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An *in silico* approach to elucidate structure based functional evolution of oxacillinase

Arijit Pal, Anusri Tripathi*

Department of Biochemistry and Medical Biotechnology, Calcutta School of Tropical Medicine, 108 C.R. Avenue, Kolkata 700073, India

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ABSTRACT

Bacterial Oxacillinases (OXAs), genetically being extremely diverse and highly versatile in hydrolyzing antibiotics of different classes, holds utmost significant clinical importance. Hence, to analyze functional evolution of this enzyme, plausible changes in drug profile, affinity and binding stability of different subclasses of OXA with their preferred drugs, viz. penicillin, ceftazidime, imipenem/meropenem were investigated. Maximum-Likelihood dendrogram was constructed and based on tree topology, the least and most divergent variants of each clade were selected. Pocket characterization, enzyme structural stability and mutational effect were analyzed *in silico*. Modes of interaction of selected OXA variants with respective antibiotics were analyzed by Autodock4.0 and LIGPLOT. Comparative mobility profiling and subsequent ΔG° and Km calculations of representative OXA variants revealed that after RSBL evolution, perhaps, two competitive strategies evolved among the OXA variants. Either loops flanking helix5 gets stabilized or it becomes more flexible. Therefore, while OXA variants (e.g. OXA-2, OXA-32, OXA-23, OXA-133, OXA-24, OXA-25, OXA-51 and OXA-75) with highly stabilized loops flanking helix5 exhibited improved binding stability and affinity towards carbapenems, especially meropenem, OXA variants (e.g. OXA-10, OXA-251, OXA-48 and OXA-247) possessing highly flexibile loops flanking helix5 revealed their catalytic proficiency towards ceftazidime. Moreover, LIGPLOT and PROMALS3D jointly identified ten consensuses/conserved residues, viz. P68, A69, F72, K73, W105, V120, W164, L169, K216 and G218 to be critical for drug hydrolysis. Hence, novel inhibitors could be designed to target these sites.

1. Introduction

There is a grave concern regarding increased incidence of antibiotic resistance among pathogenic Gram negative bacteria worldwide. Among several antibiotics, β-lactams can inhibit polymerization of bacterial peptidoglycan cell wall (Rasmussen and Høiby, 2006). Consequently, emergence of bacterial β-lactam resistance through expression of several β-lactamases, members of peptidoglycan transpeptidases of Penicillin Binding Protein (PBP) family, has been considered to be one of the serious and threatening drug-resistance issues. Amongst them, Oxacillinase (OXA), a class D β-lactamase, is significant for its noteworthy sequence diversity and versatility in choice of drugs. Starting from penicillin, cloxacillin, oxacillin, they have the unique property to hydrolyze even third generation cephalosporins and carbapenems, notably imipenem and hence include penicillinase/oxacillinase (Subclass 2d), restricted and extended spectrum β-lactamase (Subclass 2de: RSBLs and ESBLs) and carbapenemase (Subclass 2df) group of enzymes (Bush and Jacoby, 2010). This gene family, considered the largest among the β-lactamases, represents two hundred and one variants with wide difference in coding sequence and 3-D architecture possibly indicates the presence of more than one ancestor of OXA family. Moreover, earlier reports of evolution of OXA-51 and OXA-48 carbapenemases from *Acinetobacter baumanii* and *Shewanella oneidensis* respectively, support their polyphyletic origin (Rasmussen and Høiby, 2006).

On the contrary, as all OXA variants have the same signature sequences: S-XX-K (residues 70–73), S-X-V (residues 118–120) and K-T/S-G (residues 217–219), their catalytic mechanisms remain essentially same for all subclasses (Poirel et al., 2010). Similar to other groups of SBLs, OXAs have active site serine residues (S^{70} and S^{118}) for nucleophilic attacks which hydrolyze the amide bond of β-lactam ring. Although amino acid substitutions in stabilization centers of OXA variants are considered to affect their drug affinity (measured by Km, Michealis constant) and binding stability with the preferred drug (evaluated by ΔG°, Gibb's free energy for substrate binding), their role in substrate specificity has been poorly characterized as of now. Till date, substrate specificity of

* Corresponding author.
E-mail address: anusri.stm@gmail.com (A. Tripathi).

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OXA is known to be solely governed by accessibility of catalytic pocket, which, in turn, is regulated by the flexibility of two loops: upper loop (105–115 loop) and lower loop (β5-β6 loop) (Kaitany et al., 2013; Luca et al., 2011). Hence, in the present study an attempt has been made to reconstruct the evolutionary trajectories of OXA gene family, analyze their structural dynamicity, topology and key mutations that followed in each subclass leading to their diverse catalytic activity.

2. Materials and methods

2.1. Sequence alignments and phylogenies

Accession numbers of all oxacillinase (OXA) (n = 201) gene variants were retrieved from Lahey's mutational table (http://www.lahey.org/Studies/). Both nucleotide & protein sequences of OXA variants and PBP were retrieved from Genbank database of National Center of Bioinformatics (http://www.ncbi.nlm.nih.gov). Signal peptides of OXA genes were predicted by LipoP1.0 server and deleted (Juncker et al., 2003). Amino acids were numbered according to DBL standard numbering system (Couture et al., 1992). Both nucleotide and protein sequences of OXA were aligned separately using CLUSTALW server.

