Effects of Simulated Atmospheres on the Growth and Persistence of Microbial Space Isolates

Kaitlyn Nielsen-Daugherty and Takara O'Brien (Undergraduate) and Dr. Alba Chavez (PHD)

EMBRY-RIDDLE Aeronautical University
DAYTONA BEACH, FLORIDA

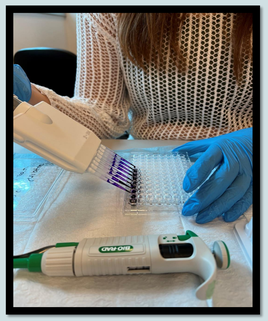
ABSTRACT

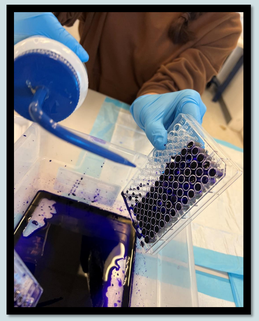
This study aimed to test the virulence and adaptive capabilities of the microorganism, Rhodotorula, isolated from the International Space Station (ISS), as well as if they could congregate and form different microbial communities under a specific environment. The results would demonstrate how the species of bacterium would develop outside of the ISS in an effort to understand microbial adaptation under space conditions. Previous research has shown that this biofilm not only poses an increased threat to the health of the astronauts but proves to be resistant to multiple antibiotic stressors. This research served to further test the adaptive abilities of biofilms under different atmospheres and the antibacterial resistance spectrum which originate from a micro-gravitational atmosphere. The hypothesis stated that if the Rhodotorula isolate was incubated in a microaerophilic chamber, then it would adapt to enable anaerobic respiration, determining whether this species of bacteria is a facultative or obligate anaerobe. Rhodotorula isolate incubated in the oxygen saturated atmosphere served as a control for undisrupted biofilm formation. The results dictated that there is no statistically significant difference between the two atmospheres, but the trends indicate a better adaptation under aerobic conditions.

METHODS

In order to most accurately record and observe our supplemental hypothesis we isolated the Rhodotorula strain of bacteria, allowing it to incubate in solution for one week. Once incubated, 100 microliters of solution were transferred into each well of a 96 well plate, rows A-H. This plate was labelled, "No Oxygen Incubation" and placed into a sealed microaerophilic chamber. The leftover solution was used to formulate a control. Rows A-D, wells 1-10 were filled and the plate was left outside of the microaerophilic chamber. Both plates were left to incubate for 2 weeks and 4 days.

After the incubation period both plates with the corresponding samples had to be washed 5 times, stained with crystal violet, rinsed, mixed with 200 microliters ethanol per well, agitated to ensure homogony, and transferred into a new 96-well plate. The two new plates were then ready for data analysis

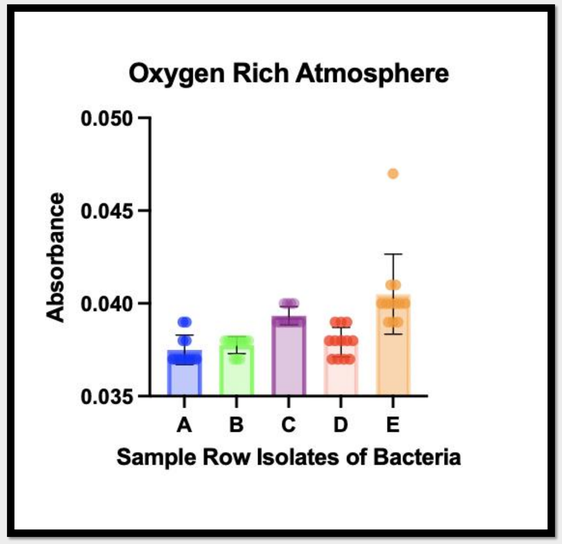




FUTURE STUDIES

Future studies will include a deeper analysis of the antimicrobial spectrum and the genetic basis of microbial adaptation in space; the inclusion of a same species strain control that has not been isolated from the ISS will be necessary is repeated trials

