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POLYPHASIC TAXONOMY OF A GROUP OF GLOBALLY DISPERSED, DESICCATION-RESISTANT STRAINS OF THE MICROVIRGA/BALNEIMONAS CLUSTER

Bradley Tolar

POLYPHASIC TAXONOMY OF A GROUP OF GLOBALLY DISPERSED, DESICCATION-RESISTANT STRAINS OF THE *MICROVIRGA*/*BALNEIMONAS* CLUSTER

by

Bradley Tolar

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Dr. Fred A. Rainey

Department of Biological Sciences

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ABSTRACT

Through the study of the diversity of heterotrophic bacteria in soils from arid environments we assembled a collection of strains related to the species *Microvirga subterranea* and *Balneimonas flocculans*. The strains were isolated on a variety of culture media from soil samples collected in the Atacama, Mojave, Negev, Oman, Sahara, and Sonoran deserts. The aim of our study was to discover the level of phenotypic and phylogenetic diversity within the strains in order to assign them to a taxonomic level within the *Microvirga/ Balneimonas* group. 16S rRNA gene sequences were determined for 60 bacterial strains and compared to each other and the current databases of organisms of the *Microvirga/ Balneimonas* cluster. Phenotypic and chemotaxonomic characteristics including growth temperature, NaCl tolerance, motility, oxidase, catalase, carbon sources utilization patterns and fatty acid profiles were determined. A subset of the isolates was selected on the basis of 16S rRNA gene sequence diversity and tested for desiccation resistance and survival after exposure to gamma and UV radiation. The strains showed 95.0-100% 16S rRNA gene sequence similarity to each other and 95.0-97.5% similarity to the type species of the genera *Microvirga* and *Balneimonas*. The strains showing 100% 16S rRNA gene sequence similarity came from both the same sample sites as well as geographically remote sites. Differences in carbon sources utilization patterns and fatty acid profiles were observed and can be used to differentiate the strains. A number of strains showed resistance to UV radiation and had D_{10} values of ~3.5 kGy when exposed to gamma radiation. Strains exposed to extended desiccation showed survival equal of greater than that displayed by *Deinococcus radiotolerans*. The polyphasic characterization of these strains from arid environments demonstrated them to

represent a phylogentically and phenotypically diverse assemblage. This study shows the close relationship between the type species of the genera *Microvirga* and *Balneimonas* which are inter-populated with these novel strains sharing similar phylogenetic and phenotypic properties. The collection of strains represents a number of novel species of these genera.

CHAPTER 1:

INTRODUCTION

Extremophilic microorganisms are of great interest to microbiologists today for a number of reasons. The first of these is that advances in technology have made their isolation and study more available, and thus more individuals are able to examine their unique properties. Additionally, the study of these organisms may lead to insights on the origin of life, since we do not fully understand the conditions under which it occurred. Examining the limits of life on Earth can only improve our knowledge of this complex process, especially since early life was subject to a more extreme environment than seen today. Since the ozone layer had not yet developed, organisms on the surface must have possessed adaptations to survive direct radiation from the sun and other cosmic rays. Lower levels of oxygen in the oceans and atmosphere also required that life be more adapted to harsh environments (Javaux, 2006).

The field of astrobiology is concerned with the origin of life in the universe, as well as its evolution and distribution. In order to better understand the possibilities for life outside our own Earth, it is necessary to study the biological properties of the extremophiles. Prime candidates for life-containing bodies within our solar system include Mars, Jupiter's moon Europa, and Saturn's Titan (Javaux, 2006). Analogous habitats to Mars have been discovered on our planet; one of these is the hyper-arid core region of the Atacama Desert around Yungay, Chile. In this region there exists very low levels of culturable bacteria and organic carbon, much like the environment of Mars observed by the Viking missions. Additionally, the rapid release of molecular oxygen was observed in Mars soils upon exposure to water vapor, which has been attributed to

the presence of a reactive oxidant. Similar findings were obtained for the core region of the Atacama, as described in Navarro-Gonzales et. al. (2003). Survival in the region surrounding Yungay is dependent upon adaptations to extreme dryness, since precipitation occurs infrequently. Thus, studying the extremophilic organisms that live in the Atacama can lead to improved methods of examining life on Mars. The presence of a Mars analog on this planet would allow for testing of instruments for future experiments in the search for life outside Earth (Navarro-Gonzales, 2003).

Another application of the study of extremophilic microorganisms lies in the field of biotechnology. The most obvious utilization of the extremophiles is a direct application; one such example is using bacteria to extract metals like cobalt and gold from ore in a process known as bioleaching. Another use for the extremophiles involves the extraction of their enzymes and other biomolecules. An example of this enzyme mining is *Taq* DNA Polymerase, a heat-stable polymerase isolated from the thermophilic bacterium *Thermus aquaticus* for use in PCR processes. Other enzymes may be used as detergents and in chemical and food industries due to their optimal activity at extremes of pH and temperature (Podar and Reysenbach, 2006). Each of these applications shows obvious economic value, but still possess scientific relevance. Future discoveries may help to optimize scientific processes or increase production in a particular industry.

Organisms from all three domains, Archaea, Bacteria, and Eukarya have adapted to life at high temperature, pressure, salt concentration, ionizing radiation, and extremes in pH. Some of these are even adapted to more than one extreme condition and are therefore are referred to as polyextremophilic (Rainey and Oren, 2006). In this study, we

focused on conditions of low moisture, or desiccation, along with its correlation to ionizing radiation resistance.

Desiccation is the process of cell drying as a result of the extreme loss of water. Such an action can cause great damage and sometimes death to a bacterium, since many microbial processes are dependent on water to function. Additionally, DNA damage can result with double-strand breaks caused by loss of stability in a dry environment (Mattimore and Battista, 1996; Potts et. al., 2005). Other cellular changes caused by desiccation include shrinkage, loss of membrane fluidity, increasing salt concentrations, growth arrest, and crowding of macromolecules (Potts et. al., 2005). The formation of reactive oxygen species (ROS) during desiccation can cause damage including the loss of electron transport chain control, elevated exposure to atmospheric oxygen, and distortion of DNA. Hydroxyl and peroxyl radicals are common ROS formed, along with more complex species derived from protein hyperoxides (Fredrickson et. al., 2008). All of these things can lead to increased stress on the bacterial cell, and make survival difficult. Some bacteria have developed means to overcome these stresses, and can thrive in extreme desiccated environments without much loss of population.

One possible method for this tolerance lies in DNA repair mechanisms. Mattimore and Battista (1996) studied correlations between desiccation and radiation resistance in *Deinococcus radiodurans*. They found that prolonged dehydration of cells resulted in extensive DNA damage on the order of at least 60 DNA double-strand breaks per cell after only 6 weeks. Survival would thus depend on the organism's ability to repair these damages. In addition they found that *Deinococcus* strains that were deficient in DNA repair capability were sensitive to desiccation (Mattimore and Battista, 1996).

Another plausible means of resistance involves repair of proteins damaged during desiccation. It has been found that a relationship exists between cellular protein damage and desiccation resistance, as well as intracellular Mn/Fe concentration. *Deinococcus* species that were highly desiccation resistant were also the least likely to have protein carbonylation occur, while *Shewanella oneidensis* showed high carbonylation levels with its sensitivity to desiccation induced stress. The ability of a cell to accumulate Mn allows for further protection of proteins from damage caused by ROS (Fredrickson et. al., 2008).

Arid regions, both hot and cold, are environments that have low relative humidity and thus little water available for life. These regions are considered naturally desiccated environments, and cover about 30% of the Earth's surface (Rainey et. al., 2005). In addition to dryness, microorganisms inhabiting desert lands experience temperature extremes, strong thermal contrasts, and the lack of nutrients, all of which contribute to stress (Chanal et. al., 2006). They important to study because of the specific adaptations that resident bacteria must possess for survival. Desiccation resistance is an apparent evolutionary adaptation, since water is low in these environments. Additionally, resistance to ionizing radiation has been shown to correspond to desiccation resistance in bacteria (Mattimore and Battista, 1996; Potts, 2001; Rainey et. al., 2005). A higher proportion of ionizing radiation-resistant organisms have been discovered from arid regions in comparison to non-arid ones, and an extensive microbial diversity also exists in these regions (Rainey et. al., 2005; Chanal et. al., 2006).

Previous studies of the diversity of communities present in arid soils show the diversity present in them, despite extreme conditions. Rainey et. al. (2005) irradiated a sample of soil from the Sonoran desert in Arizona to isolate radiation-resistant bacteria.

They succeeded in finding a number of ionizing radiation-resistant genera previously described as such, including *Deinococcus*, *Methylobacterium*, *Hymenobacter*, *Kocuria*, and *Kineococcus*. In addition, they found the first examples of radiation resistance in the taxonomic groups *Bosea*, *Chelatococcus*, *Geodermatophilus*, *Planococcus*, *Corbulabacter*, and *Spirosoma*. To further the link between stress resistance and arid environments, a non-arid soil sample from the forests of Louisiana was used for comparison, and contained species of *Bacillus*, *Nocardia*, and *Micrococcus*, as well as the dominant genus present, *Streptacidophilus*. The diversity of ionizing radiation-resistant bacteria from the non-arid soil was significantly less than those found in the Sonoran desert. It is important to note that no *Deinococcus* species were isolated from the Louisiana soils, even after exposure to greater than 11 kGy (Rainey et. al., 2005).

Chanal et. al. (2006) also examined microbial diversity in a desert, this time in south Tunisia, known as Tatouine. Initial sampling from the desert sands showed 11 divisions of bacteria; a majority of 83% were members of the phyla *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* (Chanal et. al., 2006). To better examine the presence of highly radioresistant micoorganisms, the sand sample was irradiated by a ^{60}Co source at a dose of 15 kGy. The genera isolated and identified from 16S rRNA gene sequences after this irradiation included *Bacillus*, *Deinococcus*, and *Chelatococcus*. Further testing of the radiation resistance of each of these isolates showed a D_{10} of 2 kGy for the *Chelatococcus* species, while the two *Deinococcus* species showed D_{10} values of 11 and 12 kGy (Chanal et. al., 2006).

Although it is readily known that adaptations do exist to overcome stress presented in arid environments, the different means of this resistance are for the most part

unknown. The analysis of the phylogenetic diversity of microbial communities residing in desert soils can be of use in defining taxons of interest, and more detailed studies on such groups could lead to increased information on bacterial resistance mechanisms (Chanal, et. al. 2006).

In this study we examined the diversity of heterotrophic bacteria in soils from two arid regions, the Atacama and Negev deserts. We also looked at the resistance of a number of phylogenetically unrelated bacteria isolated from surface soils of the Atacama desert to desiccation, UV, and gamma radiation stresses.

CHAPTER 2:

DESICCATION STUDIES

Introduction

Water loss in a bacterial cell can often result in a lethal situation, since water is crucial for life. However, certain organisms possess structural, molecular, or physiological adaptations that prevent cell death due to the absence of moisture; these organisms are designated as desiccation tolerant. Prokaryotic organisms possessing this resistance to drying enter a state of suspended metabolism that allows for survival during the period of high stress, followed by recovery of metabolic functions upon cell rehydration. Any damages incurred during the initial drying period throughout the time spent in desiccation must be repaired in order for the cell to survive (Potts, 2001).

Bacteria that live in arid lands can be thought to exhibit desiccation resistance because of the low relative humidity in such environments. It is thus important to observe the types of microorganisms that inhabit desert soils, to discover desiccation-tolerant bacteria. This type of study is most easily performed by examining the viable population counts of a soil sample, followed by the isolation and identification of bacterial strains obtained from the soils. Two soil samples were chosen for comparison in this study: the first from the Atacama Desert in northern Chile, while the second was collected in Israel's Negev Desert. The ultimate goal of this project was to observe the population numbers and diversity of the two samples from different environments, and to make comparisons between them.

The Atacama is considered one of the driest places on earth. In some areas of the Atacama Desert, rainfall is actually measured in centimeters per decade. This dryness is

due to a combination of factors, namely the rain shadow effect caused by the Andes Mountains to the east, which block moisture from passing over them and into the desert. Low levels of culturable bacteria exist in the Atacama Desert, especially in the hyper-arid region known as Yungay (Navarro-Gonzales et al, 2003). However, the sample AT05-22 (S 28° 7' 4.5", W 69° 55' 8") comes from the "vineyard", an area that contains more vegetation as compared to the rest of the Atacama (Navarro-Gonzales et. al., 2003). The Negev Desert is in southern Israel and comprises>60% of the country's land surface. It has a semi-arid toarid climate, with regions ranging from 50 to 300 millimeters of rainfall per year. The sample NG05-4 (N 29° 59' 39.6", E 34° 58' 44.4") comes from the Arava Valley region of the Negev, in southern Israel. This arid region is characterized by poor soils and little rainfall, an average 50 mm per year (Negev Foundation, 2008).

Figure 2.1: Location of Atacama and Negev Soil Sample Sites AT05-22 and NG05-4

Figure 2.2: The Atacama Sample Site, AT05-22

Figure 2.3: The Negev Sample Site, NG05-4

Five test organisms were used in this experiment. The first of these is a strain related to the genera *Rubrobacter– Solirubrobacter* – that is a rod-shaped, Gram-positive aerobe (Kim et. al., 2007). It belongs to the Phylum *Actinobacteria*, and *Rubrobacter* species have been shown resistance to gamma radiation (Rainey et. al., 2005). Cells of the species *Sphingomonas jaspsi* are Gram-negative, pleomorphic short rods with deep yellow colonies that bud. They are strictly aerobic and members of the alpha *Proteobacteria*, and are typically found in soils (Asker et. al., 2007). Thirdly, a species of the genus *Hymenobacter* was utilized (Figure 2.6). *Hymenobacter* species are a group of Gram-negative rod-shaped bacteria that belong to the Bacteriodetes group (*Cytophaga*/*Flavobacterium*/*Bacteroides*). They are aerobic heterotrophs, and some are psychrophilic with an optimum growth temperature between 10 and 27°C (Hirsch et. al., 1998). Some strains of this genus have been shown to survive after exposure to gamma radiation (Rainey et. al., 2005). *Blastococcus saxobsidens*, also used in this experiment, is a Gram-positive, aerobic coccus that occurs typically in singles, pairs, or tetrads, often forming aggregates. Budding is also observed in certain strains of the genus *Blastococcus*. This species is a member of the Phylum *Actinobacteria* (Urzì et. al, 2004). Each of these four organisms can be seen in Figure 2.4. Finally, *Escherichia coli* – a Gram-negative, gamma *Proteobacteria* – was used as a control for this experiment. *E. coli* is a good control organism that does not survive desiccation (Mattimore and Battista, 1996). An image of the *E. coli* control can be seen in Figure 2.5.

Figure 2.4: DIC microscopy images of the four organisms studied. These are (clockwise from left): *Rubrobacter* sp., *Sphingomonas jaspsi*, *Blastococcus saxobsidens*, and *Hymenobacter* sp.

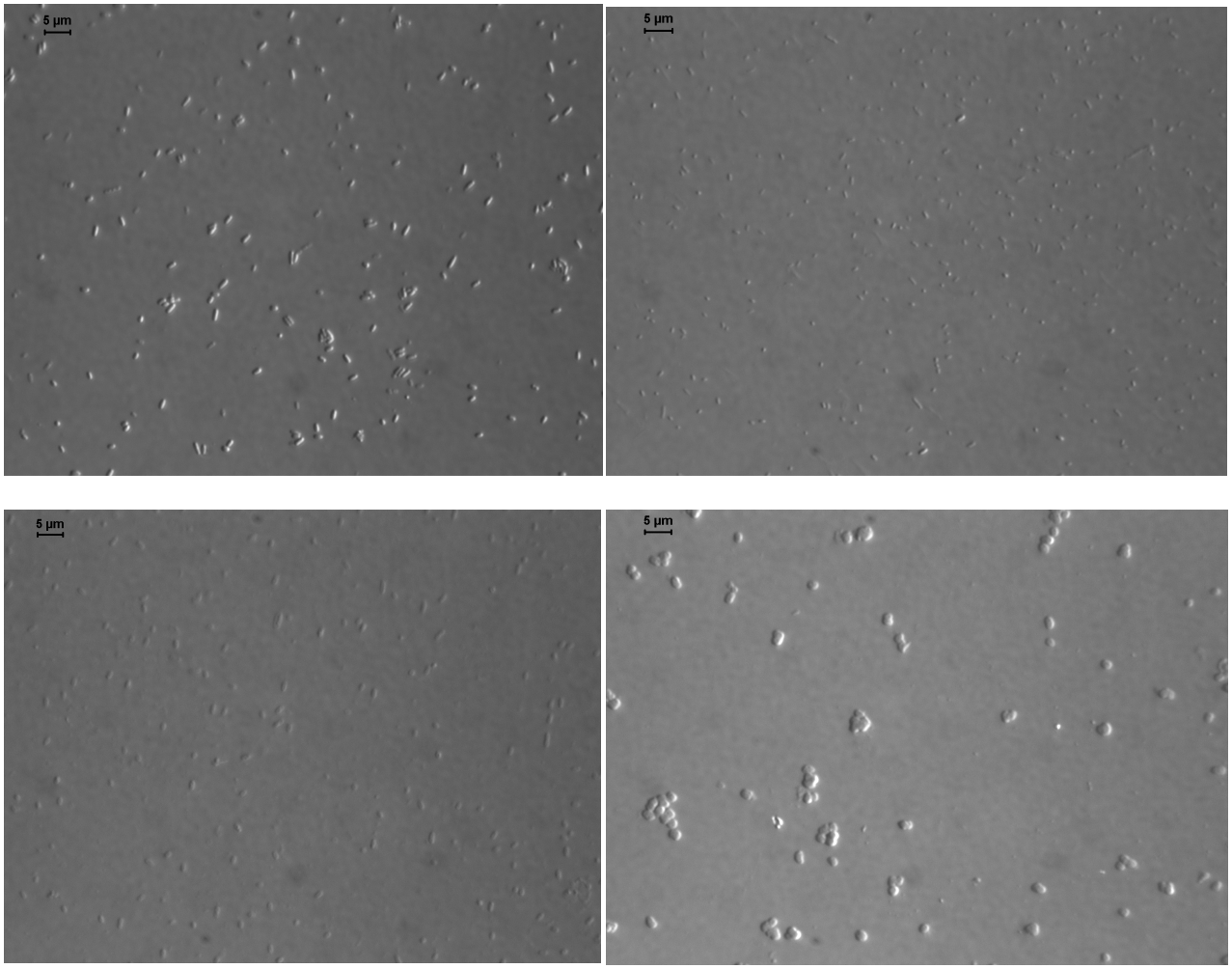
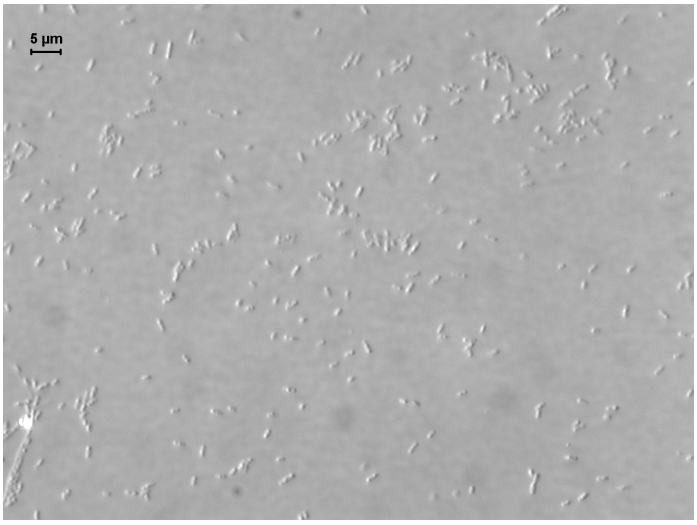


Figure 2.5: DIC microscopy image of the *E. coli* control



Materials and Methods

Sample collection.

Two desert soils samples were collected – one each from the Atacama Desert in Chile and the Negev Desert in Israel. Five organisms were selected from previous strains collected from soil samples or used in instructional laboratories on campus – these included *Rubrobacter* sp. (AT03-58-52), *Sphingomonas jaspsi* (AT04-164-45), *Hymenobacter* sp. (AT04-159-30), and *Blastococcus saxobsidens* (AT04-164-22), as well as an *E. coli* control.

All of these organisms, with the exception of *E. coli*, were obtained from the soils of the Atacama Desert. The site AT04-164 (S 24º 3’ 66”, W 69º 52’ 56”), from which *Sphingomonas jaspsi* and *Blastococcus saxobsidens* were isolated, is a surface site in the core hyper arid region of the Atacama, around Yungay. The *Hymenobacter* strain was isolated from a soil pit in the Altamira region of the Atacama Desert, designated as AT04-159 (S 25º 45’ 54.3”, W 70ºW 11’ 75.8”). Finally, the *Rubrobacter* isolate came from AT03-58, which was a sub-surface sample in the core region of the Atacama.

Sample Preparation.

Two Petri dishes each containing 2g soil were prepared from the sample sites AT05-22 and NG05-4, and designated AT05-22A, AT05-22B, NG05-4A, and NG05-4B. A micro-titer plate, containing 24 wells, was utilized for the organism aspect of the experiment. One loopful of bacterial culture was suspended in 1 mL distilled water and transferred via pipette into a well. Three different samples were used for each of the five organisms. This collection of four soil plates and one micro-titer plate will hereafter be referred to as a "set".

Desiccation Resistance Testing.

Three different conditions were prepared for survival testing – one at room temperature, one in a freezer set to -20°C, and one in a Secador™ desiccation chamber (Bel-Art) containing 160g silica gel at room temperature. Four sets were prepared for each environment: one each for time periods of 28, 42, and 56 days, and a last for an unspecified period of time. This final set was collected after 203 days. A HOBO®H8 Pro RH/Temp Data Logger (Onset) was used to measure the temperature and relative humidity of both the room temperature and desiccation environments. After the time period of one set expired, samples were removed from their respective environments. These samples were diluted using Plate Count Broth (Difco) and 1/10 strength Plate Count Broth for the soils, and distilled water for the organisms. Dilutions were plated on Plate Count Agar (Difco) (*E. coli*), 1/10 strength Plate Count Agar (*Rubrobacter* sp. AT03-58-52, *Sphingomonas jaspsi* AT04-164-45, *Hymenobacter* sp. AT04-159-30), and Marine Agar (Difco) (*Blastococcus saxobsidens* AT04-164-22). Preliminary plating was also performed prior to experimentation, using the same samples.

Viable Count Studies.

Cfu/mL and Cfu/g were measured for the organisms and soils, respectively. These numbers were obtained from each sample after incubation at 28°C for 20 days. Colonies from the soil samples plated onto 1/10 strength Plate Count Agar and Plate Count Agar were isolated and preserved for future studies.

Results and Discussion

Data were compiled from the samples taken from each of the three conditions (two temperatures and desiccation) and all five of the time periods, including the preliminary samples. Calculations for the 56-day experiment were made based on counts at day 15 after plating. All other data resulted from counts made on or near 20 days post-plating. Errors were discovered in the dilutions planned for the 28-day plating, which resulted in almost no countable data; it was thus repeated, and the data shown is an average of these sets.

Due to experimental error and miscalculations during the plating of the organism portion of the experiment, many organism samples lacked a countable plate. The reason for such errors was likely the mistaken anticipated growth patterns for each organism, as well as the lack of a standard concentration of each organism when initially introduced into the micro-titer plates. That aspect of this experiment should be repeated in order to get sufficient data from which to draw conclusions. Regardless, a few patterns can still be observed in the data collected.

A steady decrease could be observed in the Cfu/mL for each of the organisms tested in room temperature (Figure 2.6). The *Rubrobacter* strain (AT03-58-52) had a preliminary count of 1.63×10^9 Cfu/mL, but a countable plate was not obtained after that

point. *Sphingomonas jaspsi* began with a 1.27 x 10^8 Cfu/mL value, which dropped to 4.07 x 10^3 after 42 days; these two data points were the only obtained from the room temperature conditions. The *Blastococcus* and *Hymenobacter* strains were the only two to possess more than two data points for the room temperature experimentation; each showed a decrease in bacterial growth. The *Hymenobacter* sp. had a preliminary count of 2.0 x 10^5, which remained stable at 2.19 x 10^5 after 28 days before dropping to 4.47 x 10^4 and 7.33 x 10^2 after 42 and 56 days, respectively. No countable plate was obtained after 203-day experimentation. The cell counts for the *Blastococcus saxobsidens* strain started at 2.17 x 10^8, but quickly fell to 7.0 x 10^5 after 28 days. Samples plated from 42, 56, and 203 days showed Cfu/mL values of 4.00 x 10^5, 3.40 x 10^3, and 6.70 x 10^2, respectively. Finally, the *E. coli* control began with 9.37 x 10^9 Cfu/mL, greater than any of the other organisms but could not be observed in any of the other platings.

Desiccation studies also show a decline in populations of all organisms tested (Figure 2.7). The culture of *Rubrobacter* sp. (AT03-58-52) had an initial population of 1.63 x 10^9 Cfu/mL, which dropped to 6.67 x 10^2 after 56 days, once a countable plate was obtained. There were no survivors after 203 days. A more gradual decline was observed in *Sphingomonas jaspsi*, which began at 1.27 x 10^8 Cfu/mL and decreased to 1.11 x 10^6 by the 42nd day. After 56 days, two log cycle decreases were observed, putting the population at 5.40 x 10^4 Cfu/mL, and *Sphingomonas* sp. was not found on a plate after the 56th day. The culture containing *Hymenobacter* sp. started with 2.00 x 10^5 Cfu/mL, but showed no survival both the 28th and 42nd day platings. However, the sample reemerged with a count of 1.20 x 10^3 Cfu/mL for the 56th day, and even survived desiccation until day 203, where it contained 5.00 x 10^2 Cfu/mL. The *Blastococcus saxobsidans* strain was

observed to decrease gradually over the time of the experiment. It began with 2.17 x 10^8 Cfu/mL and declined to 7.0 x 10^5 after 28 days, then increased slightly to 7.85 x 10^5 at the 42-day plating before decreasing once again to 3.53 x 10^4 after 56 days. The 203-day plating showed 1.57 x 10^5 Cfu/mL for the *B. saxobsidans* strain, the greatest survival after desiccation of the five species tested. *E. coli* once again did not survive past the preliminary plating, where 9.37 x 10^9 Cfu/mL were present.

