# Characterizing Carboxyguanidine Deiminase, an Enzyme with Promising Bioremediation Applications

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## Introduction

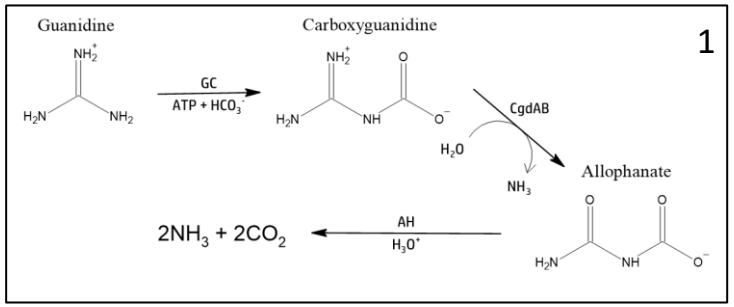


**Figure 1:** Carboxyguanidine deiminase (CgdAB) is involved in the breakdown of guanidine in *P. syringae*. Guanidine carboxylase (GC) converts guanidine into carboxyguanidine, which CgdAB converts into allophanate and ammonia. Allophanate Dehydrogenase (AH) then breaks allophanate down into ammonia and carbon dioxide.

- Carboxyguanidine deiminase (CgdAB) is a heteromeric enzyme that aids in the catabolism of guanidine in the bacteria *P. syringae*. The metabolic pathway it is involved in allows for guanidine (which is toxic in large concentrations) to be broken down into ammonia, a valuable compound for bacteria in nitrogen-poor conditions.
- CgdAB is a member of the DUF1989 protein family and the first to be studied extensively. A comprehensive understanding of this enzyme would serve as a point of reference for future studies of the other DUF1989 proteins, which could potentially also be involved in breaking down other dangerous nitrogenous compounds and pollutants.
- This project focused on analyzing the activity of CgdAB, discovering the optimal pH for its activity, and identifying the catalytic metal ion that is hypothesized to be integral to CgdAB's activity.

## Methods

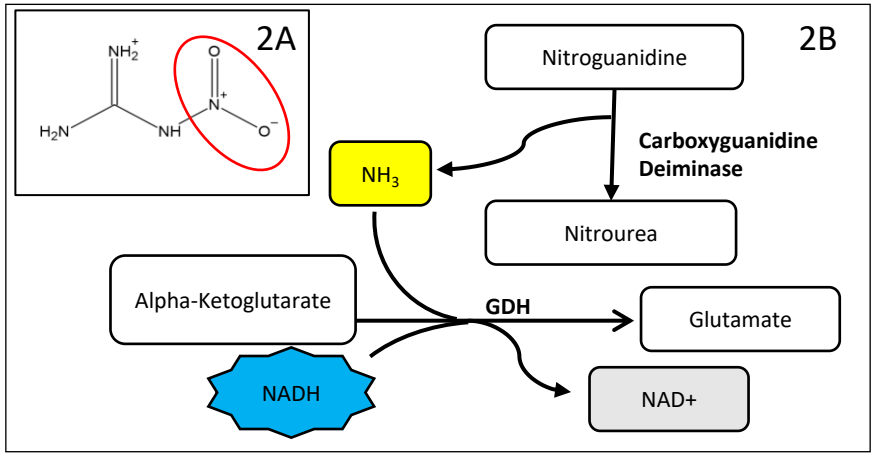


**Figure 2A:** Carboxyguanidine quickly is hydrolyzed into bicarbonate and guanidine. As a result, it cannot be stored for use. Nitroguanidine, pictured above, is currently the only other confirmed substrate for CgdAB, and is stable in solution. The only difference is the nitro group (circled in red) in place of the carboxyl group seen in carboxyguanidine.
**Figure 2B:** The activity of CgdAB was measured using a GDH-coupled assay and a spectrophotometer. The ammonia produced by the activity of CgdAB is then used in a reaction with the enzyme glutamate dehydrogenase (GDH). This reaction oxidizes NADH into NAD+. NADH absorbs light at 340nm while NAD+ does not. As such, the rate of the decrease in absorbance corresponds to the rate at which NADH is oxidized. This in turn corresponds to the rate that ammonia is produced by CgdAB, which is the enzyme's activity.

## Conclusions and Future Directions

Further experiments need to be performed solve the problems identified in these experiments, such as what is causing the inconsistent CgdAB activities measured at pH's 9.0 and 9.5. For the dialysis procedure, it is possible that the cysteine residues in the enzyme are coordinated to the metal ion and preventing its removal. Oxidizing these cysteines could solve this issue. These experiments also confirmed that nitroguanidine is acted on by CgdAB. As a toxic byproduct of the military munitions manufacturing process, nitroguanidine's removal from contaminated areas could potentially be assisted with by bacteria expressing CgdAB.

## Results and Discussion

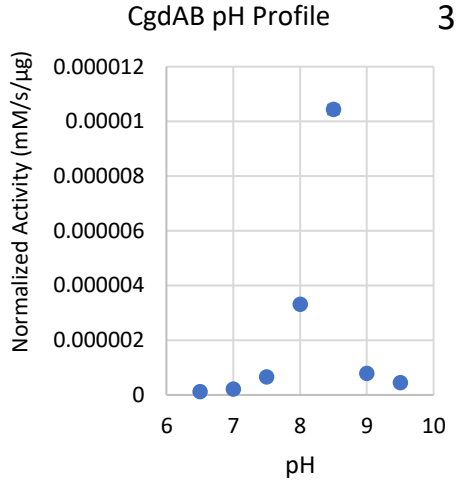


**Figure 3:** The activity of CgdAB was measured between pH's 6.5-9.5 in increments of 0.5. Most results demonstrated that (as seen in this example) pH 8.5 is optimal for CgdAB activity. Later results, however, suggested that pH 9.0 and 9.5 had the highest activities. The activity at these pH's is still uncertain.

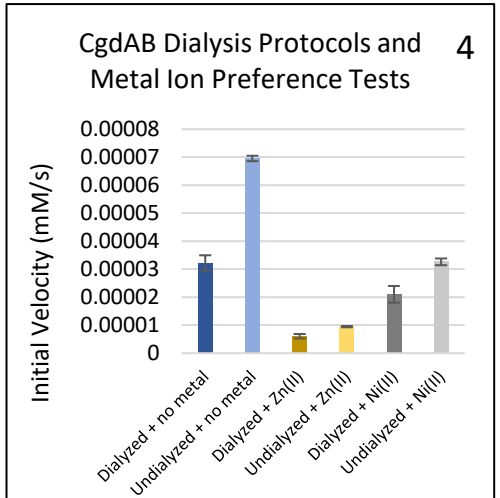


**Figure 4:** CgdAB was dialyzed to remove the unidentified metal ion. Other metals were then reintroduced, and the activity was measured. The results suggested that the dialysis was unsuccessful, and that the reintroduced metal ions might have also inhibited CgdAB or some aspect of the GDH-coupled assay.