Protein alignment file was used for phylogenetic analyses by Maximum-Likelihood (ML) method through MEGA version5.0 (Tamura et al., 2011). Whelan and Goldman substitution model, selected with the model test program according to Akaike Information Criterion, was used as evolutionary models that included gamma distribution with six rate categories to account for substitution rate and pattern of homogeneity among sites. Close Neighbor Interchange had been used as ML heuristic model during phylogenetic tree constructions.

2.2. Ancestor reconstruction of OXA variants

In order to infer most likely ancestral amino acid sequence at each node of the obtained maximum-likelihood tree, Maximum-Likelihood analyses were performed using MEGA version5. Additionally, GC contents of OXA variants were calculated by EMBOSS 6.3.1 (http://bioweb2.pastuer.fr/) and compared with GC content of several related bacterial genera available at UCSC Microbial Genome Browser (http://microbes.ucsc.edu) to predict the primary organism of origin of OXA.

2.3. Selection of OXA variants for molecular docking

Similar to our previous work, the OXA variants present at bases with no/least number of substitutions and those at apices with maximum number of substitutions of different clades, indicating minimum and maximum genetic diversity respectively, were selected for molecular docking studies (Pal and Tripathi, 2013).

2.4. Molecular docking studies

2.4.1. Template identification and protein homology modeling

RCSB Protein Data Bank (http://www.rcsb.org/pdb) was searched by PSI-BLAST program for three dimensional structures of proteins similar to the selected OXAs. Most similar proteins (i.e. proteins with highest percentage of sequence identity) were selected as templates for query protein homology modeling (Table 1). Modeller 9v7 (http://salilab.org/modeller/9v7/) was

Table 1
Homology Modeling & Model validation data of selected OXA variants.