A comparison of the three conditions tested shows that there was a greater survival rate in the -20°C samples than at room temperature or in the desiccation chamber (Figure 2.8). All of the organisms survived 203 days of -20 C, although each of them declined from the preliminary plating. *Rubrobacter* sp. declined two log cycles, from 1.63 x 10^9 to 1.67 x 10^7 Cfu/mL, with its lowest point at 56 days with 4.83 x 10^7 Cfu/mL; it showed the best survival at lower temperatures, since the other four species showed at least a two log cycle loss of population. *Sphingomonas* sp. decreased from 1.27 x 10^8 Cfu/mL to 7.45 x 10^5, while *Hymenobacter* sp. got down to 2.67 x 10^2 from 2.0 x 10^5 Cfu/mL initially. The *Blastococcus* sp. decreased from 2.17 x 10^8 Cfu/mL to 1.43 x 10^6. *E. coli* survived up to 203 days in this experiment, maintaining a population of 4.00 x 10^5 Cfu/mL; the preliminary count showed 9.37 x 10^9 Cfu/mL.

These findings reinforce the use of cold environments to slow growth and death rates of bacteria. The rate of death seemed also to correlate to the cell counts present in each sample initially. Future experimentation would create a standard concentration for each bacterial culture for improved comparisons.

The complete data for the organism portion of this experiment is shown below (Figures 2.6, 2.7, and 2.8).

Figure 2.6: Cfu/mL of strains plated from the room temperature study

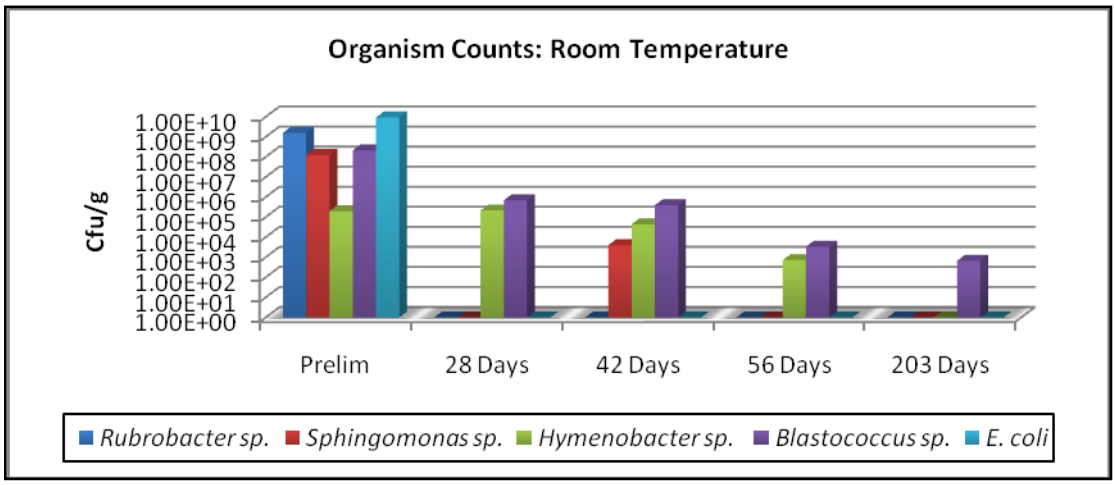


Figure 2.7: Cfu/mL of strains plated from the desiccation study

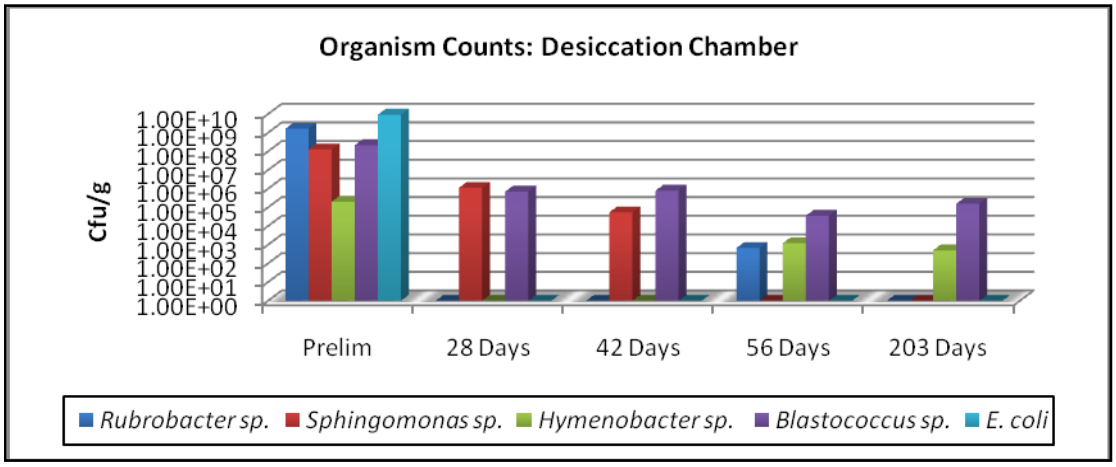
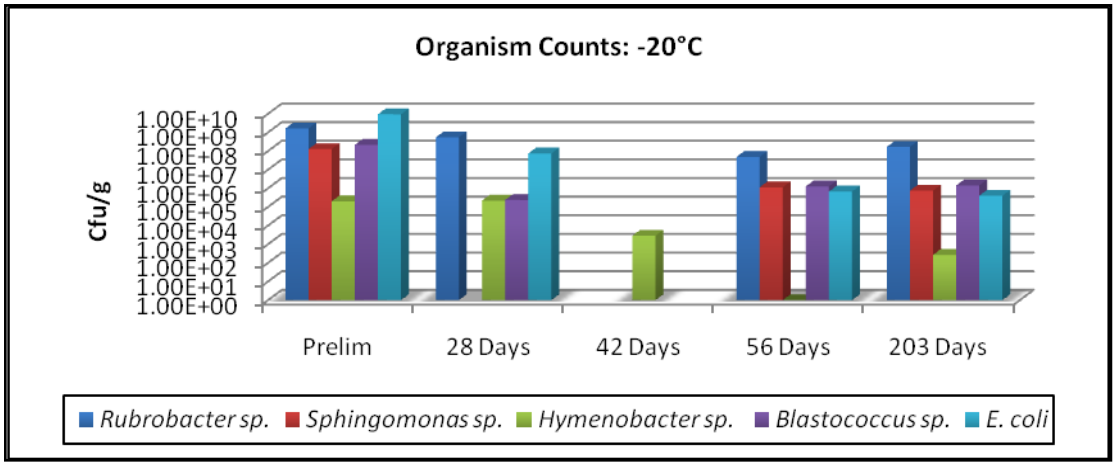


Figure 2.8: Cfu/mL of Organisms plated from the low temperature study



An interesting note is that the Cfu/mL of samples in the desiccation chamber (Figure 2.7) tended to be higher than those tested at room temperature (Figure 2.6). There are a number of potential explanations for this observation. Perhaps the available humidity in the non-desiccated environment (~50% RH at room temperature) prevented the stress response of the desiccation-tolerant organisms from becoming fully active. It may have even been the result of a lesser concentration of cells being placed in one environment versus another.

Data collected from the soil sample experimentation is shown in Figures 2.9, 2.10, and 2.11, and shows more steady population counts than the five organisms studied.

Figure 2.9: Cfu/mL of soil samples plated from the room temperature study

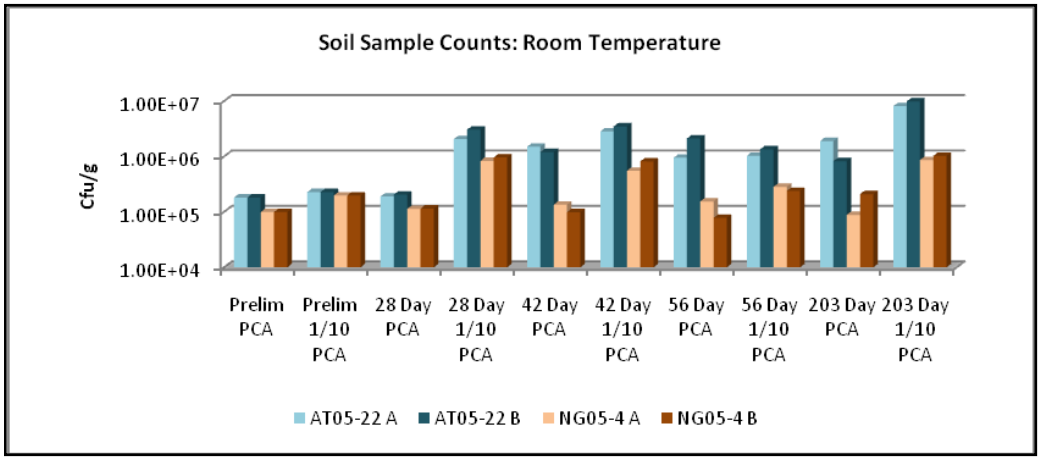


Figure 2.10: Cfu/mL of soil samples plated from the desiccation study

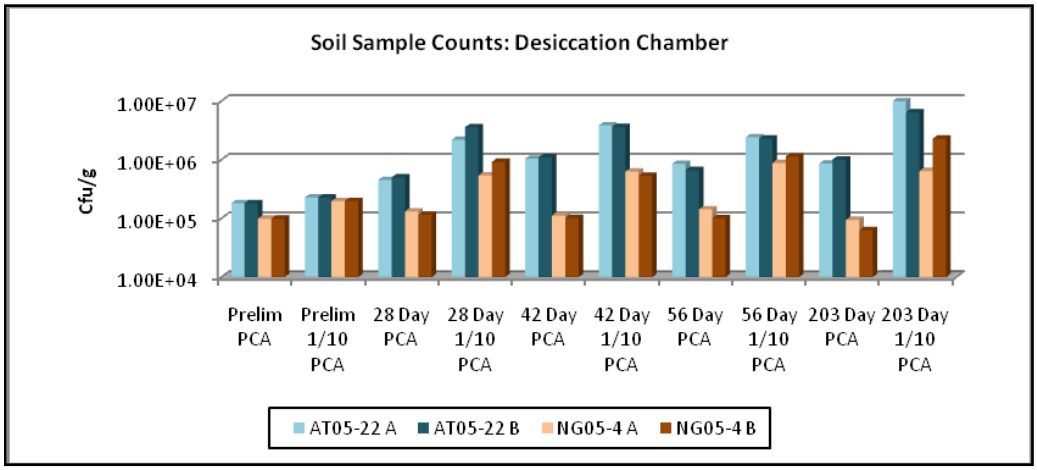
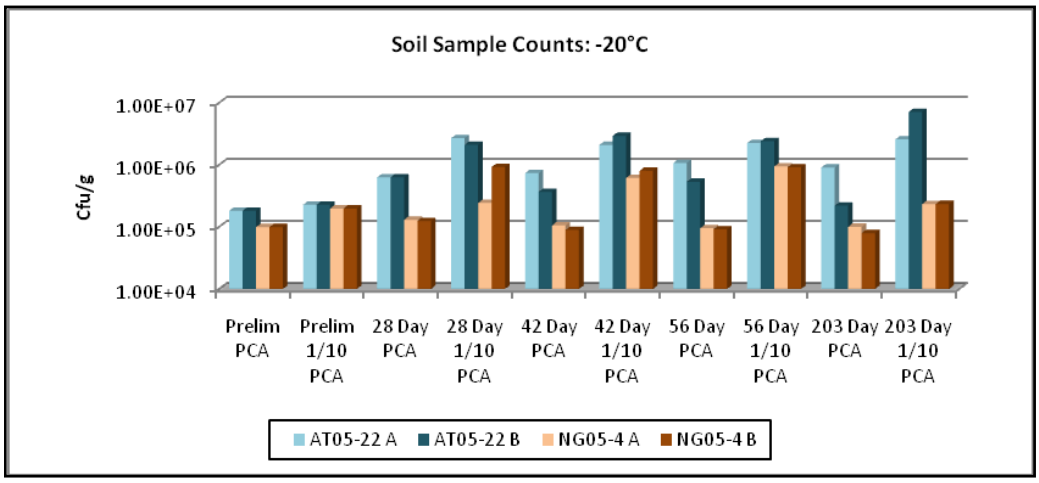


Figure 2.11: Cfu/mL of soil samples plated from the cold temperature study



For the soil sample data, the large amount of available cells in the samples prevented errors in dilution that would result in a loss of a countable plate. A vast number of microorganisms inhabit a single gram of soil, including ones that are not culturable. Because of this, the results from this aspect of the experiment showed consistently high Cfu/mL for all time points and in each environment. In some instances, an increase in the amount of growth was shown even after 203 days of exposure to conditions of desiccation (Figure 2.10). This is likely due to the desiccation-resistant properties of the newly growing organisms, or the death of a species that inhibited growth of the others. However, further experimentation and determination of the identities of isolates will be needed to confirm this. It is also interesting to note that the Atacama samples showed more survivors than those from the Negev. This is perhaps due to a more microbial-rich environment in site AT05-22 from the Atacama or the higher desiccation resistance of the strains in the more desiccated Atacama environment.

Further analysis and experimentation should provide a more complete set of data for future studies. Many more conclusions can be drawn, and these with more certainty, once isolates from each time period and environment can be identified and compared.

CHAPTER 3:

ISOLATE COLLECTION AND IDENTIFICATION

Introduction

Prokaryotic populations have been studied classically using a culture-dependent approach involving artificial media in the laboratory. This method can be used for the isolation of individual colonies or enrichment of a group of organisms inhabiting an environmental sample. Although it has been established that a majority of microorganisms cannot be cultured in this manner, studies with artificial media can be used to gather basic information about the culturable inhabitants of a microbial community.

A fraction of the microbes residing in a particular soil sample can be obtained using the plate count method, which is very useful in comparisons with other sample sites. Furthermore, viable cells can be recovered from such plates and isolated into a pure culture for identification and characterization. This data provides information about the composition of the microbial community in a sample, and allows for a more in-depth comparison between soils. It also serves as a solid foundation for future research using culture-independent techniques to gain a more complete knowledge of the microbial community under study (Amann et. al., 1995).

In order to discover the identity of a purified culture, DNA must first be extracted from the cell. This is achieved by lysing the cell wall with physical and/or chemical processes. Physical actions include freeze/thaw and bead beating protocols, while lysozyme and sodium dodecyl sulfate are used for chemically induced cell lysis. The combination of these two processes is most effective in extracting DNA; extraction kits

typically combine bead beating and chemical lysis e.g. Mo Bio Laboratories' UltraClean™ Microbial DNA Isolation Kit. After extraction is complete, the nucleic acids of the sample must be purified for further use.

Specific regions of the DNA can then be amplified via polymerase chain reaction (PCR) to gain a product suitable for sequencing. The 16S rRNA gene is the most commonly used target for bacterial identification, for a number of reasons. The first is that it is a highly conserved sequence due to its slow evolutionary rate. This allows for examination of ancient evolutionary events, as well as the construction of nearly universal primers. Secondly, all 16S rRNA sequences in prokaryotes have a similar length (1500 nucleotides), easing comparison and confirmation of amplified product (Hillis and Dixon, 1991).

The sequence reaction and resulting purification via ethanol/sodium acetate precipitation provide a finished product that can be evaluated using a genetic analyzer, like the ABI 3130XL. Such a device takes the purified DNA product, along with a BigDye Terminator, and places it into a 36 cm capillary array that loads polymer, separates, and detects nucleotides in the sequence. Data is subsequently generated in the form of a relative fluorescent quantification assay (Applied Biosystems, 2008). This data can be analyzed using a sequence editing software, such as BioEdit (Hall, 1999). The edited sequence is then entered into a sequence database for identification purposes. It is generally accepted that for 16S rRNA samples, a sequence similarity above 97% for an isolate confirms its identity to a particular genus.

Materials and Methods

Sample isolation.

Single, distinct colonies were selected from soil dilution plates of PCA and 1/10 strength PCA, and transferred to a new plate of the same media. Morphological characteristics, medium type, and date isolated were recorded for each colony selected. The streak plate technique was used to isolate individual colonies and check for purity. All plates were incubated at 28°C until pure culture growth was observed. The growth plate was divided into four sections; cells from three of the sections were used for preservation, while the remaining cells were used for DNA extraction.

Isolates were given a strain designation according to the source of the soil sample from which the organism was isolated, sample number, and conditions under which the organism was subjected, along with the duration of those conditions. For example, the first culture isolated from the Negev soil sample "A" plated after six weeks (42 days) of desiccation would be designated as NG05-4-D6A-1. Note that the two soil samples, NG05-4 and AT05-22, begin with two letters used to identify the arid region (Negev or Atacama) followed by two digits for the year it was collected, then a dash followed by a number representing the sample site.

Isolate Preservation.

Preservation solutions were prepared using broth media – PCB or 1/10 strength PCB – with 15% (v/v) glycerol. For each isolate, 750 µL of preservation solution was aliquoted into three sterile Eppendorf® 1.5 mL Safe Lock Tubes. For DNA extractions, a saline EDTA solution was prepared and 300 µL added to a sterile Eppendorf® 1.5 mL Safe Lock Tube. Cell material was then transferred from plates to each of the four tubes

containing culture medium plus 15% (v/v) glycerol or saline EDTA. The tubes containing bacterial cell cultures plus the preservation medium were labeled and preserved in a Thermo Forma® ultra low temperature freezer at -80ºC. Those tubes containing saline EDTA were labeled and stored in the -20°C freezer for future DNA extraction.

DNA Extraction.

The UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc.) was used to extract and purify DNA from bacterial cells. The manufacturer's instructions were followed except for a few minor modifications in the first five steps. The samples, suspended in saline EDTA, were thawed from -20°C and centrifuged at 16,100 x g for 5 min to form a pellet of cells. The supernatant was then removed and discarded using a pipette. Cells were resuspended in 300 µL MicroBead solution and transferred to a MicroBead tube. 50 µL of solution MD1 was added to each tube. Using a Mo Bio vortex adaptor, MicroBead tubes were vortexed at maximum speed for 5 min. The tubes were removed from the vortex and placed in a 65°C water bath for a 10 min incubation period. After incubation, the MicroBead tubes were returned to the vortex adaptor and vortexed at maximum speed for an additional 5 min. Manufacturer's instructions were followed precisely from this point forward. The final DNA-containing solution was transferred to a clean sterile Eppendorf® 1.5 mL tube for storage at 4°C. DNA was verified via agarose gel electrophoresis before use in 16S rRNA gene PCR amplification.

Polymerase Chain Reaction (PCR) and PCR Purification.

PCR was utilized to amplify the 16S rRNA genes from each isolate. Between 20 and 200 ng of DNA was amplified in reaction mixtures totaling 50 µL, containing (as

final concentrations): 5 µL of 10X PCR Buffer (Applied Biosystems), 3.0 µL of 25 mM $MgCl_2$ solution (Applied Biosystems), 50 mM each deoxynucleoside triphosphate, 0.5 µL of each forward and reverse primer (0.5 µg/µL), and 35.8 µL sterile distilled water. Reaction mixtures were initially incubated in a BioRad iCycler® at 98°C for 6 min. (for DNA denaturation). Upon reduction of temperature to 95°C, 0.2 µL (5 U/µL) of AmphiTaq Gold™ *Taq* polymerase (Applied Biosystems) was added. Reaction mixtures were returned to the BioRad iCycler® for 28 cycles at 52°C for 30 sec, 72°C for 1 min, and 94°C for 30 sec. A final extension period of 7 min at 72°C was then performed, and completed reactions were stored at 4°C.

The primers used for 16S rRNA PCR were specific to Bacteria and included forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1525R (5'-AGAAAGGAGGTGATCCAGCC-3'). Products of PCR were confirmed using agarose gel electrophoresis before purification using the Invitek Invisorb® Spin PCRapid Kit. The protocol followed was as per manufacturer's guidelines. The resulting products were transferred to clean sterile 1.5 mL Eppendorf® tubes for storage at 4°C.

Sequencing and Sequence Purification.

Purified PCR products were sequenced using the Big Dye Terminator reagent Version 3.1 (Applied Biosystems) and the 27F Primer. Reactions occurred in 10 µL volumes, containing: 1 µL 16S rRNA gene PCR product, 2.5 µL Big Dye Terminator reagent, 1.25 µL 27F primer (0.5 µg/µL), and 5.75 µL sterile distilled water. Incubation occurred in a BioRad iCycler® at 94°C for 30 sec., followed by 25 cycles at 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. Reaction tubes were removed from the BioRad iCycler® and stored at 4°C until purification.

Purification of sequence reactions was performed using ethanol precipitation. To begin, 10 µL sterile distilled water was added to each reaction tube. This volume was then transferred to a new 0.6 mL tube containing 50 µL of 100% ethanol and 2 µL sodium acetate (3M pH 5.0). Tubes were mixed lightly using a vortex, and then placed on ice. After 10 min., the tubes were removed from ice and centrifuged for 30 min at 16,100 x g. Upon removal from the centrifuge, about 75 µL of supernatant was removed from each tube using a pipette and discarded. Next, 250 µL of 75% (v/v) ethanol was added to each tube and centrifuged for 10 min. at 16,100 x g. Supernatant was removed twice by pipette, approximately 175 µL each time, and discarded. Tubes were placed in a 60°C drying oven for ~30 min. or until completely dry. Purified DNA was stored in the -20°C freezer until ready for resuspension. DNA was resuspended in HiDi Formamide (Applied Biosystems) and analyzed using an ABI 3130XLGenetic Analyzer.

Sequence Data Analysis

The resulting sequence data was compiled using the BioEdit™ program version 7.0.0 (Hall, 1999). Sequences were analyzed using the BLAST search genetic sequence database GenBank®, managed by the National Center for Biotechnology Information (NCBI) of the National Institutes of Health (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). From these BLAST searches, described by Altschul et. al. (1990), the closest phylogenetic relatives were determined for each isolate. These results were checked against type strains using the EzTaxon Server (http://www.eztaxon.org), as described by Chun et. al. (2007).

Results and Discussion

Isolates from the serial dilution plates of the 42-day plating of both the desiccation and room temperature environments investigated in Chapter 2, as well as the initial plating, for both soil samples were further studied. A total of 576 colonies were described and isolated from the serial dilution plates and transferred to new agar plates. Of these, 408 were successfully isolated, preserved, and prepared for further studies. Descriptions for each of the preserved isolates can be found in Appendix A. A total of 119 isolates were identified using 16S rRNA gene sequence analysis. A majority of these isolates were from the Negev desert (110), with only nine from the Atacama used for this study. The Negev isolates were primarily collected from conditions of desiccation and room temperature. Of the 110 isolates from the Negev, 51 were isolated after being subjected to desiccation for 42 days (D6A and D6B), while 48 were left on the lab bench for six weeks at room temperature (R6A and R6B).

From these isolates, a variety of bacterial taxa were identified. A majority of 88 of the isolates (74%) fell within the radiation of the Actinobacteria, with most of these belonging to the genus *Arthrobacter*. Firmicutes was represented by 18 isolates, primarily from the class Bacilli. Six organisms were identified as *Deinococcus* species. Additionally, a group of 12 isolates identified as α-Proteobacteria were present in the form of species of the genera *Microvirga*, *Methylobacterium, Sphingomonas,* and *Belnapia*. One isolate was determined to be a member of the Bacteriodetes/Chlorobi group, identified as *Adhaeribacter aquaticus*. Figure 3.1 shows the diversity by division, and Figure 3.2 presents diversity at the genus level. The BLAST and EzTaxon results for

the 119 isolates for which 16S rRNA gene sequences were determined can be found in Appendix B.

