Optimizing the Expression of Nitronate Monooxygenase in *E. coli*

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Nitronate monooxygenase (NMO) is a flavin-dependent enzyme that catalyzes the denitrification of alkyl nitronates (1,2). Propionate 3-nitronate (P3N), the physiological substrate of NMO, is a powerful toxin found in many plants and bacteria that inhibits succinate dehydrogenase and fumarase and thereby inactivates the Krebs cycle (1, 3). Bacterial and fungal NMOs oxidize the P3N to malonic semialdehyde with k_{cat}/K_m values in the order of 10^6-10^7 $M^{-1}s^{-1}$, thus providing protection against this toxin (3). Poisoning by P3N has been reported on livestock and humans and leads to neurological disorders and even death (1). It is therefore important to study the catalytic mechanism of NMO.

The first step in characterizing NMO recombinant enzymes is to optimize the expression and purification in a practical expression system. This study aims to test multiple conditions for the expression of NMO from *P. aeruginosa* PAO1. Small cultures have been induced with differing concentrations of isopropyl β-D-1-thiogalactopyranoside (IPTG) at varying OD_{600} values and incubation times, and the expression has been assessed by using enzymatic assays and SDS-PAGE. With the results found in this study, the expression of NMO has been improved for future mechanistic studies of this detoxifying enzyme.

References:

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