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Review

Promiscuous Enzyme Activity as a Driver of Allo and Iso Convergent Evolution, Lessons from the B-Lactamases

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Abstract: The probability of the evolution of a character depends on two factors: the probability of moving from one character state to another character state and the probability of the new character state fixation. More the evolution of a character is probable more convergent evolution will be witnessed, consequently, convergent evolution could mean that the convergent character evolution result as a combination of these two factors. We investigate this phenomenon by studying the convergent evolution of biochemical functions. We use for the investigation the case of β-lactamases. β-lactamases hydrolyzes β-lactams which are antimicrobials able to block the DD-peptidases involved in bacterial cell wall synthesis. β-lactamase activity is present in two different superfamilies: the metallo-β-lactamase and the serine β-lactamase superfamily. The mechanism used to hydrolyze the β-lactam is different for the two superfamilies. We named this kind of evolution an allo-convergent evolution. We further show that the β-lactamase activity evolved several times within each superfamily, a convergent evolution type that we named iso-convergent evolution. Both types of convergent evolution can be explained by the two evolutionary mechanisms discussed above. The probability of moving from one state to another is explaining the promiscuous β-lactamase activity present in the ancestral sequences of each superfamily, while the probability of fixation is explained in part, by positive selection as the organisms having β-lactamase activity allows them to resist to organism secreting β-lactams. Indeed a mutation increasing the β-lactamases activity will be selected as the organisms having this activity will have an advantage over the others.

Keywords: β-lactamase; convergent evolution; antibiotic resistance

1. Introduction

The concepts: iso and allo convergent evolution, evolutionary shift and maintain of apomorphies. In order to gain a finer scale understanding of the dynamics of convergence, we proposed to use the concepts and corresponding terms "iso-convergent" and "allo-convergent" evolution [1]. Iso-convergent traits have converged from the same ancestral state (traditionally "parallel evolution" but parallel evolution also has a different meaning), whereas allo-convergent traits have converged from different ancestral states. In the case of iso-convergent evolution, because we could define the ancestral and the derived state, it could be possible to modelize the process that allows the shift. The probability to go from one state to another is better explained in the case of amino acid substitutions where the evolutionary model has been well studied. The substitution (shift from an ancestral to a derived state) depends on two factors: the mutation rate and the fixation rate. Regarding the mutation rate, some shifts are more probable than others. For example, the

transition is more probable than transversion, and in the genomes of some vertebrates' taxa, another important form of mutation bias involves changes at CpG dinucleotides: if the DNA nucleotide cytosine (C) is immediately 5' to a guanine (G) on the same coding strand, then depending on methylation status point mutations at both sites occur at an elevated rate relatively to mutations at the non-CpG site [2]. Regarding the fixation rate, besides the importance of positive selection, an important factor has to be taken into an account: the pleiotropy. Indeed amino acid substitution usually has pleiotropic effects on protein biochemistry. Indeed, a substitution that improves one aspect of a protein function may also compromise other structural or functional properties. Within a set of mutations that have functionally the same effects on the phenotype the mutations that display the lower deleterious pleiotropic effect should have a higher fixation probability [3]. The dichotomy: mutation rate and fixation rate can be found at higher biological levels. However, usually at higher biological levels, scholars discuss constraint and selection [4] whereas the probability of shift (mutation rate) is not discussed. For example, Losos (2011) [4] pointed out that the wings allowing flight in vertebrates have been built convergently in different ways in birds, pterosaurs, and bats. In all these cases the wings represent modified forelimbs. The combination of wings and forelimbs, in theory, could be very useful but is not found in real life. The author concluded that this was due to a lack of constraints. We underline here that besides the constraint, the probability to modify forelimbs to get wings is likely higher than that to start from nothing. Note that the same reasoning could be applied for the DDE transposon co-option as sequence-specific recombination activating systems that evolved via iso-convergent evolution; many times explained in part by the fact that the biochemical shift from a transposase to sequence-specific recombination activating endonuclease is an easy evolutionary step [5]. We think that it is mandatory to clarify the participation of the shift and fixation probability and we will show that this clarification is important regarding the process of iso-convergent and allo-convergent evolution. Further, we describe a property that increases the probability of shift: the enzymatic promiscuity.

2. Enzyme convergent evolution via allo-convergent and iso-convergent evolution: the case of β-lactamases

2.1. Allo-convergent evolution of enzyme function: the case of β-lactamases

The allo-convergent evolution of enzyme function results in two distinct mechanisms: 1) non-homologous enzymes deliver the same transformation as expressed by the same four-digit enzyme commission (EC) number [6,7] but with a different mechanism; 2) the other case of allo-convergent evolution corresponds to the one where enzyme transformation is realized by a similar disposition of residues in the active site; the active site occurring then in an independent manner [6,7]. The enzyme commission (EC) number is a numerical classification scheme for enzymes based on the catalyze chemical reactions. In enzymes exhibit multispecificity or substrate ambiguity, EC number for the various substrates should be the same/differ only by the 4th digit between enzymes of the same class. Catalytic promiscuity refers to cases in which the EC numbers of the various substrates and reactions catalyzed by the same enzyme differ in the 2nd or the 3rd digits that refer to different chemistries, and different classes of substrates, or even by the 1st digit that indicates a completely different reaction category [8].

The β-lactamases correspond to the former case have the same EC number 3.5.2.6 and they correspond to two distinct families: the serine β-lactamases (class A/C/D) superfamily and the metallo-β-lactamases (class B) superfamily with therefore two distinct ancestors. These two superfamilies can hydrolyze the antibacterial β-lactams. The β-lactams inhibit penicillin-binding proteins, which are involved in the cell wall synthesis of bacteria, by performing cross-linking of peptide chains to form peptidoglycan. The inhibition is done by acylating an active-site serine that is essential for penicillin-binding protein activity [9].