Figure 3.1: Bacterial Divisions of Isolates

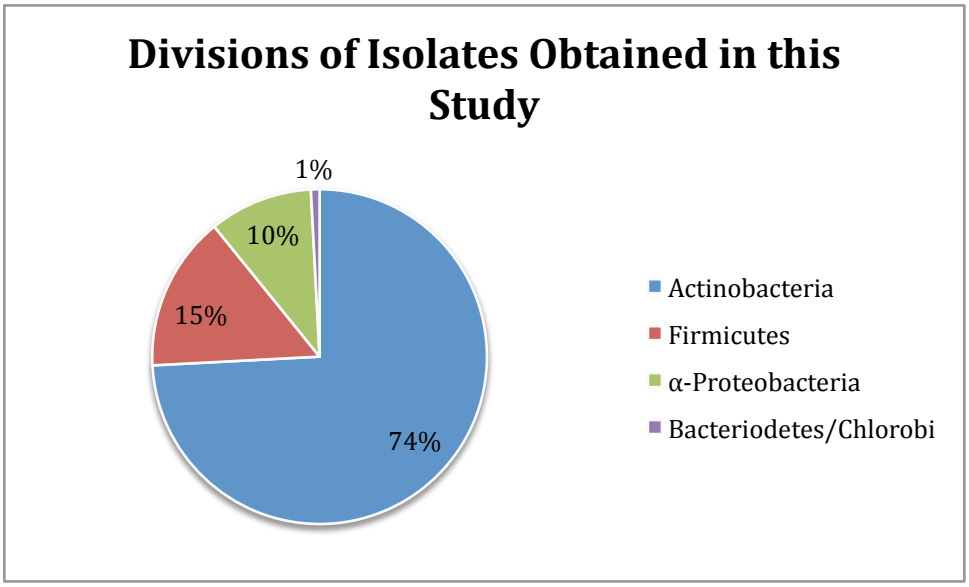
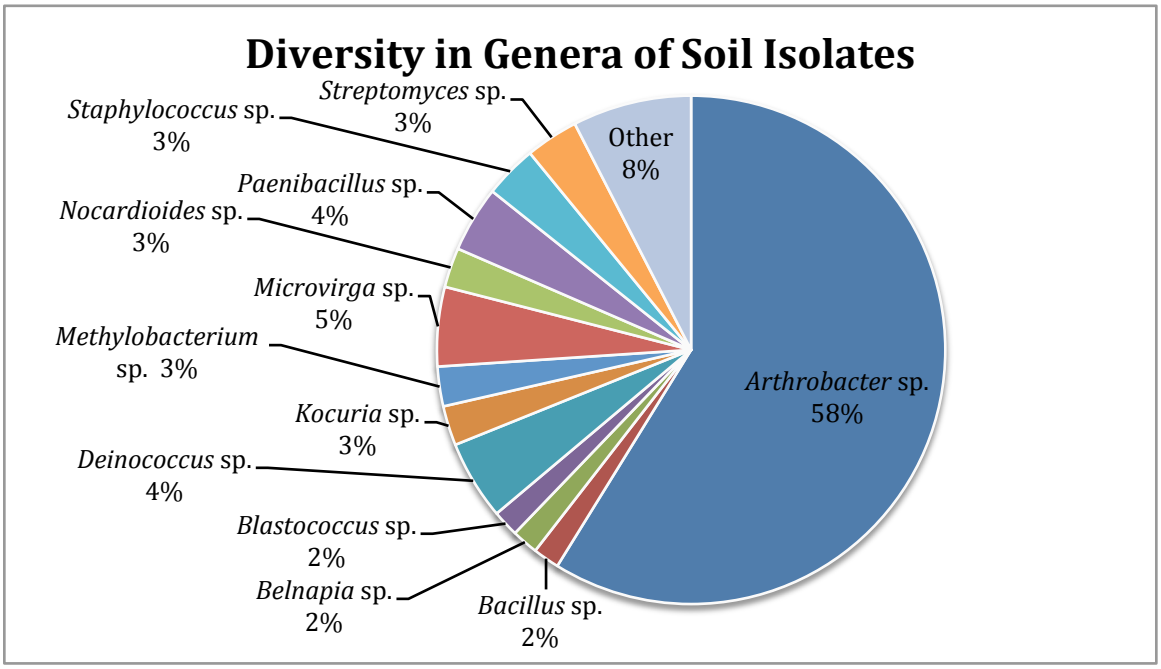


Figure 3.2: Diversity of Genera Identified as Closest Relative to Soil Isolates



Within the Actinobacteria isolated from this study, a number of different genera were present. A majority of these, however, comprised the genus *Arthrobacter* (79%).

Of the 70 isolates identified as *Arthrobacter* sp., 29 were most closely related to *Arthrobacter agilis*. The species *A. oryzae*, *A. ramosus*, *A. scleromae*, and *A. tumbae* were each represented by five isolates from the soil samples. *Arthrobacter humicola* and *A. oxydans* both had four strains identified, while three isolates were associated with *A. crystallpoietes* and with *A. polychromagenes*. The remaining species within the genus *Arthrobacter* were represented by one isolate each and include *A. koreensis*, *A. luteolus*, *A. monumenti*, *A. parietis*, *A. tecti*, and *A. ureafaciens*.

Other Actinobacteria identified from the soil isolates include members of the genus *Streptomyces*, as well as *Blastococcus, Nocardioides*, and *Kocuria*. Four species of *Streptomyces* were discovered, each with one isolate – *Streptomyces candidus*, *enissocaesilis*, *helvaticus*, and *roseolilacinus*. Two species of *Blastococcus – B. aggregatus* and *B. saboxidans* – were found in samples from the Negev. *Nocardiodes* was represented in this study by three isolates, two of the species *N. terrigena* and one *N. oleivorans*. Two isolates were identified as *Kocuria polaris*, with a third as *K. rosea*. Additionally, a few genera belonging to Actinobacteria were found that only had one species represented. These included *Cellulomonas cellasea*, *Leukobacter komagate, Micrococcus lylae*, *Modestobacter versicolor*, *Nesterenkonia jeotgall*, and *Saccharothrix texasensis*.

The next most common bacterial division was Firmicutes, which contained 18 isolates. Class Bacilli within Firmicutes contained the generas *Bacillus*, *Paenibacillus*, *Staphylococcus*, and *Planomicrobium*, and comprised 12 of the 18 Firmicutes isolates. Three species of *Bacillus – anthracis*, *megaterium*, and *murialis* – were identified from the isolates, as well as three *Paenibacillus* species (*lautus*, *lactis*, and *larvae*).

Paenibacillus lautus was the closest relative of three of the isolates, while only one isolate was close to *P. lactis* and *P. larvae*. *Staphylococcus* was represented by three genera, with two isolates as *S. epidermidis* and the other two isolates related to *S. nepalensis* or *S. pasteuri*. Class Bacilli also contained one isolate related to *Planomicrobium okeanokoites*. Six isolates were determined to belong to the genus *Deinococcus*, which is a class of Firmicutes. *Deinococcus deserti* was the closest relative of four of these isolates, while *D. hohokamensis* and *D. navajonensis* were identified in only one isolate each.

In regards to the α-Proteobacteria, four genera were determined to be present in its 12 isolates. *Microvirga* was the most common of these, having six isolates identified as the species *M. subterranea*. Additionally, three strains were determined to be most closely related to *Methylobacterium iners*, a relatively new species in the genus. Two isolates were found to be *Belnapia moabensis*, which was originally isolated from a biological soil crust in Colorado (Reddy et. al., 2006). The last of the 12 Firmicutes isolated from this study was *Sphingomonas kaistensis*, isolated from the Atacama soil sample (AT05-22-27).

Adhaeribacter aquaticus was identified in one isolate, NG05-4-D6B-29, and is the only one of the 119 isolates to belong to the Bacteriodetes/Chlorobi division of Bacteria.

CHAPTER 4:

CHARACTERIZATION OF ISOLATES OF THE *MICROVIRGA/BALNEIMONAS* LINEAGE

Introduction

Microvirga subterranea was isolated from the Great Artesian Basin (GAB), a deep geothermal aquifer that covers an area of 1.7 x 10^6 km^2 and lies below the surface of Australia. The water contained within the GAB aquifer is over 2 million years old, and provides a source of drinking water for the area. The sample site from which *M. subterranea* was isolated is the Failea bore in Longreach, Queensland. It has a flow rate of 9 L per year and a water temperature of 42°C (Kanso and Patel, 2003). The isolate that became the type strain for the genus *Microvirga* was obtained from this sample and is known as FaiI4^T (Figure 4.1). It was found to be Gram-negative, rod-shaped, and motile by a single polar flagellum. On agar, colonies of FaiI4^T were light-pink (Figure 4.2) and required yeast extract for growth, and had an optimal growth temperature of 41°C. Additionally, it produced catalase and reduced nitrate to nitrite. Its phylogenetic relatives were found to be members of the *Methylobacteriaceae*, more specifically the species *Chelatococcus asaccharovarans*, and *Bosea thiooxidans*, which showed 93% sequence similarity to the newly discovered isolate (Kanso and Patel, 2003). On the basis of these characteristics, strain FaiI4^T was described as a new species of a novel genus, *Microvirga subterranea* (Kanso and Patel, 2003).

Balneimonas flocculans was cultivated from the runoff of the Kazawa hot spring in the Gunma Prefecture of Japan (Takeda et. al., 2004). The isolate, TFB^T (Figure 4.1), was Gram-negative, aerobic, and had a rod-shaped morphology with flagella for motility.

It appeared non-pigmented on nutrient media (Figure 4.2). Optimal growth was observed at temperatures in the range 40-45°C. *B. flocculans* was found to utilize only three of the 48 carbon sources tested when used as sole carbon and energy sources in media; growth occurred only with tryptone, peptone, or yeast extract. It was also observed to produce both catalase and oxidase, in addition to possessing a cellulose synthase gene. When examining sequence similarities, closest relatives were found to be *Microvirga subterranea*, *Bosea thiooxidans*, and strains of the genus *Methylobacterium*. It was determined that the isolate TFB^T should be established into a new genus, *Balneimonas*, due to phylogenetic analysis and phenotypic characterization (Takeda et. al., 2004).

A group of 60 isolates from arid soils were selected from the RaineyLab collection, all showing 16S rRNA gene sequence similarities in the 94-100% to Balneimonas sp. or *Microvirga* sp. These isolates were designated as members of the *Microvirga*/*Balneimonas* group, and showed extensive diversity on the basis of colony morphology alone. The need for further characterization of these 60 isolates was apparent due to the high 16S rRNA gene sequence similarities and phylogenetic relationships to the two type strains, *M. subterranea* (strain FaiI^T) and *B. flocculans* (strain TFB^T). Additionally, this phylogenetic analysis brought into focus the possible requirement for emendation of the description of the genus *Microvirga* and reclassification of *B. flocculans* as a species of the genus *Microvirga.*

The strains included in this study were isolated by serial dilution plating over the past decade from arid soil samples around the world. They sample sources represent seven distinct arid regions on four continents. An additional strain was collected from an air sample in the hyper-arid core region of the Atacama Desert, Chile.

Figure 4.1: Microscopy images of *Microvirga subterranea* (top) and *Balneimonas flocculans* (bottom)

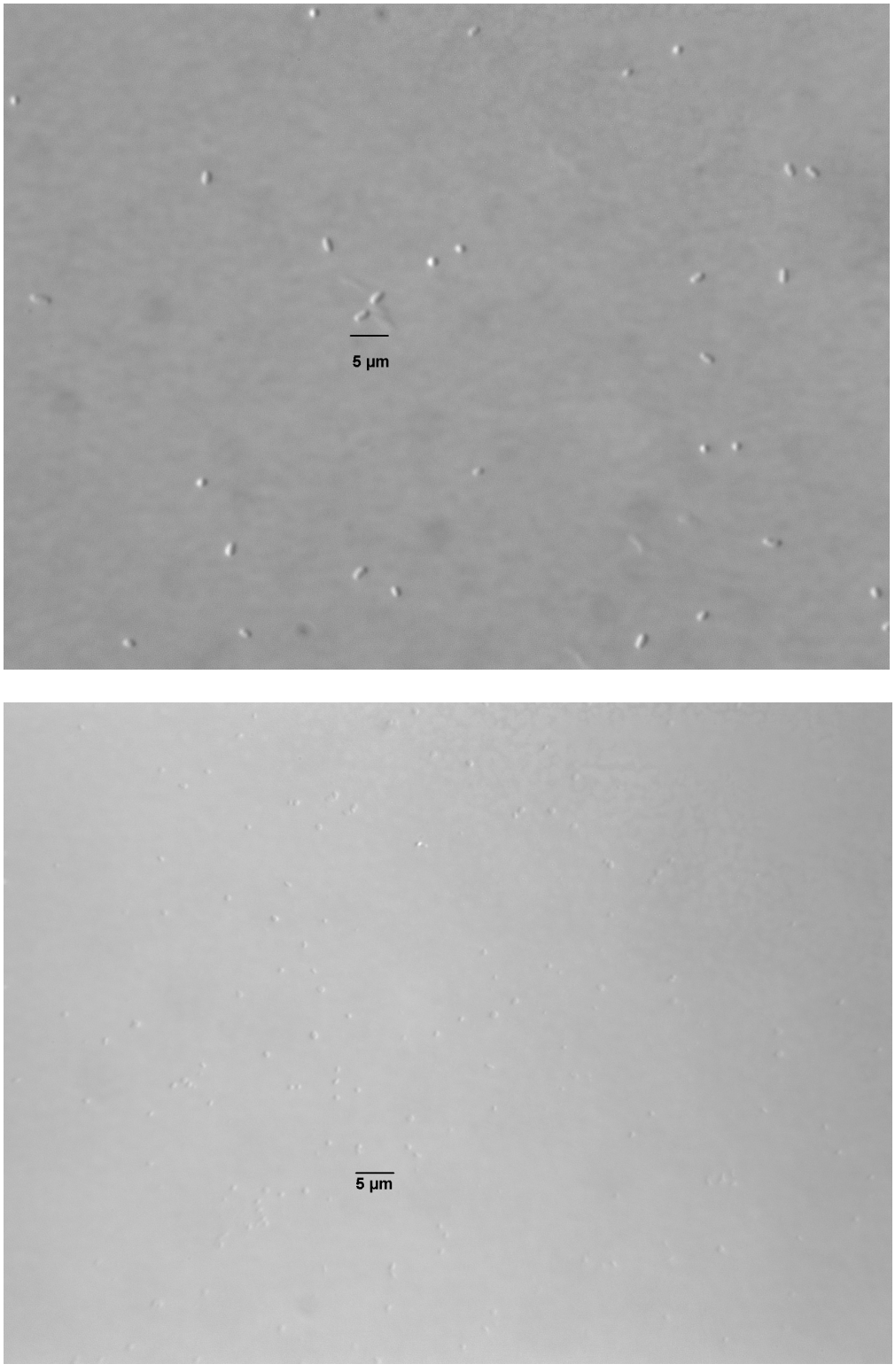
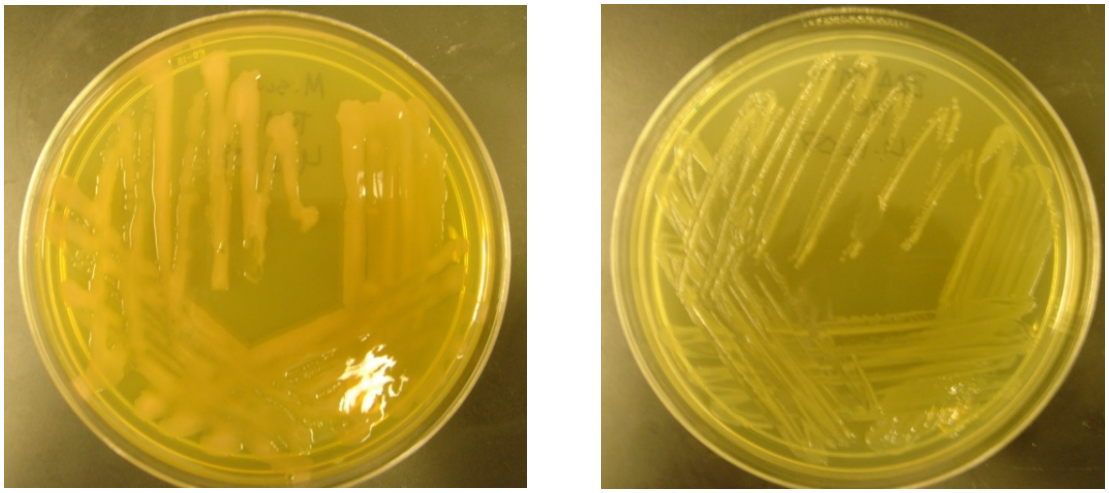


Figure 4.2: Colony morphology of *Microvirga subterranea* (left) and *Balneimonas flocculans* (right)



Materials and Methods

Isolate Collection.

For this study, a collection of 60 environmental isolates from soil samples was assembled; the elimination of one isolate due to contamination reduced the collection to 59 isolates. These samples were obtained from seven distinct arid regions, namely deserts, from across the globe – the Atacama Desert in Chile, the Negev Desert in Israel, the Sahara Desert in Africa, the Oman Desert, the Sonoran Desert in Arizona, the Mojave Desert in California, and the Nevada Desert.

Additionally, a pair of root nodule symbionts isolated from the legume *Lupinus texensis* was included. The isolates, designated Lut5 and Lut6, show 16S rRNA sequence similarity of 95% to the type strains (*M. subterranea* and *B. flocculans*) and can be considered relatives of the other 59 isolates included in this study. Strains Lut5 and Lut6 were provided by Dr. Matthew Parker of the State University of New York in Binghampton, whose lab originally isolated and described some of the characteristics of these two strains (Andam and Parker, 2007).

The two type strains were obtained for use in this study from culture collections;

Microvirga subterranea from the German Collection of Microorganisms and Cell Cultures (DSM 14364^T) and *Balneimonas flocculans* from the American Type Culture Collection (ATCC-BAA 817).

The sample locations for the 63 isolates studied are outlined in Table 4.1. All of these isolates shared greater than 94% 16S rRNA gene sequence with the type strains of the species *Microvirga subterranea* and *Balneimonas flocculans*.

Table 4.1: Source of samples from which the strains were isolated

Sample Location	Country/Area of Origin	Designation	# of Isolates
Atacama Desert	Chile	AT04-150/159, AT05-22, 68J	6
Negev Desert	Israel	NG05-4	5
Sahara Desert	Northern Africa	SS04-2	2
Mojave Desert	USA – California	2LRH	1
Oman Desert	Oman	33-40Q, 10-39Q, 20-40R	25
Sonoran Desert	USA – Arizona	KR-S97	9
Nevada Desert	USA – Nevada	KR-N97	11
Lupinus texensis	USA – Texas	Lut5, Lut6	2
GAB Aquifer	Australia	DSM 14364^T	1
Kazawa Spring	Japan	ATCC-BAA 817	1

Media Composition.

Four nutrient media were used for growth of the isolates studied from preserves, including RM, PCA, 1/10 strength PCA, 1/100 strength PCA, and NA. RM agar is made with 10.0 g peptone, 5.0 g yeast extract, 5.0 g casamino acids, 2.0 g beef extract, 5.0 g malt extract, 2.0 g glycerol, 1.0 g $MgSO_4 \bullet 7H_2O$, and 15 g agar, all added to 1 L of sterile, deionized water. PCA is composed of 5.0 g tryptone (pancreatic digest of casein), 2.5 g yeast extract, 1.0 g dextrose, and 15.0 g agar in 1 L deionized water. 1/10 strength and

1/100 strength PCA is made by the dilution of PCA. Finally, NA contains 3.0 g beef extract, 5.0 g peptone, and 15.0 g agar in 1 L of deionized water.

Colony Morphology.

Strains were inoculated onto RM media using the streak plate technique in order to isolate individual colonies. After 7 days of incubation at 30°C (or optimum temperature), colony morphology was recorded for each of the strains.

Optimal Growth Temperature.

To determine the optimal growth temperature for each isolate, strains were streaked on RM agar plates and incubated at 30°C, 37°C, 40°C, and 45°C. A few isolates were also tested at 25°C, since they did not grow well at 30°C. After 5 days of incubation, isolate growth at the different temperatures was compared and scored.

Growth in Liquid Media.

One loopful of each isolate was inoculated in 2 mL nutrient broth of both 1/10 strength PCB and RM broth. Tubes were then incubated at the temperature optimum for growth and scored after 5 days. The presence of aggregated cells in the liquid media was noted as flocculation along with the amount of growth.

Phenotypic Characterization.

Phenotypic tests were carried out for each of the 63 isolates, including the type strains, to determine the phenotypic diversity of the isolates and assist with identification and differentiation. All methods employed are as described in Smibert and Krieg (1994).

Oxidase Activity.

The activity of the oxidase enzyme was determined using ashless filter paper (Whatman no. 40) placed into a Petri dish. To this filter paper, 0.5 mL of 1% (w/v)

tetramethyl-*p*-phenylenediamine in DMSO was added. For each isolate, a small loopful of cell material was placed onto the filter paper. A positive result was noted if a blue-purple color developed within 15 sec.

Urease Activity.

Urease activity was determined using Christensen urea agar. Agar slants were inoculated for each strain and incubated at the optimum growth temperature. The appearance of a red-violet color on the media denoted a positive test; results were read after 5 days incubation. *Bacillus megaterium* was used as a positive control, and *E coli* was used as a negative control.

Christensen urea agar is made by adding the following to 1L of distilled water: 3.0 g sodium citrate, 0.2 g glucose, 0.5 g yeast extract, 0.1 g cysteine hydrochloride, 0.4 g ferric ammonium citrate, 1.0 g KH_2PO_4, 5.0 g NaCl, 0.08 g sodium thiosulfate, 0.012 g phenol red, and 15.0 g agar. The ingredients were combined and pH adjusted to 6.7 before sterilization by autoclaving; media was cooled in a slanted position in test tubes.

Starch Hydrolysis.

The ability to hydrolyze starch was tested using RM media with 0.2% (w/v) soluble starch added prior to boiling. Isolates were streaked in a single line across the center of each plate, and incubated at their optimum growth temperature. After 5 days, plates were flooded with Gram's Iodine; plates that showed a zone of clearing area around the bacterial colonies were marked positive for starch hydrolysis. A culture of *Bacillus subtilis* was used as a positive control for starch hydrolysis.

Catalase Activity.

To determine the presence of catalase in a particular isolate, a loopful of bacteria from that isolate was suspended in a drop of 3% (v/v) hydrogen peroxide on a glass slide. A positive reaction was determined by the formation of bubbles within 5 min.

Motility.

The motility of each isolate was determined by stab inoculation into an agar deep containing Motility Test Medium with tetrazolium chloride (TTC). Agar deeps were incubated after inoculation at the optimum growth temperature for 5 days. The appearance of a red color within the medium radiating from the center stab indicates a motile organism. The positive control for this experiment was *Bacillus subtilis*, and *E. coli* was used as the negative.

Motility Test Medium with TTC was made by adding the following to 1 L of distilled water: 22.0 g Motility Test Medium (Bacto), 2.0 g beef extract, 6.0 g gelatin, 2.62 g $K_2HPO_4 \bullet 3H_2O$, 2.0 g KNO_3, and 1.0 g agar. Ingredients were combined and mixed, and the pH was adjusted to 7.2 before sterilization via autoclave. After media tempered, 1% (v/v) filter sterilized TTC solution was added, and 8 mL of media was dispensed into each sterile 10mL test tube. Tubes were refrigerated until use.

NaCl Tolerance.

The NaCl tolerance of each isolate was tested using 1/10 strength PCB with 0.5% (w/v) 1.0% (w/v), 1.5% (w/v), and 2.0% (w/v) sodium chloride. Isolates were first inoculated into tubes containing 4 mL 1/10 strength PCB, with growth scored after 5

days. From these broth cultures, 100 µL was transferred to four sterile test tubes, each containing 2 mL of the PCB with different NaCl concentrations. Growth in each saline solution was scored following another 5d incubation.

Carbon Source Utilization: Glucose.

To determine the utilization of glucose as a carbon source by each of the isolates, cultures were inoculated onto two plates of a minimal media (Rouf's Medium) one with glucose and one without. Both plates were incubated at the inoculated isolate's optimal growth temperature. After 5 days incubation, culture growth was scored and compared between the media containing and lacking glucose. A positive result for glucose as a carbon source was determined if growth on non-glucose media was less than that on glucose-containing media; negative results were obtained if growth on glucose-containing Rouf's Medium was greater than or equal to that on media lacking glucose.

Rouf's Medium was prepared in 1 L of sterile deionized water, to which the following were added: 5.0 g yeast extract, 5.0 g peptone, 0.2 g $MgSO_4 \bullet 7\ H_2O$, 0.05 g $CaCl_2 \bullet 6\ H_2O$, 0.15 g $Fe(NH_3)$ citrate, 0.05 g $MnSO_4 \bullet 4\ H_2O$, 0.01 g $FeCl_3 \bullet 4\ H_2O$, 10 mL vitamin solution, 1 mL trace elements solution, and 17 g agar. After all ingredients had been added, Rouf's Medium was adjusted to pH 7.1 prior to autoclaving. Glucose was added to Rouf's Medium for carbon sources testing in a concentration of 200 mg/mL.

Vitamin solution contained 4 µg/mL each of the following vitamins: thiamine, riboflavin, pyridoxine, biotin, choline, folic acid, inositol, nicotinic acid, pantothenic acid, and aminobenzoic acid. Trace elements solution was made by first dissolving 12.8 g nitrilotriacetic acid in 1 L deionized water and adjusting the pH to 6.5 with KOH. The following minerals were then added: 0.17 g $CoCl_2 \bullet 6\ H_2O$, 0.1 g $CaCl_2 \bullet 2\ H_2O$, 0.1 g

$FeSO_4 \bullet 7\ H_2O$, 0.1 g $MnCl_2 \bullet 4\ H_2O$, 0.1 g NaCl, and 0.1 g $Na_2MoO_4 \bullet 2\ H_2O$. The trace elements solution was then adjusted to pH 7.0 with KOH.

DNA Extraction.

Extraction and purification of DNA from the isolates was performed using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc.). Instructions were followed as provided by the manufacturer, except for a few minor modifications listed in Chapter 3. Solutions containing DNA were stored in sterile Eppendorf® 1.5 mL tubes and stored at 4°C. Agarose gel electrophoresis was used to verify the size of the 16S rRNA gene band prior to PCR.

Polymerase Chain Reaction (PCR) and PCR Purification.

Amplification of the 16S rRNA gene for each isolate was achieved through PCR of DNA samples. Protocol was followed as described in Chapter 3, and completed reactions were stored at 4°C. Primers used were specific to the Bacterial 16S rRNA gene, and again included forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1525R (5'-AGAAAGGAGGTGATCCAGCC-3'). Products were confirmed by agarose gel electrophoresis, and samples were purified with the Invisorb® Spin PCRapid Kit (Invitek). The protocol followed was as per manufacturer's guidelines. Resulting DNA was transferred to sterile 1.5 mL Eppendorf® tubes for storage at 4°C.

Sequencing and Sequence Purification.

Purified PCR products were initially sequenced using a Big Dye Terminator reagent (Applied Biosystems) and the 27F primer (5'-AGAGTTTGATCCTGGCTCAG-3'). In order to sequence the entire 16S rRNA gene for each isolate, additional primers were used. These included: 1525R (5'-AGAAAGGAGGTGATCCAGCC-3'), 357F (5'-

CTCCTACGGGAGGCAGCAG-3'), 357R (5'-CTGCTGCCTCCCGTA-3'), 530F (5'-CAGC[C/A]GCCGCGGTAAT[T/A]C-3'), 519R (5'-GWATTACCGCGGCKGCTG-3'), 1100F (5'-GCAACGAGCGCAACCC-3'), and 803F (5'-ATTAGATACCCTAGGTAG-3'). These primer sequences were all obtained from Rainey et. al. (1996).

