July 16-21, 2017 at UC San Diego, CA, USA

Elucidating the evolution of metallo-β-lactamases through ancestral gene reconstruction

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Introduction: Proteins are the core of an organism's survival and adaptation, therefore understanding their evolutionary history is critical to knowing their ability to survive [1]. Enzymes, a type of protein, are the catalysts for biochemical reactions and the crux for all cellular processes. The metallo-β-lactamases are a family of enzymes that hydrolyse the commonly prescribed β-lactam antibiotics, rendering them ineffective [2]. The evolutionary history and origins of this family of enzymes is unknown due to the low sequence homology in the clade [3]. I created an ancestral library of the metallo-β-lactamase BcII to understand the evolution of this enzyme and how it may affect antibiotic resistance and disease pathogenesis. I accomplished this by reconstructing ancestral sequences at nodes along the metallo-β-lactamase phylogeny and producing the corresponding recombinant proteins. I determined the phenotype of these enzymes, through their catalytic efficiency with nitrocefin, their zinc content and estimated binding affinities, to illuminate the evolutionary history of this enzyme. From the reconstructions I was able to determine that all six zinc binding residues are conserved. The homology modelling of the 4 ancestral states shows that the metallo-β-lactamase structure evolved before the node where the B1 B2 subclasses join on the metallo-β-lactamase phylogeny. This indicates that the origin of the zinc binding residues and the structure requires delving into the metallo hydrolase super family. From this I have not only created a complete phylogeny differing from those previously published, but I have also shown that while the stability has increased in the extant state of the enzyme, activity has not. While this still requires further experimental verification, this study brings the field one step closer to understanding the origins and evolution of metallo-β-lactamases.

References:

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