The mechanisms by which the two superfamilies of β-lactamase perform the hydrolysis, and thus the resistance to β-lactam are different. In the case of the A, C, and D serine β-lactamase family, the hydrolysis occurs through the formation of an acyl-enzyme with an active-site serine and in the

case of metallo-β-lactamases, this occurs via a hydrolytic reaction facilitated by one or two essential zinc ions in the active sites of metallo-β-lactamases. The independent evolution of the same function, most often using different mechanisms, is well documented for proteins of different families of nonhomologous enzymes[7].

The case of iso-convergence has been less documented, one of the best described examples being the "HAD (haloacid dehalogenase) superfamily" of proteins [10]. In the case of the different families of β-lactamase [11], the iso-convergent evolution of β-lactamases were found.

While Keshri et al [11] focused on the convergent (iso-convergent) evolution of the β-lactam' specificities in the four different classes of β-lactam. We will focus our analysis on the independent evolution of the generic β-lactamase function in regards to the evolution of the metallo-β-lactamases superfamily and the serine-β-lactamases superfamily.

2.2. Iso-convergent evolution of the metallo-β-lactamases functional family

2.2.1. Evolutionary analyses of the metallo-β-lactamases family

In general, superfamily based upon a structural fold is organized in structural families. Each family could have its own function. However this is not always the case, this is due, as we will see, to iso convergent evolution. This is why we use the terms functional family and structural family since these two sentences have a different meaning.

The metallo-β-lactamase family is a superfamily, the superfamily is defined based on a structural fold and the eponymic family being the metallo-β-lactamases family. At least 23 other functional families have been identified including enzymes involved in DNA and RNA nucleotide processing, detoxification, quorum quenching, and pesticide hydrolysis. Most of these functions involved hydrophilic reactions and target different substrates with different chemical properties such as phosphodiester, phosphotriester, choline phosphoester, thiol ester, sulfonate ester, and β-lactam bond. Other functions involve non-hydrolytic reactions such as nitric oxydoreduction and sulfur dioxygenation as well as non-enzymatic functions [12,13].

Because the metallo-β-lactamases have the same fold they likely arose from a common ancestor. Even if the metallo-β-lactamases fold is conserved, the identity level could be very low between the different members of this superfamily (less than 5%). The members share structural features such as the MBL fold and a mononuclear or binuclear active site center with a unique metal-binding motif (H-X-HX-D-H). Even on the functional group of β-lactamases, these sequences are highly divergent for example only 11% of the amino acids are conserved between MBL B1 and B2, and only 9% are conserved between MBL B3 versus B1or B2. In addition, when aligning all the sequences, 1 or 3 amino acids will be shared (hopefully in the active sites). Because very few amino acids are conserved, the rate of phylogenetic artifacts due to the sharing of the same amino acid in the alignment increase. Thus it is likely that the phylogenetic analysis will be robust at the subfamily level but not reliable between subfamilies. We can also hypothesize that the sequences share a common ancestor but the ancestor is so old that very few amino acids are found in common. In that case, we could define subgroups based on network similarity as described by Baier and Tokuriki [13] (of course in that case we have a phenetic analysis and therefore the evolutionary history is only a guess). Because to different members belonging to the different superfamilies are not well conserved and because more than 30,000 sequences are for example available in the Pfam database, a phylogenetic tree is not possible and a pre-classification must be done. This pre-classification can be obtained via a similarity network [14] that is the most comprehensive clustering on the β-lactamases fold so far described (Figure 1). This analysis as well as other analyses [15] including ours [16–18] helps us to define several subfamilies allowing us to perform precise analysis for some of them. We redefine the subfamily metallo-β-lactamase B3, Glyoxalase 2, sulfur dioxygenase, metallo-β-lactamase B1/B2, TNP dehalogenase, and the archaea metallo-β-lactamase like [13,15–17]. To perform a robust phylogenetic analysis, we used a distant based phylogenetic analysis since very few positions are conserved.

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The phylogenetic analysis shows that MBLB1, MBLB2, and TNP dehalogenase form a group while MBLB3 forms a group with the MBL Like of archaea; the NJ analyses were unable to classify Glyoxalase 2 sulfur dioxygenase families.

The other families include for example SNM, Ribonuclease Z, B -CASP RNases [13,19].