The same sequencing reaction protocol was followed as previously described in Chapter 3, except for the substitution of the 27F primer with any one of the seven additional primers mentioned above. Purification of the sequence reaction products with ethanol precipitation was also followed as described in Chapter 3.

Sequence Data Analysis and Dendrogram Construction.

Sequence data was compiled using the BioEdit™ program and searched with NCBI BLAST or the EzTaxon search tool (Chun et. al., 2007) as mentioned in Chapter 3. The closest phylogenetic relatives were found for each isolate to check that the sequence data results remained in the *Microvirga/Balneimonas* cluster.

The phylogenetic analysis of the aligned sequences was carried out using the MEGA 4 program (Tamura et. al., 2007). Sequences were aligned on the basis of secondary structure. Dendrograms showing phylogenetic relationships were constructed by the neighbor-joining method from distance matrices (Tamura et. al., 2007).

Fatty Acid Analysis.

From the collection of 63 isolates, a group of 18 were selected for fatty acid analysis based on the diversity revealed in the 16S rRNA phylogenetic tree. Sample plates from each isolate were shipped to the laboratory of Dr. Milton S. da Costa at Universidade de Coimbra in Coimbra, Portugal, for analysis. Results were obtained from the da Costa Lab as percent abundances for each fatty acid found in a given culture.

The following methods were used at the daCosta lab: All strains were grown on RM agar for 5 days at 37°C. Fatty acid methyl esters (FAMEs) were obtained and separated, identified, and quantified with the standard MIS Library Generation Software (Microbial ID, Inc.) as described previously in Chung et. al. (2000).

Results and Discussion

Of the 60 original environmental isolates designated for use in this study, excluding the type strains and Lut strains, 59 were carried through and have complete characterization profiles. One isolate, 10-39Q-45, was removed from the study because of contamination. All other isolates were seen as pure and continued in the study.

Near the end of the characterization process, the isolate NG05-4-1 was discovered to be growing in tandem with a yeast culture. These two strains were separated using the streak plate method, and subsequently designated NG05-4-1B (bacterium) and NG05-4-1Y (yeast). Characterization tests were repeated for the bacterium, since its growth was subdued by competition from the yeast.

The remainder of the isolates, including the two type strains and the Lut5 and Lut6 root nodule symbionts, were carried through the characterization process to completion. All of these 63 strains showed great diversity based on the results obtained from the tests performed, especially with respect to their phenotypic characteristics. Similarities were discovered among some isolates that grouped together phylogenetically, thus the characterization will aid in the designation of the isolates into separate species of the genus *Microvirga*.

At the basic level, the temperature profile obtained from the isolate collection possessed many similarities. A majority of isolates showed a high amount of growth at

30°C (45 of 63 isolates; 71.43%), as well as at 37°C (41 isolates; 65.08%). At the higher temperatures of 40°C and 45°C, a large number of cultures showed weak growth. At 45°C, 17 of the isolates (26.98%) showed no growth at all, while 30 (47.62%) showed a miniscule amount of growth. The type strains of *M. subterranea* and *B. flocculans* have optimum temperatures described as 41°C and 40-45°C, respectively (Kanso et. al., 2003; Takeda et. al., 2004); these temperatures held true in experimentation with both strains exhibiting high growth at their published optimum temperatures. Overall, the isolates do show some tolerance of higher temperatures, but many of them are not able to grow well above 40°C. These results are shown in Table 4.2.

Table 4.2: Percentage of isolates growing at temperatures between 30 and 45 °C

Incubation Temperature	Isolate Growth				
	No growth	+	++	+++	++++
30°C	0%	0%	6.35%	22.22%	71.43%
37°C	1.59%	1.59%	17.46%	14.28%	65.08%
40°C	4.76%	22.22%	20.64%	14.28%	38.10%
45°C	26.98%	47.62%	6.35%	6.35%	12.70%

The salt tolerance of each isolate was determined using 1/10 strength PCB with 0.5-2.0% (w/v) added NaCl. The results indicate that there is not much variation in the growth of isolates in the 0.5% to 2.0% NaCl range. For all NaCl concentrations, 3-7 isolates did not grow, 12-15 showed a small amount of growth, 17-24 possessed adequate growth, and 23-27 displayed excellent growth; these results are shown in Table 4.2.

Table 4.3: NaCl tolerance of the isolates

% NaCl (in 1/10 strength PCB)	Isolate Growth			
	No growth	+	++	+++
0.5%	6.35%	23.81%	26.98%	42.86%
1.0%	4.76%	19.04%	38.10%	38.10%
1.5%	7.94%	23.81%	31.74%	36.51%
2.0%	11.11%	23.81%	26.98%	38.10%

It was determined that the isolates do not show any more or less tolerance to added NaCl. About 38% of isolates, however, did show adamant growth in the presence of up to 2.0% NaCl, including *M. subterranea*. Additionally, seven of these isolates (AT05-22-20, AT05-22-21, 33-40Q-8, 10-39Q-41, 10-39Q-57, 10-39Q-94, 10-39Q-95) did not grow at low NaCl concentrations, but showed increased growth with increasing NaCl concentration. Also, two isolates (NG05-4-1B and 33-40Q-27) showed growth only in 1/10 strength PCB containing 1.0% (w/v) NaCl. Three isolates 33-40Q-60, 20-40R-25, and 10-39Q-110, did not show significant growth when NaCl was added to 1/10 strength PCB in the range of 0.5-2.0% (w/v). Further testing at higher salt concentrations may allow for a more complete study of salt tolerance in these organisms.

For each phenotypic test performed on the isolates, various similarities and differences were revealed among the strain collection. The major characteristics shared between the isolates and the type strains were a positive test for catalase activity (only KR-S97-47 and KR-N97-447 tested negative) and a negative test for urease activity (only KR-N97-477 and 2LRH1B-19 tested positive). Both type strains were urease negative and oxidase positive, showing a strong correlation between *Microvirga subterranea* and *Balneimonas flocculans* and the experimental results for the isolates.

The remainder of the tests showed equal positive and negative results, and the two type strains showed differences in starch hydrolysis, oxidase activity, and flocking upon growth in liquid media. These results are highlighted in Table 4.4, and a complete listing of the results of all phenotypic tests can be found in Appendix C. These phenotypic differences between the type strains of the species *M. subterranea* and *B. flocculans*, along with their distinct phylogenetic positions (Figure 4.3), support their species status

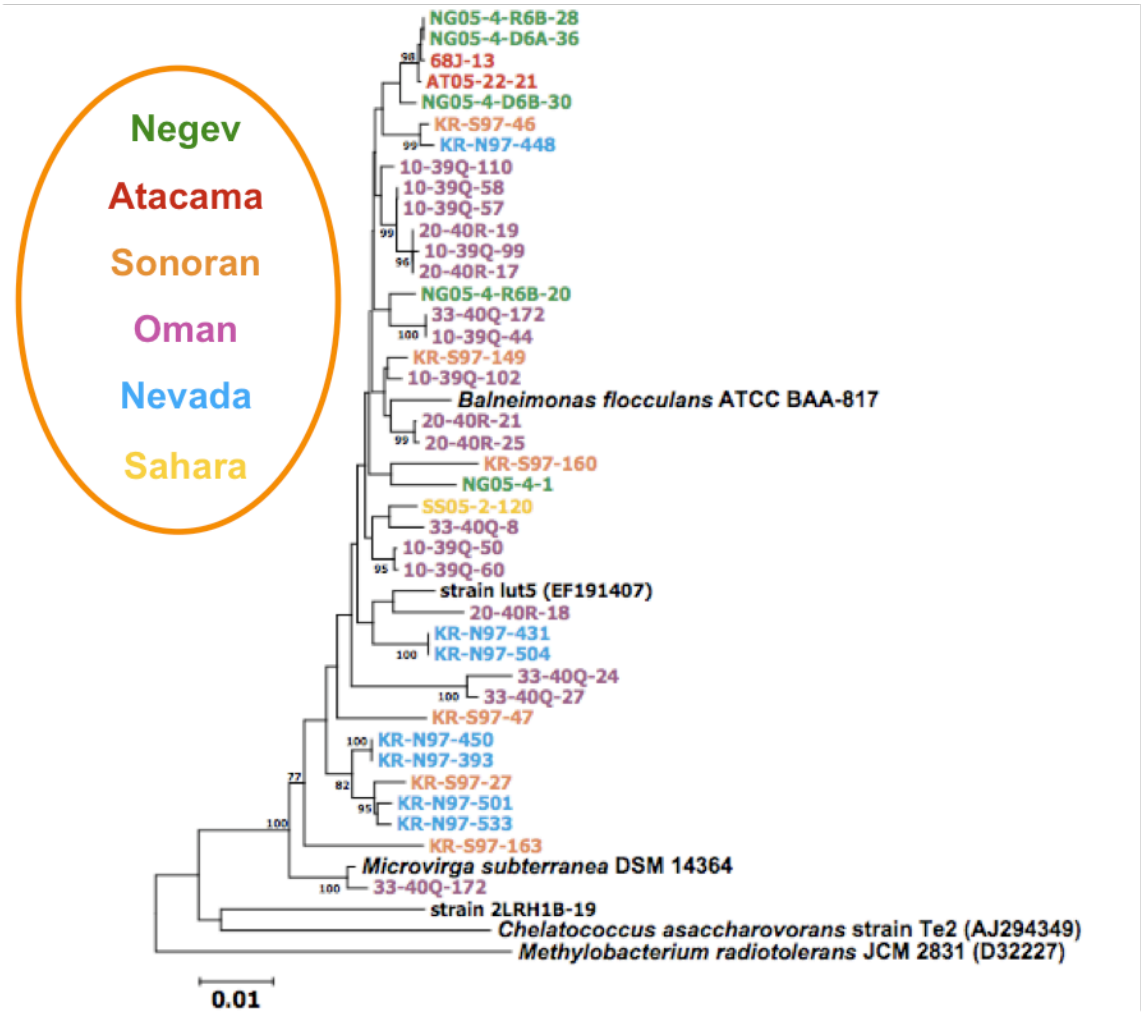
within a given genus. However, the phenotypic diversity of the isolates would point to the establishment of more species than the two existing species.

Table 4.4: Phenotypic test results for the isolates and type strains.

Phenotypic character	**Isolates (+ / -)**	***Microvirga subterranea***	***Balneimonas flocculans***
Liquid Flocking	36 / 25	-	+
Glucose Utilized	31 / 30	-	-
Motility	35 / 26	+	+
Oxidase Activity	42 / 19	-	+
Urease Activity	2 / 59	-	-
Catalase Activity	59 / 2	+	+
Starch Hydrolysis	34 / 27	+	-

Additional primers were used to fully sequence the 16S rRNA gene of 40 isolates plus the type strains of *M. subterranea* and *B. flocculans*, to obtain a more complete picture of their phylogenetic relatedness. These almost complete (>1450 nucleotides) sequences were used to construct a phylogenetic dendrogram indicating their phylogenetic relationships (Figure 4.3). The names of the isolates are shown in color to differentiate between the arid regions from which each was collected. The type strains are shown on the tree, as well as the root nodule symbiont Lut5. *Chelatococcus asaccharovarans* and *Methylobacterium radiotolerans* are included as outgroup organism in the analysis.

Figure 4.3: 16S rRNA gene sequence-based phylogeny indicating relationships of the isolates and type strains.



Note: The scale bar represents 1 inferred nucleotide substitution per 100 nucleotides.

The 16 S rRNA gene sequences of the isolates share between 94 and 100% pairwise sequence similarity resulting in them branching in different clusters containing isolates from different sources or the same source. It is interesting to notice how closely related some isolates are, despite their isolation from geographically isolated regions. The first cluster at the top of the phylogenetic dendrogram contains isolates from the Negev and Atacama regions which share >99.8% 16S rRNA gene sequence similarity. As was mentioned in Chapter 2, the two sample sites were very different, especially with regard to vegetation cover and annual precipitation. The same is true for the grouping of

isolates the Oman desert with the KR strains from Nevada and Arizona, both in the southwestern United States. The close relationship between strain SS05-2-120 from the Sahara and strain 33-40Q-8 from Oman is not entirely surprising, given the close geographical distance between the two locations in Northern Africa and the Middle East.

This phylogenetic analysis shows the isolate 2LRH1B-19 from the Mojave Desert to be distantly related to the other isolates, which corresponds with its unique properties in regard to it being negative for catalase and positive for urease. It could be considered that this isolate may be a member of a different and novel genus based on its phylogenetic distance from the other isolates and type strains included in the analysis.

Fatty acid analysis was carried out on 18 isolates for additional characterization. These results show the predominance of the C18:1ω7c and C18:1ω6c fatty acids for all strains. The amounts ranging from 53.1% for strain KR-N97-501 to 83.1% for strain Lut5, and an overall average of 68.3% total composition for all strains. The next most common fatty acid seemed to be C16:0, which ranges from 3.8% in strain 68J-13 to 13.8% in strain KR-N97-431, with an average of 8.3% of the fatty acids for all isolates. These and other average percentages of total fatty acid are shown in Table 4.4. Complete fatty acid results can be seen in Appendix D.

Table 4.5: Percentage of specific fatty acids for isolates analyzed

Fatty Acid(s) Present in Isolate	Average Total %	# of Isolates
C16:1iso I/C14:0 3OH	3.0%	18
C16:1ω7c/C16:1ω6c	4.7%	18
C16:0	8.3%	18
C17:0	2.0%	15
C18:1ω7c/C18:1ω6c	68.3%	18
C18:0	3.5%	18
C19:0 cyclo ω8c	5.6%	14
C18:0 3OH	1.1%	16

The results of the phylogenetic analysis and phenotypic characterization provide a starting point for the designation of new species within this genus cluster, as well as the emendation of the description of the genus *Microvirga* and the transfer of the species *Balneimonas flocculans* to the genus *Microvirga*. This is because taxonomic priority is given to the name *Microvirga*, having been validly published prior to the name *Balneimonas*. Considering the high similarity values that exist between many of the isolates from geographically remote sources, as well as isolates that are phenotypically distinct, there is a need to carry out DNA-DNA re-association studies between representative strains from the clusters recovered in the 16S rRNA gene sequence based phylogenetic analysis. There are many examples in other genera, for example the genus *Bacillus* were species share >99.9% 16S rRNA gene sequence similarity but have DNA-DNA re-association values of <70% (Fox et. al., 1992)

CHAPTER 5:

DESICCATION AND RADIATION RESISTANCE PROFILES OF SELECT ISOLATES

Introduction

Organisms that possess the ability to readily repair DNA damage have a selective advantage for survival in arid environments (Rainey et. al., 2005). These unique environments possess a number of less than ideal traits for life, ranging from dryness, lack of nutrients to temperatures extremes and fluctuations in those temperatures over time (Chanal et. al., 2006). These conditions can lead to stress of the microorganisms that inhabit arid lands. In this study, we specifically looked at the effect of the stresses of desiccation and radiation, both ultraviolet (UV) and ionizing or gamma radiation, on bacterial strains from arid lands.

Desiccation involves the removal of water from a cell, and results in stress that is often lethal to organisms. Bacterial stasis or dormancy is a major means of tolerating desiccation, which also includes the subsequent recovery of metabolic function (Potts, 2001; Billi and Potts, 2002; Chanal et. al. 2006). Organisms that are able to tolerate desiccation are referred to as anhydrobiotic, and can survive water content less than 0.3 g water/g dry weight. The absence of water in a cellular system can have negative effects on protein folding, membrane structure and function, as well as gene expression. DNA damage can occur in the form of chemical modification, especially alkylation or oxidation, depurination, and cross-linking (Billi and Potts, 2002).

Energy emitted from the sun travels to the earth in the form of electromagnetic radiation and can affect the organisms that inhabit it. One such type of radiation – ultraviolet (UV) radiation – falls outside the visible spectrum, with wavelengths ranging

from 190-315 nm. DNA can absorb short-wavelength UV radiation efficiently because of the aromatic rings present on its bases. Such absorption leads to damage in the form of pyrimidine dimers, especially with thymine, and (6-4)-photoproducts, both of which create crosslinks between adjacent DNA bases (Latonen and Laiho, 2005).

Gamma radiation is a type of ionizing radiation that can cause DNA double-strand breaks in a bacterial genome. Additionally, single-strand breaks, DNA-protein crosslinks, and many types of base damage can occur after exposure to ionizing radiation (Battista et. al. 1999). Most species would be unable to overcome such damages to their genome; however, *Deinococcus radiodurans* and other radiation tolerant organisms possess adaptations that make survival possible. Examples of such adaptations could include enhanced DNA repair ability or a means of preventing radiation damage from occurring *in vivo* (Zimmerman and Battista, 2006).

DNA damage in any form can be detrimental to the cell without repair. On the basic level, it can result in cell cycle arrest or cell death. DNA replication is altered by damage, since error-prone DNA polymerases are utilized to copy damaged DNA. Lesions in the DNA strands can also inactivate transcription, which essentially causes gene expression to be halted in affected genes. Repeated stress incurred by blocked transcription can also cause p53-induced apoptosis. Lastly, mutations may cause unarrested cell growth, which leads to cancer in eukaryotic cells (Hoeijmakers, 2001). With such negative effects in mind, it becomes apparent that organisms would need to adapt to harsh conditions for survival of DNA damage.

Deinococcus radiodurans is only one example of a bacterium that can survive ionizing radiation, with a D_{10} value of 10 kGy. Another microorganism with comparable

resistance to *D. radiodurans* is *Rubrobacter radiotolerans* (D_{10} of 11 kGy). However, these two organisms show the high end of the resistance spectrum. Other bacteria have been shown to survive radiation doses ranging from 1 to 5 kGy, indicating a lower level of gamma radiation resistance. Such organisms include: *Methylobacterium radiotolerans* (1 kGy), *Kocuria rosea* (2 kGy), *Acinetobacter radioresistens* (2 kGy), *Kineococcus radiotolerans* (2 kGy), *Hymenobacter actinosclerus* (3.5 kGy), *Chroococcidiopsis* spp. (4 kGy), and *Rubrobacter xylanophilus* (5.5 kGy), each of which show more resistance to radiation than bacteria like *E. coli* (Cox and Battista, 2005).

Resistance of bacteria isolated from arid soils to ionizing radiation appears to be an incidental consequence of the organism's ability to survive periods of desiccation (Rainey et. al., 2005). Since there is no part of Earth in which organisms are subjected to a high enough flux of ionizing radiation, there is no reason for resistance to this type of radiation to evolve (Mattimore and Battista, 1996). Thus, it can be assumed that the DNA repair mechanisms found in cultures resistant to ionizing radiation developed as a result of their desiccation tolerance, and parallels can be drawn between them (Mattimore and Battista, 1996;Potts, 2001; Rainey et. al., 2005). It is therefore important to study the survival rates of isolates from arid environments when exposed to radiation and desiccation to further comparisons at the ecological level between the two mechanisms.

Materials and Methods

Isolate Selection.

Of the 63 isolates in this study belonging to the *Microvirga/Balneimonas* group, ten were selected for desiccation and radiation resistance profiling due to their diverse properties, phylogenetic position, and source. The ten isolates studies wereAT04-150-34

and AT05-22-20 from the Atacama Desert, NG05-4-1 from the Negev, KR-S97-11 and KR-S97-46 from the Sonoran Desert, KR-N97-393 and KR-N97-501 from the Nevada Desert, 20-40R-23 from the Oman Desert, SS05-2-117 from the Sahara, and 68J-13, an air sample isolate from the Atacama Desert.

In addition both type strains, *Microvirga subterranea* and *Balneimonas flocculans* were also used for comparison. The root nodule symbiont known as Lut5, isolated from the plant *Lupinus texensis*, was selected for inclusion in this study due to its phylogenetic position within the *Microvirga/Balneimonas* cluster. Finally, strains of *Deinococcus radiodurans* and *E. coli* were included as positive and negative controls, respectively. All isolates were incubated at 30°C for growth, with the exception of *B. flocculans* which was incubated at 37°C.

Standard Suspension of Bacterial Cultures.

The select bacterial cultures were inoculated onto 1/10 strength PCA or RM agar plates for fresh growth. After 24-48 hours, individual colonies were transferred from each plate into tubes containing their respective media broth – 1/10 strength PCB or RM broth. The colonies were resuspended until an OD_{600} of 0.2 was reached. This value was used as a standard comparison for each of the resistance profiles studied.

Desiccation Resistance Profiles.

For desiccation studies, a polystyrene 24-well micro-titer plate was used. An aliquot of 100 µL was taken from each of the standard OD_{600} cell cultures and placed in a different well in the micro-titer plate. These cultures were dried completely before placement in a Secador™ desiccation chamber (Bel-Art). A HOBO®H8 Pro RH/Temp Data Logger (Onset) was used to monitor relative humidity, which was maintained using

silica gel desiccant that was replaced weekly. Samples were taken from the micro-titer plates in the desiccation chamber at 2, 7, 14, 28, and 42 days by rehydration with 1 mL of the appropriate media broth. Cells were resuspended for 30 min, and mixed vigorously before being transferred to a sterile 1.5 mL tube. From this tube, dilutions were made in corresponding liquid media and 100 µL aliquots were taken for triplicate plating from each sample. Plates were incubated for 7 days and Cfu/mL enumerated. Surviving fractions were calculated by comparison to the non-desiccated control plates.

Ultraviolet (UV) Radiation Resistance Profiles.

Cultures were transferred in aliquots of 1 mL from the standard OD_{600} tubes into sterile Petri dishes, which were then exposed to UV radiation from a 254 nm UVLMS-38 (UVP) light source. Each culture was exposed to dosages of 200, 400, 600, 800, and 1000 Joules/m^2. Calibration of the light source was done using a J225 (UVP) radiometer for delivery of a dose equal to 630 µW·s/cm^2. Immediately after exposure, the culture was transferred to a sterile 1.5 mL tube, where it was diluted using the corresponding liquid media. The dilutions were then plated in triplicates, using 100 µL of liquid for each plate. Special care was taken to minimize exposure to light in order to avoid photo repair processes of the samples. Plates were incubated for 7 days, after which the colonies were enumerated. The fraction of survivors was calculated for each sample by comparison to the cultures that had not been irradiated.

A higher dose of UV radiation dosage was applied after two of the strains – the type strain *M. subterranea* and NG05-4-1 – were found to be highly resistant to lower doses. For these higher doses the same protocol was followed, with the dosages increased to 500, 1000, 1500, and 2000Joules/m^2.

Gamma Radiation Resistance Profiles.

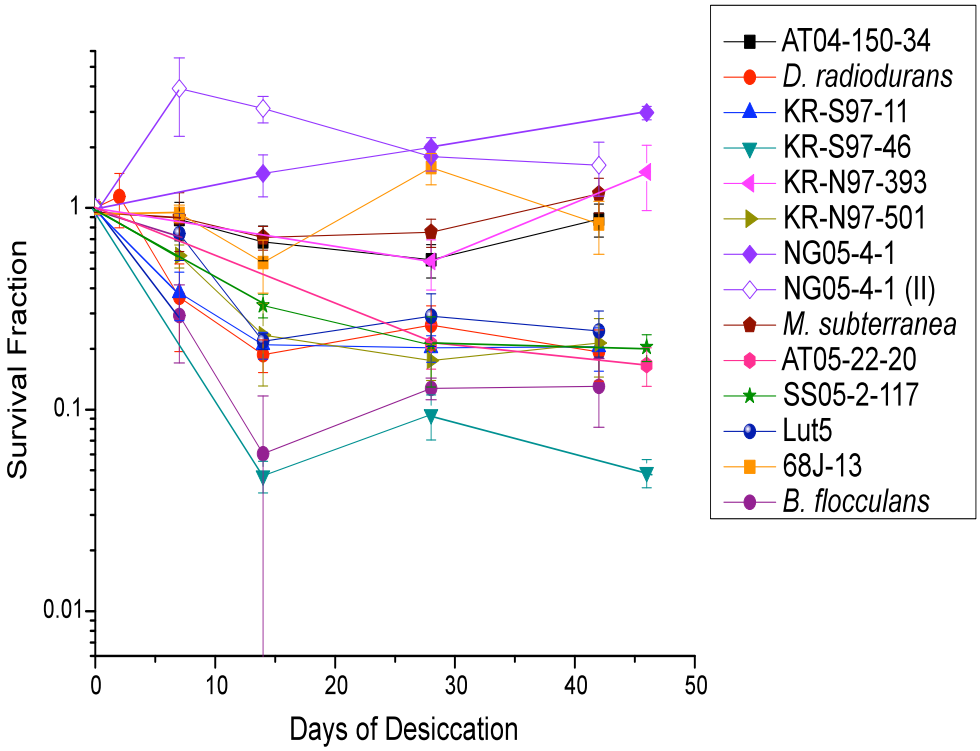
Cultures were grown in RM broth to determine resistance of cells in log phase of growth. For each isolate tested, 50 µL of glycerol preserve was inoculated into 5 mL of RM broth and placed in a shaking incubator until growth was observed. These cultures were again inoculated 24 hours before exposure to gamma radiation by transferring 500 µL from each tube in the shaker to a fresh tube of 4.5 mL RM broth. After incubation overnight, a standard 0.2 OD_{600} solution was obtained and used for experimentation.

Standardized 0.2 OD_{600} cultures were subjected to gamma radiation exposure from a cobalt-60 source, using the methods described in Zimmerman and Battista (2006). The dose rate for this radiation was 16.7 Gy/min (approximately 1 kGy/hr). Samples were transferred to sterile Eppendorf® 1.5 mL Safe Lock Tube and placed in the radiator for 1, 3, and 5 hours corresponding to doses of 1, 3, and 5 kGy of radiation.

