groups in human home environments (Fig 5.1, quadrant D).

. In natural environments resources for microbial growth occur as mixed substrates, Luria Bertani media which is nutrient rich and complex was used to model resources in natural environments for the study described in chapter two. The *Pseudomonas* strains studied in chapter two were isolated from one house to control for house to house variation. Chapter two shows that the growth traits (total growth, growth rate and CUC at 24 hours) are influenced by environments of isolation, providing evidence for local resource adaptation of *Pseudomonas* species groups to human home environments. Chapter two also shows that viability at 24 and 48 hours are influenced by the environments of isolation and the phylogenetic history of the strains.

There were significant species group by environment by time point interactions in viability at 24 and 48 hours. These significant interactions indicate that species groups based on deep phylogenetic history have different abilities and/strategies for dealing with the same ecological conditions. These results provided evidence for the effects of environment and phylogenetic history on the distribution of household *Pseudomonas* strains. Chapter two results supports Fig 5.1, quadrant D; results also supports previous studies that *Pseudomonas* strains exhibit some form of biogeographic patterns in

household environments (Remold et al. 2011; Purdy-Gibson et al. 2014; Remold et al. 2015). This study also highlights the importance of evaluating multiple traits in biogeographic studies. This research used 5 different characteristics of growth to provide evidence for the effects of phylogenetic history and current environment on the biogeography of *Pseudomonas* species in human home environments.

In chapter three I continued to explore the hypotheses started in chapter two with the same strains and 31 different resources. These resources included those associated with root exudates such as amino acids, malic acid, and xylose (Rovira, 1965). There were significant species group by resource guild and environment by resource guild interactions in the carbon utilization profiles. These significant interactions indicate species group are different with respect to their carbon profiles in a resource guild specific way. It also suggest that strains from different environment types are different with respect to their carbon profiles in a guild specific way. These results could be indicative of local resource adaptation in space.

Chapter three results also support Fig 5.1, quadrant D; carbon utilization and oxidative profiles of *Pseudomonas* species groups (*P. aeruginosa*, *P. fluorescens,* and *P. putida*) were influenced by environments of isolation and species groups. These results are consistent with previous studies that microbes exhibit some form of biogeographic patterns in different environments (Belottle et al. 2003; Dumbrell et al. 2010; Remold et al. 2011; Lennon et al. 2012; Larouche et al. 2012; Elliot et al. 2014; Purdy-Gibson et al. 2014; Remold et al. 2015). This study utilized different resources to test for effects of environment and species groups on the biogeographic patterns of household

Pseudomonas strains. This also reiterates the importance of using multiple traits in studies addressing the biogeography of microbes.

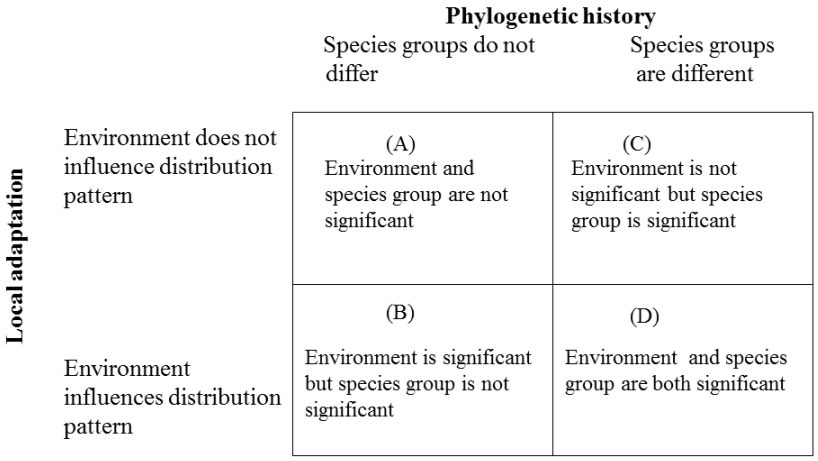
In chapter four, some resources from the chapter three study in which the strains showed variability were studied; to understand if household *P. aeruginosa* strains could be differentiated based on their habitats of isolation. The strains showed variability in their carbon utilization and growth profiles, strains utilized similar resources. This suggest effect of phylogenetic history on the carbon utilization and growth profiles of strains of *P. aeruginosa* strains isolated from multiple human homes. Chapter 4 suggest support for Fig 5.1, quadrant C for these household *P. aeruginosa* strains. In chapter 4, there was no support for effect of environment. According to Martiny et al., (2006), over small spatial scales the environment has been shown to influence biotic composition, but becomes negligible over large spatial scales (distance effect).

By using a framework modified from Martiny et al., (2006) on the contributions of environments of isolation and phylogenetic history (species group) on the biogeography of household *Pseudomonas* strains; I have demonstrated that *Pseudomonas* species group exhibit biogeographic patterns in human home environments and that a combination of environment and phylogenetic history influences their distribution patterns in human home environments. This research contributes to our understanding of what drives the patterns of diversity and/or the biogeography of microbes.

