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Chemical Engineering
Skin Thinning Mediated Bacterial Penetration During Space Travel
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Staphylococcus aureus, *S. aureus,* is a bacteria known to be one of the leading causes of soft tissue infections. *S. aureus* is an opportunistic pathogen that belongs to the human skin microbiome. It does not infect healthy skin naturally [1]; however, once penetrated through human skin, it can colonize underlayer tissues or enter the bloodstream to cause infections. This entryway could be an open wound, puncture, or skin thinning due to aging or space traveling. These infections can range from cellulitis, boils, and severe rashes [2]. This research project was based on the idea that skin thinning during space traveling could be directly related to the changes in the characteristics of certain bacteria through space travel, including *S. Aureus*.

Skin thinning is a very common phenomenon present in humans. Skin thinning is the act of the skin stretching past its elongation point, therefore decreasing the skin's Young Modulus, and making the appearance of the skin to look older and contain more wrinkles [3]. There are various reasonings behind why skin thinning occurs. The most common being as a person ages, the elasticity in their skin begins to decrease tremendously. However, there are various other reasons such as side effects from medication, sun damage, and even traveling to space [4]. Astronauts are very likely to experience skin thinning for several reasons. Weightlessness, which is the experience of microgravity, causes the body's fluids to not be distributed evenly, the epidermis to shrink, and a decrease in the regeneration of cells. This reduction of cells has the biggest impact on the skin thinning present in astronauts. Specifically, during space travel, the body tends to have a reduction in the proteobacteria microbe in the human microbiome. This microbe is especially important in the protection against skin sensitivity-related issues [5]. Therefore, the purpose of this research project was to develop a procedure to study the effects of gravity on *S. aureus* activity by changing the bacterium mass density. Based on the results, preventative measures could be determined to hopefully help the astronauts protect themselves against skin-thinning-mediated infections.

The innovation for this project involved the development of a strategy to replicate the different levels of gravity that astronauts experience during space travel. We achieved this through two approaches. We used microcentrifugation to generate the G-force ranging from 170 to 1252 xg (Approach 1) and cell-to-liquid density contrast (Approach 2) to replicate the G-force that astronauts and bacteria can experience during space traveling (from 7-times of gravitational forces to microgravity). This innovation was inspired by the design principle of centrifugations for separation that is commonly used in Chemical Engineering. We systematically changed cell-to-liquid density contrast using various sucrose solutions with different mass densities including 1000 kg/m^3, 1018 kg/m^3, 1038 kg/m^3, 1081 kg/m^3, 1176 kg/m^3, and 1230 kg/m^3. The lightest density was a 0.1M NaCl solution, which has a very similar density to water. Once the sucrose solutions were created in microcentrifuge tubes, they were placed on ice for at least 15 minutes, so that they were chilled. Along with the sucrose solutions, a diluted bacteria solution was

developed by adding 100µL of the stationary bacteria solution and 900µL of the 0.1 M NaCl solution to a microcentrifuge tube. After all the solutions were chilled, 100µL of the diluted bacteria solution was slowly transferred on top of each sucrose and NaCl solution. The solutions in the microcentrifuge tubes were placed into the centrifuge for ten minutes. Only three of the solutions could be placed into the centrifuge at a time, to decrease the likelihood of the bacteria and the chilled solutions mixing. At first, the microcentrifuge tubes were placed at 4°C with a rcf (or xg) of 170 for the first five minutes, then increased to 1250 rcf (or xg) for the last five minutes. After the ten minutes were done, 100 µL from the top of the microcentrifuge tube and 100 µL from the bottom of the microcentrifuge tube were placed into a 96-well plate. Using the 96-well plate and the 0.1M NaCl solution, each of the samples were diluted and then the drop plate method was used, to count the number of bacteria cells present in each of the solutions. The results from the separation method can be shown in Figure 1 and Figure 2.