Results and Discussion

Twelve strains were subjected to conditions of desiccation and the surviving fraction calculated over a period of 48 days. It was found that for 11 of these strains the surviving fraction did not reach the D_{10} level throughout the experiment; only KR-S97-46 showed sensitivity to desiccation (Figure 5.1). *Balneimonas flocculans* initially decreased below the D_{10} level by 14 d, but had recovered in the 28 d sample. Additionally, nine isolates showed more desiccation resistance than *Deinococcus radiodurans*, with strains AT04-150-34, 68J-13, NG05-4-1, KR-N97-393, and *Microvirga subterranea* showing either an increase or no change in surviving population as compared to the pre-desiccation Cfu levels (Figure 5.1).

Figure 5.1: 48-Day desiccation tolerance of select isolates



An extended, 90-day desiccation experiment was performed to obtain a more complete picture of desiccation tolerance of these isolates. The result was a more complete division among the isolates studied into levels of tolerance. The two isolates obtained from the Sonoran Desert in Arizona, KR-S97-11 and KR-S97-46, showed the most sensitivity to desiccation. The most resistant of the isolates were 20-40R-23 from Oman, the Atacama air sample isolate 68J-13, and NG05-4-1 from the Negev in Israel. Comparisons between the surviving organisms for this extended desiccation are shown in Figures 5.2 and 5.3.

Figure 5.2: 90-Day desiccation tolerance of select isolates

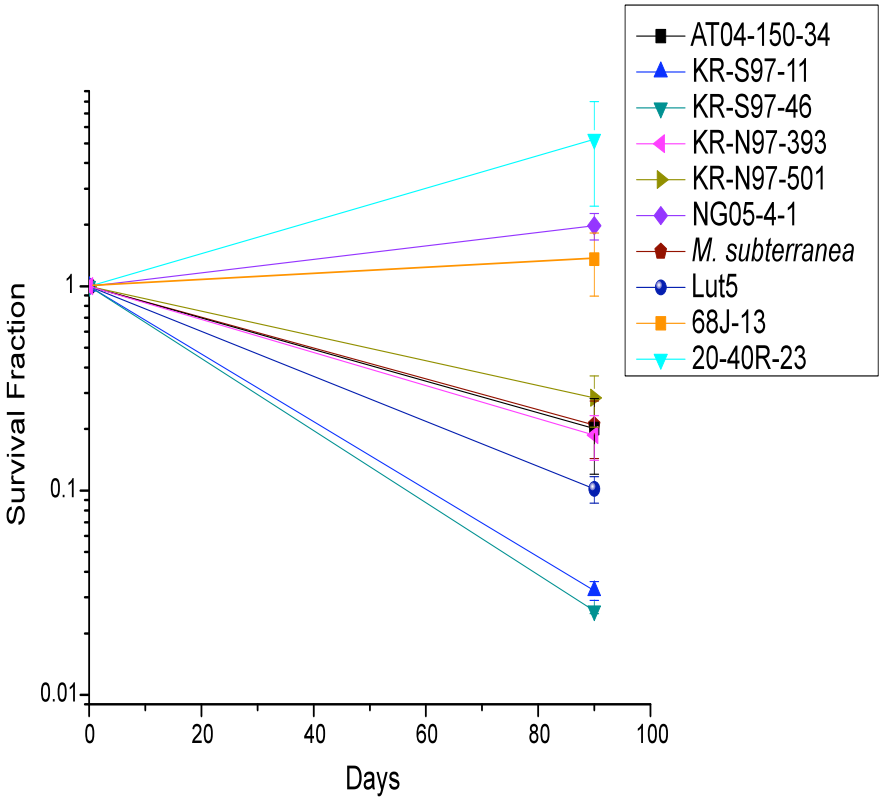
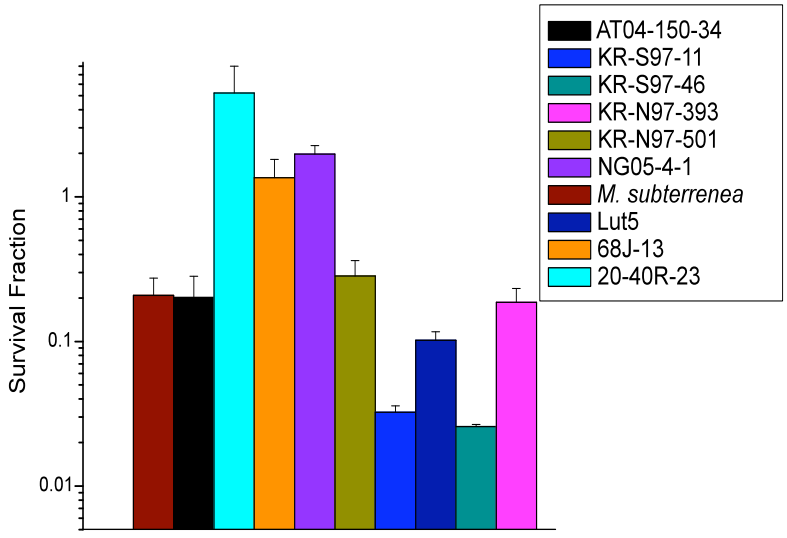


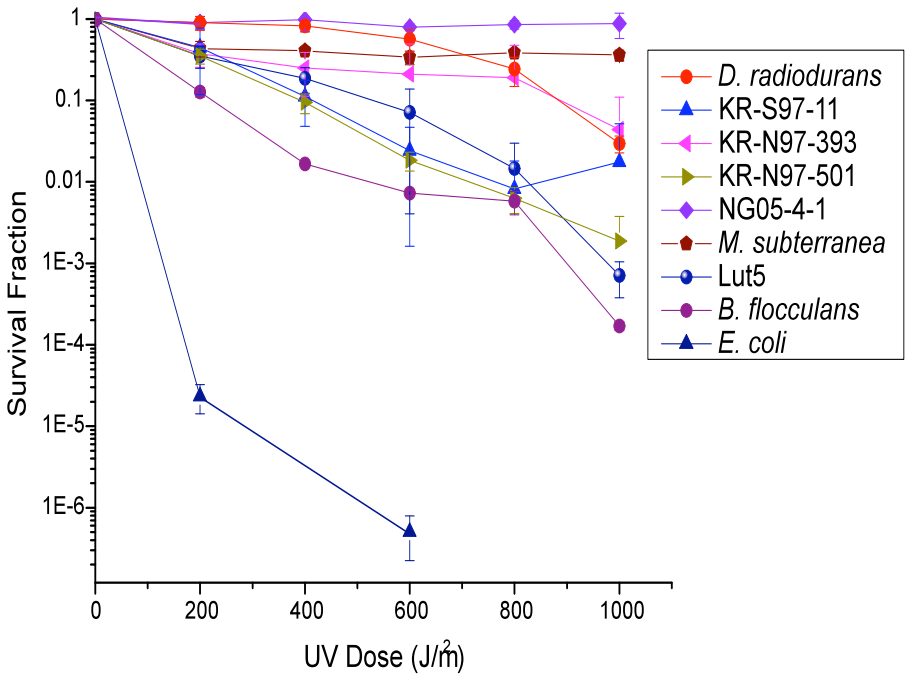
Figure 5.3: 90-Day desiccation survival fraction of select isolates



It is important to mention that the culture of isolate NG05-4-1 was discovered to contain both yeast and bacteria. Upon separation of the two, resistance profiles were repeated (with the exception of desiccation) to determine the tolerance of the bacterium. The original values for the isolate can be attributed to the yeast since it both outgrew the bacterial culture and was the only microorganism observed upon microscopy of surviving colonies after radiation exposure. The bacterial culture was renamed NG05-4-1B for future studies, while the yeast became NG05-4-1Y.

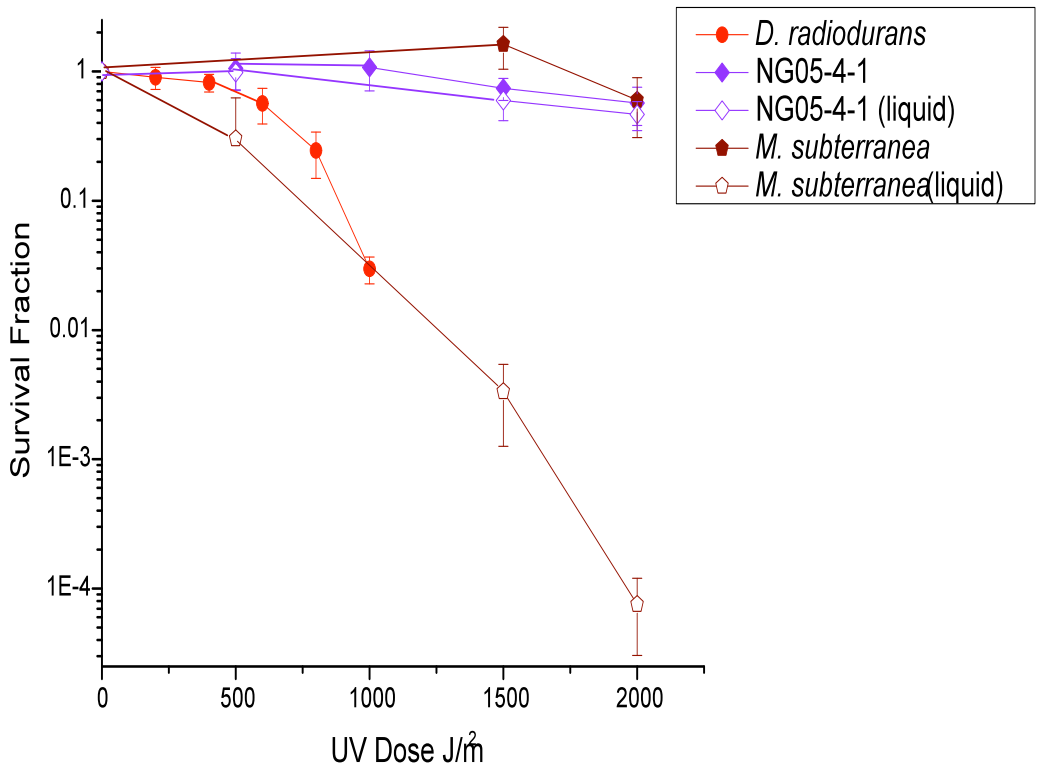
In studies of ultraviolet radiation resistance, some of the isolates were found to be resistant. Notably, both *M. subterranea* and NG05-4-1 were more resistant of UV radiation than *D. radiodurans*. The isolates KR-N97-393 and KR-S97-11 were found to have similar surviving fractions after exposure to 1000 J/m^2 radiation. The UV survival profiles of the isolates tested are shown below (Figure 5.4).

Figure 5.4: UV resistance profiles of select isolates



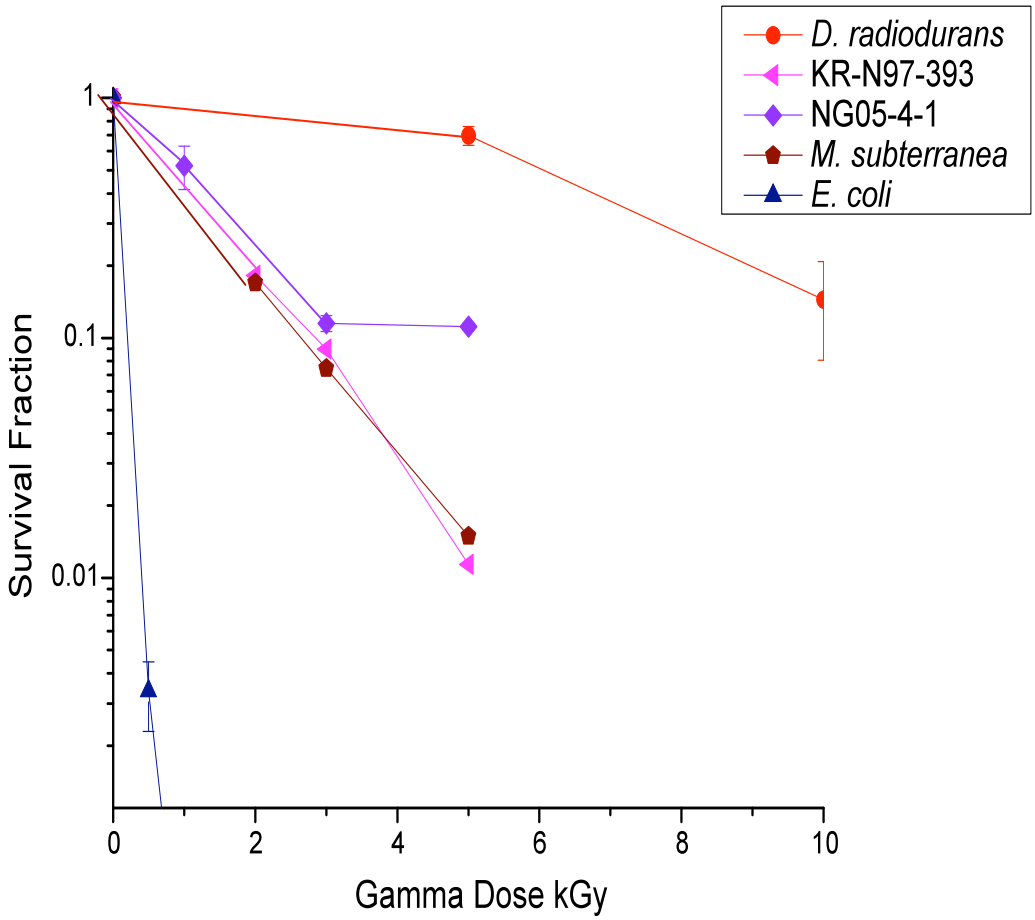
An additional UV experiment was performed for the resistant isolates, to determine at which point they would begin to lose resistance. Each of the two isolates (NG05-4-1 and *M. subterranea*) was exposed to 2000 J/m^2 of UV radiation double the highest previous exposure. Additionally, both isolates were grown in liquid culture prior to exposure to draw comparisons between the growth phase and level of tolerance. Both cultures were resistant, with little to no decrease in the numbers of surviving cells(Figure 5.5). In liquid culture, only *M. subterranea* showed a rapid decrease in population after exposure to 500 J/m^2. The yeast, NG05-4-1, therefore shows resistance to UV radiation in addition to desiccation. *D. radiodurans* was also included as a comparison, although it was not subjected to more than 1000 J/m^2.

Figure 5.5: Extended-dose UV resistance profiles of select isolates



In order to investigate further correlations between desiccation and ionizing radiation resistance, isolates were exposed to gamma radiation up to 5 kGy. In comparison to *E. coli*, strains KR-S97-393 and *M. subterranea* are resistant and have D_{10} values in the range 2-3 kGy; however, this does not compare with the D_{10} value of *D. radiodurans*, which is 10 kGy (Figure 5.6). The yeast, NG05-4-1 was the closest to this high resistance, with a D_{10} at 5 kGy.

Figure 5.6: Gamma radiation resistance in select isolates



The D_{10} values of two of the strains (KR-S97-393and *M. subterranea*) fall between 2-3 kGy, which correspond to D_{10} values for a number of bacteria also found to be resistant to ionizing radiation. These include *Kocuria rosea*, *Acinetobacter radioresistens*, and *Kineococcus radiotolerans*, which all have D_{10} values of ~2 kGy. Additionally, KR-S97-393 and *M. subterranea* show more resistance to gamma radiation than their phylogenetic relative, *Methylobacterium radiotolerans*, which has a D_{10} of 1 kGy (Cox and Battista, 2005). The yeast isolate NG05-4-1 showed the highest gamma radiation resistance of the three with a D_{10} value of 5 kGy. This is higher than both *Hymenobacter actinosclerus* (3.5 kGy) and *Chroococcidiopsis* spp. (4 kGy), and very close to the D_{10} value of 5.5 kGy for *Rubrobacter xylanophilus* (Cox and Battista, 2005).

This study has demonstrated that the isolates from arid lands that fall within the radiation of the *Microvirga*/*Balneimonas* group show resistance to desiccation and UV radiation, and gamma radiation. This corresponds with their original source and thus their ability to survive in arid environments, which gives the isolates a selective advantage for surviving radiation exposure levels that *E. coli* cannot tolerate. However, there is a need for further experimentation in order to obtain more complete radiation and desiccation tolerance/resistance profiles for a larger number of these isolates.

CHAPTER 6:

CONCLUSION

As has been shown in this study, diverse groups of microorganisms inhabit arid lands, some of which can possess adaptations that help them survive the harsh conditions of their surroundings. The main stress factor addressed in this study was desiccation, and a number of bacterial isolates were shown to be resistant to this lack of water, both in individual isolate studies as well as ones involving entire soil populations.

In soil population diversity assessments, isolates were obtained from room temperature, desiccation, and low temperature (-20°C) conditions, regardless of the length of time spent in each. This shows most prominently the variety of microorganisms present in a given sample of soil, and the high numbers of bacteria from arid lands that are able to survive desiccation for extended periods of time. Although a majority of the isolated organisms from soils were of a single genus, *Arthrobacter*, there were a wide variety of genera represented from all of the samples.

A number of these isolates were also discovered to show resistance to radiation, which has been said to correlate to desiccation tolerance (Mattimore and Battista, 1996). This study furthers this parallel between the two types of resistance and provides more examples of desiccation and radiation resistant isolates. This was shown most prominently in studies of the isolate collection related to *Microvirga subterranea* and *Balneimonas flocculans*.

The extensive characterization of a group of 61 isolates related with greater than 94% sequence similarity to the type strains *Microvirga subterranea* and *Balneimonas flocculans* showed how truly diverse a group of related microorganisms can be. It was

quickly discovered that grouping of isolates into species by 16S rRNA gene sequences would not be practical alone, and that more comparisons needed to be made between the isolates in the collection. After a number of phenotypic and biochemical tests, the question of which species should be created within the genus *Microvirga* to include all of the isolates studied still has not been answered. Further analysis of fatty acids, carbon sources, desiccation resistance, and radiation resistance will likely give an even more complete picture of the characteristics possessed by the *Microvirga*/*Balneimonas* cluster.

Even without the emendation, reclassification, and speciation of this group of bacteria that has not yet occurred, this study has further demonstrated diversity amongst microorganisms that inhabit arid regions. It has also shown an additional group of bacteria that possess resistance to gamma radiation, with D_{10} values of ~2 kGy for the *Microvirga subterranea* type strain, as well as discovering a yeast isolate resistant to both desiccation and ionizing radiation. Finally, a number of different genera have been isolated following extended desiccation of desert soil samples, which may help to give a more complete picture of desiccation and radiation resistance from these environments in future studies. All of these aspects make this thesis valuable to the study of microorganisms in arid lands, more specifically those involving resistance to environmental stress factors.

REFERENCES

Altschul, SF, Gish, W, Miller, W, Myers, EW, and Lipman, DJ (1990). Basic local alignment search tool. Journal of Molecular Biology, 215: 403-410.

Amann, RI, Ludwig, W, and Schleifer, KH (1995). Phylogenetic Identification and *in situ* Detection of Individual Microbial Cells without Cultivation. Microbiological Reviews, 59: 143-169.

Andam, CP, and Parker, MA (2007). Novel Alphaproteobacterial Root Nodule Symbiont Associated with *Lupinus texensis*. Applied and Environmental Microbiology, Sept. 2007: 5687-91.

Applied Biosystems, Inc (2008). 3130xl Genetic Analyzer Literature and Resources. <https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=601644>.

Asker, D, Beppu, T, and Ueda, K (2007). *Sphingomonas jaspsi* sp. nov., a novel carotenoid-producing bacterium isolated from Misasa, Tottori, Japan. International Journal of Systematic and Evolutionary Microbiology, 57: 1435-41.

Battista, JR, Earl, AM, and Park, M-J (1999). Why is *Deinococcus radiodurans* so resistant to ionizing radiation? Trends in Microbiology, 7: 362-5.

Billi, D, Friedmann, EI, Hofer, KG, Caiola, MG, and Ocampo-Friedmann, R (2000). Ionizing-Radiation Resistance in the Desiccation-Tolerant Cyanobacterium *Chroococcidiopsis*. Applied and Environmental Microbiology, 66: 1489-92.

Billi, D, and Potts, M (2002). Life and death of dried prokaryotes. Research in Microbiology, 153: 7-12.

Chanal, A, Chapon, V, Benzerara, K, Barakat, M, Christen, R, Achouak, W, Barras, F, and Heulin, T (2006). The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. Environmental Microbiology, 8: 514-25.

Chun, J, Lee, J-H, Jung, Y, Kim, M, Kim, S, Kim, BK, and Lim, YW (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. International Journal of Systematic and Evolutionary Microbiology, 57: 2259-61.

Chung, AP, Rainey, FA, Valente, M, Nobre, MF, and da Costa, MS (2000). *Thermus igniterrae* sp. nov. and *Thermus antranikianii* sp. nov., two new species from Iceland. International Journal of Systematic and Evolutionary Microbiology, 50: 209-17.

Cox, MM, and Battista, JR (2005). *Deinococcus radiodurans* – the consummate survivor. Nature Reviews in Microbiology, 3: 882-92.

Fox, GE, Wisotzkey, JD, and Jurtshuk, P, Jr. (1992). How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity International Journal of Systematic Bacteriology, 42: 166-70.

Fredrickson, JK, Li, SW, Gaidamakova, EK, Matrosova, VY, Zhai, M, Sulloway, HM, Scholten, JC, Brown, MG, Balkwill, DL, and Daly, MJ (2008). Protein oxidation: key to bacterial desiccation resistance? The International Society for Microbial Ecology Journal, 2: 393-403.

Hall, TA (1999). Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95-8.

Hillis, DM, and Dixon, MT (1991). Ribosomal DNA: Molecular Evolution and Phylogenetic Interference. The Quarterly Review of Microbiology, 66: 411-453.

Hirsch, P, Ludwig, W, Hethke, C, Sittig, M, Hoffmann, B, and Gallikowski, CA (1998). *Hymenobacter roseosalivarius* gen. nov., sp. from continental Antarctic soils and sandstone: Bacteria of the *Cytophaga*/*Flavobacterium*/*Bacteroides* line of phylogenetic descent. Systematic and Applied Microbiology, 21: 374-83.

Hoeijmakers, JHJ (2001). Genome maintenance mechanisms for preventing cancer. Nature, 411: 366-74.

Javaux, EJ (2006). Extreme life on earth – past, present, and possibly beyond. Research in Microbiology, 157: 37-48.

Kanso, S, and Patel, BKC (2003). *Microvirga subterranea* gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. International Journal of Systematic and Applied Microbiology, 53: 401-6.

Kim, MK, Na, J-R, Lee, T-H, Im, W-T, Soung, N-K, and Yang, D-C (2007). *Solirubrobacter soli* sp. nov., isolated from soil of a ginseng field. International Journal of Systematic and Evolutionary Microbiology, 57: 1453-55.

Latonen, L, and Laiho, M (2005). Cellular UV damage responses – Functions of tumor suppressor p53. Biochimica et Biophysica Acta, 1755: 71-89.

Mattimore, V, and Battista, JR (1996). Radioresistance of *Deinococcus radiodurans*: Functions Necessary To Survive Ionizing Radiation Are Also Necessary To Survive Prolonged Desiccation. Journal of Bacteriology, 178: 633-7.

Navarro-Gonzalez, R, Rainey, FA, Molina, P, Bagaley, D, Hollen, B, de la Rosa, J, Small, A, Quinn, RC, Grunthaner, FJ, Caceres, L, Gomez-Silva, B, and McKay, CP (2003). Mars-like soils in the Atacama Desert, Chile, and the dry limit of microbial life. Science, 302: 1018-21.

Negev Foundation (2008). Israel's Negev Desert. <http://www.negev.org/About/negev_desert.htm>.

Podar, M, and Reysenbach, A-L (2006). New opportunities revealed by biotechnological explorations of extremophiles. Current Opinion in Biotechnology, 17: 250-5.

Potts, M (1994). Desiccation Tolerance of Prokaryotes. Microbiology Reviews, 58: 755-805.

Potts, M (2001). Desiccation tolerance: a simple process? TRENDS in Microbiology, 9: 553-9.

Potts, M, Slaughter, SM, Hunneke, F-U, Garst, JF, and Helm, RF (2005). Desiccation Tolerance of Prokaryotes: Application of Principles to Human Cells. Integrative and Comparative Biology, 45: 800-9.

Rainey, FA, Ward-Rainey, N, Kroppenstedt, RM, and Stackebrandt, E (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. nov. International Journal of Systematic Bacteriology, 46: 1088-92.

Rainey, FA, Ray, K, Ferreira, M, Gatz, B, Nobre, MF, Bagaley, D, Rash, BA, Park, M, Earl, A, Shank, N, Small, A, Henk, M, Battista, J, Kämpfer, P, and da Costa, MS (2005). Extensive Diversity of Ionizing-Radiation-Resistant Bacteria Recovered from Sonoran Desert Soil and Description of Nine New Species of the Genus *Deinococcus* Obtained from a Single Soil Sample." Applied and Environmental Microbiology, Sept. 2005: 5225-35.

Rainey, FA, and Oren, A (2006). Extremophilic Microorganisms and the Methods to Handle Them. Methods in Microbiology, 35: 1-25.

Reddy, GSN, Nagy, M, and Garcia-Pichel, F (2006). *Belnapia moabensis* gen. nov., sp. nov., an alphaproteobacterium from biological soil crusts in the Colorado Plateau, USA. International Journal of Systematic and Evolutionary Microbiology, 56: 51-83.

Smibert, RM, and Krieg, NR (1994). Chapter 25: Phenotypic Characterization. In: Methods for General and Molecular Bacteriology. P Gerhardt, RGE Murray, WA Wood, and NR Krieg (ed). ASM, Washington: 607-54.

Takeda, M, Suzuki, I, and Koizumi, J (2004). *Balneimonas flocculans* gen. nov., sp. nov., A New Cellulose-producing Member of the α-2 subclass of *Proteobacteria*. Systematic and Applied Microbiology, 27: 139-45.