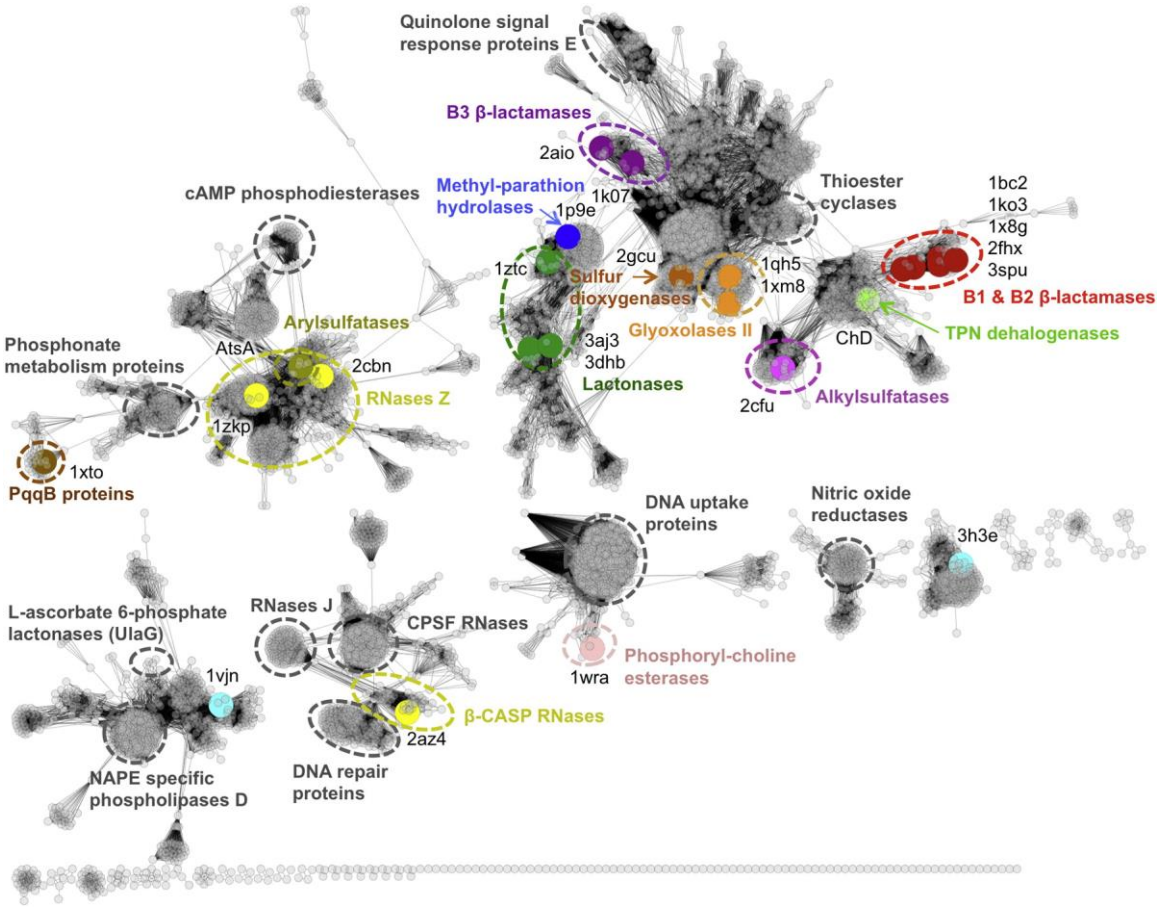

Figure 1. Sequence similarity network of MBL superfamily members. A total of 6233 sequences (nodes) and lines (edges) show sequence relationship at a BLAST e-value cutoff of $1 \times e^{-14}$. Large colored nodes show sequences that were experimentally characterized in this study with colored broken circles indicating their approximate functional family cluster. Pale-blue-colored nodes (PDB IDs 1vjn and 3h3e) are experimentally characterized sequences with unknown function and from clusters with unknown function. The functional families clusters of three experimentally characterized sequences and have not been encircled due to the small number of functional homologues (methyl-parathion hydrolase and TPN dehalogenase) and annotation ambiguity (sulfur deoxygenase). Gray broken circles indicate functional sequence clusters that have been experimentally characterized and reported in the literature but have not been included in this study. For unassigned gray sequence clusters (not encircled), no confident functional information could be retrieved from the databases or literature. This figure has been reprinted with permission from Baier and Tokuriki [13].

2.3. Evolution of the metallo-β-lactamase activity via an increase of the probability of shift and fixation of the new character

The metallo-β-lactamases evolved via iso-convergent evolution due to the increase of the probability of shift (mutation rate) and to the promiscuous activity. Most and maybe all enzymes, besides their physiological reaction, are able of catalyzing secondary reactions termed promiscuous reactions [20,21]. The ratio *k*cat/*KM*, an index that is usually used for comparing the relative rates of an enzyme acting on the alternative substrate, show a value around 10 5 to 10 8 M-1s-1 for the "native" substrate of a given enzyme and lower activity of several orders for the promiscuous

activities [8,13]. It has been proposed by Jensen [22], more than forty years ago, that this ability to catalyze multiple chemically distinct reactions in addition to their primary function constitutes a functional repertoire from which the enzyme can be co-opted and further enhanced by mutations. The phylogenetic analysis above shows that the archaea MBL forms a monophyletic clade with B3 metallo-β-lactamases. The archaea MBL Like have a promiscuous activity [23]. The *k*cat/*K*M is around 20 in the case of nitrocefin while this activity is 10 000 fold higher in the case of MBL B3 β-lactamases 1 (B3 β-lactamase) [24–27]. The phylogenetic analysis shows also that TPN dehalogenases, which form a monophylogenetic group with MBL1 and B2, display also a promiscuous activity [13].

All the other families are less phylogenetically related but some of them display lactamase activity, such as 1VJN (Uniprot ID: Q9WY50) family [13], whose physiological activity is unknown, display a good β-lactamase activity with a *k*cat/kM around 10,000. Furthermore, a promiscuous activity is found in different structural families.

The Glyoxalase 2 sulfur dioxygenase, TPN dehalogenases families PqqB display also a promiscuous β-lactamases activity [13]. This is also the case for Ribonuclease Z [13,18,28], MBLAC1 [17] as well as other enzymes with unknown function [13]. Therefore promiscuous β-lactamase activity is found in most of the members of the metallo-β-lactamases superfamily and was likely to be present in the common ancestor of the superfamily. Thus, *a* bona fide -β-lactamases activity evolved at least 3 times from a promiscuous activity: in B1/B2 MBL, B3 MBL, and 1VJN.

2.4. The metallo-β-lactamases evolved via iso-convergent evolution because possibly also of positive selection

Besides the increase of probability shift, the iso-convergence evolution of the β-lactamases activity is likely to be due to an increase of fixation; in that case, positive selection can be a possibility. The secretion of antimicrobial compounds by microbes including lactamin could be an ancient strategy to improve the survival of microbes competing for space and nutrients with other microorganisms. Thus, the emergence of resistance mechanisms to antimicrobials could be also an ancient natural response process [29,30]. β-lactams are antimicrobials able to block the DD-peptidases involved in bacterial cell wall synthesis. Therefore a mutation increasing the β-lactamases activity from a weak activity can be selected and the species having this activity will have an advantage over the others.

