Isolating Secondary Metabolites that Induce Bacillithiol S-transferases in *Bacillus subtilis*

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Introduction

As antibiotic resistance is increasing in bacteria and key pathogens are becoming more and more resistant to most common antibiotics, new drugs are needed. This is why it is critical that we characterize the mechanisms by which bacteria detoxify antibiotics they encounter in nature. The goal of this project was to isolate secondary metabolites from soil bacteria that induce the S-transferases used in the process of defense and detoxification in *Bacillus subtilis.* Studying detoxification in *B. subtilis* will help to take a step forward in the process of designing new drug therapies.

Background Information

Secondary metabolites are molecules produced by bacteria in order to protect themselves from invasion by other species of possibly harmful bacteria. These metabolites are not always necessary for the bacteria's growth, but rather are secreted in order to protect the colony. Due to high competition in such small areas, *B. subtilis* and other soil bacteria secrete secondary metabolites in order to increase their chances of survival. If the harmful metabolite is indeed able to pass through the bacteria's cell membrane, bacillithiol is thought to be the key component to detoxification of the toxic element [1]. The constant exposure of bacteria in nature to antibiotics has led to the build up of resistance in these detoxification pathways, which is why it is imperative that we study bacteria and their methods of self-defense.

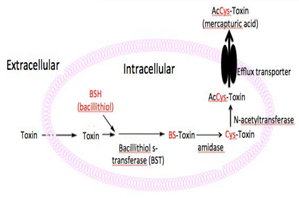


Figure A[2]*: BSH-dependent detoxification* (diagram of the dtoxification pathway in *B.* subtilis) (A) A hydrophobic toxin enters the cell passively. BSH is conjugated to the toxin in a BST-dependent manner. A BSH conjugate amidase then cleaves the conjugate to Cysteine-toxin (Cys-toxin). An N-acetyltransferase enzyme acetylates the conjugate (AcCys-Toxin). This detoxified AcCys-Toxin is transported out of the cell via an efflux transporter.

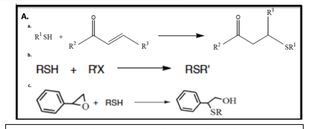


Figure B: Thiol-reactivity of molecules (the chemistry behind detoxification) (A) Reactions of LMWTs and thiol-reactive molecules (RSH=LMWT) (a) Michael addition to an α/β unsaturated bond (b) Halogen replacement (X=halogen) (c) opening of strained ring

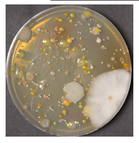


Figure C: Different types and colors of bacteria have formed from one of our soil samples, displaying the versatility in the shapes and colors of the bacillus family

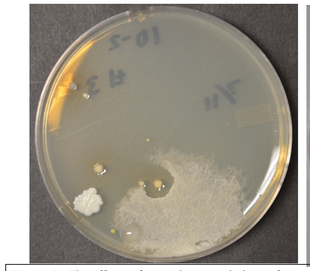


Figure D: The effects of secondary metabolites of environmental samples can be seen on this plate. The small yellow bacterial colony is clearing the neighboring white bacterial colony. The secondary metabolites being sent out by the yellow bacteria are preventing the white bacteria from growing near it because it sees the yellow bacteria as a threat.

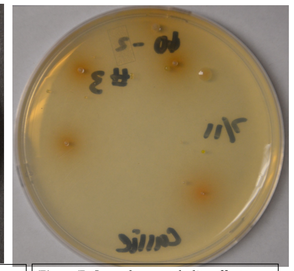


Figure E: Secondary metabolite effects can also be seen on this plate. The halos around the bacteria are secondary metabolites being sent out that are changing the color of the agar due to their effects.

Procedure

Gather and prepare unique environmental soil samples from various locations on the UCSD campus.

Resuspend the samples in T-Base and freeze portions of each in glycerol to test if the freezing will affect the number of colonies.

Dilute the *B.subtilis* strains down in order to obtain 500 to 1,000 colonies per plate.

Test the *B. subtilis* strains at various stages of growth using S-transferase GFP promoter fusions in order to identify bacillithiol S-transferase inducers.

Purify colonies of interest in order to identify the organism or perhaps the antibiotic that is inducing *B. subtilis*.

GFP's Under the Microscope

Many antibiotics are secondary metabolites isolated from bacteria. We chose a handful of candidate antibiotics to study S-transferase induction.

Figure F: DinB alone: This figure shows what DinB by itself under the microscope with the GFP promoters looks like. As you can see, there is no fluorescence, showing that on our control plate, when alone, DinB does not fluoresce.

Figure G: DinB, when spotted with Ciprofloxacin, fluoresces along the edges of where the antibiotic was spotted. This shows that there is something about this antibiotic that is inducing this specific S-transferase.

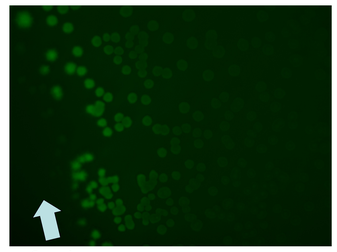
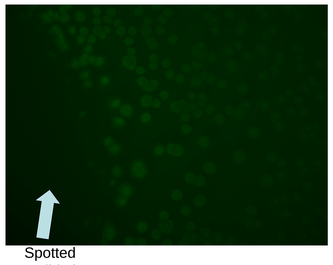


Figure H: The S-transferase YizA alone is shown in this figure. No fluorescence can be seen, which makes this an ideal control plate.

Figure I: YizA with Cipro again is induced, as shown by the fluorescence around the edges as the B. *subtilis* approaches the antibiotic.



Findings

Our findings were inconclusive, but we did learn that one specific antibiotic, Ciprofloxacin, seems to induce a few of our *B. subtilis* S-transferase genes. Another antibiotic, Cephalexin, seemed to 'turn off' the GFP reporters in our S-transferases, making them appear to gradually stop fluorescing as they approached the area where the antibiotic was spotted. This could be a sign of the antibiotic suppressing the gene, but further research will need to be done in this area to be conclusive.

Conclusion

As we stated previously, our results were inconclusive at this point, but further research is going to be conducted in these areas. Future directions for this research would be to optimize the assays that will be used and to test alternate organisms and antibiotics with all of the S-transferase genes.

Acknowledgements

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References

1. Helmann, JD (2011) Bacillithiol, a New Player in Bacterial Redox Homeostasis. Antioxidants & Redox Signaling 15, 123-131.
2. Newton et *al.* (2011) The DinB Superfamily Includes Novel Mycothiol, Bacillithiol, and Glutathione *S-Transferases.* Biochemistry 49, 10751–10760.