Subclass	Variants	Template(s) (% identity)	SAVES Scores & PROSA Score	Ramachandran Plot data
Oxacillinase	PBP	1M6K, 3ISG and 2XO2 (36%, 37% and 31%)	Verify-3D = 98.79%; Z score = −8.13; Errat = 90.756	A = 83.2%; B = 14.9%;C = 1.4%; D = 0.5%
	OXA-59	1M6K, 3ISG and 2XO2 (37%, 37% and 31%)	Verify-3D = 100.00%; Z score = −8.16; Errat = 90.336	A = 85.5%; B = 12.1%;C = 1.4%; D = 1.0%
	OXA-43	1M6K, 3ISG and 2XO2 (33%, 33% and 30%)	Verify-3D = 90.69%; Z score = −7.37; Errat = 92.857	A = 84.5%; B = 13.6%;C = 1.9%; D = 0.0%
	OXA-1	1M6K (94%)	Verify-3D = 99.60%; Z score = −7.75; Errat = 96.708	A = 86.4%; B = 11.4%; C = 1.3%; D = 0.9%
	OXA-47	1M6K (92%)	Verify-3D = 90.87%; Z score = − 7.75; Errat = 95.062	A = 89.1%; B = 10.5%; C = 0.4%; D = 0.0%
	OXA-136	3HBR, 1H8Z and 1K38 (42%, 34% and 36%)	Verify-3D = 73.91%; Z score = − 7.01; Errat = 93.033	A = 85.4%; B = 12.4%; C = 1.3%; D = 0.9%
	OXA-63	3HBR, 1H8Z and 1K38 (41%, 33% and 35%)	Verify-3D = 81.35%; Z score = −6.56; Errat = 93.827	A = 88.5%; B = 11.1%; C = 0.4%; D = 0.0%
RSBL	OXA-258	4GN2, 3ISG and 1M6K (45%, 33% and 33%)	Verify-3D = 98.00%; Z score = −7.90; Errat = 91.286	A = 87.4%; B = 8.3%; C = 3.4%; D = 1.0%
	OXA-243a	4GN2, 3ISG and 1M6K (45%, 32% and 32%)	Verify-3D = 94.80%; Z score = −6.70; Errat = 87.500	A = 86.4%; B = 10.7%; C = 2.9%; D = 0.0%
	OXA-119	1K38, 3ISG and 3HBR (83%, 28% and 43%)	Verify-3D = 89.43%; Z score = −6.94; Errat = 94.937	A = 86.8%; B = 11.8%; C = 0.9%; D = 0.5%
	OXA-46	1K38, 3ISG and 3HBR (82%, 29% and 43%)	Verify-3D = 95.53%; Z score = −7.20; Errat = 96.203	A = 87.3%; B = 10.9%; C = 1.8%; D = 0.0%
ESBL	OXA-2	1K38 (100%)	Verify-3D = 94.90%; Z score = − 7.66; Errat = 93.878	A = 86.3%; B = 12.4%; C = 0.4%; D = 0.9%
	OXA-32	1K38 (99%)	Verify-3D = 94.51%; Z score = −7.36; Errat = 91.020	A = 85.8%; B = 11.1%; C = 2.2%; D = 0.9%
	OXA-10	2XO2 (100%)	Verify-3D = 97.98%; Z score = −8.57; Errat = 98.745	A = 90.5%; B = 8.6%; C = 0.5%; D = 0.5%
	OXA-251	2XO2 (98%)	Verify-3D = 97.58%; Z score = −8.58; Errat = 96.653	A = 88.2%; B = 10.0%; C = 0.9%; D = 0.9%
	OXA-35	1H8Z (99%)	Verify-3D = 95.16%; Z score = −8.50; Errat = 98.326	A = 88.6%; B = 11.0%; C = 0.0%; D = 0.5%
	OXA-7	1H8Z (97%)	Verify-3D = 95.47%; Z score = −7.71; Errat = 97.071	A = 89.0%; B = 10.0%; C = 0.0%; D = 0.9%
Carbapenemase	OXA-48	3HBR (100%)	Verify-3D = 98.36%; Z score = − 8.51; Errat = 97.021	A = 90.5%; B = 8.6%; C = 0.9%; D = 0.0%
	OXA-247	3HBR (97%)	Verify-3D = 97.08%; Z score = −7.90; Errat = 94.372	A = 90.4%; B = 9.2%;C = 0.4%; D = 0.0%
	OXA-309	3G4P, 4JF5 and 3ISG (62%, 62% and 26%)	Verify-3D = 87.55%; Z score = −8.04; Errat = 93.145	A = 87.6%; B = 10.7%;C = 1.3%; D = 0.4%
	OXA-212	3G4P, 4JF5 and 1K38 (61%, 61% and 26%)	Verify-3D = 87.16%; Z score = −8.12; Errat = 88.259	A = 90.7%; B = 8.4%;C = 0.9%; D = 0.0%
	OXA-237	3G4P, 3HBR and 1K38 (61%, 35% and 30%)	Verify-3D = 94.23%; Z score = −8.35; Errat = 93.574	A = 85.2%; B = 13.5%;C = 0.4%; D = 0.9%
	OXA-134a	3G4P, 3HBR and 1K38 (61%, 35% and 30%)	Verify-3D = 89.88%; Z score = −7.81; Errat = 92.713	A = 89.4%; B = 8.9%; C = 0.4%; D = 1.2%
	OXA-230	3G4P, 1K38 and 3ISG (60%, 35% and 26%)	Verify-3D = 80.93%; Z score = − 7.62; Errat = 87.097	A = 85.5%; B = 11.1%; C = 2.4%; D = 1.0%
	OXA-229	3G4P, 1K38 and 3ISG (60%, 36% and 26%)	Verify-3D = 84.05%; Z score = − 7.27; Errat = 89.919	A = 90.7%; B = 6.6%; C = 1.8%; D = 0.9%
	OXA-58	3G4P, 3HBR and 1K38 (53%, 36% and 34%)	Verify-3D = 73.38%; Z score = −7.86; Errat = 93.701	A = 89.6%; B = 10.4%; C = 0.0%; D = 0.0%
	OXA-97	3G4P, 3HBR and 1K38 (53%, 36% and 33%)	Verify-3D = 83.65%; Z score = −8.89; Errat = 97.933	A = 93.5%; B = 5.2%; C = 1.3%; D = 0.0%
	OXA-23	4JF5 (100%)	Verify-3D = 82.49%; Z score = −7.80; Errat = 89.256	A = 87.7%; B = 10.5%; C = 1.3%; D = 0.4%
	OXA-133	4JF5 (97%)	Verify-3D = 77.43%; Z score = −8.84; Errat = 94.694	A = 88.7%; B = 9.6%; C = 1.3%; D = 0.4%
	OXA-143	3G4P, 1K38 and 3ISG (88%, 29% and 28%)	Verify-3D = 82.88%; Z score = −7.69; Errat = 87.755	A = 87.2%; B = 10.6%; C = 1.3%; D = 0.9%
	OXA-255	3G4P, 1K38 and 3ISG (89%, 29% and 29%)	Verify-3D = 90.27%; Z score = − 8.44; Errat = 89.113	A = 90.3%; B = 8.8%; C = 0.9%; D = 0.0%
	OXA-24	2JC7 (100%)	Verify-3D = 92.61%; Z score = −8.23; Errat = 95.122	A = 89.4%; B = 9.3%; C = 0.9%; D = 0.4%
	OXA-25	2JC7 (99%)	Verify-3D = 91.05%; Z score = − 8.24; Errat = 94.332	A = 90.3%; B = 9.3%; C = 0.4%; D = 0.0%
	OXA-51	3G4P, 1K38 and 3ISG (67%, 29% and 26%)	Verify-3D = 92.64%; Z score = −7.97; Errat = 86.345	A = 88.4%; B = 10.7%;C = 0.4%; D = 0.4%
	OXA-75	3G4P, 1K38 and 3ISG (65%, 29% and 28%)	Verify-3D = 84.88%; Z score = −7.70; Errat = 93.469	A = 86.7%; B = 12.9%;C = 0.0%; D = 0.4%

A = Most favoured region; B = additionally allowed region, C = generously allowed region; D = disallowed region.