The β-lactams include five naturally occurring families. Four of them block the DD-peptidase: carbapenems, penicillin/cephalosporin, monocyclic β-lactams, and sulfazecin/monobactam. These families are synthesized by different biosynthetic pathways [31] and therefore evolved via allo-convergent evolution. It's possible that the β-lactamases activity evolved many times (from a promiscuous activity) in response to one of these β-lactams synthesis pathways.

In the case of a mutation giving rise to an increase of the β-lactamase activity two scenarios are possible: (ii) a decrease of the original function, in that case, the mutation will be counter selected; (ii) the original function is preserved in that case the mutation will not be counter selected. In the first case, a gene duplication event could help with the fixation [32].

2.5. Iso-convergent evolution of the serine β-lactamases family

As mentioned above, β-lactams inhibit penicillin-binding proteins(PBP), which are involved in the cell wall synthesis of bacteria, by performing cross-linking of peptide chains to form peptidoglycan. The inhibition is done by acylating an active-site serine which is an essential penicillin-binding protein activity. PBPs were likely the precursors of the serine β-lactamases, with the rate for deacylation being increased dramatically for serine β-lactamases family, compared to PBPs that exhibit a fast acylation step compared to a slow deacylation step. Formation of the PBP-acyl enzyme complex has half-lives ranging from around 10 min to more than 24 h depending on the PBPs and the β-lactam [33–35].

Some PBPs have evolved to function as weak β-lactamases with a slow turnover of the β-lactam substrate [36]; for example, cefotaxime deacylation rates are 70- to 80-fold higher for PBP2x variants than that for the wild-type enzyme in *Streptococcus pneumoniae* [37]. Diene et al showed [23]

that an archaea DD peptidase like has a promiscuous β-lactamases activity (Kcat/ Km=16.57 s-1.M-1). Therefore the PBPs can develop the β-lactamases activity in an iso-convergent manner due in part to their β-lactamases promiscuous activity. The discussion concerning the positive selection end the role of duplication on the fixation of the events is the same as above.

2.6. Hypothesis: Allo-convergent evolution should be linked to iso-convergent evolution

Because of the promiscuous activity of the different protein folds (MBL lactamases fold and DD peptidase fold) positive selection and lack of constraint, both folds evolved a bona fide activity via allo-convergent evolution. Furthermore, inside each family, the β-lactamase activity occurred not only on time but several times (iso-convergent evolution). We propose here that when allo-convergent evolution is evidenced iso-convergent evolution should be also found. Of course, this will depend on the size of the superfamilies where the allo-convergent event occurs. Unfortunately, very few enzymatic families have been studied as well as the β-lactamases families. Another problem that prevented such observation is that authors do not make a clear distinction between allo-convergent and iso-convergent evolution even if the difference is sometimes mentioned by using the term of parallel evolution. However, as discussed by Pontarotti and Hue [1] the term is confusing as some authors use this term to explain a morphological shift with the same genetic mechanisms.

Many cases of allo-convergent evolution have been reported [7]. One interesting case to start with could be the one of paraoxonase that evolves twice, via allo-convergent evolution, from two different folds exhibiting ancestral promiscuous paraoxonase activity [38]. We propose that the paraoxonase activity evolve also inside each family and this can be tested.

Finally, the β-lactamases functional/ structural evolutionary analysis allows to identify two distinct evolutionary processes: exaptation and coevolution. In the case of MBL fold, we witness an exaptation. This term is used for a character that evolved a given function and is then used for another one [39] which is the case for the MBL lactamase. However, in the case of A, C, D serine β-lactamases we evidenced a specific case of exaptation that corresponds to a coevolutionary arms race (Van Valen,1973). Indeed the β-lactams used the DD peptidase active site to block the DD peptidase. The DD peptidase, in turn, evolved its hydrolysis activity against the β-lactams.

Furthermore, concerning the MBL fold based on our analysis, it is likely that the ancestral MBL fold had a hydrolase activity against an unknown organic compound and a promiscuous β-lactamase activity or other promiscuous hydrolase activity. This information is important regarding the understanding of the early phase of life evolution. Two main hypotheses aim to explain on the earliest phases of evolution: the autotrophic origins and the heterotrophic origins. Theories for autotrophic origins propose that the first cells satisfied their carbon needs from CO2 [40] while heterotrophic origin theories propose that the first cells lived from the fermentation of reduced organic compounds present in some kind of rich organic soup [41]. B-lactamase fold function could have been important for the two scenarios. In the case of the heterotrophic hypothesis, the ancestral β-lactamase fold could have been involved in hydrolysis reaction on organic compounds already present on earth and was able to provide energy to the prototo cell. In the case of autotrophic hypothesis, the next step could have been fermentations [42], and there the β-lactamase could have been extremely useful in such a process for its hydrolysis capacity of specific organic components. The promiscuous activity of ancestral MBL fold could have then evolved to a more efficient activity against new organic compounds found in the environment. Regarding the DD-transpeptidases (PBP)/serine β-lactamase families they belong to a single clan (fold) with serine hydrolase properties [43], the enzymatic reaction of the PBP which has the function ancestral to all the group has already a complex function D-alanine carboxypeptidase, peptidoglycan transpeptidase, and peptidoglycan endopeptidase. We do not have a hint about the previous functions, this prevents a discussion similar to that for the Metallo-β-lactamase.

