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Directed evolution of desiccation tolerance in Escherichia coli

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by

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ABSTRACT

The lack of water on Mars has rendered the red planet's environment one of extreme desiccation, and whether microbial life can exist within these conditions remains a mystery. Directed evolution is a methodology that has been used to study the development of microbial resistance to stresses such as ionizing radiation and low pressure. This methodology is applied in this study to investigate desiccation tolerance within *Escherichia coli.* Results display an overall 3-fold increase in desiccation tolerance over 50 iterative cycles of dehydration and re-hydration. Desiccated populations showed increased survival when desiccated under low pressure. Understanding the process of desiccation tolerance in microorganisms facilitates the investigation of components necessary to microbial survival on Mars.

INTRODUCTION

Of all the planets in the solar system, it is not strange that Mars has gained the most attention in the search for extraterrestrial habitats that could potentially support microbial life. Many similarities exist between Mars and Earth, including similarities in land surface area, length of day, the occurrence of seasonal changes, and the presence of polar ice caps. Although Earth has a diameter nearly two times greater than Mars' diameter, the planets have a similar land surface area because the majority of Earth's surface is covered in ocean [1] . Seasonal changes occur on both planets due to similar tilts in their rotational axes, and both share historical evidence of drastic climate change [2]. Even the length of Mars' day is similar to Earth's at 24 hours and 39 minutes [1]. Polar ice caps are also present on both planets [2]. It is believed that the northern ice cap is composed mostly of frozen water, and the southern ice cap is composed of frozen water and carbon dioxide with the northern ice cap being significantly larger

than the southern [2]. Despite these major similarities, the Martian atmosphere is anything but Earth-like.

Mars' atmosphere is cold, desiccated, and irradiated. The planet's surface experiences several stressful conditions, including UV irradiation, extreme desiccation, low pressure (1 – 14mbar), and extremely low temperatures (-61°C) (Table 1) [3]. The Martian atmosphere is considered anoxic, composed largely of carbon dioxide [3]. All of these conditions would likely make microbial growth difficult on Mars [3]. Any terrestrial organism that could potentially survive such harsh conditions would be expected to possess tolerance to these globally occurring factors.

Tolerance to Mars' low pressure and aridness are traits that a microorganism must possess if expected to survive. *Deinococcus radiodurans*, an extensively studied inherently desiccation-tolerant bacterium, is known to survive months in a desiccated environment [4]. This bacterium's tolerance to desiccation is partly attributed to an ability to repair double-stranded DNA breaks, an effect caused by extreme desiccation that can be otherwise lethal to other species [4]. Desiccation can cause several other effects to a cell, such as damage to external layers, increases in intracellular salt levels, shrinkage of capsular layers, and reduced membrane fluidity (Table 2) [5]. Changes in cell compartment volumes and physiological processes are also possible effects of desiccation [5]. On the microbial community level, changes in pigment, shape, and texture can occur [5]. Another condition that might constrain microbial growth on Mars is low pressure. Previous studies conducted to investigate microorganisms' response to low pressure do not duplicate the low pressures found on Mars with experimental pressure conditions remaining above 100mbar [6]. It is unlikely that a terran organism could survive Martian conditions without some genetic modification. By mimicking natural evolutionary

processes, directed evolution can be used to select for desired traits within existing terrestrial organisms.

Directed evolution is a method that has been successfully used to develop ionizing radiation-resistant (IR) organisms as well as tolerance to high pressure. For IR resistance, directed evolution was performed on the ionizing radiation-sensitive organism, *Escherichia coli* K-12 MG1655. Twenty iterative cycles of ionizing radiation and outgrowth led to an increase in radio-resistance in *E. coli* [7]. Directed evolution was also applied to a study involving the development of high pressure-resistant *E. coli*. Both studies involved repeated exposure of *E. coli*, strain K12 MG1655, to a particular stress and outgrowth of surviving populations in attempt to isolate tolerant populations [8]. The success of these studies has rendered directed evolution a plausible method for the development of desiccation tolerance in desiccation-sensitive species and possibly the coevolution of tolerance to low pressure as well.

In a previous study, directed evolution was applied to Founder, a clonal derivative of *E. coli* strain K12 MG1655 that differs by seven base pairs. After 50 cycles of iteration, increased desiccation tolerance was not observed. It is possible that desiccation, unlike ionizing radiation, does not cause sufficient genetic changes within the surviving populations, thereby limiting the selection for desiccation tolerance. In this study, Founder was modified by deleting the *mutS* gene, responsible for base mismatch repair, which created a means of increasing genetic diversity within the strain used for directed evolution.

This study uses directed evolution to develop a desiccation-tolerant population of *E. coli*. We performed iterative cycles of desiccation and outgrowth to the desiccation-sensitive organism, *E. coli* K-12 MG1655. The use of directed evolution to generate a desiccation-tolerant organism has not been previously reported. The development of desiccation tolerance in *E. coli*

would allow further insight into the processes necessary for an organisms' self-preservation against such stresses and aid in the investigation of terran microorganisms that could potentially survive on Mars.

METHOD

Strains used for cycles of directed evolution:

Two derivatives of *Escherichia coli,* strain K-12 MG1655 were developed. The first derivative, called *E. coli* MG1655 Founder (Founder), was created from an isolate of strain K-12 MG1655. Founder differs from its parent strain by seven base pairs. The second derivative of strain K-12 MG1655 was a knockout of the *mutS* gene. The *mutS* gene was removed from Founder by the Warner method. Mutation frequency was calculated for the *ΔmutS* strain by plating on LB^{rif} and determining survival. The mutation frequency increased 10-fold upon removal of the *mutS* gene. In order to accumulate potential beneficial mutations, *ΔmutS* was repeatedly grown for 10 populations. The last population generated was used for the first cycle of directed evolution.

