The Growth and Characterization of *Psychromonas aquimarina*, a New Model Organism for Climate Change

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Introduction

Climate change is currently affecting the Earth and will only increase with time[1]. A change to the environment means that wildlife will need to adapt. Adaptation occurs when an organism changes physiologically or otherwise to permit continued growth in the environment in which it resides. Scientists do not completely understand adaptation mechanisms. *Psychromonas aquimarina* is a novel bacterium, with little known. This bacteria will first be characterized and studied before conducting temperature studies. *Psychromonas aquimarina* is a psychrophile, a bacterium able to survive in colder regions where most bacteria would not. *P. aquimarina* is an ideal bacterium to study because climate change is affecting the poles two times the rate that it is affecting elsewhere [1]. This means colder regions are experiencing harsher effects. The study of adaptation in cold tolerant organisms can give better insight to the mechanisms of thermotolerance.

Hypothesis: *Psychromonas aquimarina* will be able to adapt to an increase in temperature due to heat shock proteins.

Methods

Bacterial Culture

Psychromonas aquimarina (ATCC BAA-1526) was grown in Marine Broth 2214 and Marine Agar 2214 (Difco). Media was prepared as directed, boiled for one minute, and autoclaved at 121˚ C for 15 mins. Bacteria were grown at room temperature, 25.8˚C.

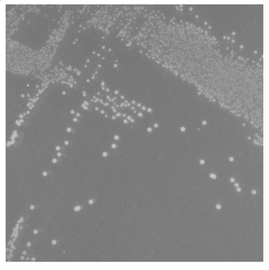

Figure 1. Colonies of *Psychromonas aquimarina on Marine Agar 2214*

Growth Analysis

Using sterile technique, a colony of *P. aquimarina* was placed into broth media. The bacteria were allowed to grow over night. Readings were taken every hour after 15 hours. Bacteria were grown at room temperature and subject to slight variation. The Spectrophotometer UV-1800 at 600 nm was used to measure scatter, which correlates to the density of broth, to obtain growth curve.

Doubling Time Calculation

ln(OD2-OD1)/(T2-T1) = rate (r)
Doubling Time = ln2/r, OD= optical density, T= time

Imaging Bacteria

Bacteria colonies were imaged using Alpha Innotech, FluorChem SP. Bacterium biofilm was imaged using an inverted microscope, Olympus IX51. Biofilm was imaged without staining or stained with cresyl violet and acridine orange.

Results & Discussion

Table 1. Comparison of *P. aquimarina* characteristics, known and newly observed.

Characteristics	Known[3]	Observed
Gram Stain	Negative	Negative
Shape	Rod- Bacillus	Spherical or coccobacillus
Length/ Width (µm)	.9-1.1/1.6-3.2	To be determined
Colony Shape	Slightly raised, circular, smooth, convex	Slightly raised, circular, smooth, convex
Colony color	cream	Opaque with hint of yellow
Motility	Single polar sheathed	nd
Doubling Time (hrs)	nd	**20.67**
Biofilm potential	nd	+
Biofilm Type	nd	Pellicle

- There is only one published study of *P. aquimarina* characteristics[3]. *P. aquimarina* exhibited most expected characteristics (Table 1). Notably, cell shape was observed to be spherical or coccobacillus rather than rod shaped. Detailed length vs. width analysis of individual cells (Figure 2) shows dimensions that are consistent, indicating cocci as a better shape descriptor.
- *P. aquimarina* was also observed producing a pellicle, air-liquid interface, biofilm (Figure 2, lower right). *P. aquimarina* stained with cresyl violet and acridine orange confirm the presence of biofilm (Figure 3).

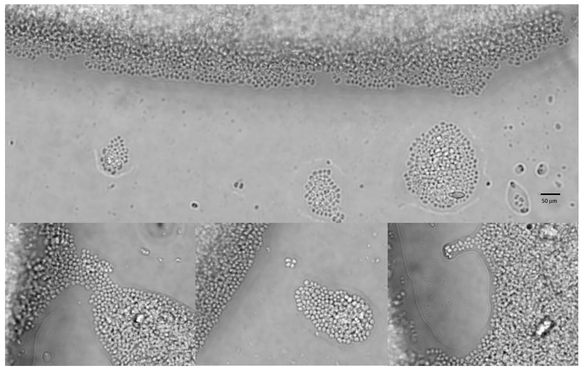

Figure 2. Light microscopy of *P. aquimarina* without stain using 100X oil-immersion objective.

