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ENZYMOLOGY AND PROTEIN ENGINEERING

Structural Insights into 6-Hydroxypseudooxynicotine Amine Oxidase from *Pseudomonas geniculata* N1, the Key Enzyme Involved in Nicotine Degradation

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ABSTRACT Bacteria degrade nicotine mainly using pyridine and pyrrolidine pathways. Previously, we discovered a hybrid of the pyridine and pyrrolidine pathways (the VPP pathway) in *Pseudomonas geniculata* N1 and characterized its key enzyme, 6-hydroxypseudooxynicotine amine oxidase (HisD). It catalyzes oxidative deamination of 6-hydroxypseudooxynicotine to 6-hydroxy-3-succinoylsemialdehyde-pyridine, which is the crucial step connecting upstream and downstream portions of the VPP pathway. We determined the crystal structure of wild-type HisD to 2.6 Å. HisD is a monomer that contains a flavin mononucleotide, an iron-sulfur cluster, and ADP. On the basis of sequence alignment and structure comparison, a difference has been found among HisD, closely related trimethylamine dehydrogenase (TMADH), and histamine dehydrogenase (HADH). The flavin mononucleotide (FMN) cofactor is not covalently bound to any residue, and the FMN isoalloxazine ring is planar in HisD compared to TMADH or HADH, which forms a 6-*S*-cysteinyl flavin mononucleotide cofactor and has an FMN isoalloxazine ring in a "butterfly bend" conformation. Based on the structure, docking study, and site-directed mutagenesis, the residues Glu60, Tyr170, Asp262, and Trp263 may be involved in substrate binding. The expanded understanding of the substrate binding mode from this study may guide rational engineering of such enzymes for biodegradation of potential pollutants or for bioconversion to generate desired products.

IMPORTANCE Nicotine is a major tobacco alkaloid in tobacco waste. Pyridine and pyrrolidine pathways are the two best-elucidated nicotine metabolic pathways; *Pseudomonas geniculata* N1 catabolizes nicotine via a hybrid between the pyridine and pyrrolidine pathways. The crucial enzyme, 6-hydroxypseudooxynicotine amine oxidase (HisD), links the upstream and downstream portions of the VPP pathway; however, there is little structural information about this important enzyme. In this study, we determined the crystal structure of HisD from *Pseudomonas geniculata* N1. Its basic insights about the structure may help us to guide the engineering of such enzymes for bioremediation and bioconversion applications.

KEYWORDS nicotine, 6-hydroxypseudooxynicotine amine oxidase, VPP pathway

Nicotine is a major tobacco alkaloid and the primary toxin in tobacco waste. It can easily spread in the environment because it is water soluble and is emerging as a public health threat (1–4). As a result, nicotine was classified as a "toxic release inventory" chemical by the U.S. Environmental Protection Agency in 1994 (5). Microbial treatment is an important tool for removing nicotine from tobacco industry waste. Many microorganisms that use various nicotine degradation pathways have been isolated from the environment, and nicotine degradation pathways have been widely

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A *Pseudomonas* sp. strain uniquely degrades PAHs and heterocyclic derivatives via lateral dioxygenation pathways

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and heterocyclic derivatives are organic pollutants that pose a serious health risk to human beings. In this study, a newly isolated *Pseudomonas brassicacearum* strain MPDS could effectively degrade PAHs and heterocyclic derivatives, including naphthalene, fluorene, dibenzofuran (DBF) and dibenzothiophene (DBT). Notably, strain MPDS is able to degrade fluorene, DBF and DBT uniquely via a lateral dioxygenation pathway, while most reported strains degrade fluorene, DBF and DBT via an angular dioxygenation pathway or co-metabolize them via a lateral dioxygenation pathway. Strain MPDS completely degraded 50 mg naphthalene (in 50 mL medium) in 84 h, and OD_{600} reached 1.0–1.1; while, it stabilized at OD_{600} 0.5–0.6 with 5 mg fluorene or DBF or DBT. Meanwhile, 65.7% DBF and 32.1% DBT were degraded in 96 h, and 40.3% fluorene was degraded in 72 h, respectively. Through genomic and transcriptomic analyses, and comparative genomic analysis with another DBF degradation strain, relevant gene clusters were predicted, and a naphthalene-degrading gene cluster was identified. This study provides understanding of degradation of PAHs and their heterocyclic derivatives, as well as new insights into the lateral dioxygenation pathway of relevant contaminants.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants that are composed of two or more benzene rings (Haritash and Kaushik, 2009), such as naphthalene and fluorene. Heterocyclic aromatic hydrocarbons are compounds with other elements composing the ring structures like dibenzofuran (DBF) and dibenzothiophene (DBT). The main anthropogenic sources of PAHs and heterocyclic derivatives are from incomplete combustion of fossil and solid biomass fuels, high-temperature industrial processes and petroleum refinery effluents (Sakshi et al., 2019). About 116,000 tons of PAHs have been produced since 2003 (Xu et al., 2006; Zhang et al., 2008), causing environmental pollution and threatening human health. Fluorene is a tricyclic aromatic hydrocarbon with a strong toxic effect, and its heterocyclic derivatives (DBF and DBT) pose a serious health risk to human beings, resulting in deformities, cancer, gene mutation and chromosome aberration through breathing or direct skin contact (Sakshi and Haritash, 2020).

Considering physical and chemical treatments of PAHs are energy, cost, chemical intensive, and even causing secondary pollutants, bioremediation is ecofriendly and sustainable, and has recently gained considerable attention in the last two decades (Sakshi et al., 2019). Several microorganisms that could utilize fluorene, DBF or DBT were screened and discovered, including *Pseudomonas* (Fortnagel et al., 1990; Grifoll et al., 1995; Li et al., 2009), *Sphingomonas* (Wilkes et al., 1996; Gai et al., 2007), *Rhodococcus* (Aly et al., 2008), *Burkholderia* (Gregorio et al., 2004), *Rhizobium meliloti* (Frassinetti et al., 1998), and *Terrabacter* (Schmid et al., 1997; Kasuga et al., 2013). However, these reported strains only utilize one of those pollutants as the sole carbon, or degrade DBF or DBT by co-metabolism with other compounds. For example, Becher et al. (2000) and Li et al. (2009) respectively found *Ralstonia* sp. strain SBUG 290 and *P. putida* B6-2, which could co-metabolize DBF in cultivation with biphenyl. *Arthrobacter* sp. P1-1 capable of co-metabolizing DBT with phenanthrene was identified (Seo et al., 2006). These strains lack the ability to utilize DBF or DBT independently, and few strains could degrade these three substrates simultaneously.

Through technologies of genomics, proteomics, transcriptomics and metabolomics, researchers analyzed the microbial degradation

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