Directed evolution for desiccation tolerance:

Founder and *ΔmutS* were grown overnight at 37°C to reach stationary phase. One hundred microliters of each overnight culture was transferred to 10mL of fresh Luria broth for 4 hours at 37°C to reach early stationary phase. Cells were centrifuged at 13,500rpm for 5 minutes, and the supernatant was removed. Cells were resuspended in 1mL of 0.9% saline. Media was removed and replaced with saline to prevent cell growth during incubation in the desiccation chamber. Ten microliters of each culture was transferred to sterile microscopic slides

in triplicates. A cell titer was performed to determine the number of viable cells placed onto each microscopic slide. Slides were transferred to a desiccation chamber with Drierite (>98% calcium sulfate, <2% cobalt chloride). Relative humidity in the desiccation chamber, measured using a hygrometer, ranged from 18 – 25%. Cells were desiccated for 50 minutes. After 50 minutes, cells were rehydrated with 1mL of 0.9% saline, and a cell titer was performed to determine number of surviving cells. An aliquot of the surviving population was grown overnight in Luria broth at 37°C. Surviving populations were stored and used for future iterative cycles of desiccation (Figure 1). Founder was desiccated non-iteratively for each cycle to serve as a control.

Naming the surviving populations:

The surviving population from cycle 1 of desiccation was termed Desiccated Population 1. Desiccated Population 1 was desiccated for cycle 2 of desiccation, and the surviving population was termed Desiccated Population 2. All remaining surviving populations were termed Desiccated Population along with associated cycle number.

Calculation of survival percentage and survival ratio:

Survival percentages were calculated by dividing the number of surviving cells by the number of cells desiccated, and the quotient multiplied by 100%. The number of surviving cells and the number of cells desiccated were determined from cell titers performed before and after desiccation. Survival percentages were generated for each triplicate. Number of surviving cells was determined for each triplicate and divided by the average number of cells desiccated. The quotient was then multiplied by 100% to calculate survival percentage.

The survival ratio was determined by dividing the survival percentage of the respective Desiccated Population by the survival percentage of its parent, Founder. Survival percentages from triplicates of the respective Desiccated Population were individually divided by the average survival percentage of Founder. The value reported was an average of survival ratios calculated from all triplicates.

Determining the mutation frequencies of *ΔmutS* and Desiccated Population 34:

The mutation frequencies of *ΔmutS* and Desiccated Population 34 were determined and compared with the mutation frequency of their parent, Founder. Cells were grown overnight in Luria broth at 37°C. Cells were spread onto Luria agar plates with rifampicin (50μg/mL). A cell titer was performed to determine the number of viable cells that were spread. Number of rifampicin-resistant cells was determined. Mutation frequency was calculated by dividing number of resistant cells by the total number of cells spread.

Desiccation of Desiccated Population 49 under low pressure:

The protocol for a cycle of desiccation, previously described, was followed and performed on Desiccated Population 49 (D49), replacing the desiccation chamber with a thermal vacuum chamber. D49 and Founder were desiccation at 28 – 30% relative humidity with an atmospheric pressure of 2 – 8mbar for 1 and 2 hours. Survival percentages and ratio of survivability were determined as described previously.

Desiccation of other organisms under low pressure:

Pseudomonas aeruginosa PAO1, *Deinococcus radiodurans* R1, Desiccated Population 50 (D50) and Founder, were desiccated under low pressure for 50 minutes at 28 – 30% relative humidity and 2-6mbar of atmospheric pressure. The protocol for a cycle of desiccation was used as previously described, replacing the desiccation chamber with a thermal vacuum chamber. Survival percentages were determined as described previously.

Performing growth curves of "slow-growing" Desiccated Population 22 and Founder isolates:

Growth curves were performed on isolates of Desiccated Population 22 (D22) and Founder. A single isolate was taken from D22 and Founder after 24 hours of growth post-desiccation. Single isolates that required 48 and 72 hours to grow post-desiccation were also selected from D22 and Founder. The OD_{600} was measured for 6 hours in 30-minute intervals. Isolates were grown in Luria broth and incubated at 37°C between measurements. Equation 1 was used to calculate generation time based on the measurements taken from the OD_{600}.

$$G = \frac{t}{3.3 \log N_o/N}$$

Equation 1. Calculation of generation time. T is the time interval between OD_{600} measurements. N_o is the absorbance measurement at the beginning of the time interval, and N is the number of cells at the end of the time interval.

Desiccation of the "slow-growing" population extracted from Desiccated Population 32:

Single isolates were chosen from Desiccated Population 32 (D32) and Founder that required 48 – 72 hours to form post-desiccation. Isolates were grid-plated on Luria agar plates. All growth from grid plates were compiled and transferred to one tube of Luria broth and incubated at 37°C to create a subpopulation. Subpopulations of D32 and Founder were desiccated for 50 minutes at 18 – 25% relative humidity, following the protocol for a cycle of desiccation as previously described. The surviving populations were plated to determine survival percentage and survival ratio as described previously.

RESULTS

Removal of *mutS* gene causes 10-fold increase in mutation frequency:

All Desiccated Populations arose from the *ΔmutS* strain. After 34 cycles of desiccation, the mutation frequencies of Desiccated Population 34 (D34) and Founder were calculated for comparison. Previously, we calculated the mutation frequencies of *ΔmutS* and Founder and noted a 10-fold increase in mutation frequency upon removal of the *mutS* gene. We tested D34 and Founder using the same method previously described. Mutation frequency of Founder and D34 were $1.20 \times 10^{-7} \pm 1.73 \times 10^{-8}$ and $6.96 \times 10^{-6} \pm 8.45 \times 10^{-7}$, respectively, indicating a 10-fold increase. This confirmed our previous results, and after 34 cycles of desiccation, the mutation frequency did not change between *ΔmutS* and D34.

