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Evaluating Four Inosine-Uridine Preferring Nucleoside Hydrolases in Bacillus Anthracis for Decontamination Strategies

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Evaluating four inosine-uridine preferring nucleoside hydrolases in *Bacillus anthracis* for decontamination strategies

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Bacillus anthracis is a spore-forming bacterium that is the infectious agent in anthrax. The spore induces disease in a host through a process called germination, which is the conversion of a dormant spore into a metabolically active vegetative cell. Given that vegetative cells and germinated spores are more easily killed than dormant spores, adding a germination step to decontamination strategies is a current idea under investigation. Specific molecules such as alanine and inosine are germinants that induce germination by binding to receptors. Inosine-uridine preferring nucleoside hydrolase (IunH) is a spore surface protein that is responsible for the breakdown of the germinant inosine into non-germinants hypoxanthine and ribose, preventing inosine from inducing germination immediately. Interestingly, there are four hydrolase paralogs in the *B. anthracis* genome, two that are spore associated, IunH and IunA, and two in the vegetative cell, BAS2236 and BAS4961. Previous work in the lab has shown *iunH* mutant spores have no detectable nucleoside hydrolase activity and germinate more completely than wt spore populations. An insertion mutant *iunA* has reduced spore-associated activity and an exosporium assembly defect. Two possibilities for the reduced activity are that IunA has weak catalytic activity or IunA impacts exosporium assembly which reduces the amount of IunH present. Therefore, we decided to express and purify all four hydrolase genes to establish which of the four proteins are functional enzymes. We incorporated this project into the Honors Microbiology course at Louisiana Tech. To date, the students have successful PCR products for three of the hydrolases and have purified the DNA inserts to clone into *E. coli* expression plasmids pBAD24 and pBAD33. Future steps include purifying protein via His-tag technology and measuring hydrolase activity of purified proteins. If a protein has catalytic activity we will screen inosine analogs for enzymatic inhibition. Identification of inosine hydrolase inhibitors may prove to be a viable target which will contribute to our long-term goal of developing novel strategies for decontamination.