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Evolution to carbapenem-hydrolyzing activity in class D β-lactamases by rational protein design

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Class D carbapenemases represent increasingly important bacterial antibiotic resistance determinants which compromise the efficacy of the last-resort carbapenem antibiotics. The 3D structures of *Acinetobacter baumannii* OXA-24 and *Klebsiella penumoniae* OXA-48 were recently obtained [1,2] and revealed significant structural heterogeneity with OXA-10 (a narrow-spectrum enzyme inactive on carbapenems), suggesting the potential role of residues of the β5-β6 loop, showing a typical conformation, in the carbapenemase activity of OXA-24 and OXA-48. To probe this hypothesis, we obtained two hybrid OXA-10 proteins bearing the structurally-equivalent loops of OXA-24 and OXA-48 [3]. Functional analysis revealed that both hybrid OXA-10 proteins acquired significant carbapenem-hydrolyzing activity. Furthermore, we obtained the X-ray crystal structures of the OXA-10 derived hybrids enzymes [3] in both the native form and as acyl intermediates with two carbapenem antibiotics, providing insight into substrate binding and catalysis in class D carbapenemases. In this work, we successfully evolved a narrow-spectrum class D β-lactamase into a carbapenemase using a rational structure-based approach and demonstrated the crucial role of the β5- β6 loop in the acquisition of carbapenemase activity and modulation of substrate specificity among class D β-lactamases.

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[2] Santillana E, Beceiro A, Bou G, Romero A. *PNAS* 2007, **104**, 5354-9
[3] De Luca F, Benvenuti M, Carboni F, Pozzi C, Rossolini GM, Mangani S, Docquier JD. *PNAS* 2011, **108**, 18424-9.