Directed evolution of *Escherichia coli* results in 3-fold increase in desiccation tolerance:

Directed evolution was performed on *Escherichia coli* K12 MG1655 in attempt to create a desiccation-tolerant strain. Removing the *mutS* gene, responsible for base mismatch repair, created a derivative of *E. coli* K12 MG1655 that possessed a 10-fold increase in mutation

frequency. *ΔmutS* was used for fifty cycles of desiccation and outgrowth to select for desiccation-tolerant populations. Founder grown from frozen stock was desiccated for each cycle to serve as a control. The survival ratio of the Desiccated Populations (DP) was determined to objectively compare performance of the DP with its parent strain, Founder, for each cycle. A ratio greater than one meant that DP survivability exceeded Founder survivability, and an increase in desiccation tolerance was observed. A ratio less than one meant that DP survivability was less than Founder survivability, and desiccation sensitivity was observed. A ratio equal to one indicated that the DP showed no difference in level of desiccation tolerance when compared to Founder.

A wave in survivability of the DP was seen throughout the 50 cycles of desiccation. Increased desiccation tolerance was seen for some cycles, albeit, not without being followed by cycles that displayed desiccation sensitivity. The first observed peak of desiccation tolerance occurred during cycle 15, which showed a 3-fold increase when compared to Founder. The second peak lasted four cycles, cycles 24 – 27, in which desiccation tolerance was 3- to 4-fold higher than Founder. The third peak also lasted four cycles, and desiccation tolerance was 5- to 6- fold higher than Founder. The last peak was seen at cycle 50, in which D50 was 3 to 4 times more desiccation-tolerant than Founder. Each observed peak in desiccation tolerance increased as cycles progressed. However, every increase in desiccation tolerance was followed by cycles of desiccation sensitivity. Desiccation sensitivity for DP was seen for cycles 4 – 5, cycle 10, cycle 18, cycle 22, cycle 28, cycle 41, and cycles 46 – 48. Survival percentage of the DP was 2-fold lower than Founder for cycles 4 – 5, 10, 18, and 22. After cycle 28, the survival percentage of the DP remained higher than Founder. An overall trend is observed throughout 50 cycles of desiccation, in which a gradual increase in desiccation tolerance occurs in the DP when

compared to Founder. Cycle 1 had no difference in tolerance when compared to Founder, and cycle 50 ended with a 3- to 4-fold tolerance to desiccation. Variance of Founder percent survival ranged from 0.002 to 1.29%, while variance of survival of the DP ranged from 0.012 to 3.31% (Figure 2).

Desiccated Population 49 is more tolerant to desiccation under low pressure than Founder:

Desiccated Population 49 (D49) survives better than its parent strain, Founder, when desiccated under low pressure. D49 and Founder were desiccated under low pressure to observe tolerance to dual stresses. D49 was a population that displayed a 2-fold increase in desiccation tolerance compared to Founder. D49 and Founder were desiccated under low pressure using a thermal vacuum chamber at 2 – 8 mbar of pressure for 1 and 2 hours. Survival percentage was calculated and shown in Figure 3. D49 survived significantly better than Founder for both 1 and 2 hours ($p < 0.05$). Survival percentage of D49 was 6-fold higher than Founder's for both time intervals. Although both Founder and Desiccated Population 49 show sensitivity to desiccation under low pressure, D49 shows significant increase in tolerance when compared to its parent, Founder, indicating that some level of desiccation tolerance was achieved through directed evolution.

***Pseudomonas aeruginosa* and *Escherichia coli* show sensitivity to desiccation under low pressure while *Deinococcus radiodurans* displays tolerance:**

Desiccation population 50 (D50) was shown to survive better than *Pseudomonas aeruginosa* and Founder when desiccated under low pressure for 50 minutes. Founder, D50, *Pseudomonas aeruginosa* PAO1 (*P. aeruginosa*), and *Deinococus radiodurans* R1 (*D.*

radiodurans) were desiccated under low pressure to compare the organisms' abilities to survive under dual stresses. Cultures of each organism were grown as previously described. Cells were desiccated in a thermal vacuum chamber for 50 minutes at 2 – 6 mbar and 28 – 30% relative humidity. Results are shown in Figure 4. D50, which was 3- to 4-fold more desiccation-tolerant than Founder, survived significantly better under the additional stress of low pressure ($p < 0.05$). Survival of *P. aeruginosa* was below the level of detection, indicating extreme sensitivity. *D. radiodurans* displayed tolerance at 74% survival, as expected since *D. radiodurans* is a known desiccation-tolerant organism. When subjected to dual stresses, desiccation and low pressure, extreme sensitivity was observed for *P. aeruginosa*, Founder, and D50 while extreme desiccation tolerance was displayed in *D. radiodurans.* D50 exhibited a significant increase in desiccation tolerance under low pressure when compared to its parent, Founder, providing evidence that desiccation tolerance can be achieved.

Desiccation causes variation in generation time within populations:

Growth rates differed among single isolates of the Desiccated Populations (DP) post-desiccation. Growth rates were determined for single isolates from Desiccated Population 22 (D22) and Founder that required 24 hours, 48 hours, and 72 hours to form colonies on Luria agar plate post-desiccation. Approximately 6.76 ± 3.07% of the surviving population of D22 formed colonies after 72 hours, while 2.90 ± 2.35% of Founder's surviving population formed colonies after 72 hours post-desiccation. Growth curves were performed on single isolates from D22 and Founder that required 24, 48, and 72 hours of growth post-desiccation. The OD_{600} was measured in 30-minute intervals for six hours and used to calculate generation time. The generation time of Founder grown from frozen stock was 42 minutes. Single isolates of Founder that required 24,

48, and 72 hours post-desiccation had a generation time of 55 – 56 minutes. The generation time of D22 grown from frozen stock was 50 minutes. A single isolate of D22 that required 24 hours of growth to form a colony post-desiccation had a generation time of 54 minutes. A single isolate of D22 that required 48 hours of growth had a generation time of 56 minutes. The single isolate that required 72 hours of growth to form a colony post-desiccation had a generation time of 65 minutes (Figure 5). Desiccation caused a 13-minute increase in generation time for single isolates of Founder, while desiccation caused a 4- to 15-minute increase in generation time for single isolates of D22. Generation time of D22 isolates increased as time required for growth increased at 4 minutes for 24 hours of growth, 6 minutes for 48 hours of growth, and 15 minutes for 72 hours of growth. Desiccation caused generation time to vary within isolates of Desiccated Population 22 and the surviving Founder population.

