

JOINT EVENT

11th International Conference on**Tissue Engineering & Regenerative Medicine**

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4th International Conference on **Synthetic Biology and Tissue Engineering**

October 18-20, 2018 Rome, Italy

Investigating the thermostability and activity of DyP1B manganese active-site mutants from *Pseudomonas fluorescens* Pf-5 towards lignolytic substrates**Austine O Ehibhatomhan**
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Lignin is an organic aromatic polymer which constitutes 15%-30% of most plant biomass as well as being one of the largest byproduct of 2nd generation biofuel production and paper/pulp manufacture. Delignification processes for lignocellulosic biomass have gathered attention for its commercial and environmental benefits like the production of organic chemicals and hydrocarbon polymers i.e. bio-ethanol, vanillin and ferulic acid. Microorganisms namely fungi and bacteria produce extracellular lignolytic peroxidases i.e. manganese peroxidases and lignin peroxidases. Fungi peroxidases produced by the white-rot fungi *P. chrysosporium* have been extensively characterised for lignin oxidation however, bacterial peroxidases have amassed more consideration due to favourable industrial characteristics for example mutagenesis studies for improved activities and specific reaction conditions i.e. thermostability. A novel bacterial peroxidase DyP catalyses the peroxide-dependent oxidation of divalent manganese (Mn²⁺) as seen with fungi MnPs, DyPs have also shown improved lignin oxidation in the presence of Mn²⁺. A DyP homologue, from *R. jostii* RHA1 displayed improved Mn²⁺ and subsequently improved lignin oxidation via mutagenesis studies of amino acid residues involved in Mn²⁺ oxidation. In this project, DyP1B from the gram-negative *P. fluorescens* Pf-5 was investigated for improved Mn²⁺ and lignin oxidation by the substitution of amino acids involved in the binding of Mn. The aim of this project was to improve the catalytic efficiency and thermostability of the enzymes production which is vital for its industrial implementation, as well as identification of novel or existing lignin by-products via HPLC. The resulting mutants were expressed as Apoproteins, purified and characterised using a range of lignolytic substrates and profiled for thermostability. Kinetic parameters were measured and analysed.

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