Tamura, K, Dudley, J, Nei, M, and Kumar, S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24: 1596-9.

Urzì, C, Salamone, P, Schumann, P, Rohde, M, and Stackebrandt, E (2004) *Blastococcus saxobsidens* sp. nov., and emended descriptions of the genus *Blastococcus* Ahrens and Moll 1970 and *Blastococcus aggregators* Ahrens and Moll 1970. International Journal of Systematic and Evolutionary Microbiology, 54: 253-9.

Zimmerman, JM, and Battista, JR (2006). Measuring Survival in Microbial Populations Following Exposure to Ionizing Radiation. Methods in Microbiology, 35:743-54.

APPENDIX A
TABLE OF SOIL ISOLATES

Name	Date	Dil	#	Media	Description
NG05-4-1	3/7/06	-2	2	PCA	beige/pink, irregular, ~op, convex, rough
NG05-4-2	3/7/06	-2	3	PCA	clear, trans, round, shiny
NG05-4-3	3/7/06	-2	3	PCA	rough/veiny, peach, ~trans
NG05-4-4	3/7/06	-2	3	PCA	beige/white, ~trans, ~umbonate, shiny
NG05-4-5	3/7/06	-2	3	PCA	brown, ~op, shiny, round, dark in center, convex
NG05-4-6	3/7/06	-2	3	PCA	yellowed center, ~op, shiny, round
NG05-4-7A	3/7/06	-2	3	PCA	white/beige, round, smooth, shiny
NG05-4-7B	3/7/06	-2	3	PCA	yellow, convex, dull, round, smooth
NG05-4-8	3/7/06	-3	1	PCA	peach/orange, shiny, op, round, convex
NG05-4-9	3/7/06	-3	1	PCA	pink, ~umbonate, dark center, ~trans
NG05-4-10	3/7/06	-3	3	PCA	white, irregular, op, shiny
NG05-4-11	3/7/06	-3	3	PCA	yellow/green, op, shiny, round
NG05-4-12	3/7/06	-3	3	PCA	peach/white, round, shiny, convex, op
NG05-4-13	3/7/06	-3	3	PCA	red/rust, round, shiny, convex, ~op
NG05-4-14	3/7/06	-4	2	PCA	beige, darker center, ~op, round
NG05-4-15	3/7/06	-4	3	PCA	white/beige, round, ~op, shiny
NG05-4-16	3/7/06	-4	3	PCA	irregular, beige, veiny, op
NG05-4-17	3/8/06	-2	1	PCA	yellowed/brownish-green, white powder, ~op
NG05-4-18	3/8/06	-2	1	1/10 PCA	white, irregular, brown center ring, op, ~umbonate
NG05-4-19	3/8/06	-3	1	1/10 PCA	beige, irregular, ~op, rough
NG05-4-20	3/8/06	-3	1	1/10 PCA	peach, irregular, rough, ~op
NG05-4-21	3/8/06	-3	2	1/10 PCA	orange, round, smooth, shiny, op
NG05-4-22	3/8/06	-3	3	1/10 PCA	yellow, round, smooth, shiny, op
NG05-4-23	3/8/06	-3	3	1/10 PCA	beige, irregular (lobate?), "rippled", shiny, op
NG05-4-24	3/8/06	-4	1	1/10 PCA	peach/clear, shiny, ~trans, ~irregular, convex

Name	Date	Dil	#	Media	Description
NG05-4-25	3/8/06	-4	1	1/10 PCA	white, yellowish center, shiny, opaque, round
NG05-4-26	3/8/06	-4	2	1/10 PCA	pink outer ring, white, umbonate, shiny, round, brown center, ~op
NG05-4-27	3/8/06	-4	2	1/10 PCA	pink, rough, ~umbonate, ~round, shiny, opaque
NG05-4-28	3/8/06	-4	2	1/10 PCA	brown/yellow, irregular, op, dull
NG05-4-29	3/8/06	-4	2	1/10 PCA	beige, irregular, rough, ~shiny, op
NG05-4-30	3/8/06	-4	3	1/10 PCA	pink/red/orange, ~round, dull, opaque
NG05-4-31	3/8/06	-3	3	PCA	concave, white/beige, powdered, rough, irregular, op
NG05-4-32	3/8/06	-2	3	1/10 PCA	white, powdered, round, op, convex, dull, clear ring, white thin edge
NG05-4-33	3/8/06	-2	2	1/10 PCA	white, irregular/lobate, yellowed center, rough, op, filaments into agar
NG05-4-34	3/16/06	-6	1	PCA	rough brown/white, irregular, op
NG05-4-35	3/16/06	-6	1	PCA	rough, yellow, op, dull, irregular
NG05-4-36	3/16/06	-5	1	1/10 PCA	white, round, smooth, shiny, convex, op
AT05-22-1	3/7/06	-2	3	PCA	brown, ~trans, round, convex, darker toward middle, shiny
AT05-22-2	3/7/06	-2	2	PCA	clear, ~op edge, ~trans center, "rough looking"
AT05-22-3	3/7/06	-2	2	PCA	red, ~trans (with op center), shiny, round, "rough" surface
AT05-22-4	3/7/06	-2	2	PCA	white/beige, shiny, convex, ~trans, smooth, round
AT05-22-5	3/7/06	-3	1	PCA	red/orange/peach, shiny, ~trans, smooth, round
AT05-22-6	3/7/06	-3	2	PCA	concave, white edge, clear center, ~op, shiny, round
AT05-22-7	3/7/06	-3	2	PCA	pinkish red, shiny, round, ~op
AT05-22-8	3/7/06	-3	2	PCA	umbonate, beige/clear (dark in center), ~trans
AT05-22-9	3/7/06	-3	2	PCA	beige irregular, shiny, opaque, "globs" on top/shapes on surface
AT05-22-10	3/7/06	-3	3	PCA	white, op, ~dull, convex, round
AT05-22-11	3/7/06	-3	3	PCA	beige, yellow bubbled center, op, round
AT05-22-12	3/7/06	-3	3	PCA	red/peach, op, bubbled/veined center, ~irregular
AT05-22-13	3/7/06	-4	3	PCA	beige/white, shiny, round, op
AT05-22-14	3/7/06	-4	3	PCA	red, umbonate, round, op, dull
AT05-22-15	3/7/06	-4	1	PCA	beige, shiny, ~convex, round, op
AT05-22-16	3/8/06	-2	1	1/10 PCA	clear, peach, round, ~trans, smooth, shiny
AT05-22-17	3/8/06	-2	2	1/10 PCA	greyish clear, ~op, shiny, irregular, rough

Name	Date	Dil	#	Media	Description
AT05-22-18	3/8/06	-2	3	1/10 PCA	yellowish center, clear outer ring, irregular, ~op, dull
AT05-22-19	3/8/06	-2	3	1/10 PCA	beige/clear, round shiny, convex, ~op
AT05-22-20	3/8/06	-2	3	1/10 PCA	red/clear, round, shiny, convex, op
AT05-22-21	3/8/06	-2	3	1/10 PCA	peach, irregular, rough, shiny, ~op
AT05-22-22	3/8/06	-2	3	1/10 PCA	orange, round, shiny, opaque
AT05-22-23	3/8/06	-3	1	1/10 PCA	yellow, round, convex, ~shiny, opaque
AT05-22-24	3/8/06	-3	1	1/10 PCA	clear, round, umbonate, translucent
AT05-22-25	3/8/06	-3	1	1/10 PCA	red, rough, opaque, irregular
AT05-22-26	3/8/06	-3	1	1/10 PCA	yellow/brown, round, op, dull
AT05-22-27	3/8/06	-3	1	1/10 PCA	orange, convex, opaque, round, dull
AT05-22-28	3/8/06	-3	1	1/10 PCA	beige, irregular, brown, powder, center enough
AT05-22-29	3/8/06	-4	3	1/10 PCA	green/white, powdered, irregular, rough
AT05-22-30	3/8/06	-3	2	1/10 PCA	beige/yellow/white alternating rings, irregular, convex, dull, opaque
AT05-22-31	3/8/06	-3	3	1/10 PCA	peach/clear, round, convex, ~op, shiny
AT05-22-32	3/8/06	-3	3	1/10 PCA	pink, rough, ~op, ~shiny
AT05-22-33	3/8/06	-5	3	PCA	tan, rough/scaly, round, filaments, browned agar around colony, dull, op
AT05-22-34	3/8/06	-4	2	PCA	irregular, lumpy, beige, dull, op
AT05-22-35	3/16/06	-6	2	PCA	beige/yellow, ~op, smooth, ~round, dull
AT05-22-36	3/16/06	-6	3	PCA	clear edge with yellow center, round, ~trans, smooth, ~shiny
AT05-22-37	3/16/06	-4	1	1/10 PCA	black, round, dull, op, smooth
AT05-22-38	3/16/06	-5	2	1/10 PCA	yellow, round, shiny, ~op, smooth
AT05-22-39	5/3/06	-2	1	1/10 PCA	orange-pink, irreg, shiny op
AT05-22-40	5/3/06	-2	1	1/10 PCA	orange, irreg, op, rough
AT05-22-41	5/3/06	-2	2	1/10 PCA	darker orange, dull, op, round
AT05-22-42	5/3/06	-2	3	1/10 PCA	light pink, ~umb, ~shiny, ~op, round
AT05-22-43	5/3/06	-2	3	1/10 PCA	reddish pink, round, ~trans, shiny
AT05-22-44	5/3/06	-2	3	1/10 PCA	clear with orange-ish pink center, ~trans, shiny, round
AT05-22-45	5/3/06	-2	3	1/10 PCA	light orange, dull, round, ~op

Name			Date	Dil	#	Media	Description
NG05-4	D6A	1	6/7/06	-2	2	PCA	clear peach, ~op, shiny, smooth, round, convex
NG05-4	D6A	2	6/7/06	-2	2	PCA	clear yellowish, trans, shiny, ~umb, round
NG05-4	D6A	3	6/7/06	-2	2	PCA	beige/yellow, op, dull, smooth
NG05-4	D6A	9	6/7/06	-2	3	PCA	pink, op, dull
NG05-4	D6A	10	6/7/06	-2	3	PCA	clear w/ rough center, trans, rough
NG05-4	D6A	11	6/7/06	-2	3	PCA	orange center, clear edge, shiny, smooth, convex
NG05-4	D6A	16	6/7/06	-3	1	PCA	red, ~shiny, umb, smooth
NG05-4	D6A	17	6/7/06	-3	1	PCA	veined, beige, op
NG05-4	D6A	18	6/7/06	-3	1	PCA	yellow-clear, round, smooth, ~trans
NG05-4	D6A	19	6/7/06	-3	1	PCA	red-pink, smooth shiny outside, rough dull inside, irreg., op
NG05-4	D6A	20	6/7/06	-3	2	PCA	clear peach, umb, shiny, round
NG05-4	D6A	22	6/7/06	-3	2	PCA	beige, shiny, smooth, irreg.
NG05-4	D6A	23	6/7/06	-3	3	PCA	clear peach, smooth, ~trans, shiny
NG05-4	D6A	24	6/7/06	-2	1	1/10 PCA	dark red, round, smooth, shiny, convex, op
NG05-4	D6A	25	6/7/06	-2	1	1/10 PCA	clear pink, round, ~trans, shiny
NG05-4	D6A	26	6/7/06	-2	1	1/10 PCA	orange-brown, dull, round, op
NG05-4	D6A	27	6/7/06	-2	1	1/10 PCA	yellow/brown, shiny, round, smooth ~op
NG05-4	D6A	28	6/7/06	-2	2	1/10 PCA	peachish, op, shiny, round, smooth
NG05-4	D6A	29	6/7/06	-2	2	1/10 PCA	clear pink, ~umb, shiny, round, ~trans
NG05-4	D6A	30	6/7/06	-2	2	1/10 PCA	peach, rough, dull, op
NG05-4	D6A	31	6/7/06	-2	2	1/10 PCA	pink/red, ~umb, shiny, round, op
NG05-4	D6A	32	6/7/06	-2	3	1/10 PCA	yellow clear, ~trans, smooth, round, shiny
NG05-4	D6A	34	6/7/06	-2	3	1/10 PCA	silvery bubbled white, rough
NG05-4	D6A	36	6/7/06	-3	1	1/10 PCA	clear pink, trans, shiny, round, ~smooth
NG05-4	D6A	37	6/7/06	-3	1	1/10 PCA	clear white, rough, irreg., ~trans, ~shiny
NG05-4	D6A	38	6/7/06	-3	1	1/10 PCA	peach, round, smooth, convex, shiny, op
NG05-4	D6A	39	6/7/06	-3	1	1/10 PCA	yellow, round, smooth, convex, shiny, op
NG05-4	D6A	40	6/7/06	-3	1	1/10 PCA	pink, rough, dull, op
NG05-4	D6A	41	6/7/06	-3	1	1/10 PCA	pink, ~op, ~umb, smooth, round

Name			Date	Dil	#	Media	Description
NG05-4	D6A	42	6/7/06	-3	1	1/10 PCA	rough, beige center, white irreg edge, op, dull
NG05-4	D6A	43	6/7/06	-3	1	1/10 PCA	orange, shiny, smooth, round, op
NG05-4	D6A	44	6/7/06	-3	1	1/10 PCA	tan, smooth, shiny, op, dark center
NG05-4	D6A	45	6/7/06	-3	2	1/10 PCA	white, op, shiny, smooth, round
NG05-4	D6A	46	6/7/06	-3	2	1/10 PCA	clear pink, rough edges, smooth, shiny, ~trans
NG05-4	D6A	47	6/7/06	-3	2	1/10 PCA	clear peach, rough edge, shiny, convex, ~trans
NG05-4	D6A	48	6/7/06	-3	3	1/10 PCA	yellow-white, shiny, smooth, op
NG05-4	D6A	49	6/7/06	-3	3	1/10 PCA	pink clear, dark center, ~trans, shiny, smooth
NG05-4	D6B	2	6/7/06	-2	1	PCA	veined, yellowish-clear, ~trans, dull, rough
NG05-4	D6B	4A	6/12/06	-2	2	PCA	red orange, dull, rough, ~round, op
NG05-4	D6B	4B	6/12/06	-2	2	PCA	beige/tan, smooth, shiny, op
NG05-4	D6B	5	6/7/06	-2	2	PCA	white clear, trans, shiny, smooth, rough edge
NG05-4	D6B	7	6/7/06	-2	3	PCA	orange, ~op, shiny, smooth, round
NG05-4	D6B	8	6/7/06	-2	3	PCA	beige/yellow, ~trans, shiny, smooth
NG05-4	D6B	13	6/7/06	-3	1	PCA	white, irreg, smooth, shiny, op
NG05-4	D6B	15	6/7/06	-3	2	PCA	white w/ pink center, op, shiny, round, smooth
NG05-4	D6B	16	6/7/06	-3	2	PCA	light orange, round, smooth, shiny, convex
NG05-4	D6B	17	6/7/06	-3	3	PCA	light pink, convex, ~dull, op, round, smooth
NG05-4	D6B	18A	6/12/06	-3	3	PCA	pink, round, smooth, shiny, op
NG05-4	D6B	18B	6/12/06	-3	3	PCA	white/beige, round, smooth, shiny, op
NG05-4	D6B	19	6/7/06	-3	3	PCA	rough, white, op, dull
NG05-4	D6B	20	6/7/06	-2	1	1/10 PCA	orange, round, smooth, shiny, op
NG05-4	D6B	21	6/7/06	-2	1	1/10 PCA	red clear, shiny, ~trans, round, smooth
NG05-4	D6B	22	6/7/06	-2	1	1/10 PCA	rough, white, powdered
NG05-4	D6B	23	6/7/06	-2	2	1/10 PCA	peach, smooth, shiny, round, op
NG05-4	D6B	24	6/7/06	-2	2	1/10 PCA	tan, smooth, ~lob, op
NG05-4	D6B	25	6/7/06	-2	2	1/10 PCA	grey, powdered, op, w/ring
NG05-4	D6B	26	6/7/06	-2	3	1/10 PCA	red, shiny, ~op, round, smooth
NG05-4	D6B	27	6/7/06	-2	3	1/10 PCA	clear with small yellow center, trans, shiny, smooth

Name			Date	Dil	#	Media	Description
NG05-4	D6B	28	6/7/06	-2	3	1/10 PCA	pink/clear, trans, shiny, round
NG05-4	D6B	29	6/7/06	-3	1	1/10 PCA	red orange, rough-looking, smooth, op, clear edge, shiny
NG05-4	D6B	30	6/7/06	-3	1	1/10 PCA	light pink, smooth, rough edge, op, shiny
NG05-4	D6B	31	6/7/06	-3	1	1/10 PCA	orange-pink, ~op, shiny, round, smooth
NG05-4	D6B	32	6/7/06	-3	1	1/10 PCA	yellow, round, convex, smooth, shiny, op
NG05-4	D6B	33	6/7/06	-3	1	1/10 PCA	light pink, smooth, shiny, round, ~op
NG05-4	D6B	34	6/7/06	-3	2	1/10 PCA	orange, dull, rough, round, op
NG05-4	D6B	35	6/7/06	-3	2	1/10 PCA	peach, shiny, smooth, ~op
NG05-4	D6B	36	6/7/06	-3	2	1/10 PCA	yellowish/beige/clear, trans, rough
NG05-4	D6B	37	6/7/06	-3	2	1/10 PCA	pink w/ darker center, shiny, smooth, ~round
NG05-4	D6B	38	6/7/06	-3	3	1/10 PCA	yellow, op, round, smooth, shiny
NG05-4	D6B	39	6/7/06	-3	3	1/10 PCA	pink/red, smooth, op, shiny, round
NG05-4	D6B	40	6/7/06	-3	3	1/10 PCA	white/clear, rough, ~trans, round
NG05-4	D6B	41	6/7/06	-3	3	1/10 PCA	light yellow center, clear edge, shiny, round, op
AT05-22	D6A	5	6/30/06	-2	2	PCA	peach, powdered, dull, op
AT05-22	D6A	7	6/30/06	-2	3	PCA	red, dull, round
AT05-22	D6A	8	6/30/06	-2	3	PCA	white, round, dull
AT05-22	D6A	11	6/30/06	-3	1	PCA	beige w/ yellow powder, rough, op
AT05-22	D6A	17	7/5/06	-2	1	1/10 PCA	white/clear, round, smooth, shiny, trans
AT05-22	D6A	18	7/5/06	-2	1	1/10 PCA	peach/clear, round, smooth, shiny, trans
AT05-22	D6A	19	7/5/06	-2	2	1/10 PCA	white powdered, rough, op, dull
AT05-22	D6A	20	7/5/06	-2	2	1/10 PCA	pink, round, smooth, shiny
AT05-22	D6A	24	7/5/06	-3	1	1/10 PCA	pink/clear, round, smooth, shiny, trans
AT05-22	D6A	25	7/5/06	-3	2	1/10 PCA	pink w/ light center, op, round, ~smooth, shiny
AT05-22	D6A	26	7/5/06	-3	2	1/10 PCA	pink w/ white edge, op, convex, shiny, smooth
AT05-22	D6A	27	7/5/06	-3	2	1/10 PCA	pink, umb, round, ~trans, shiny
AT05-22	D6A	28	7/5/06	-3	3	1/10 PCA	red clear, smooth, shiny, convex, round
AT05-22	D6A	29A	7/11/06	-3	3	1/10 PCA	clear, round, trans, smooth, shiny
AT05-22	D6A	29B	7/11/06	-3	3	1/10 PCA	white, round, trans, smooth, shiny

Name			Date	Dil	#	Media	Description
AT05-22	D6A	30	7/5/06	-3	3	1/10 PCA	yellowish, umb, round, shiny, ~op
AT05-22	D6B	13	6/30/08	-3	2	PCA	green/brown, round, ~dull, op, convex
AT05-22	D6B	18	6/30/08	-2	1	1/10 PCA	pink clear, round, smooth, shiny, trans
AT05-22	D6B	19	6/30/08	-2	1	1/10 PCA	yellow, shiny, ~rough, ~trans, irreg, mucoid
AT05-22	D6B	20	6/30/08	-2	2	1/10 PCA	grey, shiny, round, smooth, lighter edge, op
AT05-22	D6B	21	6/30/08	-2	2	1/10 PCA	yellow, rough edge, ~trans, mucoid
AT05-22	D6B	22	6/30/08	-2	3	1/10 PCA	white powdered w/ clear bubbles, rough, op
AT05-22	D6B	23	6/30/08	-2	3	1/10 PCA	orange/clear w/ dark specks, rough, shiny
AT05-22	D6B	24	6/30/08	-3	1	1/10 PCA	pink, rough, ~round, op, shiny
AT05-22	D6B	25	6/30/08	-3	1	1/10 PCA	black, dull, veined, op, rough
AT05-22	D6B	26	6/30/08	-3	1	1/10 PCA	dark yellow, dull, round, ~rough
AT05-22	D6B	27	6/30/08	-3	2	1/10 PCA	beige/clear, shiny, round, ~op, smooth
AT05-22	D6B	28	6/30/08	-3	2	1/10 PCA	pink w/ clear edge, shiny, round, smooth
AT05-22	D6B	29	6/30/08	-3	2	1/10 PCA	peach powdered w/ ring, rough, dull, op
AT05-22	D6B	30A	7/11/06	-3	2	1/10 PCA	pink, ~op, round
AT05-22	D6B	30B	7/11/06	-3	2	1/10 PCA	yellow, trans, round
AT05-22	D6B	31	7/5/06	-3	3	1/10 PCA	light pink, round, smooth, shiny, ~op
AT05-22	D6B	32A	7/11/06	-3	3	1/10 PCA	orange, round, smooth, shiny, op
AT05-22	D6B	32B	7/11/06	-3	3	1/10 PCA	yellow/orange, small, round, ~trans, shiny, smooth
AT05-22	D6B	33A	7/11/06	-3	3	1/10 PCA	pink, round, smooth, shiny, op
AT05-22	D6B	33B	7/11/06	-3	3	1/10 PCA	pink/clear, round, smooth, shiny, trans
NG05-4	R6A	3	6/8/06	-2	1	PCA	tran, smooth, round, shiny, op
NG05-4	R6A	4	6/8/06	-2	1	PCA	yellow, smooth, round, shiny, op, w/ white edge
NG05-4	R6A	5	6/8/06	-2	1	PCA	red-orange, convex/umb, smooth, ~lob, op, shiny
NG05-4	R6A	8	6/8/06	-2	1	PCA	light pink, ringed, ~smooth, shiny, round, ~op
NG05-4	R6A	9	6/8/06	-2	2	PCA	white/clear, round, convex, smooth, shiny, ~trans
NG05-4	R6A	12	6/8/06	-2	3	PCA	white, ~dull, smooth, op
NG05-4	R6A	13	6/8/06	-2	3	PCA	yellow-clear, trans, smooth, round, shiny
NG05-4	R6A	16	6/8/06	-2	3	PCA	peach/orange, smooth, round, shiny, op, convex

Name			Date	Dil	#	Media	Description
NG05-4	R6A	17	6/8/06	-2	3	PCA	yellowish beige, white powder atop, dull, op, round
NG05-4	R6A	19	6/8/06	-3	1	PCA	clear off-white/yellow, veined, round, shiny, op
NG05-4	R6A	20	6/8/06	-3	1	PCA	orange, smooth, shiny, op
NG05-4	R6A	21	6/8/06	-3	1	PCA	yellow , rough, ~op, ringed, shiny
NG05-4	R6A	23	6/8/06	-3	2	PCA	white, ~op, shiny, round, smooth
NG05-4	R6A	24	6/8/06	-3	2	PCA	peach/orange, smooth, round, shiny, op, convex
NG05-4	R6A	27	6/8/06	-3	2	PCA	yellow, veined, dull, irreg
NG05-4	R6A	28	6/8/06	-3	2	PCA	yellow/tan, veined, dull, irreg
NG05-4	R6A	30	6/8/06	-3	3	PCA	yellow, op, shiny, smooth, round, entire
NG05-4	R6A	31	6/8/06	-3	3	PCA	pink/white, dull, op, rough
NG05-4	R6A	32	6/8/06	-2	1	1/10 PCA	clear pink, ~umb, smooth, round, shiny, trans
NG05-4	R6A	33	6/8/06	-2	1	1/10 PCA	yellow clear, shiny, smooth, ~op
NG05-4	R6A	34	6/8/06	-2	1	1/10 PCA	tan, powdered, umb, op, dull, round
NG05-4	R6A	35	6/8/06	-2	1	1/10 PCA	yellowish beige, irreg, dark center, shiny, op
NG05-4	R6A	36	6/8/06	-2	2	1/10 PCA	dark red, ~op, round, shiny, smooth
NG05-4	R6A	37	6/8/06	-2	2	1/10 PCA	yellow/clear w/ dark center, ~op, clear edge, shiny, smooth
NG05-4	R6A	39	6/8/06	-2	3	1/10 PCA	pinkish, powdered, dull, rough, ~roud, op
NG05-4	R6A	40	6/8/06	-2	3	1/10 PCA	white, clear, ~trans, shiny, smooth, round
NG05-4	R6A	41	6/8/06	-2	3	1/10 PCA	yellow clear, ~trans, shiny, smooth, round
NG05-4	R6A	42	6/8/06	-2	3	1/10 PCA	red, ~op, shiny, smooth, round
NG05-4	R6A	43	6/8/06	-3	1	1/10 PCA	white clear, ~trans, smooth, shiny, round
NG05-4	R6A	44	6/8/06	-3	1	1/10 PCA	yellow/white/clear, lobate/irreg, smooth, shiny, ~op
NG05-4	R6A	45	6/8/06	-3	1	1/10 PCA	red/peach, op, shiny, round, smooth
NG05-4	R6A	46	6/8/06	-3	2	1/10 PCA	brown, smooth, shiny, round, ~op
NG05-4	R6A	47	6/8/06	-3	2	1/10 PCA	deep red, rough, irreg, shiny, smooth center, op
NG05-4	R6A	48	6/8/06	-3	2	1/10 PCA	peach, smooth, op, shiny
NG05-4	R6A	49	6/8/06	-3	3	1/10 PCA	pink clear, appears rough, shiny, round, ~trans
NG05-4	R6A	50	6/8/06	-3	3	1/10 PCA	red, round, ~umb, shiny, op
NG05-4	R6A	51	6/8/06	-3	3	1/10 PCA	pink, ~trans, smooth, shiny, round