Cells with slower growth rates do not show increased tolerance to desiccation:

Subpopulations made from isolates of Desiccated Population 32 (D32) and Founder that required 48 and 72 hours of growth post-desiccation were desiccated with their parent populations to compare survivability. Isolates were extracted and transferred to Luria broth and incubated overnight to create a subpopulation. Subpopulations were created for both D32 and Founder and desiccated under the same conditions as their parent populations. The survival percentage of the "slow-growing" Founder subpopulation was the same as the survival percentage of its parent population with overlapping error bars. The survival percentage of the "slow-growing" D32 subpopulation was slightly lower than survival percentage of its parent population with non-overlapping error bars. These "slow-growing" isolates do not possess increased survivability when compared to their parent populations (Figure 6).

DISCUSSION

The difficulty in the search for a terrestrial organism capable of surviving the harsh Martian environment lies in the fact that Martian conditions do not exist on Earth. Due to the drastic differences in atmospheric temperature, pressure, and humidity, it is unlikely that current terrestrial organisms are capable of survival on Mars. Although many terrestrial organisms possess an inherent ability to withstand certain stresses like desiccation and ionizing radiation, the degree in which these stresses exist on Earth is much less than their magnitude on Mars. Simulated Martian conditions are necessary to test the response of current terrestrial organism to the stresses within the Martian environment.

It has not been previously reported that an inherently desiccation-sensitive species can be forced to evolve into a desiccation-tolerant species. The use of directed evolution was applied to *E. coli* K12 MG1655 derivatives to obtain a desiccation-tolerant population. The last population generated after 50 cycles of desiccation and outgrowth displayed a 3- to 4-fold increase in desiccation tolerance (Figure 2). Desiccation induces double-strand breaks and in turn can cause mutations (Mattimore *et al.*, 1996). Throughout the fifty cycles of desiccation, there were cycles in which the DP was 5-fold more desiccation-tolerant than its parent, Founder. Despite this increase in desiccation tolerance, some cycles thereafter showed desiccation sensitivity within the DP when compared to Founder. The increased mutation frequency in the DP could lead to the accumulation of harmful mutations, which can partially explain the increase and sudden decreases in desiccation tolerance. If there are beneficial mutations that are accumulating in the population during one cycle, a secondary mutation could occur that would hinder the bacterium's survival for the next cycle, leading to a decrease in desiccation tolerance. Overall, a gradual

increase in desiccation tolerance was observed for the DP. A similar trend was seen when directed evolution was used to generate an ionizing radiation-resistant population in Founder (Harris *et al*., 2009). When directed evolution was applied for selection of ionizing radiation tolerance, a 4,000- to 5,000-fold increase in radio-resistance was observed. It is possible that desiccation is a stress that more often causes cell death than mutations. The effects of desiccation can alter the availability of water and subsequently lead to a decrease in the organisms' ability to repair damage.

Desiccation is not the only stress present on the Martian surface. Mars' atmosphere also harbors extremely low pressure, a stress microorganisms must overcome to survive. Desiccated Population 49 (D49) was tested for its response to combined low pressure and desiccation for one and two hours (Figure 3). For both time increments, D49 survived significantly better than Founder ($p < 0.05$). It is possible that desiccation tolerance can aid in tolerance to low pressure as well. D49 displayed a 2-fold increase in desiccation tolerance when compared to Founder, and the increased desiccation tolerance observed might have helped D49's tolerance to low pressure. The large variation observed could be attributed to the diversity of mutations within the population tested, and this diversity might be responsible for the range in degree of desiccation tolerance observed. Mutations attributed to desiccation tolerance and tolerance to low pressure would require further examining.

Several terrestrial organisms were tested for their ability to withstand desiccation and low pressure. Founder, D50, *Pseudomonas aeruginosa* PAO1, and *Deinococcus radiodurans* R1 were desiccated under low pressure for 50 minutes, and survival percentage was calculated (Figure 4). D50 had a significantly higher survival percentage when desiccated under low pressure than Founder and *P. aeruginosa* ($p< 0.05$). A 3- to 4-fold increase in desiccation

tolerance was observed in D50 when compared to Founder. This increase in desiccation tolerance could have attributed to its performance when desiccated under low pressure. *D. radiodurans* is known to be extremely desiccation-tolerant, and our results coincide with previous studies (Mattimore *et al.*, 1996). To develop a terrestrial organism capable of surviving Martian conditions, a method of directed evolution can be applied to obtain tolerances to stresses as they exist on Mars.

Mutations that were accumulated during directed evolution could affect the growth rate of isolates within Founder and the DP. Colony formation in *E. coli* typically takes 24 hours, while some colonies took up to 72 hours to form post-desiccation. Growth curves were performed on these isolates, and an increased generation time was observed for colonies that required 48 and 72 hours to form colonies (Figure 5). The increased generation time could be attributed to the way in which the cells repaired DNA damage before division. Subpopulations were created of the "slow-growing" isolates of cycle 32. Desiccated Subpopulation 32 was more desiccation-tolerant than Founder's subpopulation but was significantly less desiccation-tolerant than its parent population, Desiccated Population 32 ($p < 0.05$). The increased generation time observed did not mean increased tolerance to desiccation.

Directed evolution has been used previously to evolve sensitive species into tolerant species under selective stresses (Harris *et al.,* 2006). Developing a desiccation-tolerant strain has not been done prior to this study. Further cycles of desiccation of *Escherichia coli* K12 MG1655 are required to allow the development of extreme desiccation tolerance. Extreme desiccation tolerance is described in this study as obtaining greater than a 10-fold increase in desiccation tolerance compared to its parent. If accomplished, this same methodology could allow a means

for the development of tolerances to other Martian stresses beyond desiccation and possibly the coevolution of tolerance to combined stresses.

