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Biochemical Characterization of AldA, a Novel Enzyme in *Pseudomonas syringae* Indole-3-acetic Scid Biosynthesis

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Plant pathogens can devastate agricultural systems by reducing crop yield and undermining crop integrity. To attenuate the negative impact of plant disease, it is critical to understand how pathogens utilize signaling pathways, regulatory mechanisms, and virulence factors to bypass plant host defenses. *Pseudomonas syringae* is a pathogenic bacterium that causes necrotic lesions and leaf coloration loss (chlorosis) in susceptible plants, including tomato and peach. Recent literature has shown that *P. syringae* has the ability to endogenously produce a key plant hormone, auxin (indole-3-acetic acid; IAA), likely as a means of concealment while infecting the plant. Initial studies indicate that *P. syringae* strain DC3000 uses a tryptophan-dependent IAA biochemical pathway in this process. To elucidate how microbial IAA confers pathogenicity, we have analyzed the NAD(H)-dependent indole-3-acetaldehyde dehydrogenase (AldA) that catalyzes the final indole-3-acetaldehyde (IAAld) to IAA conversion step in the *P. syringae* strain DC3000 IAA biosynthetic pathway. Based on the AldA X-ray crystal structure and known aldehyde dehydrogenase behavior, we hypothesize that a catalytic cysteine residue performs an essential nucleophilic attack on the aldehyde moiety in IAAld. A subsequent hydride transfer from the covalent intermediate to NAD+ and nucleophilic attack by an activated water molecule releases the final IAA product. To test this catalytic mechanism proposal, we have determined the activity profiles of AldA active site mutants, and compared them to initial velocity studies on wild-type enzyme. This study has identified two active site residues, Cys302 and Glu267, that are absolutely essential for AldA function. Further research directions could involve generating and characterizing minimally active AldA varieties that retain normal catalysis but have attenuated substrate binding. Knowing AldA structure and activity can direct us to novel ways to counteract pathogen virulence, including designing targeted inhibitors that act on essential residues.