Name			Date	Dil	#	Media	Description
NG05-4	R6A	52	6/8/06	-3	3	1/10 PCA	yellow, round, op, shiny, smooth
NG05-4	R6B	2	6/8/06	-2	1	PCA	red/orange, clumped, dull, rough, op
NG05-4	R6B	4	6/8/06	-2	1	PCA	yellow, shiny, op, smooth
NG05-4	R6B	7	6/8/06	-2	1	PCA	clear, rough, shiny, trans
NG05-4	R6B	8	6/8/06	-2	2	PCA	white, smooth, op, shiny, round
NG05-4	R6B	11	6/8/06	-2	2	PCA	clumped/veiny, beige/clear, dull
NG05-4	R6B	15	6/8/06	-2	2	PCA	yellow, rough, ~shiny, irreg, ~op
NG05-4	R6B	18	6/8/06	-3	1	PCA	pink/red, smooth, ~shiny, round, op
NG05-4	R6B	19	6/8/06	-3	1	PCA	yellow/white, round, smooth, shiny, ~op
NG05-4	R6B	20	6/8/06	-3	1	PCA	light pink, dark center, round, smooth, op
NG05-4	R6B	21	6/8/06	-3	1	PCA	white/clear w/ rough powder center, ~dull, ~op
NG05-4	R6B	22	6/8/06	-3	2	PCA	white, smooth, ~lob, op, shiny
NG05-4	R6B	24	6/8/06	-3	3	PCA	orange/pink/white, rough, dull, irreg, ~op
NG05-4	R6B	25A	6/14/06	-3	3	PCA	orange, shiny, op, smooth
NG05-4	R6B	25B	6/14/06	-3	3	PCA	white, shiny, op, round, smooth
NG05-4	R6B	26A	6/27/06	-3	3	PCA	red, shiny, smooth, round, ~op
NG05-4	R6B	26B	6/27/06	-3	3	PCA	pink, shiny, op, round, smooth
NG05-4	R6B	27	6/8/06	-2	1	1/10 PCA	red/clear, ~trans, shiny, round, smooth
NG05-4	R6B	28	6/8/06	-2	1	1/10 PCA	orange-brown clear, rough, shiny, round, trans
NG05-4	R6B	29	6/8/06	-2	1	1/10 PCA	clear pink, round, smooth, trans, shiny
NG05-4	R6B	30	6/8/06	-2	1	1/10 PCA	yellow, trans, smooth, round, shiny
NG05-4	R6B	31	6/8/06	-2	1	1/10 PCA	rough, white, lobate, powdered
NG05-4	R6B	32	6/8/06	-2	2	1/10 PCA	white, shiny, op, irreg, smooth
NG05-4	R6B	33	6/8/06	-2	2	1/10 PCA	brown/peach, clear, shiny, round, smooth, ~trans
NG05-4	R6B	34	6/8/06	-2	2	1/10 PCA	pink, dull, op, round
NG05-4	R6B	35	6/8/06	-2	3	1/10 PCA	tan, shiny, round, ~op, smooth
NG05-4	R6B	36	6/8/06	-2	3	1/10 PCA	yellow, shiny, round, ~trans, smooth
NG05-4	R6B	37	6/8/06	-2	3	1/10 PCA	black, round, shiny, op, smooth
NG05-4	R6B	38	6/8/06	-2	3	1/10 PCA	peach, powdered, rough, op

Name			Date	Dil	#	Media	Description
NG05-4	R6B	39	6/8/06	-3	1	1/10 PCA	red, round, op, smooth, shiny, convex
NG05-4	R6B	40	6/8/06	-3	1	1/10 PCA	brown/orange, rough, dull, op
NG05-4	R6B	41	6/8/06	-3	1	1/10 PCA	pink w/ orangeish center, smooth, round, ~op
NG05-4	R6B	42	6/8/06	-3	1	1/10 PCA	orange w/ white edge, shiny, smooth, round, op
NG05-4	R6B	43	6/8/06	-3	1	1/10 PCA	peach, dull, rough, ~round, op
NG05-4	R6B	44	6/8/06	-3	2	1/10 PCA	peach/orange, op, shiny, smooth, round, white edge
NG05-4	R6B	45	6/8/06	-3	2	1/10 PCA	brown, rough, ~op, shiny, round
NG05-4	R6B	46	6/8/06	-3	2	1/10 PCA	yellow/clear w/ dark yellow center, ~trans, shiny, round
NG05-4	R6B	47	6/8/06	-3	2	1/10 PCA	pink clear, rough, shiny, irreg
NG05-4	R6B	48	6/8/06	-3	3	1/10 PCA	pale yellow, op, shiny, smooth, round
NG05-4	R6B	49	6/8/06	-3	3	1/10 PCA	clear red, umb, round, ~rough, ~trans
NG05-4	R6B	50	6/8/06	-3	3	1/10 PCA	rust/orange w/ powdered center, dull, op
NG05-4	R6B	51	6/8/06	-3	3	1/10 PCA	brown w/ dark center, shiny, round, smooth, op
AT05-22	R6A	1	7/7/06	-2	1	PCA	white edge w/ dark center, smooth, ~round, shiny, op
AT05-22	R6A	16	7/13/06	-2	1	1/10 PCA	clear/yellowish, umb, trans, shiny, ~rough
AT05-22	R6A	17	7/13/06	-2	2	1/10 PCA	grey, flat, smooth, ~op, round
AT05-22	R6A	18	7/13/06	-3	1	1/10 PCA	light pink, round, smooth, shiny, ~op
AT05-22	R6A	19	7/13/06	-3	1	1/10 PCA	orange, round, dull, ~rough, op
AT05-22	R6A	20	7/13/06	-3	1	1/10 PCA	white, round, smooth, shiny, trans
AT05-22	R6A	22	7/13/06	-3	1	1/10 PCA	peach, round, smooth, shiny, ~op
AT05-22	R6A	23	7/13/06	-3	2	1/10 PCA	pink w/ light pink edge, round, smooth, shiny, op
AT05-22	R6A	25	7/13/06	-3	2	1/10 PCA	clear yellow, round, ~smooth, trans
AT05-22	R6A	26A	7/13/06	-3	2	1/10 PCA	pink/red, round, smooth, shiny, op
AT05-22	R6A	26B	7/13/06	-3	2	1/10 PCA	dark yellow, round, smooth, shiny, op
AT05-22	R6A	27	7/13/06	-3	3	1/10 PCA	peach, round, smooth, shiny, op
AT05-22	R6A	28	7/13/06	-3	3	1/10 PCA	yellow, dull, ingrowth, round, smooth, op
AT05-22	R6A	29	7/13/06	-3	3	1/10 PCA	dark red, round, smooth, shiny, trans
AT05-22	R6A	30	7/13/06	-3	3	1/10 PCA	pink w/ darker center, round, smooth, shiny, op
AT05-22	R6B	4	7/13/06	-2	2	PCA	peach, rough, shiny, lumped, op

Name			Date	Dil	#	Media	Description
AT05-22	R6B	6	7/13/06	-2	3	PCA	pale yellow, round, smooth, shiny
AT05-22	R6B	18	7/13/06	-2	1	1/10 PCA	yellow, umb, ~rough, round, ~op, shiny
AT05-22	R6B	19	7/13/06	-2	2	1/10 PCA	clear/yellow, ~round, smooth, shiny, trans
AT05-22	R6B	20	7/13/06	-2	3	1/10 PCA	clear/yellow, raised, ~rough, ~veined
AT05-22	R6B	21	7/13/06	-3	1	1/10 PCA	pink/clear, umb, round, shiny, trans
AT05-22	R6B	22	7/13/06	-3	1	1/10 PCA	pale yellow/clear, round, smooth, shiny, trans
AT05-22	R6B	23	7/13/06	-3	1	1/10 PCA	white, round, smooth, shiny, ~op
AT05-22	R6B	24	7/13/06	-3	2	1/10 PCA	yellow, raised, ringed, round, shiny, trans
AT05-22	R6B	25	7/13/06	-3	2	1/10 PCA	pink/clear, round, smooth, shiny, ~trans
AT05-22	R6B	27	7/13/06	-3	3	1/10 PCA	yellow/clear, ~rough, round, shiny, trans
AT05-22	R6B	28	7/13/06	-3	3	1/10 PCA	peach, smooth, shiny, op
AT05-22	R6B	29	7/13/06	-3	3	1/10 PCA	white/beige/clear, rough, dull, ~trans
AT05-22	R6B	30	7/13/06	-3	3	1/10 PCA	orange, round, smooth, shiny, op
AT05-22	R6B	31	7/13/06	-3	3	1/10 PCA	grey w/ white powdered center, ring, dul
NG05-4	C6A	1	6/9/06	-2	1	PCA	yellow, rough, shiny, irreg, ~op
NG05-4	C6A	2	6/9/06	-2	1	PCA	light pink, op, shiny, round, white center
NG05-4	C6A	3	6/9/06	-2	1	PCA	white w/ yellow center, ~trans, shiny
NG05-4	C6A	6	6/9/06	-2	2	PCA	white, ~trans, round, smooth, shiny
NG05-4	C6A	7	6/9/06	-2	2	PCA	dark pink/red, round, smooth, shiny, ~op
NG05-4	C6A	8	6/9/06	-2	2	PCA	clear yellow, rough, ~shiny, trans
NG05-4	C6A	9	6/9/06	-2	2	PCA	beige, smooth, shiny, ~round, ~op
NG05-4	C6A	15	6/9/06	-3	1	PCA	pink/red, rough, veiny, dull, op
NG05-4	C6A	17	6/9/06	-3	2	PCA	orange, rough, ringed, ~dull, op
NG05-4	C6A	19	6/9/06	-3	3	PCA	orange, veined, clumpy, op, dull, irreg.
NG05-4	C6A	20	6/9/06	-3	3	PCA	peach, round, smooth, op, shiny, convex
NG05-4	C6A	21	6/9/06	-3	3	PCA	white, umb, round, smooth, shiny
NG05-4	C6A	22	6/9/06	-3	3	PCA	peach, rough, dull, op, irreg/lob
NG05-4	C6A	23	6/9/06	-2	1	1/10 PCA	yellow, convex, op, dull, round, smooth
NG05-4	C6A	25	6/9/06	-2	1	1/10 PCA	pink, ringed, op, round, shiny

Name			Date	Dil	#	Media	Description
NG05-4	C6A	26	6/9/06	-2	1	1/10 PCA	peach, ~smooth, op, round, shiny
NG05-4	C6A	27	6/9/06	-2	2	1/10 PCA	white, round, smooth, shiny, op
NG05-4	C6A	28	6/9/06	-2	2	1/10 PCA	red w/ white border, rough, op, shiny
NG05-4	C6A	29	6/9/06	-2	3	1/10 PCA	red orange, ~shiny, umb, round, smooth, op
NG05-4	C6A	30	6/9/06	-2	3	1/10 PCA	beige, ~rough, shiny, round, op
NG05-4	C6A	31	6/9/06	-2	3	1/10 PCA	red, shiny, smooth, round
NG05-4	C6A	32	6/9/06	-3	1	1/10 PCA	pink, ~umb, ~round, shiny, smooth, op
NG05-4	C6A	33	6/9/06	-3	1	1/10 PCA	red w/ white/clear center, shiny, ~op, round
NG05-4	C6A	34	6/9/06	-3	1	1/10 PCA	white w/ orange/brown center, op, shiny, ~round
NG05-4	C6A	35	6/9/06	-3	2	1/10 PCA	yellow clear, ~umb, trans, shiny, round
NG05-4	C6A	36	6/9/06	-3	2	1/10 PCA	pale yellow, round, shiny, convex, smooth
NG05-4	C6A	37	6/9/06	-3	2	1/10 PCA	peach, rough, dull, irreg.
NG05-4	C6A	38	6/9/06	-3	2	1/10 PCA	white/clear, rough, ~op, shiny
NG05-4	C6A	39	6/9/06	-3	2	1/10 PCA	light pink, ~rough, shiny, round, op
NG05-4	C6A	40	6/9/06	-3	2	1/10 PCA	peach, round, smooth, shiny, op
NG05-4	C6A	41	6/9/06	-3	2	1/10 PCA	dark yellow, round, shiny, ~smooth, op
NG05-4	C6A	42	6/9/06	-3	3	1/10 PCA	pink w/ dark center, round, smooth, shiny, op
NG05-4	C6A	43	6/9/06	-3	3	1/10 PCA	peach w/ white edge, round, convex, smooth, shiny
NG05-4	C6B	2	6/9/06	-2	1	PCA	beige w/ mosaic/veined center, op, ~round
NG05-4	C6B	4	6/9/06	-2	1	PCA	yellow, round, ~op, smooth, shiny, convex
NG05-4	C6B	5	6/9/06	-2	1	PCA	beige, globbed, umb, rough, op
NG05-4	C6B	6	6/9/06	-2	2	PCA	beige, round, dull, op, smooth
NG05-4	C6B	8	6/9/06	-2	2	PCA	white, convex, shiny, ~op, round, smooth
NG05-4	C6B	12	6/9/06	-2	3	PCA	peach w/ white edge, dull, smooth, op
NG05-4	C6B	13	6/9/06	-2	3	PCA	yellow rough, ~op, irreg, ~dull
NG05-4	C6B	14	6/9/06	-2	3	PCA	orange, ~rough, irreg, op
NG05-4	C6B	15	6/9/06	-3	1	PCA	pink, ~veined, ~rough, ~op, irreg
NG05-4	C6B	16	6/9/06	-3	1	PCA	veined, white, round, rough, op
NG05-4	C6B	17	6/9/06	-3	2	PCA	orange/clear, trans, smooth, shiny, round

Name			Date	Dil	#	Media	Description
NG05-4	C6B	18	6/9/06	-3	2	PCA	peach, round, convex, smooth, shiny, op
NG05-4	C6B	19	6/9/06	-3	3	PCA	tan, rough, round, shiny, ~convex, op
NG05-4	C6B	20	6/9/06	-3	3	PCA	red/orange, round, shiny, smooth, op
NG05-4	C6B	21	6/9/06	-3	3	PCA	yellow/clear, trans, round, shiny, smooth
NG05-4	C6B	23	6/9/06	-2	1	1/10 PCA	beige yellow, dull, rough, op, ~round
NG05-4	C6B	24	6/9/06	-2	1	1/10 PCA	clear orange/peach, ~smooth, round, shiny, ~op
NG05-4	C6B	25	6/9/06	-2	1	1/10 PCA	red/brown, convex, shiny, round, smooth
NG05-4	C6B	26	6/9/06	-2	2	1/10 PCA	red/pink, smooth, convex, shiny, round, op
NG05-4	C6B	27	6/9/06	-2	2	1/10 PCA	yellow, ~trans, smooth, round, shiny, convex
NG05-4	C6B	28	6/9/06	-2	3	1/10 PCA	white, ~rough, filamentous, op, shiny
NG05-4	C6B	29	6/9/06	-2	3	1/10 PCA	red, trans, round, smooth, shiny
NG05-4	C6B	30	6/9/06	-3	1	1/10 PCA	peach, ~rough, shiny, irreg, op
NG05-4	C6B	31	6/9/06	-3	1	1/10 PCA	orange clear, round, convex, shiny, smooth, trans
NG05-4	C6B	32	6/9/06	-3	1	1/10 PCA	black, round, shiny, convex, smooth, op
NG05-4	C6B	34	6/9/06	-3	2	1/10 PCA	white, round, rough, dull, op
NG05-4	C6B	35	6/9/06	-3	2	1/10 PCA	red/pink, round, shiny, smooth, op
NG05-4	C6B	36	6/9/06	-3	2	1/10 PCA	white, ~round, ~op, shiny, smooth
NG05-4	C6B	37	6/9/06	-3	3	1/10 PCA	yellow w/ clear edge, round, smooth, shiny,~ op
NG05-4	C6B	38	6/9/06	-3	3	1/10 PCA	red/pink, rough, op, shiny
NG05-4	C6B	39	6/9/06	-3	3	1/10 PCA	orange, smooth, shiny, round, op
NG05-4	C6B	40	6/9/06	-3	3	1/10 PCA	yellow, round, smooth, shiny, op
NG05-4	C6B	41	6/9/06	-3	3	1/10 PCA	peach, rough, op, shiny
NG05-4	C6B	42	6/9/06	-3	3	1/10 PCA	orange, dull, rough, op
AT05-22	C6A	15	7/18/06	-3	2	PCA	bright yellow, round, smooth, ~op, shiny
AT05-22	C6A	16	7/18/06	-3	2	PCA	peach, rough, dull, op
AT05-22	C6A	22	7/26/06	-2	1	1/10 PCA	yellow clear, ~rough, shiny, trans, convex
AT05-22	C6A	23	7/26/06	-2	1	1/10 PCA	clear, convex, round, smooth, shiny
AT05-22	C6A	24	7/26/06	-2	2	1/10 PCA	white, dull, rough, flat, op
AT05-22	C6A	25	7/26/06	-2	3	1/10 PCA	red/orange, clear, ~trans, dull

Name			Date	Dil	#	Media	Description
AT05-22	C6A	26	7/26/06	-2	3	1/10 PCA	yellow, shiny, round, smooth, op
AT05-22	C6A	27	7/26/06	-3	1	1/10 PCA	clear, yellow, round, ~smooth, shiny, trans
AT05-22	C6A	28	7/26/06	-3	1	1/10 PCA	light pink, round, op, smooth, shiny, convex
AT05-22	C6A	31	7/26/06	-3	1	1/10 PCA	yellow/white, irreg, cflat, dull, op
AT05-22	C6A	32	7/26/06	-3	2	1/10 PCA	pale yellow, round, op, smooth, shiny
AT05-22	C6A	33	7/26/06	-3	2	1/10 PCA	pale pink, round, smooth, shiny, op
AT05-22	C6A	34	7/26/06	-3	2	1/10 PCA	pink, round, smooth, shiny, op
AT05-22	C6A	35	7/26/06	-3	2	1/10 PCA	yellow/beige, round, ~rough, trans
AT05-22	C6A	36	7/26/06	-3	3	1/10 PCA	yellow, rough, round, ~convex, shiny, trans
AT05-22	C6A	37	7/26/06	-3	3	1/10 PCA	pink/orange, round, smooth, shiny, op
AT05-22	C6A	38	7/26/06	-3	3	1/10 PCA	white, ~rough, ~trans, shiny, round
AT05-22	C6A	39	7/26/06	-3	3	1/10 PCA	light peach, round, smooth, shiny, op
AT05-22	C6A	41	7/26/06	-3	3	1/10 PCA	light pink, round, smooth, shiny, op
AT05-22	C6A	42	7/26/06	-3	3	1/10 PCA	clear pink, round, smooth, shiny, trans
AT05-22	C6B	18	7/18/06	-3	3	PCA	red, round, smooth, shiny, op
AT05-22	C6B	19	7/18/06	-3	3	PCA	blackish ingrowth w/ beige and clear edge, rough, op
AT05-22	C6B	20	7/26/06	-2	1	1/10 PCA	orange/clear, round, shiny, flat, trans
AT05-22	C6B	21	7/26/06	-2	2	1/10 PCA	white/clear, round, smooth, shiny, ~trans
AT05-22	C6B	22	7/26/06	-2	2	1/10 PCA	yellow clear, ~rough, trans, round, shiny
AT05-22	C6B	24	7/26/06	-2	3	1/10 PCA	yellowish clear, smooth, round, glossy, trans
AT05-22	C6B	25	7/26/06	-2	3	1/10 PCA	pink, round, smooth, shiny, ~trans
AT05-22	C6B	26	7/26/06	-3	1	1/10 PCA	red/clear, irreg, shiny, ~smooth, ~op
AT05-22	C6B	27	7/26/06	-3	1	1/10 PCA	white/clear, rough, round, flat, trans
AT05-22	C6B	28	7/26/06	-3	1	1/10 PCA	orange/red, round, shiny, ~smooth, op
AT05-22	C6B	29	7/26/06	-3	1	1/10 PCA	dull, yellow, round, op
AT05-22	C6B	30	7/26/06	-3	1	1/10 PCA	pink, rough, ~trans, ~shiny, veined
AT05-22	C6B	31	7/26/06	-3	2	1/10 PCA	red/pink, ~umb, round, ~smooth, shiny, op
AT05-22	C6B	32	7/26/06	-3	2	1/10 PCA	white/clear, round, smooth, shiny, trans
AT05-22	C6B	34	7/26/06	-3	2	1/10 PCA	yellow, rough, trans, ~dull

Name			Date	Dil	#	Media	Description
AT05-22	C6B	35	7/26/06	-3	2	1/10 PCA	beige, round, smooth, shiny, op
AT05-22	C6B	36	7/26/06	-3	3	1/10 PCA	clear/yellow, round, ~smooth, shiny, trans
AT05-22	C6B	37	7/26/06	-3	3	1/10 PCA	red/orange, rough, round, ~trans, shiny
AT05-22	C6B	38	7/26/06	-3	3	1/10 PCA	orange/clear, rough, ~round, shiny, trans
AT05-22	C6B	39	7/26/06	-3	3	1/10 PCA	pink, ~umb, round, smooth, shiny, ~op
AT05-22	C6B	40A	7/26/06	-3	3	1/10 PCA	orange, powdered, strep
AT05-22	C6B	40B	7/26/06	-3	3	1/10 PCA	pink/clear, rough

APPENDIX B
EZ TAXON RESULTS OF 16S rRNA GENE SEQUENCES

ISOLATE	SEQ PRIMER	CLOSEST PHYLOGENETIC RELATIVE (16s ribosomal RNA gene)	%	SEQ LENGTH	ACCESSION #
AT05-22-5	27F	*Belnapia moabensis*	96%	562	AJ871428
AT05-22-7	27F	*Arthrobacter agilis*	98%	725	X80748
AT05-22-12	27F	*Arthrobacter agilis*	97%	481	X80748
AT05-22-16	27F	*Microvirga subterranea*	89%	685	AY078053
AT05-22-20	27F	*Microvirga subterranea*	93%	879	AY078053
AT05-22-21	27F	*Microvirga subterranea*	97%	764	AY078053
AT05-22-25	27F	*Arthrobacter agilis*	99%	660	X80748
AT05-22-27	27F	*Sphingomonas kaistensis*	96%	541	AY769083
AT05-22-32	27F	*Arthrobacter agilis*	99%	520	X80748
NG05-4-1	27F	*Methylobacterium iners*	97%	696	EF174497
NG05-4-3	27F	*Belnapia moabensis*	97%	694	AJ871428
NG05-4-13	27F	*Paenibacillus larvae*	99%	778	AY530294
NG05-4-19	27F	*Deinococcus navajonensis*	99%	394	AY743259
NG05-4-20	27F	*Planomicrobium okeanokoites*	97%	694	D55729
NG05-4-21	27F	*Arthrobacter agilis*	89%	493	X80748
NG05-4-23	27F	*Deinococcus hohokamensis*	93%	656	AY743256
NG05-4-24	27F	*Kocuria rosea*	99%	631	X87756
NG05-4-26	27F	*Arthrobacter humicola*	97%	652	AB279890
NG05-4-27	27F	*Arthrobacter agilis*	98%	312	X80748
NG05-4-30	27F	*Blastococcus saxobsidens*	98%	297	AJ316571
NG05-4-D6A-1	27F	*Nesterenkonia jeotgall*	74%	780	AY928901
NG05-4-D6A-2	27F	*Arthrobacter oryzae*	97%	1054	AB279889
NG05-4-D6A-3	27F	*Arthrobacter humicola*	77%	423	AB279890
NG05-4-D6A-9	27F	*Arthrobacter agilis*	96%	800	X80748
NG05-4-D6A-10	27F	*Streptomyces roseolilacinus*	73%	698	AY999879
NG05-4-D6A-16	27F	*Arthrobacter agilis*	96%	837	X80748
NG05-4-D6A-18	27F	*Leucobacter komagatae*	71%	468	AB007419