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FIGURE LEGENDS

Table 1. Global temperature, pressure, and atmospheric composition of Mars and Earth. Surface pressure on Mars is 6.36 mbar, while surface pressure on Earth is 1014 mbar. Average temperature on Mars is -63° C, while average temperature on Earth is 15° C. The

atmospheric composition on Mars consists mostly of carbon dioxide, with nitrogen and argon, and trace amounts of oxygen, carbon monoxide, and water. The atmospheric composition on Earth consists mostly of nitrogen, oxygen, and some water. Conditions on the Martian surface are much colder than conditions on Earth and also harbor an arid, low-pressure environment (Williams, 2013).

Table 2. Gross responses of cells to desiccation. On the community level, desiccation can cause many effects, including changes in color, shape, and texture, as well as an increase in surface area, shrinkage, and salt precipitation. On the cellular level, changes in physiological and biophysical properties can occur, as well as shrinkage of capsular layers, increase in intracellular salt levels, crowding of macromolecules, and reduced fluidity. Changes in cell compartment volumes and damage to external layers can also occur (Potts, 1994). *Table originated from Table 1 of "Desiccation of Prokaryotes" (Potts, 1994)

Figure 1. Overview of method for creating a desiccation-tolerant organism. Protocol describes the methodology of each cycle of desiccation. Respective Desiccated Population and its parent strain, Founder, were grown to early stationary. An aliquot was placed on a microscopic slide and desiccated for 50 minutes. Cells were then recovered and plated. Survival percentage was calculated by dividing number of survivors by number of cells desiccated and multiplying by 100%. The surviving population was regrown and stored for the next desiccation cycle.

Figure 2. Survivability of *Escherichia coli* over iterative cycles of desiccation compared to its parent strain. Cycles of desiccation and outgrowth were performed fifty times over a span of six months. The surviving Desiccated Population generated after cycle 1 served as the population to be desiccated for cycle 2. The ratio was calculated by dividing survival

percentage of DP by survival percentage of the parent strain, Founder. Over several cycles, the DP's ability to tolerate desiccation steadily increases. Error bars are from standard deviations of three studies in triplicates (n=8 for cycle 8; n=6 for cycles 11, 12, and 14; n =9 for all other cycles).

Figure 3. Desiccation of Desiccated Population 49 under low pressure compared to its parent, Founder. D49 and Founder were desiccated under low pressure for 1 hour and again for 2 hours (2 – 8mbar and 28 – 30% relative humidity). Survival percentage of D49 was significantly higher than Founder. Results indicate that desiccation tolerance may help organisms survive low pressure. Error bars are from standard deviations of three studies in triplicates.

Figure 4. *Escherichia coli* derivatives, Founder and Desiccated Population 50, *Pseudomonas aeruginosa* PAO1, and *Deinococcus radiodurans* R1 desiccated under low pressure (6 – 7 mbar). Each strain was desiccated for 50 minutes at 28 – 30% relative humidity. Results show that survival of *P. aeruginosa* was below the level of detection. Founder and D50 survived less than 0.10%. *D. radiodurans,* a naturally desiccation tolerant organism, had a survival percentage of 74%. D50 was significantly more tolerant than Founder ($p < 0.05$). Results indicate that desiccation tolerance may help organisms survive low pressure. Error bars are from standard deviations of three studies in triplicates.

Figure 5. **Colonies that formed past 48 hours of growth post-desiccation show difference in generation time.** Single isolates were extracted after 24, 48, and 72 hours of growth post-desiccation from the surviving population of Founder for cycle 22 and Desiccated Population 22 (D22). Growth curves were done on isolates to compare generation times. Results indicate that generation time of DP22 isolates increased as time required for colony formation

increased. A similar trend was observed for Founder isolates. Longer generation time may be necessary for cells to repair damage caused by desiccation.

Figure 6. "Slow-growing" Desiccated Subpopulation 32 (32) and Founder subpopulation were desiccated to compare with survivability of parent population. Subpopulations were made from isolates that took over 48 hours to form colonies for both D32 and Founder. These subpopulations were desiccated with their parent populations, D32 and Founder for comparison. Survival percentages of Founder and its subpopulation matched. Survival percentage of D32 was higher than its subpopulation. Results indicate that isolates that possess increased generation times do not have heightened survivability when compared to their parent counterparts. Error bars are from standard deviations of three studies in triplicates.

FIGURES

Atmosphere	Mars	Earth
Surface Pressure	6.36 mbar	1014mbar
Average Temperature	-63° C	15° C
Atmospheric composition (by volume)	Carbon dioxide (95.32%) Nitrogen (2.7%) Argon (1.6%) Oxygen (.13%) Carbon monoxide (.08%) Water (210ppm)	Nitrogen (98.08%) Oxygen (20.95%) Water (1%) (highly variable)

Table 1. Global temperature, pressure, and atmospheric composition of Mars and Earth.

Level of effect	Response
Community	Change in color Change in shape Change in texture Salt precipitation Shrinkage Increase in surface area
Cell	Change in physiological processes Damage to external layers Reduced fluidity Changes in biophysical properties Changes in volumes of cell compartments Crowding of macromolecules Increase in intracellular salt levels Shrinkage of capsular layers

Table 2. Gross responses of cells to desiccation.

