PUTTING THE SPOTLIGHT ON TOLUENE O-XYLENE MONOOXYGENASE
"A GOOD BIOCATALYST CANDIDATE FOR BIOTECHNOLOGICAL APPLICATIONS"

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The biocatalyst toluene *o*-xylene monooxygenase (ToMO) of *Pseudomonas* sp. OX1 belongs to the great family of bacterial multicomponent monooxygenases and has been shown to have a potential for biotechnological and environmental applications. Hydroxylation of aromatics is an important metabolic process for bacteria, is a difficult reaction for organic chemistry, and is industrially significant for the synthesis of fine chemicals. Whole cells expressing ToMO performs aromatic hydroxylations at room temperature in water (Tris·HNO_3, pH 7.0) using only molecular oxygen and the NADH cofactor which is provided by the cells. Using protein engineering techniques, several ToMO variants have been isolated with enhanced oxidation activities as well as fine-tuned regiospecificities, which makes direct microbial hydroxylations even more attractive. Given the engineering-friendly properties of ToMO and its excellent chemistry, we began our journey with the goal of creating efficient whole cell ToMOs for a variety of oxidative transformations. Here, we will discuss our latest results centered around probing the plasticity of ToMO through protein engineering as well as expanding the substrate repertoire of this wonderful enzyme. We will also present our recent efforts showing the potential of non-human ToMO and its variants in drug metabolism applications.

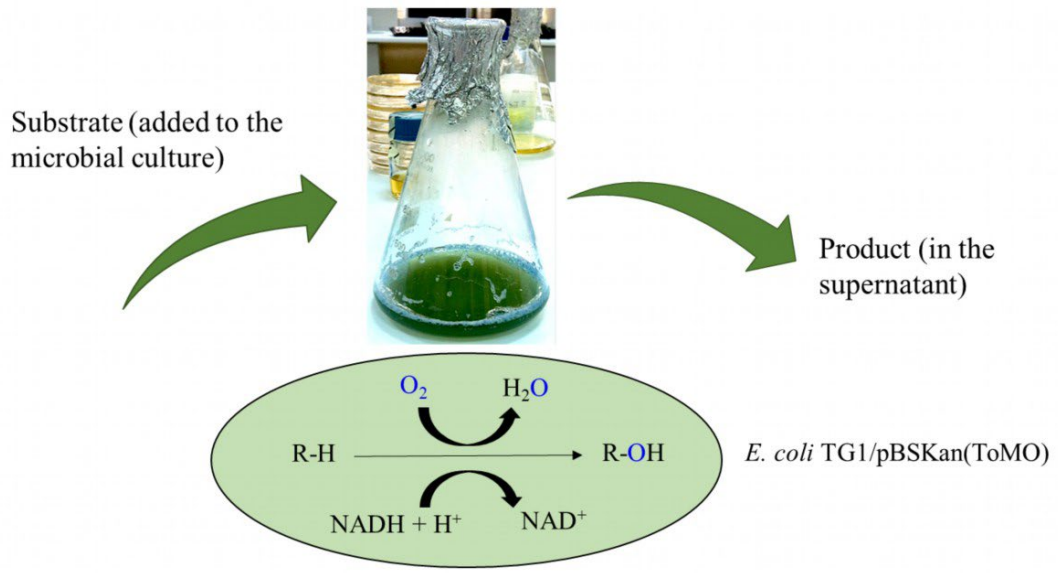

Figure 1 – Escherichia coli strain TG1 expressing ToMO in pBS(Kan)ToMO produces blue colored indigo resulting from the oxidation of indole generated by tryptophanase activity in TG1 cells. The aromatic substrate (R-H) is added to the culture expressing ToMO and enters the cell. ToMO incorporates a single atom of molecular oxygen (O_2) into the aromatic substrate while the other O atom is reduced to water with electrons from NADH which is provided by the cell. The product (R-OH) leaves the cell and can be detected from the culture supernatant.

Reference

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