Figure Legend

Figure 5.1: Effects of environments of isolation and phylogenetic history (species group) on the biogeography of household strains of *Pseudomonas* species groups (Modified from Martiny et al. 2006).

Figure 5.1


Phylogenetic history
Species groups do not differ
Species groups are different
Local adaptation
Environment does not influence distribution pattern
Environment influences distribution pattern
(A)
Environment and species group are not significant
(C)
Environment is not significant but species group is significant
(B)
Environment is significant but species group is not significant
(D)
Environment and species group are both significant

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APPENDIX

A table showing information on the strains used in Chapter 4

SRP	Environment of isolation	House #	group	species
1004	Human Ext	134	PA	*P. aeruginosa*
1027	Sink/Tub drains	134	PA	*P. aeruginosa*
1045	water	134	PA	*P. otitidis*
1062	surfaces	106	PA	*P. aeruginosa*
1068	Sink/Tub drains	106	PA	*P. aeruginosa*
1078	sponge/rag	280	PA	*P. aeruginosa*
1087	Sink/Tub drains	280	PA	*P. aeruginosa*
1091	Sink/Tub drains	280	PA	*P. aeruginosa*
1106	water	280	PA	*P. aeruginosa*
1162	Sink/Tub drains	195	PA	*P. aeruginosa*
1169	Human Mucus Membrane	274	PA	*P. aeruginosa*
1172	sponge/rag	274	PA	*P. aeruginosa*
1173	Sink/Tub drains	274	PA	*P. aeruginosa*
1175	Sink/Tub drains	274	PA	*P. aeruginosa*
1177	Sink/Tub drains	274	PA	*P. aeruginosa*
1256	Sink/Tub drains	296	PA	*P. aeruginosa*
1258	Sink/Tub drains	296	PA	*P. aeruginosa*
1260	Sink/Tub drains	296	PA	*P. aeruginosa*
1276	wet surfaces	106	PA	*P. aeruginosa*
1290	soils	106	PA	*P. citronellosis*
1321	Sink/Tub drains	195	PA	*P. aeruginosa*
1370	Sink/Tub drains	183	PA	*P. nitroreducens*
1412	soils	134	PA	*P.nitroreducens*
1421	water	134	PA	*P.aeruginosa*
1427	Human Mucus Membrane	106	PA	*P.aeruginosa*
1435	Sink/Tub drains	106	PA	*P. aeruginosa*
1445	soils	106	PA	*P.citronellosis*
1460	Sink/Tub drains	280	PA	*P.nitroreducens*
1476	Sink/Tub drains	274	PA	*P.nitroreducens*
1505	Sink/Tub drains	296	PA	*P.aeruginosa*
1577	Sink/Tub drains	183	PA	*P.nitroreducens*
1582	surfaces	183	PA	*P.aeruginosa*

Table showing information on the strains used in Chapter 4 continued.

1607	Sink/Tub drains	215	PA	*P. aeruginosa*
1609	Sink/Tub drains	215	PA	*P.aeruginosa*
1618	trash container	106	PA	*P.aeruginosa*
1632	soils	106	PA	*P. aeruginosa*
1642	Human Lg Intestine	280	PA	*P.aeruginosa*
1813	Human Ext	183	PA	*P.nitroreducens*
1822	water	183	PA	*P.nitroreducens*
1831	water	183	PA	*P.aeruginosa*
1845	Sink/Tub drains	296	PA	*P. aeruginosa*
1872	wet surfaces	129	PA	*P. nitroreducens*
1904	Human Mucus Membrane	134	PA	*P. aeruginosa*
1943	water	134	PA	*P. aeruginosa*
1952	Sink/Tub drains	106	PA	*P. aeruginosa*
2004	trash container	119	PA	*P. aeruginosa*
2049	wet surfaces	129	PA	*P. aeruginosa*
2088	Human Ext	220	PA	*P. aeruginosa*
2091	Human Mucus Membrane	220	PA	*P. aeruginosa*
2110	soils	220	PA	*P. nitroreducens*
2115	mouth device	220	PA	*P. aeruginosa*
2147	trash container	215	PA	*P. aeruginosa*
2230	Sink/Tub drains	129	PA	*P. aeruginosa*
2299	trash container	280	PA	*P.aeruginosa*
2304	wet surfaces	280	PA	*P. aeruginosa*
2361	water	134	PA	*P. aeruginosa*
2415	Sink/Tub drains	220	PA	*P. aeruginosa*
2425	surfaces	220	PA	*P. nitroreducens*
2427	surfaces	220	PA	*P.nitroreducens*
2444	surfaces	296	PA	*P. nitroreducens*
2470	Pet Mucus Membrane	119	PA	*P. nitroreducens*
2490	water	119	PA	*P. nitroreducens*
2499	Human Lg Intestine	280	PA	*P. aeruginosa*
2500	Sink/Tub drains	280	PA	*P. aeruginosa*
2521	Sink/Tub drains	134	PA	*P. aeruginosa*
2536	water	134	PA	*P. otitidis*
2540	wet surfaces	134	PA	*P.otitidis*
2544	Sink/Tub drains	215	PA	*P. aeruginosa*

Table showing information on the strains used in Chapter 4 continued.