3. Materials and methods

3.1. Sequence selection:

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The protein sequences were collected from the ARG-ANNOT [44] and the NCBI database. A total of 205 protein sequences were considered in this study. These sequences belong to serine β-lactamases, metallo-β-lactamases, dehalogenase, dioxygenases, and archaeal encoded β-lactamases. A total of 205 sequences selected in this study.

3.2. Functional Domain Identifications:

The functional domain (proteins are generally composed of one or more functional regions, commonly termed domains) of these sequences were identified and extracted through NCBI Conserve Domain Database (CDD) [45] and Pfam release 32.0 database [46]. CDD offers both an archive of pre-computed domain annotations as well as live search services for both single protein or nucleotide queries and larger sets of protein query sequences. The Pfam database is a collection of protein families, each represented by multiple sequence alignments and hidden Markov models (HMMs). The Pfam and CDD were executed with the default settings on their respective websites/servers.

3.3. Phylogenetic Tree Construction:

To explore the phylogenetic tree of the selected sequences, we performed multiple sequence alignment using the MUSCLE algorithms. Multiple sequence alignment trimming was performed using the trimAL [47], which removes poorly aligned regions. Next, the phylogenetic tree was constructed in FastTree [48] and visualized with FigTree (http://tree.bio.ed.ac.uk/software/figtree).

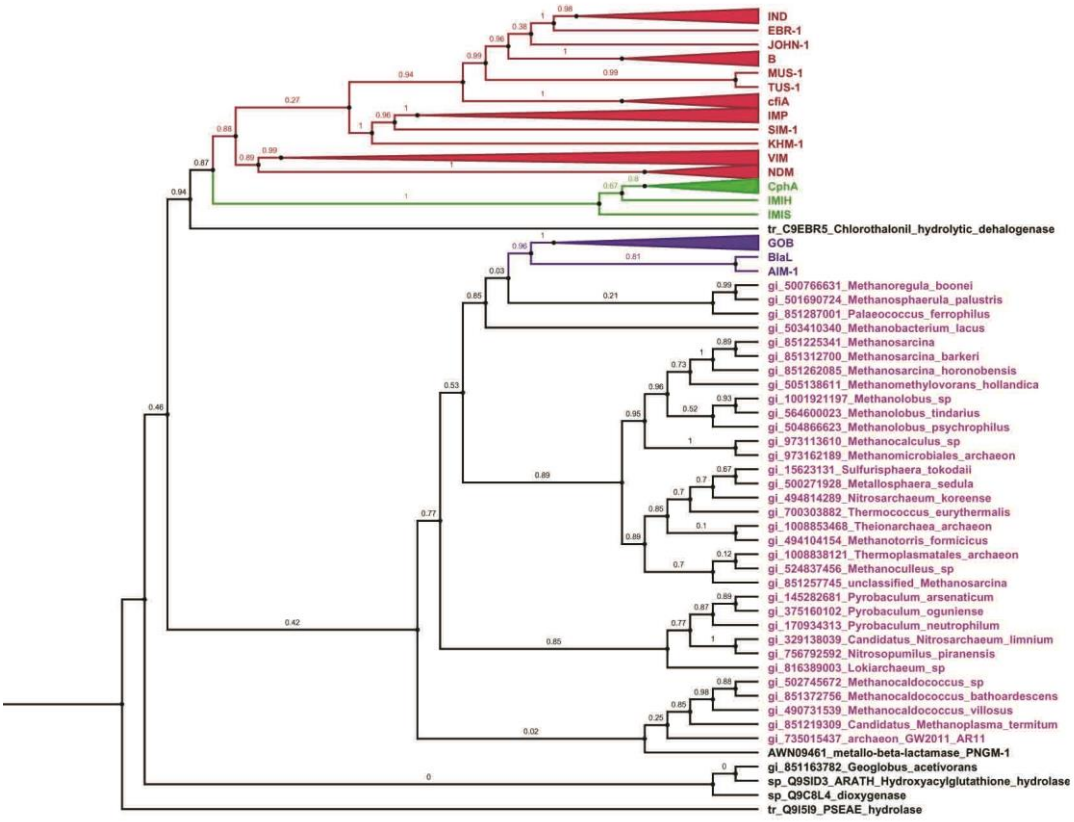

Figure 2. New phylogeny: This phylogenetic tree contains a total of 205 sequences (clades has been collapsed). The tree was constructed in FastTree and visualized in (midpoint rooted increasing order) FigTree. The coloring scheme of the leaves indicates sequences belongs to different group/ family- Red, Green and Blue indicates Metallo-β-lactamase B1, B2, and B3, Magenta color indicates archaeal sequences while black indicates diverse function. The complete figure can be seen in Supplementary Fig S1.

Authors Contributions: Analysis and wrote the manuscript, V.K.; Review and comments, E.C., L.P., P.C., S.D., JM.R., D.R.; Conceived the original idea, wrote the manuscript, and supervised the finding of this work, P.P.