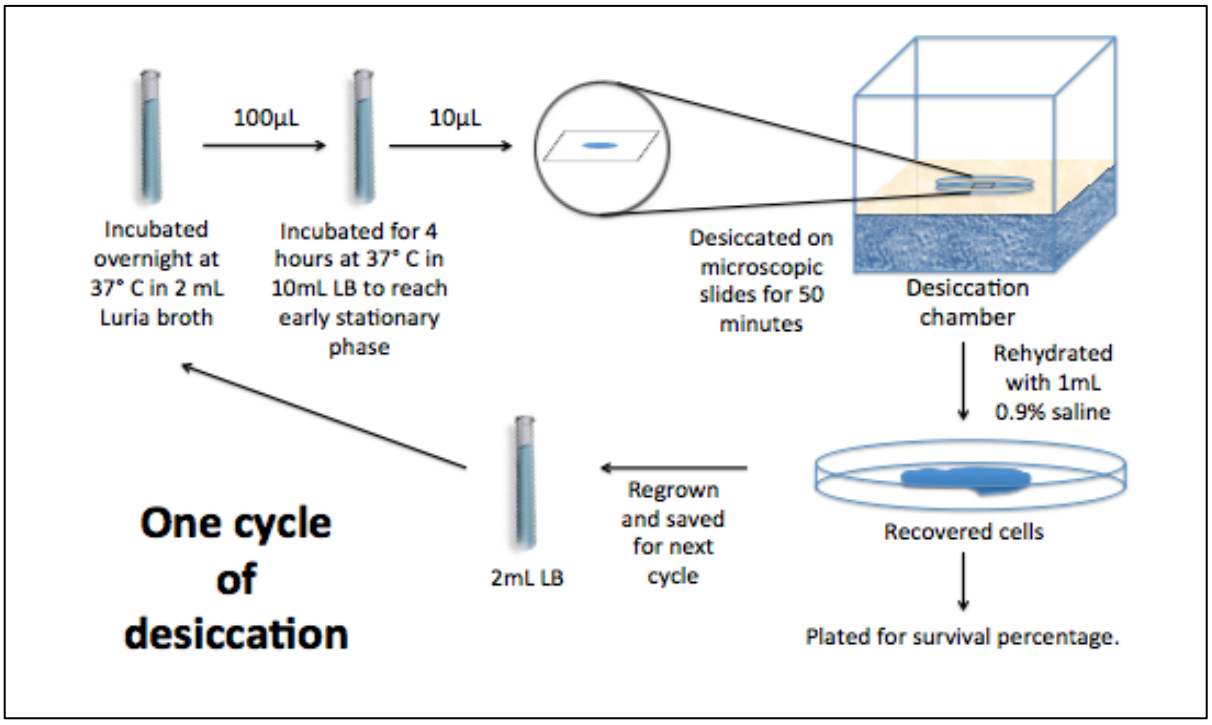


Figure 1. Overview of method for creating a desiccation-tolerant organism.

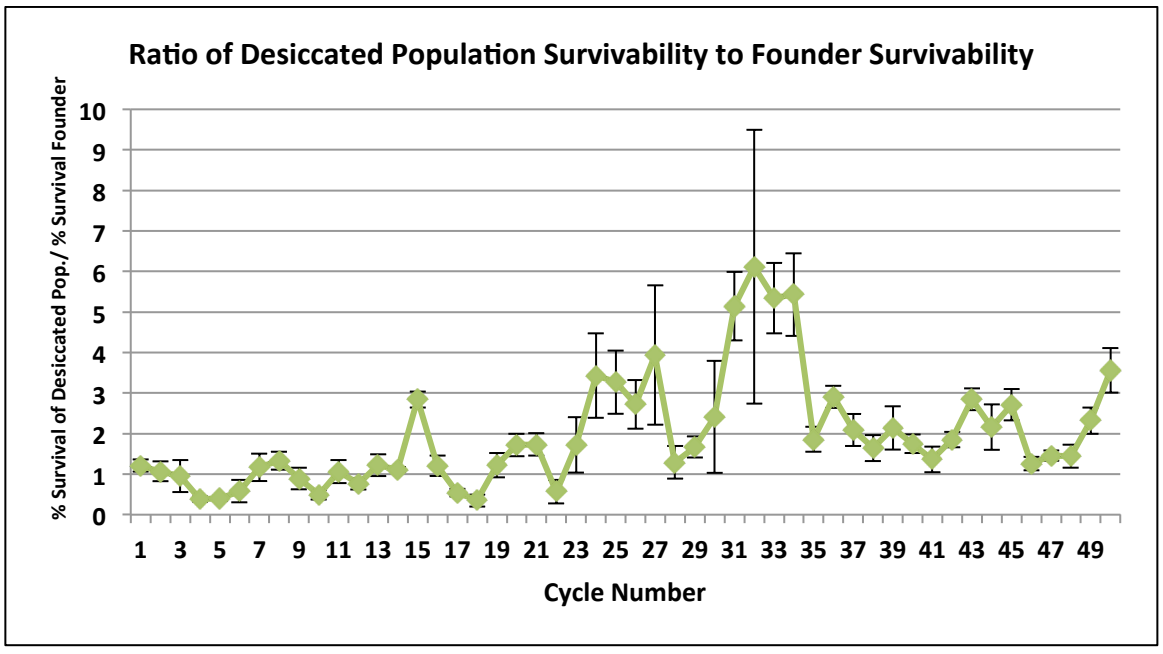


Figure 2. Survivability of *Escherichia coli* over iterative cycles of desiccation compared to its parent strain.

