FITNESS LANDSCAPE OF METALLO-β-LACTAMASE-MEDIATED ANTIBIOTIC RESISTANCE

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Protein evolution is crucial for organismal adaptation and fitness, and relies on a subtle interplay between sequence, structure, functionality and stability, that is manifested in a particular phenotype. The identification of structural traits acquired through evolution allows the prediction of future events. This perspective could be immensely valuable for anticipating the molecular features that enhance bacterial resistance to ß-lactam antibiotics. The main mechanism of this resistance is the expression of ß-lactamases, enzymes whose evolution is continuously challenged by the indiscriminate use of antibiotics. Metallo-ß-lactamases (MßLs) are the latest generation among these enzymes, whose structural diversity and broad substrate profile, allows them to inactivate most ß-lactam antibiotics. Since MßLs may have not yet achieved optimal fitness, the investigation of their future evolutionary potential is extremely important clinically. Directed evolution on the metallo-beta-lactamse BcII from *Bacillus cereus* yielded an optimized variant, that harbors four mutations remote from the active site, which is able to confer greater antibiotic resistance. Two of these mutations display sign epistasis. One of them improves the hydrolysis rate by stabilizing a catalytic intermediate. This mutation has already been observed in clinical isolates from pathogenic bacterium, demostrating the predict power of this approach. The second mutation bestows a more flexible scaffold, resulting in a enzyme with broader substrate spectrum. The fitness landscape of metallo-beta-lactamases is such that the stability threshold has not been exhausted. We have also identified structural determinants of the evolution, therefore this approach can be exploited to design mechanism-based inhibitors with an evolutionary perspective.

Keywords: protein evolution, antibiotic resistance, metallo-beta-lactamase