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References

1. Pontarotti, P.; Hue, I. Road map to study convergent evolution: A proposition for evolutionary systems biology approaches. In *Evolutionary Biology: Convergent Evolution, Evolution of Complex Traits, Concepts and Methods*; Springer International Publishing: Cham, 2016; pp. 3–21 ISBN 9783319413242.
2. Yampolsky, L.Y.; Stoltzfus, A. Bias in the introduction of variation as an orienting factor in evolution. *Evol. Dev.* **2001**, *3*, 73–83, doi:10.1046/j.1525-142X.2001.003002073.x.
3. Streisfeld, M.A.; Rausher, M.D. Population genetics, pleiotropy, and the preferential fixation of mutations during adaptive evolution. *Evolution (N. Y).* **2011**, *65*, 629–642, doi:10.1111/j.1558-5646.2010.01165.x.
4. Losos, J.B. Convergence, adaptation, and constraint. *Evolution (N. Y).* **2011**, *65*, 1827–1840, doi:10.1111/j.1558-5646.2011.01289.x.
5. Zhang, Y.; Cheng, T.C.; Huang, G.; Lu, Q.; Surleac, M.D.; Mandell, J.D.; Pontarotti, P.; Petrescu, A.J.; Xu, A.; Xiong, Y.; et al. Transposon molecular domestication and the evolution of the RAG recombinase. *Nature* **2019**, *569*, 79–84, doi:10.1038/s41586-019-1093-7.
6. Doolittle, R.F. Convergent evolution: the need to be explicit. *Trends Biochem. Sci.* **1994**, *19*, 15–18, doi:10.1016/0968-0004(94)90167-8.
7. Gherardini, P.F.; Wass, M.N.; Helmer-Citterich, M.; Sternberg, M.J.E. Convergent Evolution of Enzyme Active Sites Is not a Rare Phenomenon. *J. Mol. Biol.* **2007**, *372*, 817–845, doi:10.1016/j.jmb.2007.06.017.
8. Tawfik, O.K. and D.S. Enzyme Promiscuity: A Mechanistic and Evolutionary Perspective. *Annu. Rev. Biochem.* **2010**, *79*, 471–505, doi:10.1146/annurev-biochem-030409-143718.
9. Spratt, B.G. Penicillin-binding proteins and the future of β-lactam antibiotics. The seventh Fleming lecture. *J. Gen. Microbiol.* 1983, *129*, 1247–1260.
10. Burroughs, A.M.; Allen, K.N.; Dunaway-Mariano, D.; Aravind, L. Evolutionary Genomics of the HAD Superfamily: Understanding the Structural Adaptations and Catalytic Diversity in a Superfamily of Phosphoesterases and Allied Enzymes. *J. Mol. Biol.* **2006**, *361*, 1003–1034, doi:10.1016/j.jmb.2006.06.049.
11. Keshri, V.; Arbuckle, K.; Chabrol, O.; Rolain, J.M.; Raoult, D.; Pontarotti, P. The functional convergence of antibiotic resistance in β-lactamases is not conferred by a simple convergent substitution of amino acid. *Evol. Appl.* **2019**, *12*, 1812–1822, doi:10.1111/eva.12835.
12. Bebrone, C. Metallo-β-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. *Biochem. Pharmacol.* 2007, *74*, 1686–1701.
13. Baier, F.; Tokuriki, N. Connectivity between catalytic landscapes of the metallo-β-lactamase superfamily. *J. Mol. Biol.* **2014**, *426*, 2442–2456, doi:10.1016/j.jmb.2014.04.013.
14. Atkinson, H.J.; Morris, J.H.; Ferrin, T.E.; Babbitt, P.C. Using sequence similarity networks for visualization of relationships across diverse protein superfamilies. *PLoS One* **2009**, *4*, e4345, doi:10.1371/journal.pone.0004345.
15. Alderson, R.G.; Barker, D.; Mitchell, J.B.O. One origin for metallo-β-lactamase activity, or two? An investigation assessing a diverse set of reconstructed ancestral sequences based on a sample of phylogenetic trees. *J. Mol. Evol.* **2014**, *79*, 117–129, doi:10.1007/s00239-014-9639-7.
16. Keshri, V.; Panda, A.; Levasseur, A.; Rolain, J.M.; Pontarotti, P.; Raoult, D. Phylogenomic analysis of b-lactamase in Archaea and bacteria enables the identification of putative new members. *Genome Biol. Evol.* **2018**, *10*, 1106–1114, doi:10.1093/gbe/evy028.

