Identifying genetic markers of gamma radiation exposure in *Lactobacillus salivarius*

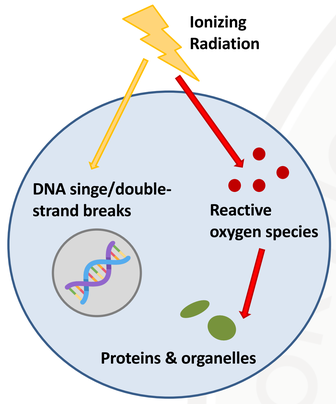
Anne Shen
Ph.D. Candidate, Massachusetts Institute of Technology
Jay Zhao[1], Eric Alm[2]
[1]Broad Institute, [2]Massachusetts Institute of Technology Bioengineering Department

Introduction and Motivation

Key Questions
1. Do bacteria carry distinct genetic signatures following recent exposure to contaminating levels of ionizing radiation?

2. If so, can we identify individual or sets of genetic markers predictive of radiation exposure?

- Ionizing radiation breaks the DNA double helix and fragments proteins inside cells.
- To survive exposure to ionizing radiation, cells must repair DNA and protect proteins from damage. Differences in the genetic code of individual bacteria determine how well each cell can repair damages.
- Genetic signatures will remain in the bacterial population longer than radiation signatures in the environment.



Mission Relevance

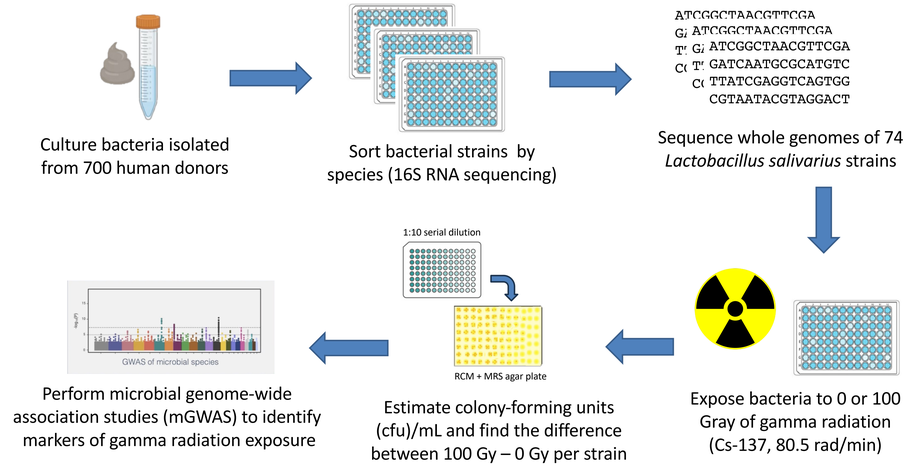
How does this work, and the problem you are trying to solve, relate to the NNSA mission?

- The NNSA seeks to prevent nuclear weapons proliferation through detection and deterrence of smuggled radioactive materials.
- Methods to identify markers of radiation exposure in bacteria increase our ability to detect the presence of radioactive materials in an environment.

MTV Impact

We have collaborated with the Radiation Protection Program at MIT to generate a protocol for irradiating our bacteria. Also, developments in mGWAS that we produce will allow researchers to bypass key bottlenecks in genetic engineering in the field of bacterial functional genetics.

Technical Approach



Results

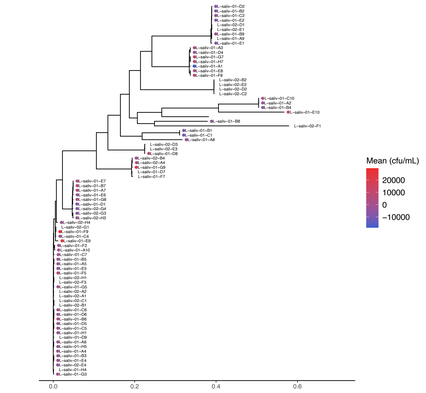


Figure 1. Radiation resistance phenotypes are not clustered on the phylogenetic tree. 74 strains of *L. salivarius* were successfully phenotyped.

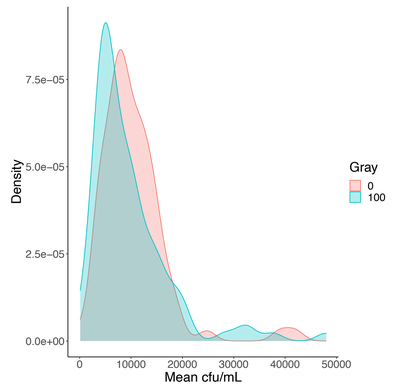


Figure 2. Distribution of mean log10(cfu/mL) among *L. salivarius* strains shifts following 100 Gy of gamma radiation. Two-sample Kolmogorov-Smirmov test: D = 0.23848, p-value = 0.01235.

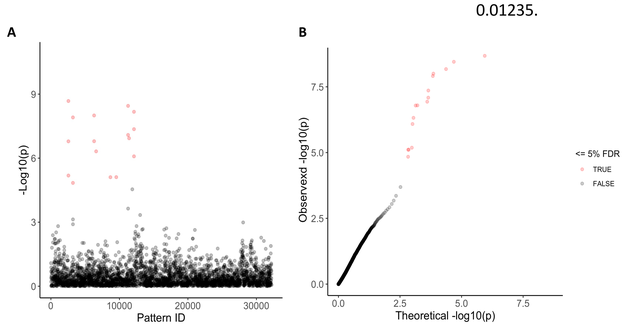
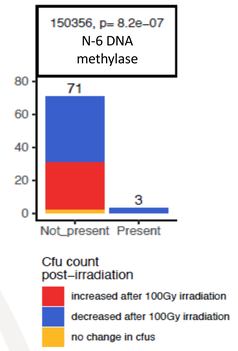


Figure 3. Pilot mGWAS reveals genetic regions linked to gamma radiation exposure. The (A) Manhattan plot and (B) Quantile-Quantile plot show some linkage among the most significant regions but not across *L. salivarius* genomes as a whole.

Results (cont.)



- Catalyzes the production of N-6 methyladenine (m6A), a methylated adenine (DNA base).
- DNA methylases protect bacteria from their own restriction enzymes produced to degrade viral DNA.
- In *E. coli*, adenine methylation is used to identify the correct DNA sequence during methyl-directed mismatch repair.

Conclusions

- We have collected and grown a large bacterial strain collection which allow us to apply mGWAS to identify genetic markers associated with bacterial resistance to environmental stressors. Prior to our work, few such collections existed.
- The results of our pilot study showed the efficacy of mGWAS as a method to identify markers of ionizing irradiation resistance and sensitivity.

How does the work presented positively impact the NNSA mission?

The results indicate a successful first step in applying microbial genetics methods to detect the presence of radioactive material within an environment.

Expected Impact

The completion of this mGWAS pilot allows us to develop a framework for identifying genetic markers enriched in bacteria with recent exposure to gamma radiation.

Next Steps

Following this pilot study, we will optimize a protocol for performing the experiment in triplicate and across a larger range of radiation doses Little is currently known about radiation resistance in bacteria, so we will test a range of radiation doses to identify the IC50 dosage of gamma radiation for *L. salivarius.*

National Nuclear Security Administration

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