2548	soils	215	PA	*P. nitroreducens*
2552	Human Mucus Membrane	296	PA	*P. aeruginosa*
2556	Sink/Tub drains	296	PA	*P. nitroreducens*
2576	wet surfaces	274	PA	*P. nitroreducens*
2581	water	274	PA	*P. nitroreducens*
2588	sponge/rag	129	PA	*P. aeruginosa*
2590	Sink/Tub drains	129	PA	*P. aeruginosa*

CURRICULUM VITA

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EDUCATION

PHD., in Biology, Univeristy of Louisville, July, 2015.
Post Master's work in sustainable agriculture, Alabama Agricultural and Mechanical Univeristy, Hunstville, AL 2007-2008.
Kentucky Initial High School Certification in Biology, Campbellsville University, 2005.
M.S., in Microbiology, Rivers State University of Science and Technology, Port Harcourt, Nigeria, 2000.
B.S., in Microbiology, Rivers State University of Science and Technology, Port Harcourt, Nigeria, 1995.

RESEARCH EXPERIENCE

Doctoral dissertation project: Metabolic Profiles of *Pseudomonas* Species Groups Isolated from Human Home Environments.
My rsearch investigated the growth traits of strains of *Pseudomonas aeruginosa, P. fluorescens* and *P. putida* groups isolated from a single human home. The variability in resource utilization profiles among strains of *P. aeruginosa, P. fluorescens* and *P. putida* groups from the same human home. The resource use diveristy among strains of *P. aeruginosa* group from various human home environments.

Post Master's research, bioavailability of heavy metals in organic agro-ecosystems, 2007-2008.
Master's thesis, bacteria community of soils contaminated with refined petroleum hydrocarbon.
Undergraduate thesis, microbial community in a waste dump site in Eagle Island, Port Harcourt, Nigeria.

PROFESSIONAL EXPERIENCE

Rsearch fellow, school of interdisciplinary and graduate school, University of Louisville, Spring 2015.
Research Assistant, Remold Lab, Univeristy of Louisville, Fall 2014.
I assumed duites of ordering of lab supplies, keeping up with lab inventories and working on other side projects.

Additional project
Utilization of extracullar DNA as carbon, nitrogen or phosphorus resource.

Teaching Assistant,
Microbiology lab for biology majors (BIO 358), University of Louisville, Department of Biology, Fall 2013-Spring 2014.

Microbiology for nursing majors (BIO 258), University of Louisville, Department of Biology, Fall 2010-Summer, 2013 and Summer, 2014.
Adjunct Instructor of biology and environmental science (traditional/in seat), Summer 2003 -Spring 2010, Campbellsville University.

Student teacher, Campbellsville High School, Spring 2005

GRANTS AND AWARDS
Dissertation completion Grant, University of Louisville, Graduate School.
Carl Underrepresented Minority Fellowship, 2011 ($600)
Graduate School Union Award, 2011 ($100)
Graduate School Union Award, 2013 ($100)
Biology Graduate Student Travel Grant, 2013 ($150)
Graduate Student Council Travel Grant, 2013 ($600)

Best graduating student in the department of biology, Rivers State University of Science and Technology, Nigeria, 1995.

PRSENTATIONS
Microbial Experimental Evolution Conference Poster Presentation, Washington, DC, June 19th – 22nd, 2014.
Title: **"**Metabolic Profiles of Human associated *P. aeruginosa* Strains"
Graduate Student Research Symposium, University of Louisville, KY, April 5th, 2014.
Title: "Metabolic profiles of *Pseudomonas* species groups isolated from a single human home"
Kentucky Academy of Science Poster Presentation at Morehead, KY from Nov 8th -9th 2013.
Title: "Utilization of Extracellular DNA as carbon, nitrogen and phosphorus resources"
Gordon Research Conference Poster Presentation, Andover, NH from July21st -26^{th,} 2013.
Title: "Metabolic profiles of *Pseudomonas* species groups isolated from a single human home"
Graduate Students Research Symposium, University of Louisville, KY, March 23rd, 2012.
Title: "Growth Kinetics of *Pseudomonas* species isolated from a single human home"
Kentucky Academy of Science Poster Presentation at Murray, KY from Nov 4th -5th 2011.
Title: "Growth Kinetics of *Pseudomonas* species isolated from a single human home"

PUBLICATIONS

Onyiri, O. 2009. Micro-pollutants: An emerging concern for aquatic organisms. The Campbellsville Review. Vol. 5: 51-60. http://www.campbellsville.edu/micro-pollutants-an-emerging-concern-for-aquatic-organisms.

Past publications were made under the last name Nwaubeta

Journal of Applied Science and Environmental Management (2002). "Bacteria Community in a Waste Dump Site" Vol. 6(1) 78-83
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Text book Review, 2006.
Environmental Science Systems and Solutions 4th ed. Publs. Jones and Bartlett.

MANUSCRIPT in progress

Onyiri, O. and S. Remold. Metabolic Profiling of *Pseudomonas* Species Groups Isolated from a Single Human Home.