"Directed Evolution of 3,4-Dioxygenase of Protocatechuic Acid: Emergence of Alternative Substrate Specificity"

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Despite the fact that the world is already heading to the end of the first quarter of the 21st century, a major problem still remains unsolved: the deterioration of the environment due to the population growth and the rapid industrialization. A vast amount of pollutants with carcinogenic, mutagenic or toxic properties, are being released each day in the environment. A major category of such pollutants consists of aromatic compounds which include Polycyclic Aromatic Hydrocarbons (PAHs), heterocycles and substituted phenols. Bioremediation is considered to be the most sustainable solution to the problem of these environmental pollutants due to its low cost, high efficiency, minimum byproducts and no secondary pollution [Garcia-Garcia et al 2016]. Despite the great variety of the aforementioned pollutants, their degradation occurs through a limited number of specific metabolic pathways to the formation of central intermediates consisting mainly of catechols and protocatechuates [Fukuda 2014). Our previous studies have demonstrated that *Pseudarthrobacter phenanthrenivorans* Sphe3, isolated from a creosote contaminated site, metabolizes phenanthrene through protocatechuate (PCA) [Vandera et al 2015].

In this study, our goal is to broaden the specificity of protocatechuate 3,4-dioxygenase (3,4-PCD) of Sphe3 (a non-heme ferric ion intradiol dioxygenase, characterized by narrow substrate specificity) in order to identify other central intermediate metabolites. Computational studies combined with literature data, lead to a set of possibly functional enzyme variants. After docking experiments and molecular dynamics simulations conducted to 3, 4-PCD (PDB ID: 2PCD) with catechol, one specific amino acid residue (R133H) was altered in order for 3,4-PCD to functionally replace 1,2-catechol dioxygenase (1,2-CAD). *In silico* experiments were followed by site-directed mutagenesis via PCR. After cloning of the mutant gene in an overexpression plasmid vector and inducing heterologous expression of the recombined enzyme kinetic studies showed that the mutant 3,4-PCD successfully acquires the ability to break down catechol through 1,2-cleavage while maintaining its native ability to break down PCA as well. Further studies may allow the construction of a handful of enzymes able to recognize and catabolize a broader range of aromatic substrates.

References

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