Examination of Phylogenetically Related β-Lactamases and their Interactions with Amoxicillin

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ABSTRACT

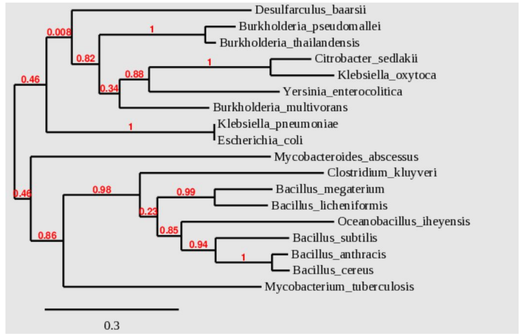
Mycobacterium tuberculosis is a major health threat that resulted in 1.5 million deaths worldwide in 2020. The enzyme BlaC, present in M. tuberculosis prior to the use of *β*-lactam antibiotics, is at least partially responsible for the resistance of tuberculosis infection to treatment by various *β*-lactams. This project describes the structure of this enzyme in comparison to proteins that have closely related sequences and structures. It relies on previously studied phylogenetic relationships of β-lactamases and the large number of lactamase structures that have been deposited into the PDB database. The VAST+ server on the NCBI website was used to provide lists of protein PDB structures with folding patterns that are closely related to that of BlaC. BLAST helped identify proteins that had similar protein sequences as that of M. tuberculosis. After protein 3D-structural alignments were prepared using Swiss Viewer, the phylogenetic trees were used to compare protein structures and the locations of active site residues. It was discovered that the phylogenetic trees weren't the most accurate method to analyze the protein structures between different β-lactamases. It seems that the evolutionary history between β-lactamase isn't necessarily as important as the amino acid sequence and structure. Two residues were found to be of interest in this study and may provide future insight to the fight against antibiotic resistance.

INTRODUCTION

- β-lactamases are enzymes that degrade β-lactam antibiotics.
- The amino acid residues around a binding pocket determine the β-lactamase's physicochemical characteristics.
- The study of β-Lactamases is extremely important as understanding more about β-lactamase's interaction with the β-lactam antibiotics may lead to more effective treatment.
- Research has been done using the phylogenetic and sequential analyses of β-lactamases to identify the existence of new members of β-lactamases. They offered a new and broadened reservoir of previously unseen β-lactamases. These extra sequences are likely to encode enzymes that provide resistance to a diverse number of antibiotics.
- Additionally, while there has been research dealing with the phylogenetic analysis of β-Lactamase, there has yet to be research that compares the binding pocket residue interactions of one β-lactam with other β-lactamases found in various bacterial species.
- *M.tuberculosis* β-lactamase, the major resistance determinant against β-lactam antibiotics against tuberculosis, was the focus of this study.
- ***Experimental Question***: How do the binding pocket residue interactions with amoxicillin differ between using *Mycobacterium tuberculosis* β-lactamase and other proteins that have closely related sequences and structures?
- The observation of binding pocket residue interactions with amoxicillin relative to that of *M. tuberculosis* and other β-lactamases allowed for a deeper understanding of the various types of β-lactamases in relation to *M. tuberculosis.*

METHODS

- The VAST+ system on the NCBI Protein Structure website was used to identify β-lactamases similar to *M. tuberculosis*. The table of 1400 proteins were sorted to reflect different bacterial species. These species were organized into a phylogenetic tree based on their names.
- A more accurate phylogenetic tree was created by taking the protein sequence of *Mycobacterium tuberculosis* and inputting it into Standard Protein BLAST, where 100 other closely related proteins were generated.
- The crystal structures of 18 different species were opened on Molegro, allowing for the observation of residue interactions with amoxicillin between *M. tuberculosis* and other β-lactamases.
- Several binding pocket residues were organized onto a table.
- Subsequently, the crystal structures were overlapped onto Swiss PDB Viewer to observe how the different β-lactamases interacted with amoxicillin.



RESULTS

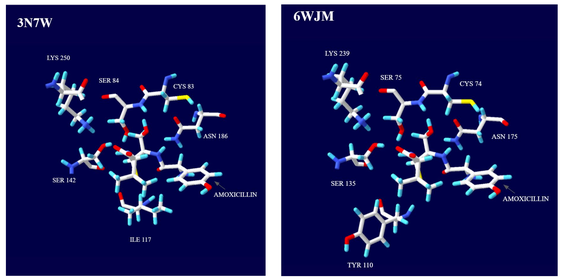


Figure 3. The residue interactions of Lys, Tyr, Cys, and Ser between the protein of bacterial species *Mycobacterium tuberculosis* and *Desulfarculus baarsii*.
The residue ILE 117 in 3N7W changes to TYR 110 in 6WJM. The other residues stay the same with a similar orientation despite the significant phylogenetic difference between 6WJM and 3N7W

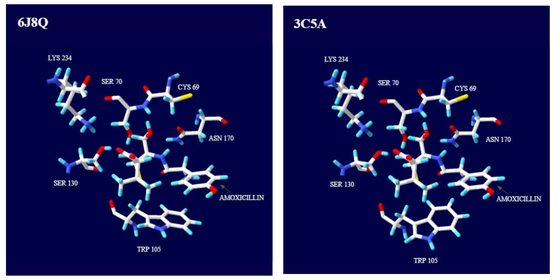


Figure 4. The residue interactions of Lys, Tyr, Cys, and Ser between the protein of bacterial species E*scherichia coli* and *Klebsiella pneumoniae*
Residue TRP 105 has a notable angle change with respect to amoxicillin from 6J8Q to 3C5A. The other residues experienced slight changes in position, but no notable changes in orientation.