17. Diene, S.M.; Pinault, L.; Armstrong, N.; Keshri, V.; Khelaifia, S.; Chabrière, E.; Caetano-Anolles, G.; Rolain, J.-M.; Pontarotti, P.; Raoult, D. Paradoxical β-lactamase activity of archaeal encoding enzymes. *bioRxiv* **2019**, 667907, doi:10.1101/667907.
18. Colson, P.; Pinault, L.; Azza, S.; Armstrong, N.; Chabriere, E.; Scola, B. La; Pontarotti, P.; Raoult, D. A metallo-beta-lactamase with both beta-lactamase and ribonuclease activity is linked with traduction in giant viruses. *bioRxiv* **2019**, 819797, doi:10.1101/819797.
19. CATH CATH Superfamily 3.40.449.10 Available online: http://www.cathdb.info/version/latest/superfamily/3.60.15.10 (accessed on Feb 15, 2020).
20. Velez Rueda, A.J.; Palopoli, N.; Zacarías, M.; Sommese, L.M.; Parisi, G. ProtMiscuity: a database of promiscuous proteins. *Database (Oxford).* **2019**, *2019*, doi:10.1093/database/baz103.
21. Copley, S.D. Shining a light on enzyme promiscuity. *Curr. Opin. Struct. Biol.* 2017, *47*, 167–175.
22. Jensen, R.A. Enzyme Recruitment in Evolution of New Function. *Annu. Rev. Microbiol.* **1976**, *30*, 409–425, doi:10.1146/annurev.mi.30.100176.002205.
23. Diene, S.M.; Pinault, L.; Keshri, V.; Armstrong, N.; Khelaifia, S.; Chabrière, E.; Caetano-Anolles, G.; Colson, P.; La Scola, B.; Rolain, J.M.; et al. Human metallo-β-lactamase enzymes degrade penicillin. *Sci. Rep.* **2019**, *9*, 12173, doi:10.1038/s41598-019-48723-y.
24. Lisa, M.N.; Morán-Barrio, J.; Guindón, M.F.; Vila, A.J. Probing the role of met221 in the unusual metallo-β-lactamase gob-18. *Inorg. Chem.* **2012**, *51*, 12419–12425, doi:10.1021/ic301801h.
25. Keshri, V.; Diene, S.M.; Estienne, A.; Dardaillon, J.; Chabrol, O.; Tichit, L.; Rolain, J.-M.; Raoult, D.; Pontarotti, P. An Integrative Database of β-Lactamase Enzymes: Sequences, Structures, Functions, and Phylogenetic Trees. *Antimicrob. Agents Chemother.* **2019**, *63*, doi:10.1128/AAC.02319-18.
26. Morán-Barrio, J.; González, J.M.; Lisa, M.N.; Costello, A.L.; Peraro, M.D.; Carloni, P.; Bennett, B.; Tierney, D.L.; Limansky, A.S.; Viale, A.M.; et al. The metallo-beta-lactamase GOB is a mono-Zn(II) enzyme with a novel active site. *J. Biol. Chem.* **2007**, *282*, 18286–93, doi:10.1074/jbc.M700467200.
27. Rodríguez, M.M.; Herman, R.; Ghiglione, B.; Kerff, F.; D'Amico González, G.; Bouillenne, F.; Galleni, M.; Handelsman, J.; Charlier, P.; Gutkind, G.; et al. Crystal structure and kinetic analysis of the class B3 di-zinc metallo-β-lactamase LRA-12 from an Alaskan soil metagenome. *PLoS One* **2017**, *12*, 1–18, doi:10.1371/journal.pone.0182043.
28. Lee, J.H.; Takahashi, M.; Jeon, J.H.; Kang, L.W.; Seki, M.; Park, K.S.; Hong, M.K.; Park, Y.S.; Kim, T.Y.; Karim, A.M.; et al. Dual activity of PNGM-1 pinpoints the evolutionary origin of subclass B3 metallo-β-lactamases: a molecular and evolutionary study. *Emerg. Microbes Infect.* **2019**, *8*, 1688–1700, doi:10.1080/22221751.2019.1692638.
29. Hall, B.G.; Barlow, M. Evolution of the serine β-lactamases: Past, present and future. *Drug Resist. Updat.* **2004**, *7*, 111–123, doi:10.1016/j.drup.2004.02.003.
30. Dcosta, V.M.; King, C.E.; Kalan, L.; Morar, M.; Sung, W.W.L.; Schwarz, C.; Froese, D.; Zazula, G.; Calmels, F.; Debruyne, R.; et al. Antibiotic resistance is ancient. *Nature* 2011, *477*, 457–461.
31. Townsend, C.A. Convergent biosynthetic pathways to β-lactam antibiotics. *Curr. Opin. Chem. Biol.* 2016, *35*, 97–108.
32. Levasseur, A.; Pontarotti, P. The role of duplications in the evolution of genomes highlights the need for evolutionary-based approaches in comparative genomics. *Biol. Direct* 2011, *6*, 11.
33. Shapiro, A.B.; Gao, N.; Gu, R.F.; Thresher, J. Fluorescence anisotropy-based measurement of Pseudomonas aeruginosa penicillin-binding protein 2 transpeptidase inhibitor acylation rate constants. *Anal. Biochem.* **2014**, *463*, 15–22, doi:10.1016/j.ab.2014.06.004.
34. Shapiro, A.B.; Gu, R.F.; Gao, N.; Livchak, S.; Thresher, J. Continuous fluorescence anisotropy-based assay of BOCILLIN FL penicillin reaction with penicillin binding protein 3. *Anal. Biochem.* **2013**, *439*, 37–43, doi:10.1016/j.ab.2013.04.009.
35. Lemaire, S.; Fuda, C.; Van Bambeke, F.; Tulkens, P.M.; Mobashery, S. Restoration of susceptibility of methicillin-resistant Staphylococcus aureus to beta-lactam antibiotics by acidic pH: role of penicillin-binding protein PBP 2a. *J. Biol. Chem.* **2008**, *283*, 12769–76, doi:10.1074/jbc.M800079200.
36. Massova, I.; Mobashery, S. Kinship and diversification of bacterial penicillin-binding proteins and β-lactamases. *Antimicrob. Agents Chemother.* 1998, *42*, 1–17.
37. Lu, W.P.; Kincaid, E.; Sun, Y.; Bauer, M.D. Kinetics of β-lactam interactions with penicillin-susceptible and -resistant penicillin-binding protein 2x proteins from Streptococcus pneumoniae. Involvement of acylation and deacylation β-lactam resistance. *J. Biol. Chem.* **2001**, *276*, 31494–31501, doi:10.1074/jbc.M102499200.