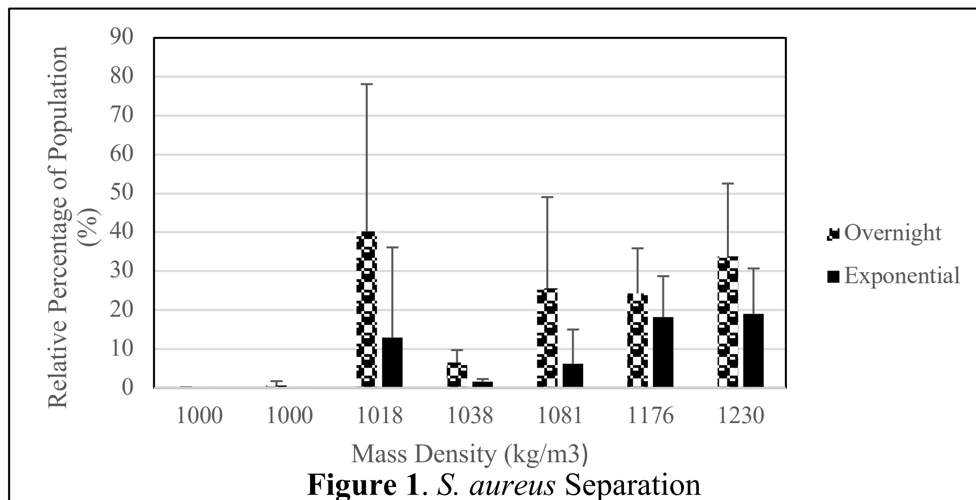

Figure 1. *S. aureus* Separation

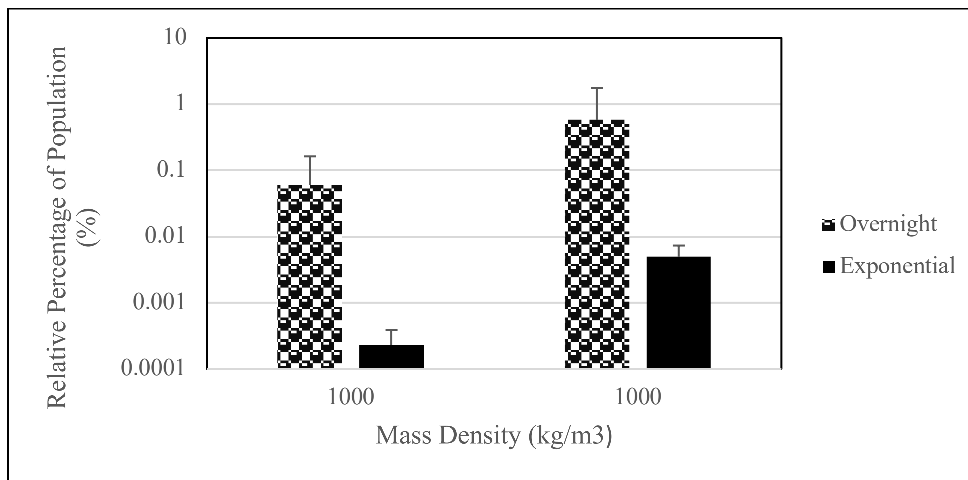

Figure 2. *S. aureus* Separation in Densities Less Than Water

The results obtained from this experiment proved that there were cells present in each of the different mass-density solutions. Through Figures 1 and 2, we found a subpopulation with a cell density similar to water. This result is consistent with a common assumption of bacterial

research or applications that bacteria has a density similar to water. What is interesting are the two new discoveries from the results summarized in Figures 1 and 2. The first new discovery is that there were cells present in densities less than water, shown in Figure 1 and further highlighted in Figure 2. This subpopulation could be the viable but nonculturable (VBNC) subpopulation that is known to have fewer cell organelles compared to normal bacterial cells [6]. This discovery suggests that this method can allow us to obtain the VBNC subpopulation without using antibiotics or killing the rest of the normal subpopulations, a method that could advance the field of antibiotic resistance and bacterial research. Another new discovery is that there is a large size of young (exponential phase) and old (stationary phase) subpopulations with cell densities higher than water, which is contradictory to the common assumption the field is currently using and could revolutionize the field.