RESULTS

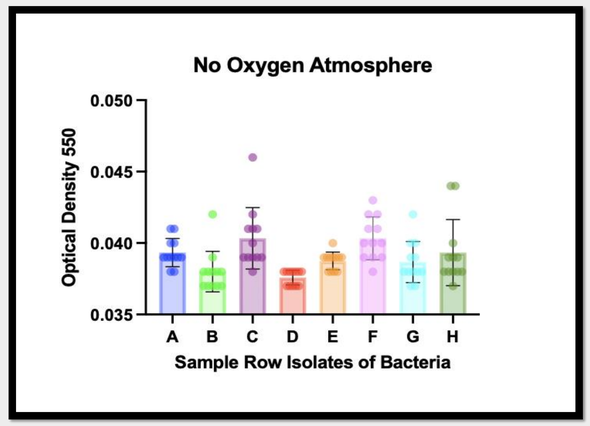


RSD%: 5.31%

Standard Deviation: 0.0021

Average Optical Density: 0.0394

Optical Density Range: 0.0370 - 0.0510



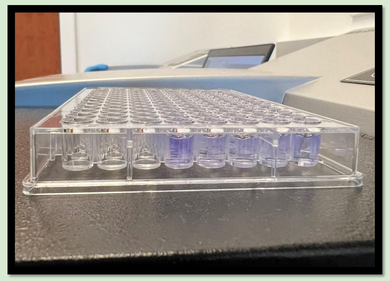
RSD%: 4.27%

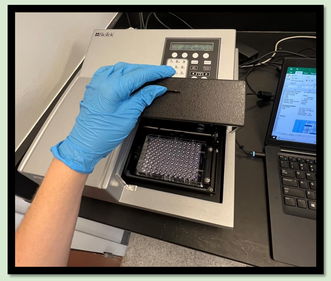
Standard Deviation: 0.0017

Average Optical Density: 0.0390

Optical Density Range: 0.0370 - 0.0460

DATA ANALYSIS & DISCUSSION





Once the samples were run through the Synergy Plate Reader, we were able to develop box and whisker plots using the data, showing mean colony growth and any outliers for each population/row. The plots how that on average, the bacteria grown in the oxygen rich atmosphere did not develop beyond the 0.040 abs line, while four out the eight populations from the no oxygen experiment grow more colonies above the 0.040 abs line, but overall, the average abs for both trials is 0.039 abs

CONCLUSION

Notably, there are bacteria that can grow and thrive in anaerobic conditions while other bacteria variants cannot; this investigation concluded on Rhodotorula space isolates that are physiologically facultative aerobes. After testing their ability to adapt under extreme atmosphere conditions without oxygen, the results found both tested variables data to be inconclusive. However, the optical density for the bacteria grown in the oxygen-rich atmosphere had a 0.0004 deviation compared to the no oxygen atmosphere, showing slight trend towards an increased adaption under aerobic conditions, suggesting better yield under an oxygen-rich environment. Albeit there were more sample rows for the no oxygen environment which consequently reduced error and increased reliability in only one condition's results.

The disproportion of sample rows from either atmosphere lacks sufficient, comparable evidence to conclude if aerobic bacteria has the capability to adapt to extreme atmospheric environments. Namely, how aerobic biofilms will behave over a long period of microgravity that simulates various atmospheres, confidence in favor or against the proposed hypothesis. Hence, a smaller standard deviation of 0.0017 and RSD% of 4.27% compared to a standard deviation of 0.0021 and RSD% 5.31% for no oxygen and oxygen-rich, respectively. If more time were allotted, multiple rows would have been tested to assess the UV absorbance to yield a more confident conclusion for the hypothesis. Moreover, it is evident that aerobic bacteria would not thrive under no oxygen conditions and would likely adapt to the new extreme environment over a period of generations. Further experimentation may provide data suitable to assess an aerobic bacteria's chances to thrive in microgravity conditions