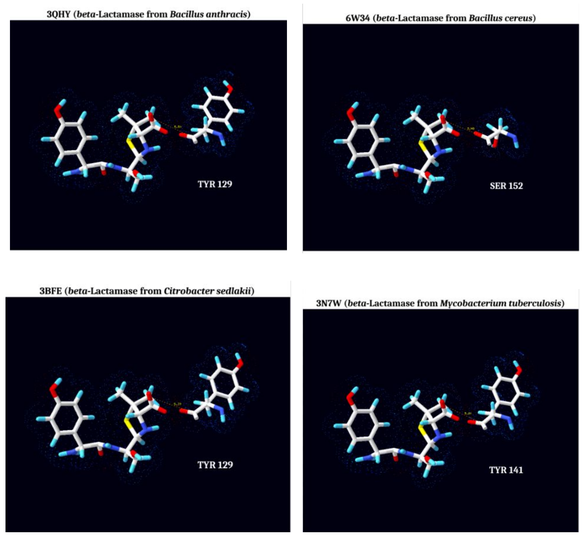


Figure 5. Residue TYR interaction comparison between protein of bacterial species *Bacillus anthracis*, *Bacillus cereus*, *Citrobacter sedlakii*, and *Mycobacterium tuberculosis.*
Although the residues, TYR 129 in 3QHY and SER 159 in 6W34, are in a similar position near the amoxicillin, their structures are completely different with serine missing the carbon ring. TYR 129 of 3BFE and TYR 141 of 3N7W are in relatively the same position and orientation.

Table 2. Residue interactions of 18 different representative bacterial species' proteins
6 key residues that were less than 4.0Å (1×10-8 Centimeters) distance from amoxicillin

6WJM	CYS 74	SER 75	TYR 110	SER 135	ASN 175	LYS 239
3W4O	CYS 69	SER 70	TYR 105	SER 130	ASN 170	LYS 234
5GL9	CYS 69	SER 70	TYR 105	SER 130	ASN 170	LYS 234
3BFE	CYS 69	SER 70	TYR 105	SER 130	ASN 170	LYS 234
3BYD	CYS 72	SER 73	TYR 108	SER 133	ASN 173	LYS 237
5E2E	CYS 46	SER 47	TYR 82	SER 107	ASN 147	LYS 211
3W4Q	CYS 69	SER 70	TYR 105	SER 130	ASN 170	LYS 234
6J8Q	CYS 69	SER 70	TRP 105	SER 130	ASN 170	LYS 234
3C5A	CYS 69	SER 70	TRP 105	SER 130	ASN 170	LYS 234
4YFM	CYS 70	SER 71	TRP 106	SER 131	ASN 171	LYS 233
6NJ1	CYS 86	SER 87	TYR 120	SER 145	ASN 185	LYS 249
6MU9	ALA 88	SER 89	TYR 122	SER 147	ASN 187	LYS 251
2WK0	ALA 69	SER 70	TYR 105	SER 130	ASN 170	LYS 234
3LEZ	THR 84	SER 85	TYR 118	SER 143	ASN 183	LYS 247
6NI1	ALA 88	SER 89	TYR 122	SER 147	ASN 187	LYS 251
3QHY	ALA 69	SER 70	TYR 105	SER 130	ASN 170	LYS 239
6W34	ALA 94	SER 95	TYR 128	SER 153	ASN 193	LYS 257
3N7W	CYS 83	SER 84	ILE 117	SER 142	ASN 186	LYS 250

DISCUSSION

- 6WJM and 3N7W are farthest apart on the phylogenetic tree, yet their residue orientations didn't show significant change with the exception of ILE and TYR. 3QHY and 6W34, two closely related β-lactamases, have a more varied residue interaction than 3BFE and 3N7W, two that are phylogenetically further apart. This indicates that the phylogenetic tree is not always an accurate way to display protein structures of two β-lactamases.
- My study shows how although some β-lactamases are similar to one another, one different residue is enough to change how the β-lactam bonds with the β-lactamase, which would therefore determine the effectiveness of the β-lactam.
- TYR/TRP 105 is a very dynamic residue while TYR 129 is relatively stable with a few stark changes in bacterial species 6WJM and 6W34, giving researchers insight into two more residues to consider.
- Additionally, results from this study shows that environmental pressures of the different bacterial species play a bigger role in protein structure than its evolutionary history. Although some bacterial species are not as closely related, their protein structures are very alike.

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