Gram Positive under Aluminum Stress: *B. altitudinis* and its survival on aluminum alloys

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Background

Aluminum is one of the most widely used non-ferrous metals due to its versatility and cost. Therefore, the deterioration of the popular metal has been a highly questioned issue in the industrial economy causing billions of dollars in damage every year. The oxidation of aluminum's toxic infrastructure is influenced by factors such as environmental conditions and the presence of certain bacteria that attach to the metal surface. Of the bacteria present in the microbial community attached to aluminum surfaces are *Bacillus* species. *Bacillus altitudinis* was isolated from an aluminum alloy and subsequently characterized to determine its metabolic potential. It is a known gram positive, rod-shaped bacteria[1] that produces spores and biofilms[2]. These characteristics help in allowing the species to survive in stressful environments such as an aluminum surface. By characterizing the isolates metabolism, we can further understand how the isolate could be affected by environmental stressors or how its metabolism influences its tolerance of stress. Future experiments will be conducted to test the isolates ability to tolerate aluminum while facing environmental stressors like variance in salinity or temperature.

How do environmental stressors affect the *Bacillus* isolate's ability to tolerate aluminum environments?

Methods

- A heat experiment was performed with 5 different temperatures:
  - 4°C
  - 10°C
  - 20°C
  - 30°C
  - 45°C
- The transferred *Bacillus altitudinis* isolate was incubated in the various temperatures for 2 weeks with observation taken every 24 hours.
- The metabolic potential of *Bacillus altitudinis* isolate was characterized by the following commonly used Microbiological media/tests:
  - Carbohydrate Catabolism Tests
    - Starch Agar
    - Spirit Blue Agar
    - OF Glucose Test
    - Carbohydrate Fermentation Tubes
    - Simmons Citrate Agar
    - Triple Sugar Iron (TSI) Slant
  - Protein Catabolism Tests
    - Gelatin Hydrolysis Test
    - Urea Broth
    - Ornithine Decarboxylation Broth
  - Respiration Tests
    - Aerobic/Anaerobic Plates
    - Thioglycollate broth

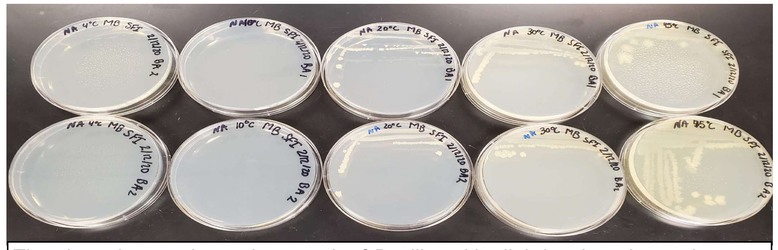
References

[1]Shivaji S, Chaturvedi P, Suresh K, Reddy GS, Dutt CB, Wainwright M, Narlikar JV, Bhargava PM. Bacillus aerius sp. nov., Bacillus aerophilus sp. nov., Bacillus stratosphericus sp. nov. and Bacillus altitudinis sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes. Int J Syst Evol Microbiol. 2006 Jul;56(Pt 7):1465-73. doi: 10.1099/ijs.0.64029-0. PubMed PMID: 16825614.

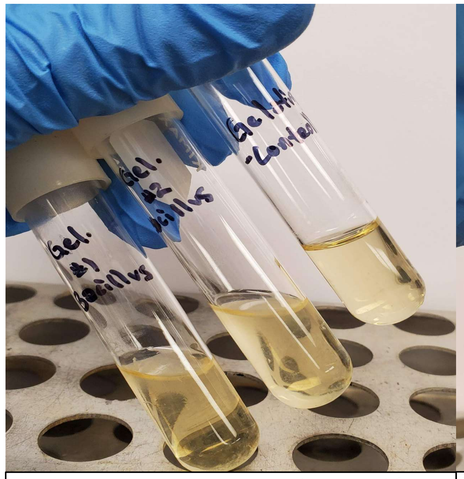
[2]Yue, Z.; Shen, Y.; Chen, Y.; Liang, A.; Chu, C.; Chen, C.; Sun, Z. Microbiological Insights into the Stress-Alleviating Property of an Endophytic Bacillus altitudinis WR10 in Wheat under Low-Phosphorus and High-Salinity Stresses. Microorganisms 2019, 7, 508.

[3]Eid, Salah & Abdallah, M. & Kamar, Eman & El-Etre, A.Y.. (2016). Gelatin as Corrosion Inhibitor for Aluminum and Aluminum Silicon Alloys in Sodium Hydroxide Solutions. Protection of metals and physical chemistry of surfaces. 52. 10.1134/S2070205116010020.

Results



The above image shows the growth of *Bacillus altitudinis* incubated at various temperatures (from left to right) 4°C, 10°C, 20°C, 30°C, and 45°C. Growth was seen only at 20°C, 30°C, and 45°C with changes in colony morphology and color occurring at the highest temperature.

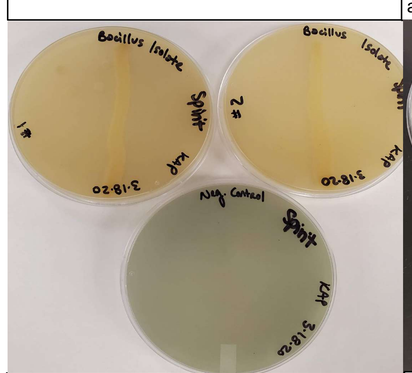


The image above shows a positive result for Gelatin Hydrolysis.

The image above shows results from a TSI Slant, positive for partial sugar fermentation and negative for H_2S and gas production.

The image above shows a negative result for Starch Agar

The above image shows results from a Thioglycolate Broth for characterization as an obligate aerobe



The image above shows a negative results for Spirit Blue Agar

The image above show a positive result for aerobic growth and negative result for anaerobic growth.

Conclusions and Future Work

- ***Bacillus altitudinis* grows optimally between 20°C and 30°C** since *Bacillus altitudinis* grew and maintained original colony morphology at these temperatures. At the highest temperature, the isolate produced a secondary metabolite that fluoresced under UV which may be used to tolerate heat stress. It may also be producing biofilm to tolerate heat due to the mucous-like appearance of colonies growing at 45°C.
- ***Bacillus altitudinis* is an obligate aerobe that can produce gelatinase and ferment sucrose.** These conclusions were made due to results in the TSI slant, gelatin hydrolysis test, sucrose fermentation test, thioglycolate broth, and aerobic test.
- ***Bacillus altitudinis* showed negative results in the OF Glucose test, Ornithine Decarboxylation Broth, Simmons Citrate test, Starch test, Spirit Blue test, Urease test, Lactose Fermentation test, and Anaerobic test**
- The isolate was found to break down gelatin, which is often located on the surface of aluminum alloys[3] and may help the isolate better survive environments limiting in nutrients or fluctuating in conditions.
- These results will be used to compare how the isolate carries out metabolic functions in aluminum environments.
- Future work will be done to understand the growth rate of *Bacillus altitudinis* under varying environmental stressors like temperature and salinity and used to determine how these stressors affect its ability to tolerate aluminum environments.

Acknowledgments

I would like to thank Dr. Erin Field and Kyra Price for their support throughout this project. I would also like to thank the coordinators of RCAW for allowing me to present my research