38. Elias, M.; Tawfik, D.S. Divergence and convergence in enzyme evolution: Parallel evolution of paraoxonases from quorum-quenching lactonases. *J. Biol. Chem.* 2012, *287*, 11–20.
39. Gould, S.J.; Vrba, E.S. Exaptation—a Missing Term in the Science of Form. *Paleobiology* **1982**, *1*, 4–15, doi:10.1017/S0094837300004310.
40. Fuchs, G. Alternative Pathways of Carbon Dioxide Fixation: Insights into the Early Evolution of Life? *Annu. Rev. Microbiol.* **2011**, *65*, 631–658, doi:10.1146/annurev-micro-090110-102801.
41. Bada, J.L.; Lazcano, A. Some like it hot, but not the first biomolecules. *Science (80-.).* 2002, *296*, 1982–1983.
42. Schönheit, P.; Buckel, W.; Martin, W.F. On the Origin of Heterotrophy. *Trends Microbiol.* 2016, *24*, 12–25.
43. Rawlings, N.D.; Barrett, A.J.; Finn, R. Twenty years of the MEROPS database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Res.* **2016**, *44*, D343–D350, doi:10.1093/nar/gkv1118.
44. Gupta, S.K.; Padmanabhan, B.R.; Diene, S.M.; Lopez-Rojas, R.; Kempf, M.; Landraud, L.; Rolain, J.M. ARG-annot, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob. Agents Chemother.* **2014**, *58*, 212–220, doi:10.1128/AAC.01310-13.
45. Marchler-Bauer, A.; Derbyshire, M.K.; Gonzales, N.R.; Lu, S.; Chitsaz, F.; Geer, L.Y.; Geer, R.C.; He, J.; Gwadz, M.; Hurwitz, D.I.; et al. CDD: NCBI's conserved domain database. *Nucleic Acids Res.* **2015**, *43*, D222–D226, doi:10.1093/nar/gku1221.
46. El-Gebali, S.; Mistry, J.; Bateman, A.; Eddy, S.R.; Luciani, A.; Potter, S.C.; Qureshi, M.; Richardson, L.J.; Salazar, G.A.; Smart, A.; et al. The Pfam protein families database in 2019. *Nucleic Acids Res.* **2019**, *47*, D427–D432, doi:10.1093/nar/gky995.
47. Capella-Gutiérrez, S.; Silla-Martínez, J.M.; Gabaldón, T. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **2009**, *25*, 1972–1973, doi:10.1093/bioinformatics/btp348.
48. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2 - Approximately maximum-likelihood trees for large alignments. *PLoS One* **2010**, *5*, e9490, doi:10.1371/journal.pone.0009490.

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Supplementary Figure

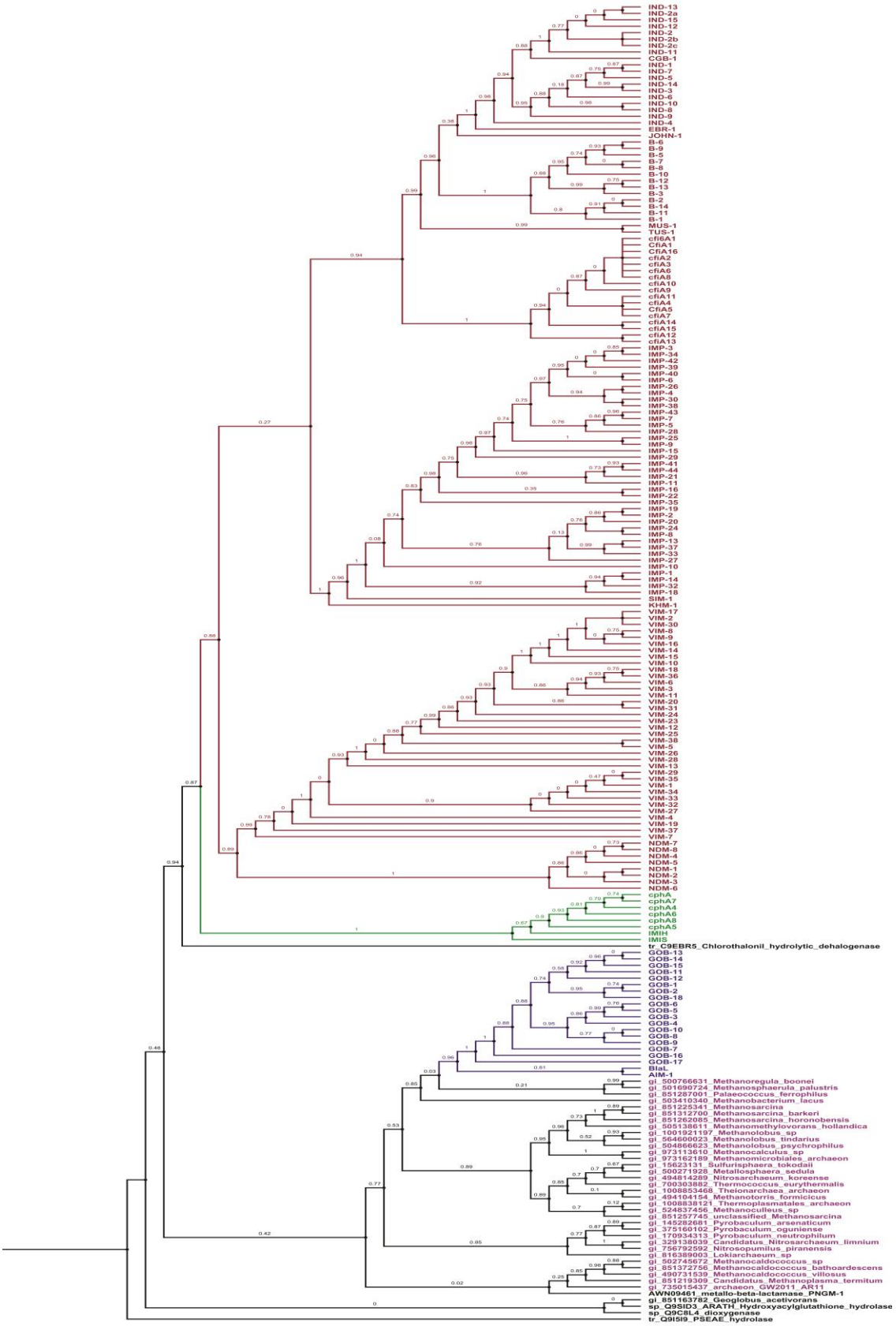

Figure S1: This phylogenetic tree contains a total of 205 sequences. The tree was constructed in FastTree and visualized in (midpoint rooted increasing order) FigTree. The coloring scheme of the leaves indicates sequences belongs to different group/ family- Red, Green and Blue indicates

Metallo-β-lactamase B1, B2 and B3, Magenta color indicates archaeal sequences while black indicates diverse function.