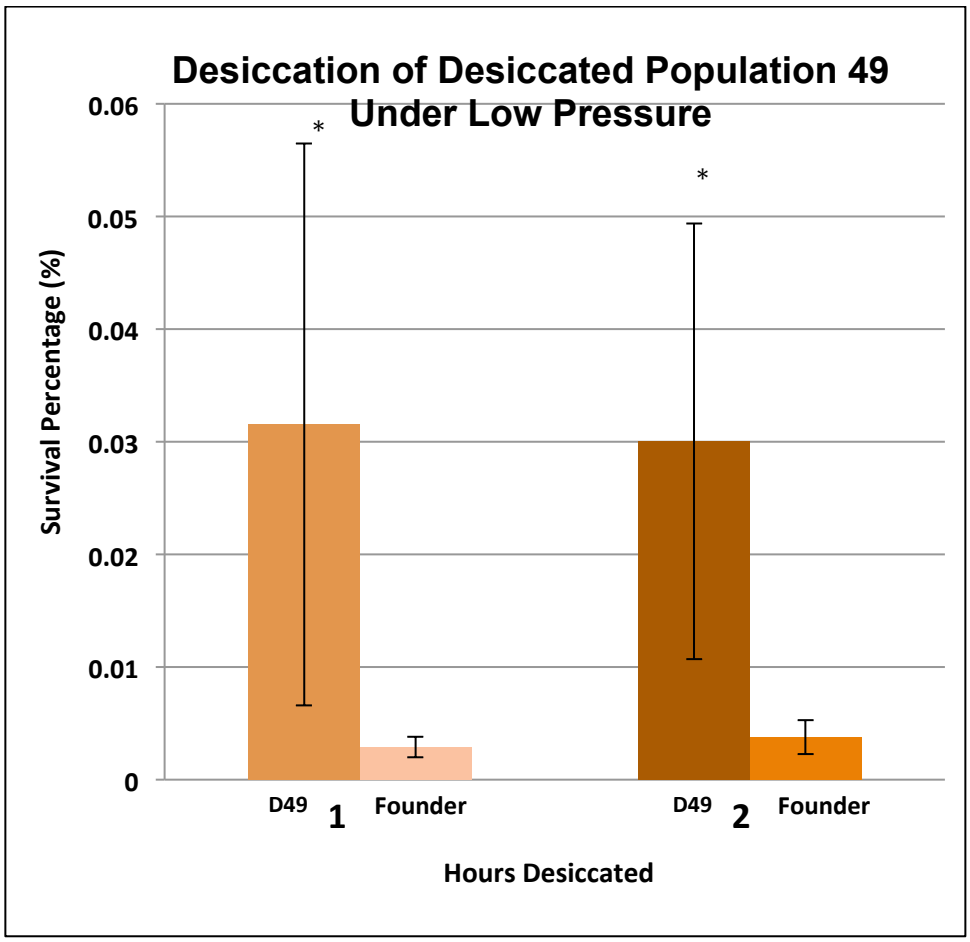


Figure 3. Desiccation of Desiccated Population 49 under low pressure compared to its parent, Founder.

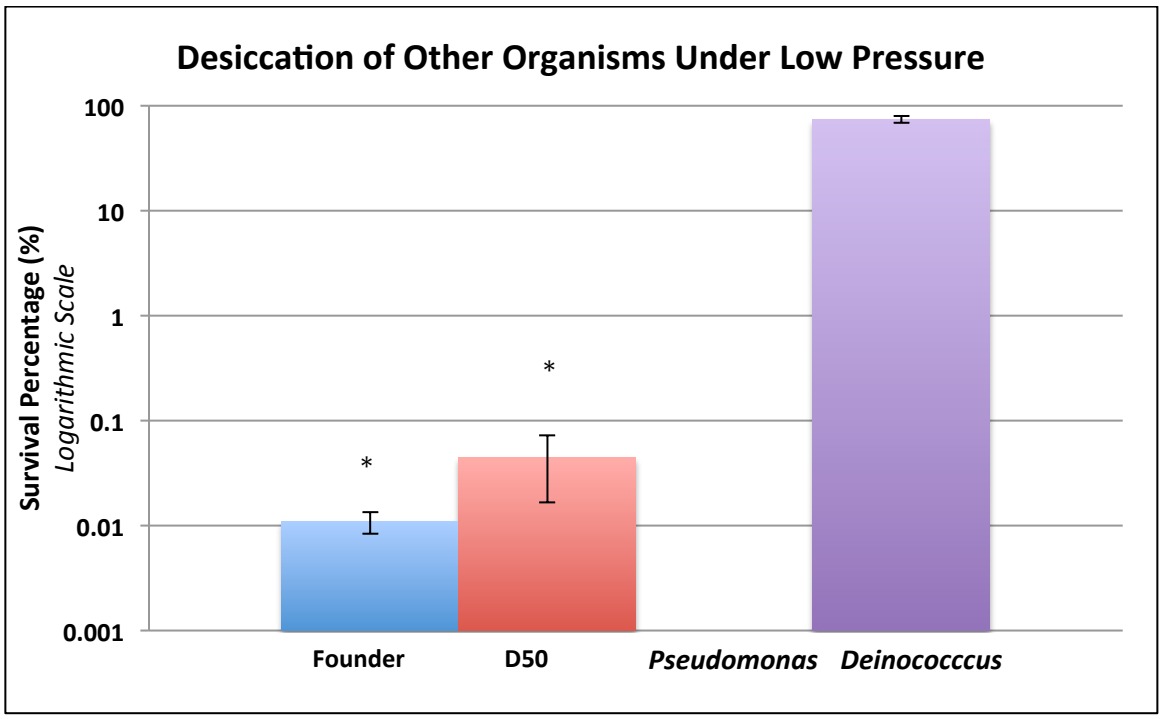
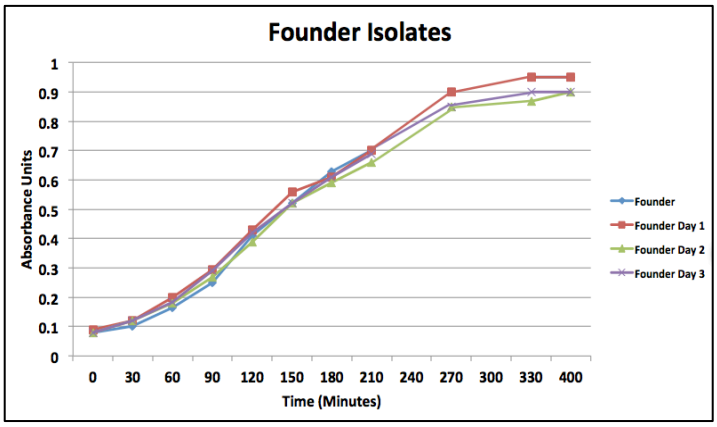
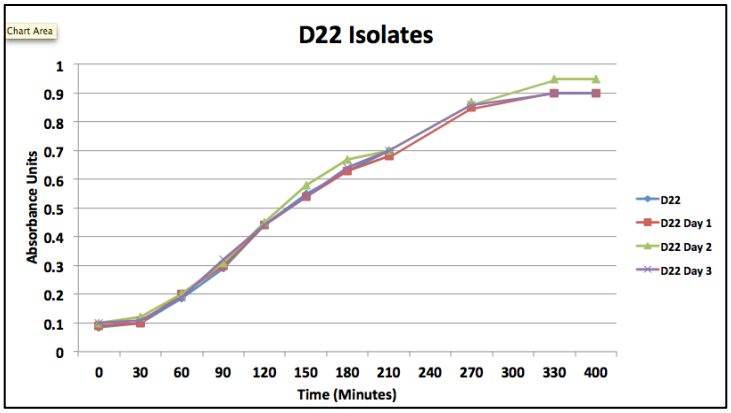
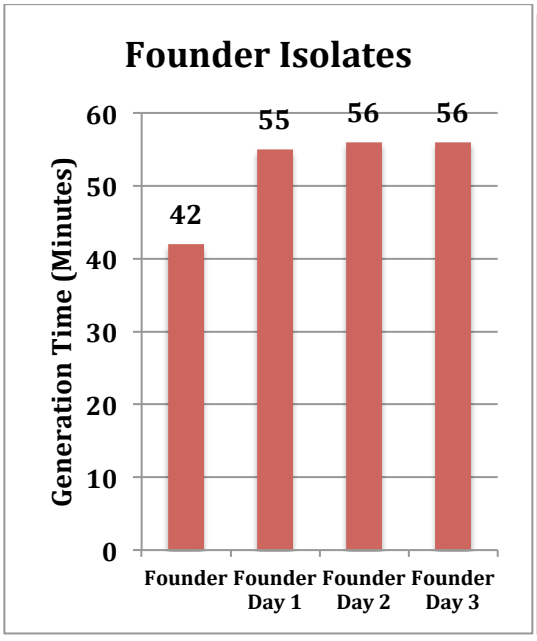