ISOLATE	SEQ PRIMER	CLOSEST PHYLOGENETIC RELATIVE (16s ribosomal RNA gene)	%	SEQ LENGTH	ACCESSION #
NG05-4-D6A-19	27F	*Arthrobacter agilis*	80%	611	X80748
NG05-4-D6A-22	27F	*Arthrobacter parietis*	89%	1056	AJ639830
NG05-4-D6A-24	27F	*Paenibacillus lautus*	83%	646	D78473
NG05-4-D6A-25	27F	*Paenibacillus lautus*	85%	1039	D78473
NG05-4-D6A-27	27F	*Paenibacillus lactis*	82%	903	AY257868
NG05-4-D6A-29	27F	*Arthrobacter koreensis*	71%	993	AY116496
NG05-4-D6A-31	27F	*Arthrobacter agilis*	83%	1031	X80748
NG05-4-D6A-32	27F	*Arthrobacter tumbae*	93%	972	AJ315069
NG05-4-D6A-36	27F	*Microvirga subterranea*	88%	947	AY078053
NG05-4-D6A-39	27F	*Nocardioides oleivorans*	86%	825	AJ698724
NG05-4-D6A-41	27F	*Arthrobacter agilis*	96%	842	X80748
NG05-4-D6A-42	27F	*Saccharothrix texasensis*	93%	627	AF114815
NG05-4-D6A-43	27F	*Modestobacter versicolor*	92%	559	AJ871304
NG05-4-D6A-45	27F	*Arthrobacter oryzae*	93%	1023	AB279889
NG05-4-D6A-46	27F	*Microvirga subterranea*	92%	860	AY078053
NG05-4-D6A-47	27F	*Deinococcus deserti*	92%	810	AY876378
NG05-4-D6A-49	27F	*Arthrobacter agilis*	89%	971	X80748
NG05-4-D6B-2	27F	*Bacillus murialis*	99%	1018	AJ628748
NG05-4-D6B-4A	27F	*Staphylococcus nepalensis*	78%	635	AJ517414
NG05-4-D6B-4B	27F	*Staphylococcus pasteuri*	99%	1004	AF041361
NG05-4-D6B-5	27F	*Arthrobacter polychromagenes*	95%	1000	X80741
NG05-4-D6B-7	27F	*Kocuria polaris*	83%	991	AJ278868
NG05-4-D6B-8	27F	*Arthrobacter scleromae*	90%	977	AF330692
NG05-4-D6B-16	27F	*Arthrobacter scleromae*	97%	1007	AF330692
NG05-4-D6B-18A	27F	*Arthrobacter agilis*	89%	850	X80748
NG05-4-D6B-18B	27F	*Arthrobacter humicola*	91%	937	AB279890
NG05-4-D6B-19	27F	*Staphylococcus epidermidis*	99%	932	L37605
NG05-4-D6B-20	27F	*Arthrobacter scleromae*	72%	788	AF330692
NG05-4-D6B-21	27F	*Arthrobacter agilis*	92%	970	X80748
NG05-4-D6B-24	27F	*Bacillus megaterium*	99%	997	D16273
NG05-4-D6B-26	27F	*Arthrobacter agilis*	99%	888	X80748
NG05-4-D6B-28	27F	*Arthrobacter agilis*	99%	949	X80748
NG05-4-D6B-29	27F	*Adhaeribacter aquaticus*	93%	1010	AJ626894
NG05-4-D6B-30	27F	*Microvirga subterranea*	96%	1016	AY078053

ISOLATE	SEQ PRIMER	CLOSEST PHYLOGENETIC RELATIVE (16s ribosomal RNA gene)	%	SEQ LENGTH	ACCESSION #
NG05-4-D6B-31	27F	*Arthrobacter tecti*	97%	966	AJ639829
NG05-4-D6B-32	27F	*Nocardioides terrigena*	97%	970	EF363712
NG05-4-D6B-33	27F	*Arthrobacter agilis*	99%	964	X80748
NG05-4-D6B-35	27F	*Deinococcus deserti*	96%	949	AY876378
NG05-4-D6B-36	27F	*Arthrobacter oxydans*	99%	979	X83408
NG05-4-D6B-37	27F	*Arthrobacter agilis*	99%	916	X80748
NG05-4-D6B-38	27F	*Arthrobacter tumbae*	97%	828	AJ315069
NG05-4-D6B-39	27F	*Arthrobacter agilis*	99%	951	X80748
NG05-4-D6B-40	27F	*Arthrobacter oxydans*	99%	991	X80748
NG05-4-D6B-41	27F	*Nocardioides terrigena*	96%	782	EF363712
NG05-4-R6A-3	27F	*Staphylococcus epidermidis*	99%	356	L37605
NG05-4-R6A-4	27F	*Arthrobacter tumbae*	99%	996	AJ315069
NG05-4-R6A-8	27F	*Arthrobacter scleromae*	99%	985	AF330692
NG05-4-R6A-9	27F	*Arthrobacter oryzae*	99%	952	AB279889
NG05-4-R6A-12	27F	*Arthrobacter crystallopoietes*	99%	981	X80738
NG05-4-R6A-13	27F	*Arthrobacter humicola*	90%	872	AB279890
NG05-4-R6A-17	27F	*Arthrobacter ramosus*	98%	898	X80742
NG05-4-R6A-19	27F	*Streptomyces helvaticus*	99%	260	AB184367
NG05-4-R6A-21	27F	*Cellulomonas cellasea*	97%	913	X83804
NG05-4-R6A-23	27F	*Arthrobacter oryzae*	96%	774	AB279889
NG05-4-R6A-24	27F	*Blastococcus aggregatus*	91%	413	L40614
NG05-4-R6A-27	27F	*Arthrobacter monumenti*	89%	377	AJ315070
NG05-4-R6A-28	27F	*Arthrobacter crystallopoietes*	95%	760	X80738
NG05-4-R6A-31	27F	*Arthrobacter agilis*	98%	310	X80748
NG05-4-R6A-32	27F	*Arthrobacter agilis*	82%	715	X80748
NG05-4-R6A-33	27F	*Paenibacillus lautus*	98%	622	D78473
NG05-4-R6A-34	27F	*Streptomyces enissocaesilis*	93%	488	AB249930
NG05-4-R6A-35	27F	*Arthrobacter oryzae*	96%	593	AB279889
NG05-4-R6A-37	27F	*Arthrobacter tumbae*	98%	1212	AJ315069
NG05-4-R6A-40	27F	*Arthrobacter scleromae*	94%	1224	AF330692
NG05-4-R6A-43	27F	*Arthrobacter polychromagenes*	99%	701	X80741
NG05-4-R6A-44	27F	*Arthrobacter oxydans*	94%	759	X83408
NG05-4-R6A-45	27F	*Arthrobacter agilis*	96%	600	X80748
NG05-4-R6A-46	27F	*Deinococcus deserti*	89%	703	AY876378

ISOLATE	SEQ PRIMER	CLOSEST PHYLOGENETIC RELATIVE (16s ribosomal RNA gene)	%	SEQ LENGTH	ACCESSION #
NG05-4-R6A-47	27F	*Arthrobacter agilis*	99%	610	X80748
NG05-4-R6A-48	27F	*Deinococcus deserti*	94%	832	AY876378
NG05-4-R6A-49	27F	*Arthrobacter agilis*	95%	317	X80748
NG05-4-R6A-51	27F	*Arthrobacter agilis*	99%	806	X80748
NG05-4-R6B-2	27F	*Arthrobacter polychromagenes*	99%	941	X80741
NG05-4-R6B-4	27F	*Micrococcus lylae*	96%	900	X80750
NG05-4-R6B-7	27F	*Arthrobacter ramosus*	96%	916	X80742
NG05-4-R6B-8	27F	*Arthrobacter crystallpoeites*	99%	968	X80738
NG05-4-R6B-15	27F	*Arthrobacter luteolus*	96%	477	AJ243422
NG05-4-R6B-18	27F	*Arthrobacter ureafaciens*	98%	332	X80744
NG05-4-R6B-19	27F	*Arthrobacter oxydans*	96%	649	X83408
NG05-4-R6B-20	27F	*Methylobacterium iners*	95%	794	EF177497
NG05-4-R6B-21	27F	*Arthrobacter ramosus*	84%	242	X80742
NG05-4-R6B-22	27F	*Arthrobacter ramosus*	99%	852	X80742
NG05-4-R6B-24	27F	*Arthrobacter agilis*	94%	867	X80748
NG05-4-R6B-25B	27F	*Arthrobacter ramosus*	99%	872	X80742
NG05-4-R6B-27	27F	*Arthrobacter agilis*	99%	891	X80748
NG05-4-R6B-28	27F	*Methylobacterium iners*	90%	858	EF177497
NG05-4-R6B-29	27F	*Arthrobacter agilis*	98%	773	X80748
NG05-4-R6B-31	27F	*Streptomyces candidus*	94%	822	AB184190
NG05-4-R6B-32	27F	*Bacillus anthracis*	80%	367	AB190217
NG05-4-R6B-34	27F	*Arthrobacter agilis*	96%	770	X80748
NG05-4-R6B-35	27F	*Kocuria polaris*	99%	901	AJ278868
NG05-4-R6B-36	27F	*Arthrobacter tumbae*	99%	872	AJ315069

APPENDIX C:
TABLE OF PHENOTYPIC TEST RESULTS

Strain	Origin	Morphology	Media	Liquid RM	Flocking	1/10 PCB
M. subterranea	DSM 14364^T	pink mucoid	RM	++	N	+++
B. flocculans	ATCC BAA 817	rough conv op white	TSA	+++	Y	+++
Lut5, Lut6	Parker Lab	conv op white	TGY	+++	Y	+++
AT05-22-20	Atacama	pink ~rough	1/10 PCA	+	Y	++
AT05-22-21	""	yellow trans	1/10 PCA	-	N	+
AT04-150-33	""	pink small	PCA	-	N	+
AT04-150-34	""	pink small	PCA	+	Y	++
AT04-159-9	""	peach/orange	1/10 PCA	+	N	++
68-J-13	Atacama Air	dk pink	PCA or 1/10	+++	N	++
NG05-4-1	Negev	white/tan	PCA	+++	N	+++
NG05-4-1B	""	pink	RM	+++	N	++
NG05-4-D6A-36	""	pink mucoid	1/10 PCA	+	N	+
NG05-4-D6B-30	""	dk pink ~trans	1/10 PCA	+	Y	-
NG05-4-R6B-20	""	dk pink ~trans	PCA	+	Y	++
NG05-4-R6B-28	""	lt pink ~mucoid	1/10 PCA	+	N	++
SS05-2-117	Sahara	orange rough	1/10 PCA	-	Y	++
SS05-2-120	""	pink mucoid ~rough	1/10 PCA	+	N	+
2LRH1B-19	Mojave	pink rough	1/10 PCA	-	Y	+
33-40Q-8	Oman	pink ~trans	1/100 PCA	-	N	-
33-40Q-24	""	pink/peach mucoid	PCA	++	N	++
33-40Q-27	""	pink mucoid ~rough	PCA	-	N	++
33-40Q-60	""	pink small trans	1/10 PCA	+++	Y	+++
33-40Q-172	""	dk pink	PCA	+++	N	++
20-40R-17	""	pink/tan ~mucoid	1/10 PCA	++	Y	++
20-40R-18	""	pink mucoid	1/10 PCA	++	Y	+++
20-40R-19	""	pink	1/10 PCA	+	Y	++
20-40R-21	""	peach	1/10 PCA	+++	Y	+++
20-40R-23	""	pink ~rough	1/100 PCA	++	Y	+
20-40R-25	""	peach mucoid rough	1/100 PCA	+++	N	+++
10-39Q-41	""	pink mucoid	1/100 PCA	+	N	++

Strain	Origin	Morphology	Media	Liquid RM	Flocking	1/10 PCB
10-39Q-44	Oman	dk pink	1/10 PCA	+	Y	++
10-39Q-50	""	dk pink	1/10 PCA	+++	Y	+++
10-39Q-56	""	dk/lt pink	1/10 PCA	+	Y	++
10-39Q-57	""	lt pink ~trans	1/10 PCA	++	N	++
10-39Q-58	""	lt pink ~trans	1/10 PCA	+++	N	+++
10-39Q-59	""	lt pink ~rough	1/10 PCA	-	N	++
10-39Q-60	""	dk/lt pink	1/10 PCA	+++	Y	+++
10-39Q-94	""	lt pink ~trans	1/10 PCA	+	Y	++
10-39Q-95	""	dk/lt pink ~trans	1/10 PCA	+	N	++
10-39Q-99	""	lt pink mucoid	1/10 PCA	++	Y	+++
10-39Q-100	""	dk pink	1/10 PCA	-	Y	++
10-39Q-102	""	dk pink small	1/100 PCA	+	Y	++
10-39Q-110	""	pink ~trans	1/10 PCA	++	Y	++
KR-6	Sonoran	orange/pink	RM	++	N	+++
KR-11	""	pink/orange	RM	++	N	+++
KR-27	""	lt pink mucoid	RM	+	Y	+++
KR-46	""	beige/pink mucoid	RM	+	Y	+
KR-47	""	beige/clear mucoid	RM	+	N	+++
KR-145	""	lt pink	1/10 PCA	+	Y	+++
KR-149	""	pink mucoid	RM	++	Y	+++
KR-160	""	dk pink	PCA	+++	N	+++
KR-163	""	beige/pink small	NA	+++	N	+++
KR-393	Nevada	lt pink mucoid	NA	++	Y	+++
KR-431	""	lt pink	NA	+	Y	+++
KR-438	""	pink/beige mucoid	NA	+++	Y	+++
KR-447	""	peach mucoid rough	PCA	+++	YY	+++
KR-448	""	peach mucoid rough	PCA	+	Y	++
KR-450	""	pink/beige mucoid	RM	++	N	+++
KR-477	""	peach mucoid	NA or RM	++	Y	+++
KR-483	""	lt pink mucoid	1/10 PCA	+	N	+++
KR-501	""	beige/white mucoid	1/10 PCA	+	Y	+++
KR-504	""	lt pink/beige mucoid	1/10 PCA	+	Y	+++
KR-533	""	pink/beige mucoid	RM	++	Y	+++

Strain	25°C	30°C	37°C	40°C	45°C	Motility	Glucose	Starch	Oxidase	Urease	Catalase
M. subterranea		++++	++++	++++	++++	+	-	+	+	-	+
B. flocculans		++	++++	+++	++++	-	-	-	+	-	+
Lut5, Lut6		+++	++++	++++	++	-	+	+	+	-	+
AT05-22-20		++++	+	+	+	-	-	-	-	-	+
AT05-22-21		++++	++	+	+	+	-	+	-	-	+
AT04-150-33		++++	++++	+	+	-	+	+	+	-	+
AT04-150-34		++++	+++	+++	0	+	-	+	-	-	+
AT04-159-9		++++	++	+	+	+	+	+	-	-	+
68-J-13		++++	+++	++	+	+	-	+	+	-	+
NG05-4-1		++++	++	++	+	-	+	-	+	+	+
NG05-4-1B		++++	++	+	+	+	+	-	-	-	+
NG05-4-D6A-36		+++	++	+	0	-	+	-	-	-	+
NG05-4-D6B-30		++++	++	+	+	-	-	+	+	-	+
NG05-4-R6B-20		++++	++++	+	0	+	-	+	-	-	+
NG05-4-R6B-28		+++	++	+	0	-	+	-	-	-	+
SS05-2-117		++	++++	+++	+	+	-	+	-	-	+
SS05-2-120		+++	++	+	0	-	-	-	-	-	+
2LRH1B-19		++++	++++	++	0	+	-	-	+	+	+
33-40Q-8		++++	++++	+++	0	-	+	+	-	-	+
33-40Q-24		++	+++	++	+	-	-	+	-	-	+
33-40Q-27		+++	+++	++++	+	-	-	-	+	-	+
33-40Q-60	+	+++	++++	++	0	+	+	+	-	-	+
33-40Q-172		++++	++++	+++	0	-	+	+	+	-	+
20-40R-17		++++	++++	+	+	-	+	+	+	-	+
20-40R-18		++++	++++	++	0	-	-	-	-	-	+
20-40R-19		++++	++++	+++	0	+	+	+	+	-	+
20-40R-21		++++	++++	+	+	+	-	+	+	-	+
20-40R-23		++++	++++	0	0	-	-	+	+	-	+
20-40R-25		+++	+++	0	0	-	+	-	-	-	+
10-39Q-41		++++	++	++	+	+	-	+	+	-	+
10-39Q-44		++++	++++	++++	+	+	+	+	+	-	+
10-39Q-50		++++	+++	+++	+	+	-	-	+	-	+

Strain	25°C	30°C	37°C	40°C	45°C	Motility	Glucose	Starch	Oxidase	Urease	Catalase
10-39Q-56		++++	++++	++++	+	-	-	+	+	-	+
10-39Q-57		++++	++++	+++	0	-	-	+	+	-	+
10-39Q-58		++++	++++	++	0	-	-	+	+	-	+
10-39Q-59		+++	++++	+++	+	-	+	-	+	-	+
10-39Q-60		++++	++++	++++	+	+	+	+	+	-	+
10-39Q-94		+++	++	+	+	+	-	+	+	-	+
10-39Q-95		++++	++	+	+	+	-	-	+	-	+
10-39Q-99		++++	++++	++	+	+	+	-	+	-	+
10-39Q-100		++++	++++	++++	+	+	-	-	+	-	+
10-39Q-102	+	++	0	0	0	+	+	+	-	-	+
10-39Q-110		++++	+++	++	+	+	+	+	+	-	+
KR-6		++++	++++	+++	+++	+	-	+	+	-	+
KR-11		++++	++++	+++	+++	+	+	+	+	-	+
KR-27		++++	++++	++++	++++	+	+	-	+	-	+
KR-46		++++	++++	++++	+++	-	-	-	+	-	+
KR-47		++++	++++	++++	++++	-	+	-	-	-	-
KR-145		+++	+++	++++	++++	-	-	+	+	-	+
KR-149		++++	+++	++	++	+	-	-	+	-	+
KR-160		++++	++++	++++	++	-	+	-	+	-	+
KR-163	+++	+++	++	++	0	-	-	+	-	-	+
KR-393		++++	++++	++++	++++	+	-	-	+	-	+
KR-431		+++	++++	++	+	+	+	-	-	-	+
KR-438		++++	++++	+++++	++++	+	+	-	+	-	+
KR-447		+++	++++	+++++	++	+	-	-	+	-	-
KR-448		++++	++++	+++	+	-	+	+	+	-	+
KR-450		++++	++++	++++	++++	+	+	-	+	-	+
KR-477		++++	++++	++++	+	+	+	-	+	+	+
KR-483		++++	++++	++++	++++	+	-	+	+	-	+
KR-501		++++	++++	++++	+++	+	+	-	+	-	+
KR-504		++++	++++	++	+	+	+	+	-	-	+
KR-533		++++	++++	++++	++++	+	+	-	+	-	+

Strain	0.5% NaCl	Flocking	1% NaCl	Flocking	1.5% NaCl	Flocking	2% NaCl	Flocking
M. subterranea	+++	N	+++	N	++	N	++	N
B. flocculans	+++	Y	+++	Y	++	Y	+	Y
Lut5, Lut6	+,++	Y	++	Y	+,++	Y	0,+	Y
AT05-22-20	++	Y	+++	Y	+++	Y	+++	Y
AT05-22-21	+	N	++	N	+++	Y	+++	Y
AT04-150-33	+	Y	++	Y	+++	Y	+++	Y
AT04-150-34	++	Y	+	Y	+	Y	++	Y
AT04-159-9	+++	Y	+++	Y	++	N	++	N
68-J-13	+++	N	+++	N	+++	N	+++	N
NG05-4-1	+++	N	-	N	+++	N	+++	N
NG05-4-1B	-	N	++	Y	-	N	-	N
NG05-4-D6A-36	++	N	++	N	++	N	-	N
NG05-4-D6B-30	++	N	+	N	++	Y	++	Y
NG05-4-R6B-20	+++	N	++	N	++	N	+++	Y
NG05-4-R6B-28	++	N	++	N	++	N	++	N
SS05-2-117	++	Y	++	Y	+	Y	+	Y
SS05-2-120	++	Y	++	Y	+++	Y	+++	Y
2LRH1B-19	+++	Y	++	Y	++	Y	++	Y
33-40Q-8	-	N	-	N	+++	N	++	N
33-40Q-24	+++	Y	+	Y	+	Y	+	Y
33-40Q-27	-	N	++	Y	-	N	-	N
33-40Q-60	+	N	+	N	-	N	-	N
33-40Q-172	++	Y	++	Y	+	N	+	N
20-40R-17	+++	Y	++	Y	++	Y	++	Y
20-40R-18	+	Y	+	Y	+	Y	+	Y
20-40R-19	+++	N	++	Y	+++	N	+++	Y
20-40R-21	+++	N	+++	N	+++	N	+++	N
20-40R-23	+	Y	+	Y	+	Y	+	Y
20-40R-25	0	N	0	N	0	N	0	N
10-39Q-41	+	N	+	N	+	N	+++	N
10-39Q-44	+	Y	+	Y	+	Y	++	Y
10-39Q-50	+++	Y	++	Y	+	Y	+	Y
10-39Q-56	++	Y	+	Y	+	Y	+	Y

Strain	0.5% NaCl	Flocking	1% NaCl	Flocking	1.5% NaCl	Flocking	2% NaCl	Flocking
10-39Q-57	+	N	+	N	++	N	++	N
10-39Q-58	+	N	++	N	+	N	+	N
10-39Q-59	++	N	+	N	+	N	+	N
10-39Q-60	+	Y	++	Y	+	Y	+	Y
10-39Q-94	+	Y	++	Y	++	Y	++	Y
10-39Q-95	+	N	++	N	++	N	++	N
10-39Q-99	+++	N	++	N	++	N	+	N
10-39Q-100	+++	N	+++	N	++	N	++	N
10-39Q-102	++	Y	+	Y	+	Y	+	Y
10-39Q-110	+	N	0	N	0	N	0	N
KR-6	+++	N	+++	N	+++	N	+++	N
KR-11	+++	N	+++	N	+++	N	+++	N
KR-27	+++	N	+++	N	++	Y	++	Y
KR-46	+	N	+++	N	++	N	+	N
KR-47	++	N	++	N	++	N	++	N
KR-145	+++	N	+++	N	+++	Y	+++	Y
KR-149	+++	N	+++	N	+++	N	+++	N
KR-160	++	N	+++	N	+++	N	+++	N
KR-163	+++	N	+++	N	+++	N	+++	N
KR-393	+++	N	+++	N	+++	N	+++	N
KR-431	++	Y	++	N	++	N	++	Y
KR-438	+++	N	+++	N	+++	N	+++	N
KR-447	+++	N	+++	N	+++	N	+++	N
KR-448	+++	Y	+++	Y	+++	Y	+++	Y
KR-450	++	N	+++	N	+++	N	+++	N
KR-477	+++	Y	+++	Y	+++	Y	+++	Y
KR-483	+++	Y	+++	Y	+++	Y	+++	Y
KR-501	+++	N	+++	N	+++	N	+++	N
KR-504	++	N	++	N	++	Y	++	Y
KR-533	+++	N	+++	N	+++	N	+++	N

APPENDIX D
TABLE OF FATTY ACID ANALYSIS RESULTS

		M. subterranea	10-39Q-44	20-40-R-18	KR-448	KR-149	*B. flocculans*	KR-163	KR-393	KR-501
Name	ECL									
14:00	13,999		0.36	1	0.65	0.31		0.62	0.54	1.24
Sum Feature 1	14,479	0.77								
15:0	15,001	0.42			0.4			0.38		0.31
Sum Feature 2	15,489	3.03	2.16	2.03	2.64	2.69	4.33	2.27	2.63	3.18
Sum Feature 3	15,819	2.16	6.53	7.47	4.89	7.27	1.25	4.76	2.7	4.18
16:0	16,000	7.35	7.99	8.63	11.09	9.91	4.4	10.98	8.28	13.38
15:0 3OH	16,505	1.04			0.29			0.22		
17:1 w8c	16,795	1.98			0.7	0.22		0.6		0.33
17:1 w6c	16,861	1.39								
17:0 cyclo	16,890				2.78			2.81	1.05	2.55
17:0	16,999	9.37			2.37	1.36	0.42	2.19	1.34	1.35
Sum Feature 5	17,720									
18:1 w9c	17,772									
Sum Feature 8	17,825	60.09	77.26	76.96	54.21	70.67	77.31	54.57	59.59	53.13
18:1 w5c	17,921									
18:0	17,999	4.48	4.32	3.41	1.67	4.39	5.99	1.52	3.65	2.2
UN 18.147	18,148									
19:0 cyclo w8c	18,901	5.6			15.06	1.67	2.79	16.33	18.11	16.03
19:0 10-methyl	19,370		0.66	0.54	1	0.53	1.05	0.82	0.45	0.85
18:0 3OH	19,553	1.39	0.61	0.45	1.22	0.99	1.75	1.12	1.65	1.46

Sum Feature 1	13:0 3OH/15:1 iso H
Sum Feature 2	16:1iso I/14:0 3OH
Sum Feature 3	16:1w7c/16:1w6c
Sum Feature 4	17:1 anteiso B/ iso I
Sum Feature 5	18:2w6,9c/18:0anteiso
Sum Feature 8	18:1w7c/18:1w6c

		20-40-R-17	Lut-5	68J-13	AT-04-150-34	SS-05-2-120	KR-46	KR-160	KR-431	AT-05-22-21
Name	ECL									
14:00	13,999	0.68	0.12	0.35		0.96	0.41	0.63	2	0.46
Sum Feature 1	14,479									
15:0	15,001	0.35				0.25				
Sum Feature 2	15,489	2.86	3.62	2	4.58	3.46	2.49	2.86	2.76	4.21
Sum Feature 3	15,819	5.2	1.65	12.1	2.51	3.64	3.62	6.77	5.89	2.24
16:0	16,000	10.61	4.52	3.81	6.22	10.44	5.98	6.51	13.78	5.84
15:0 3OH	16,505	0.27			1.02					0.96
17:1 w8c	16,795	0.67			1.27			0.31		1.27
17:1 w6c	16,861				1.24					1.15
17:0 cyclo	16,890	1.73					0.23	0.58	0.72	
17:0	16,999	2.29	0.46		6.65	1.02	0.36	0.82	0.33	6.28
Sum Feature 5	17,720									
18:1 w9c	17,772					0.46				
Sum Feature 8	17,825	60.13	83.1	79.24	67.06	74.5	78.79	74.61	65.49	63.36
18:1 w5c	17,921			0.27						
18:0	17,999	1.94	3.16	1.24	5.73	4.31	4.33	2.5	2.79	5.21
UN 18.147	18,148									4.73
19:0 cyclo w8c	18,901	11.51	1.36		1.35		1.94	2.56	5.76	1.25
19:0 10-methyl	19,370	0.8	1.01	0.99		0.33	1.01	1.04	0.68	0.8
18:0 3OH	19,553	1.3	0.94		2.35	0.86	0.82	0.69		2.24

Sum Feature 1	13:0 3OH/15:1 iso H
Sum Feature 2	16:1iso I/14:0 3OH
Sum Feature 3	16:1w7c/16:1w6c
Sum Feature 4	17:1 anteiso B/ iso I
Sum Feature 5	18:2w6,9c/18:0anteiso
Sum Feature 8	18:1w7c/18:1w6c