Generating diverse bacterial strain collections for identifying genetic markers of radiation exposure

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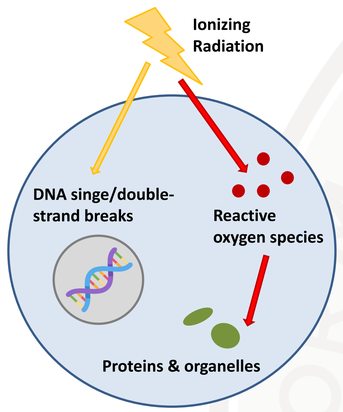
Introduction and Motivation

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 is known to induce breaks in the DNA double helix and to fragment proteins inside cells.
- To survive exposure to ionizing radiation, cells must be able to repair DNA and protect proteins from damage. Differences in the genetic code of individual bacteria determine how well each cell is equipped to overcome damages generated by radiation exposure.
- Identifying high-frequency genetic markers of resistance to the effects of radiation may indicate recent exposure to ionizing radiation.
- Genetic signatures will remain in the bacterial population longer than radiation signatures in the environment.

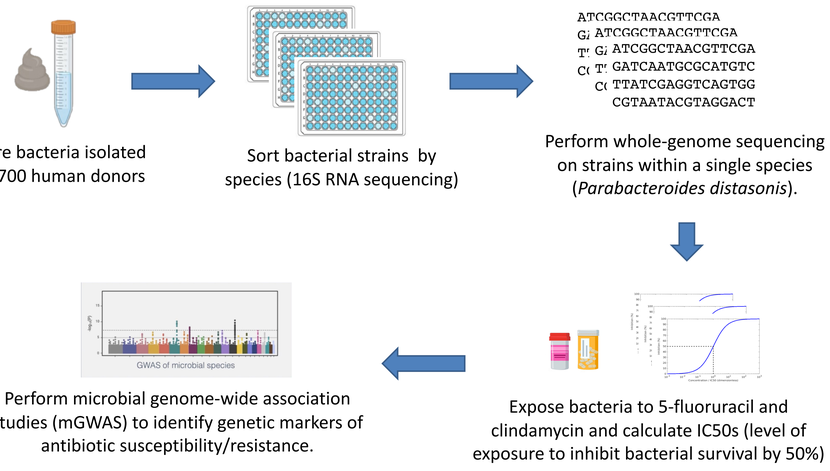


Mission Relevance

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

- One of the primary avenues through which the NNSA seeks to prevent nuclear weapons proliferation is the detection and deterrence of smuggled nuclear and radioactive materials.
- Because bacteria are ubiquitous in the environment and within animal hosts, they have the potential to serve as readily available indicators of recent nuclear presence.
- By developing methods to identify genetic markers of radiation exposure and resistance in bacteria, we strengthen our ability to detect the presence of radioactive materials within an environment.

Technical Approach



Culture bacteria isolated from 700 human donors

Sort bacterial strains by species (16S RNA sequencing)

Perform whole-genome sequencing on strains within a single species (*Parabacteroides distasonis*).

Expose bacteria to 5-fluoruracil and clindamycin and calculate IC50s (level of exposure to inhibit bacterial survival by 50%)

Perform microbial genome-wide association studies (mGWAS) to identify genetic markers of antibiotic susceptibility/resistance.

Results

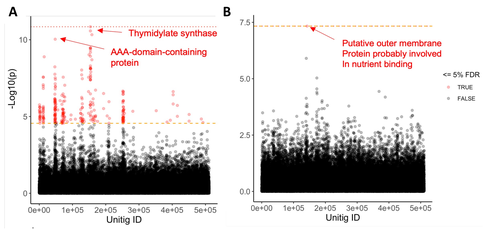


Figure 1. Genetic regions associated with resistance to (A) 5-fluorouracil and (B) clindamycin in *P. distasonis*.

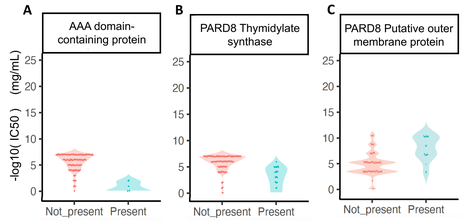


Figure 2. Distribution of resistance according the presence/absence of genetic regions discovered through mGWAS.

mGWAS successfully identified novel markers of resistance to 5-fluorouracil and clindamycin.

- A mutation in the gene encoding thymidylate synthase, which is a target of 5-fluorouracil, and a variant of the gene encoding an AAA-domain-containing enzyme are strongly associated with 5-fluorouracil resistance (Figure 1A, Figure 2A,B)
- A genetic region thought to encode a membrane protein is associated with clindamycin resistance (Figure 1B, Figure 2C). Notably, genes known to confer clindamycin resistance were not present in these *P. distasonis* strains.

Expected Impact

The completion of this mGWAS pilot allows us to develop a framework for identifying genetic markers of important phenotypes or life histories, such as exposure to ionizing radiation, in bacteria, which can be used to test for environmental contamination or human exposure

MTV Impact

- Through MTV, I have gain presentation skills by preparing for the monthly workshops and annual conferences. Furthermore, MTV has connected me to researchers at other labs who are working in the field of nuclear detection.
- We have collaborated with the Radiation Protection Program at MIT to generate a protocol for irradiating our bacteria. In addition, the mGWAS developments we produce will be of interest to the microbiology community as mGWAS bypasses some key bottlenecks in genetic engineering in the field of functional genetics.

Conclusion

- 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 find novel genetic markers of drug resistance and provides strong evidence that it can also identify markers of radiation exposure and resistance.

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.

Next Steps

- Following this pilot study, we will optimize a protocol for exposing >200 strains of *Lactobacillus fermentum* isolated from the human gut to gamma radiation. Little is currently known about radiation tolerance and resistance in bacteria, so we will test a range of radiation doses to identify the IC50 dosage of gamma radiation for *L. fermentum.*
- We will use the IC50 measurements of the *L. fermentum* strains to conduct a mGWAS and identify genetic markers associated with resistance to ionizing radiation in bacteria.

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