Figure 4. *Escherichia coli* derivatives, Founder and Desiccated Population 50, *Pseudomonas aeruginosa* PAO1, and *Deinococcus radiodurans* R1 desiccated under low pressure (6 – 7 mbar).







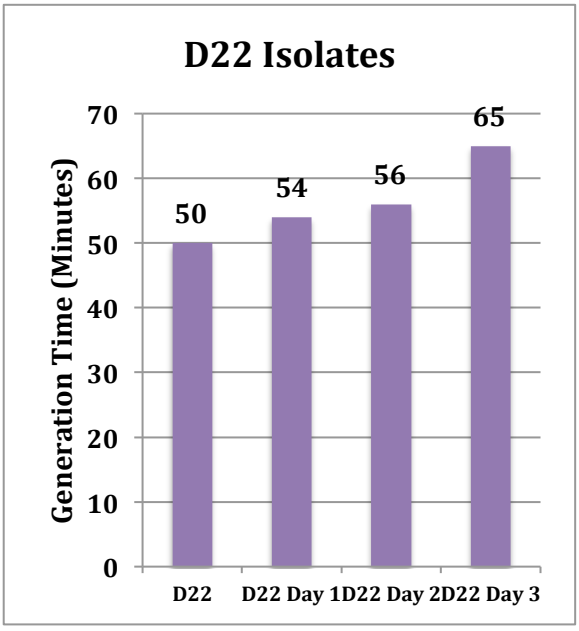


Figure 5. Colonies that formed past 48 hours of growth post-desiccation show difference in generation time.

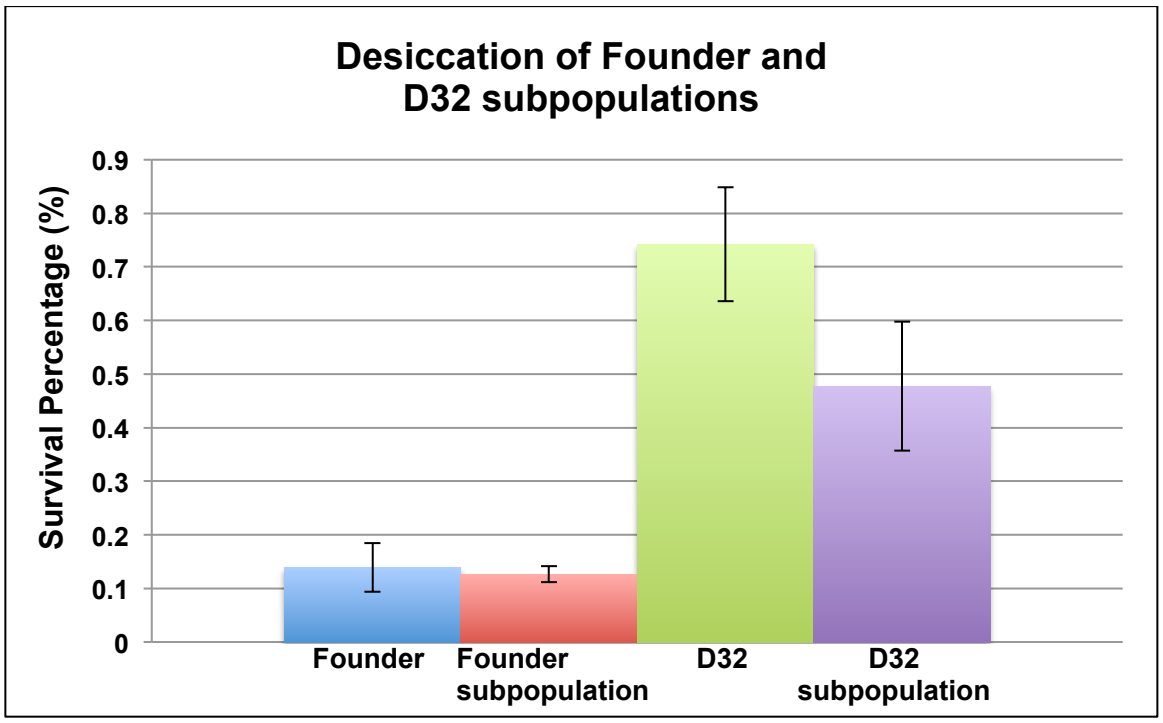


Figure 6. "Slow-growing" Desiccated Subpopulation 32 (32) and Founder subpopulation were desiccated to compare with survivability of parent population.