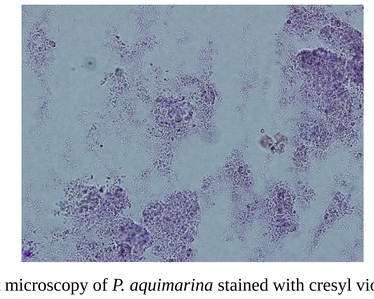

Figure 3. Light microscopy of *P. aquimarina* stained with cresyl violet and acridine orange using 100X oil-immersion objective.

Results and Discussion

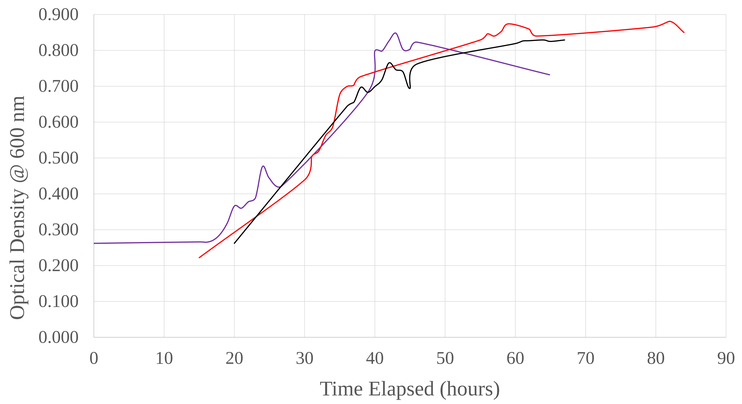

Figure 4. Comparison of *P. aquimarina* growth curves at room temperature. Purple represents growth study over a temperature range of 25-28°C. Red represents growth study over a temperature range of 18-19.8°C and displayed results were shifted x+15. Black represents growth study over a temperature range of 18.3-19.8°C and displayed results were shifted x+20. Growth curve shifts were performed to better display the overall sigmoidal shape of each.

- *P. aquimarina* was found to have a doubling time of 20.67 hours.
- *P. aquimarina* reaches log phase at about 15 hours after initiation of growth and enters death stage between 60-80 hours after initiation of growth.
- Temperature appears to affect growth. Further analysis is needed to determine the exact effect of temperature on growth and doubling time.

Future Research

P. aquimarina will be the subject of various temperature dependent growth studies. These temperature studies will be modeled to simulate climate change. Test 1: Starting at 20˚C, temperature will be raised by 1˚C every hour for a total of ten hours. Test 2: Starting at 20˚C, temperature will be raised 1˚C every 3 days for a total of 30 days. Test 3: Starting at 20˚C, temperature will be raised by 1˚ every 5 hours for a total of 50 hours and then temperature will return to 20˚C. The living cells from these studies will then undergo mRNA isolation, reverse transcription, and quantitative PCR to determine which genes have been upregulated during the thermal stress.

References

[1] IPCC, 2007: Summary for Policymakers. In: Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson, Eds., Cambridge University Press, Cambridge, UK, 7-22.

[2] Jansen, M., Geerts, A. N., Rago, A., Spanier, K. I., Denis, C., Meester, L. D., & Orsini, L. (2017). Thermal tolerance in the keystone species *Daphnia magna*-a candidate gene and an outlier analysis approach. *Molecular Ecology, 26*(8), 2291-2305.

[3] Miyazaki, M., Nogi, Y., Fujiwara, Y., & Horikoshi, K. (2008). Psychromonas japonica sp. nov., Psychromonas aquimarina sp. nov., Psychromonas macrocephali sp. nov. and Psychromonas ossibalaenae sp. nov., psychrotrophic bacteria isolated from sediment adjacent to sperm whale carcasses off Kagoshima, Japan. *International Journal Of Systematic And Evolutionary Microbiology, 58*(7), 1709-1714. doi:10.1099/ijs.0.65744-0