To confirm if the subpopulation of cells, with a cell density smaller than that of water, are VBNC cells, we evaluated the antibiotic susceptibility of this subpopulation because VBNC cells are known to be less susceptible to clinically used antibiotics, such as Vancomycin [6]. Cells with a density smaller than that of water were separated by following the procedures described above. Once the bacteria was separated, a similar transfer of the bacteria was used to place the bacteria into the 96-well plate. However, for the antibiotics experiment, there had to be two empty columns and rows surrounding the bacteria samples. Once the 96-well plate was filled, it was incubated in the incubator for an hour, then the antibiotic was added to each of the samples. The antibiotic, Vancomycin (100 µg/mL), was added to each of the samples, then they were diluted using LB. The drop plate method was used again to plate individual samples of the dilutions. In Figure 3, the percentage of survival of the bacteria is shown.

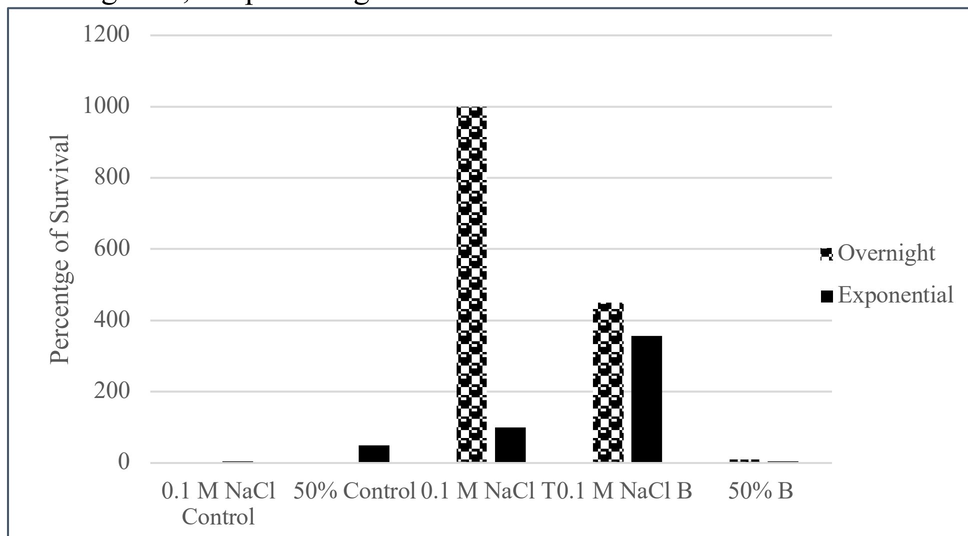

Figure 3. Percentage of Survival

These results showed that the highest rate of survival was present in the spent or overnight culture that was in the 0.1 M NaCl solution. There was an over 100% chance of survival in that specific sample, meaning that no bacteria cells were killed by the antibiotic. There is a low percentage of cells with a cell density higher than water (1230 vs. 999 kg/m^3), indicating that those cells are more susceptible to Vancomycin compared to cells with a density

smaller than water. This proves that with microgravity, the resistance of antibiotics increases in bacterial composition.

Based on the results from the previous separation method, the biofilm formation of these bacteria cells was required. The previous separation method was used before the biofilm experiment could be conducted. For the biofilm formation, fresh soft agar plates were required for the day of the experiment. The hypothesis for this experiment was that the cells with a higher density would grow a better biofilm by swarming to a greater diameter. This swarming would be a direct correlation to how the *S. Aureus* bacteria would penetrate the skin. However, the biofilm did not swarm in the three biological replicates that were conducted. The concentration of the soft agar plates was decreased twice to see if there would be any change, however, there was still no swarming present. The reasoning behind this was that the bacteria that was used was almost two weeks old, which is the life span of bacteria on a plate, therefore if younger bacteria was used, there may have been a different outcome.

Throughout this experiment, the microgravity application was used to mimic the weightlessness experienced in space. Through the separation method and the antibiotic susceptibility testing, it was determined that the bacteria become more resistant to the treatment of the antibiotic as weightlessness was experienced. In the future, this research can be taken a step further to fully determine the bacteria's effects on skin thinning. In future experiments, porcine skin will be penetrated with a bacteria culture. Once the bacterium has conducted swarming on the outer layer of the skin, the manual stretcher will be used to stretch the skin, so that elongation can be measured.

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