Oxidative Stress in Perennially Ice Covered Environments

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INTRODUCTION

Lake Vostok is the largest and deepest subglacial lake identified beneath the Antarctic ice sheet. An ice core was drilled to 3623 m stopping ~ 120 m above the lake's surface due to the concerns of contaminating this pristine lake that has been isolated from the atmosphere for at least 15 million years. This ice core contains ice >420,000 years old, and the deepest at ~80 m represents water from Subglacial Lake Vostok accreted to the bottom of the ice sheet. Lake Vostok represents one of the most extreme environments found on earth and provides an analog to examine microbial longevity and survival in icy extra-terrestrial habitats (i.e., Mars and Europa).

Perennial ice covers have been implicated to be the causative agents of accumulating O2 in both lakes in the Dry Valleys and other perennially ice covered lakes such as Lake Vostok. Lake Vostok is said to have 50 times higher oxygen levels than those found in ordinary freshwater lakes on Earth.

Oxygen can be harmful to organisms because of its toxic by-products; super oxide, hydrogen peroxide, and hydroxyl radicals are generated within the cell that is growing aerobically, these by- products damage all macromolecules such as DNA, lipids and proteins.

Here we address whether microbial populations can exist in Lake Vostok at such supersaturated oxygen levels and what effects this super saturation has on the growth of these populations in Lake Vostok.

We hypothesized that bacterial isolates from environments with high oxygen concentrations have developed ways to tolerate oxygen super-saturation and increased levels of oxygen will have no effect on the growth of these organisms.

To test our hypothesis we grew isolates from both super-saturated oxygen environments (Lake Vostok) and isolates from environments where the oxygen concentration is very low (bottom waters of Lake Bonney) in gas tight test tubes and subjected them to increased amounts of oxygen and also grew them in atmospheric oxygen.

Our results indicate that oxygen has little or no effect on the growth of isolates from environments where oxygen is super-saturated. Increased oxygen concentrations inhibited the growth of isolates from environments where the oxygen concentration is low.

These results document that life can exist in environments where the oxygen concentration is supersaturated such as Lake Vostok.

Figure 1a. A satellite image of Lake Vostok.

Figure 1b. Photograph of Lake Bonney.

METHODS

Sample collection:
In 1998, a 3623 m ice core was drilled from Vostok Station, Antarctica. Lake Bonney water samples were collected at various depths using a niskin bottle.

Decontamination:
Upon arrival the outside of the core was decontaminated using a verifiable protocol to rid the core of unwanted materials introduced during handling and recovery. Bacterial isolates were cultivated from the accretion ice.

Cultivation of Microorganisms from samples:
Lake Bonney bacterial isolates were cultivated on nutrient agar from the oxygen maxim in the water column of the East and West lobes of Lake Bonney.

Characterization of isolates:
Isolates from both lakes were characterized by color, morphology, growth at 10 and 25 degrees Celsius and growth on different type of media.

O2 experiments:
Isolates were grown to mid-log phase, inoculated into hungate tubes, then flushed with air or pure oxygen at 20 psi for 10 minutes. The sample tubes were shaken on an orbital shaker at 4500 rpm.

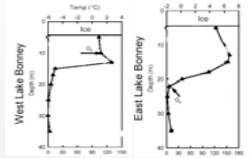


Figure 2a. WLB Oxygen profile.

Figure 2b. ELB Oxygen profile.

Turbidity measurements:
Turbidity was measured with a spectrophotometer 20 at ~ 620 nm, data was recorded at various time intervals over the course of the experiment.

Data Analysis:
Microsoft Excel was used to plot and compare the experiments.

Figure 3a. This is 3-phase streak of a Lake Vostok isolate to ensure a pure culture.

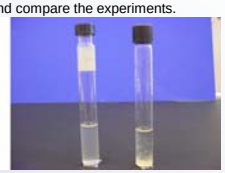


Figure 3b. Isolates from both lakes were inoculated into R2A liquid media, grown to Mid-log phase, and then re-inoculated into anaerobic test tubes.

Figure 4a, 4b, 4c. Anaerobic test tubes were flushed using 20 psi oxygen or air for 10 minutes.

Figure 5. Tubes and serum vials were shaken on an orbital shaker at 4500 rpm throughout the experiment

Figure 6. Turbidity was measured using a spectrophotometer 20 and grow curves were made for the isolates subjected to either air or oxygen.

RESULTS

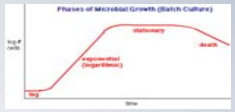
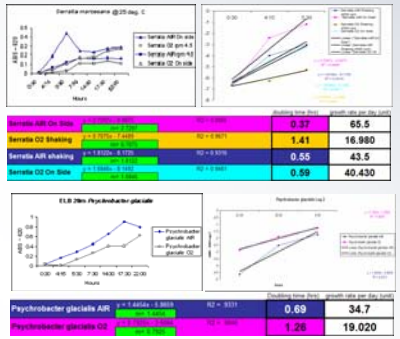
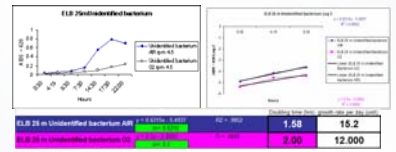


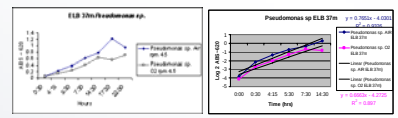
Figure 7. Typical growth curve a bacterial population.

After the experiment and data collection, we used the data of each sample to make a graph displaying the growth curve of the isolate.

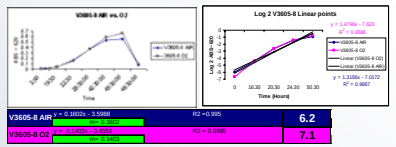
Control organisms: Microbes isolated from low oxygen environments, we wouldn't expect them to withstand high O2 levels

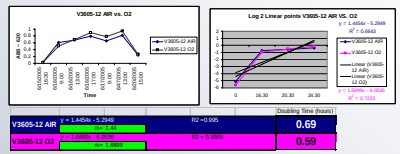






Organisms isolated from super saturated oxygen environments





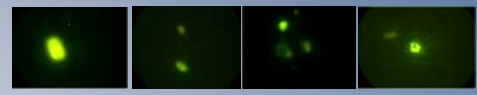


Figure 10. V3605-8 Unidentified isolate that exhibits auto fluorescence under microscope

CONCLUSIONS

Isolates from Environments with Low Oxygen Concentrations

Strain	Isolated from	Doubling Time AIR (hrs)	Doubling Time O2 (hrs)	Grew better in AIR or O2
ELB #35 Unidentified bacterium	ELB 25m Low concentrations of O2	1.58	2	AIR
ELB #55Psychrobacter glacialis	ELB 20m Low concentrations of O2	0.69	1.26	AIR
ELB #59Pseudomonas sp.	ELB 37m Low concentrations of O2	1.26	2.13	AIR
Serratia marcesans	N/A	0.55	1.44	AIR

Isolates from Environment with High Oxygen Concentrations

Strain	Isolated from	Doubling Time AIR (hrs)	Doubling Time O2 (hrs)	Grew better in AIR or O2
V3605-8	Lake Vostok Accretion Ice	7.1	6.2	O2
V3605-12	Lake Vostok Accretion Ice	0.59	0.69	Either

- **Control organisms from environments with low levels of oxygen were inhibited by increased oxygen headspace**
- **Isolates from environments with high levels of oxygen were not effected by the increased levels of oxygen in the headspace**
- **Microorganisms can exist in environments with super-saturated oxygen concentrations such as Lake Vostok**

Acknowledgements