**EVOLUTION OF NICOTINIC ACID CATABOLISM IN AEROBIC BACTERIA**

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### *Background*

Nicotinate (niacin, vitamin $B_3$) serves as the source of the nicotinamide moiety of $NAD^+$ and $NADP^+$, redox coenzymes central to metabolism in all living species. Nicotinate can also serve as a source of carbon, nitrogen and energy for a variety of bacteria (*1-7*). Nicotinate catabolism occurs by multiple routes, dependent on oxygen availability, in which 6-oxo-nicotinate is a common first intermediate (*8,9*). 6-Oxo-nicotinate is subsequently reduced to 1,4,5,6-tetrahydro-6-oxo-nicotinate and further degraded to pyruvate and propionate by anaerobic bacteria (*10,11*), or oxidized to either 3-hydroxy-2-pyridone (in *Pseudomonas* and *Bordetella* species) (*12*) or 2,6-dihydroxynicotinate (in *Bacillus* species) (*13*) where it is finally degraded to the common metabolite, fumarate [Figure 1]. The gene cluster that codes for the enzymes of nicotinate catabolism have recently been identified in bacteria that inhabit soil rhizhospheres (e.g. *Pseudomonas (14), Eubacterium barkeri and several proteobacteria (15)*). In contrast, the genes encoding the enzymes of aerobic catabolism of nicotinate in *Bacillus* have not yet been identified, nor has there been a complete description of the biochemical pathway(s) leading from 2-hydroxy-6-oxo-nicotinate to fumarate [middle pathway shown in Figure 1].

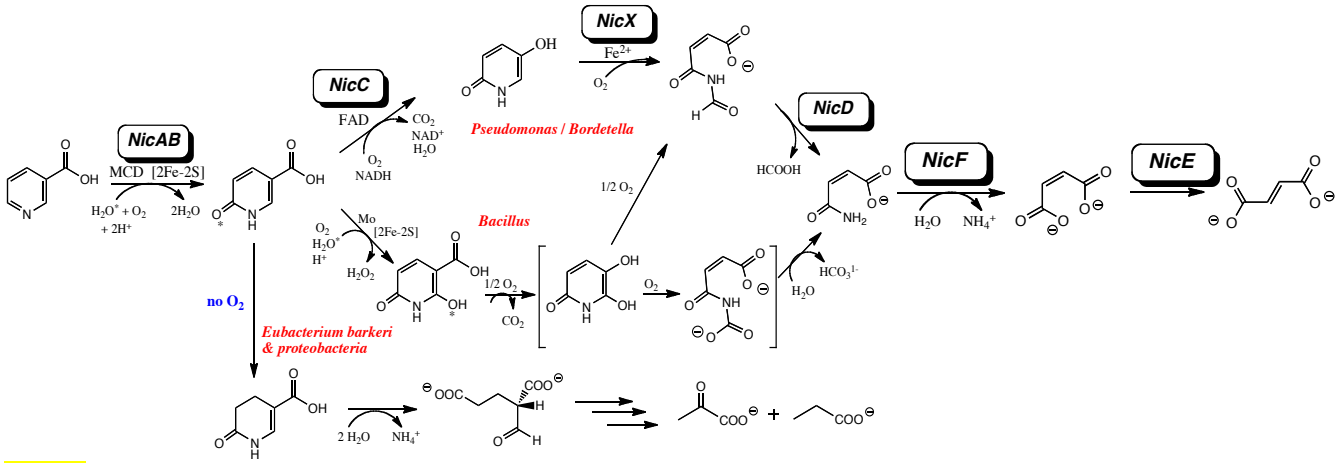


**Figure 1: Nicotinate degradation pathways in aerobic (e.g. *Pseudomonas, Bordetella, Bacillus*) and anaerobic bacteria (e.g. *Eubacterium barkeri*). Bracketed intermediates of the *Bacillus* pathway have only been hypothesized.**

### *Project Goals*

Using a subsystem based approach (*16*) for annotating novel genes within bacterial genomes, research in my lab focuses on the evolutionary differences of metabolic pathways. Our recent work on the characterization of the enzymes involved in aerobic catabolism of nicotinate in *Bordetella bronchiseptica* has lead to the first crystal structure (Figure 2) and proposed mechanism of maleamate amidohydrolase (NicF) (*17*). Results of that work, and more recent functional characterization of NicF variants (*18*) (both supported by previous HHMI Research Awards), provides evidence that, in addition to a catalytic triad (C150, D29, K117) necessary for catalyzing amide-bond hydrolysis in a number of amidohydrolases, R175 and K190 in NicF are important for substrate specificity. There are several additional residues in proximal juxtaposition to the substrate binding site that could also play key roles in substrate specificity, including F83, F34 and T146. Determining the structural basis for how NicF recognizes its substrate is key to being able to identify novel (i.e. undiscovered and currently mis-anotated) nicF genes within the *Bacillus* genome. Identifying those potential nicF genes may lead us to hypothesizing and experimentally determining the genetic determinants of nicotinate degradation in *Bacillus*.

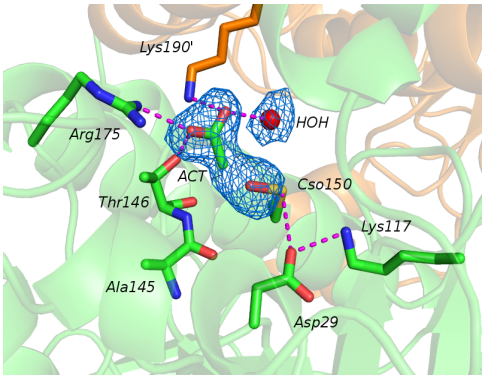


**Figure 2: Active site of NicF bound with acetate (ACT) showing the alignment of residues believed to be important for catalytic activity.**

**What would a Summer HHMI Research Student Accomplish?**

The first goal would be to generate NicF variants (F83A, F34A and T146A) using standard site-directed mutagenesis. We now have experience and excellent systems to make this genetic engineering efficient. Once a student has confirmed the mutation(s), he/she would then express and purify the enzyme(s) using standard chromatographic techniques. Once purified, the enzyme would be characterized using a variety of kinetic assays which we have developed recently, to determine the effects of the mutations on the catalytic parameters $k_{cat}$ and $K_M$. Such data is necessary for comparing the results to the effects of previously successful mutagenesis studies of other active-site residues complete in my lab to build the story for how NicF achieves substrate binding specificity. Last, the student can utilize his/her data to mine bioinformatics sequence databases to identify currently unknown nicF genes in *Bacillus*. The ultimate goal is to discover the novel genes involved in that unique pathway. The results of these predictions could then form the basis of a senior independent study thesis project in the fall.

**Potential Outcomes:**

***Educational:*** the student involved in this project would either learn standard techniques in BCMB that will help them in coursework and I.S. at Wooster and into the future, or allow them to apply the techniques they have learned in courses/labs to a complex project. The student will build confidence in working independently in the lab, and in discussing and writing about their ideas and results.

***Scholarship:*** this project builds upon the successful work of previous HHMI-sponsored students and is an important piece for publishing another paper that builds the story of the mechanism of this enzyme. Success in this project also initiates additional student-based projects in this research area that have high potential for leading to exciting new discoveries in vitamin $B_3$ metabolism.

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