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MODELING AND COMPUTATIONAL STRUCTURAL STUDIES OF THE UDP-N-PROTEIN ACETYLMURAMOYL-L-ALANYL-D-GLUTAMATE-2,6-DIAMINOPIMELATO LIGASE *Pseudomonas aeruginosa*

COMPUTATIONAL MODELING AND STRUCTURAL STUDIES OF THE UDP-N-ACETYLMURAMOYL-L-ALANYL-D-GLUTAMATE-2,6-DIAMINOPIMELATE PROTEIN LIGASE FROM PSEUDOMONAS AERUGINOSA

Flavio Teodoro – State University of Ponta Grossa (UEPG)

SUMMARY:
The hospital relevance of *Pseudomonas aeruginosa* is considerable; therefore, studies should be conducted on proteins present in the bacterium that are therapeutic targets for the discovery of new antimicrobial drugs. Thus, enzymes such as UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase (MurE), which play an important metabolic role and are not homologous in mammals, are valuable potential targets for the development of new drugs (AMERA *et al.,* 2019). MurE participates in the synthesis of murein, a substance involved in the formation of the bacterial cell wall, an essential element for the microorganism's survival. Therefore, this study performed computational modeling by homology with the bioinformatics programs Modeller and T-Coffee. It is hoped that this theoretical information can be added to biochemical knowledge, so that, in a future study, it will help in the identification of ligands for MurE as a therapeutic target.

Keywords: *Pseudomonas aeruginosa.* Homology Modeling. MurE.

ABSTRACT:

The hospital relevance of *Pseudomonas aeruginosa* is considerable, therefore, studies should be conducted on proteins present in the bacterium that serves as therapeutic targets for the discovery of new antimicrobial drugs. Thus, enzymes such as UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase (MurE), which play an important metabolic role and are not homologous in mammals, represent valuable potential targets for the development of new drugs (AMERA et al., 2019). MurE participates in the synthesis of murein, a substance involved in the formation of the bacterial cell wall, an indispensable component for the microorganism's survival.
In this study, computational homology modeling was performed using the bioinformatics programs Modeller and T-Coffee. It is expected that these theoretical findings may be integrated with biochemical knowledge so that, in a future study, they may assist in the identification of ligands for MurE as a therapeutic target.
Keywords: *Pseudomonas aeruginosa.* Homology Modeling. MurE.

1. INTRODUCTION

Pseudomonas aeruginosa is among the main bacteria that present great clinical relevance (NEVES *et* al., 2011), especially in hospital infections, as it is resistant to multiple antimicrobials (BORDIGNON; LIMA, 2017). Thus, structural studies of

specific enzymes of this species may become valuable for the research of new antimicrobials. Among them is UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase (MurE), an essential enzyme in the biosynthesis of the bacterial cell wall and which has no homologues in humans (AMERA *et* al., 2019). Structural information can be obtained through experimental techniques, however, when experimental failure occurs, it can be use computational methods to obtain a prediction of the protein structure, for example, based on previously determined homologous protein structures (VERLI, 2014).

2 THEORETICAL FRAMEWORK

MurE is responsible for adding mesodiaminopimelic acid to the nucleotide precursor, called UDP-N-acetylmuramoyl-L-alanyl-D-glutamate, during the synthesis of murein (peptidoglycan) in the cytoplasm. Given this fact, MurE has a notable impact on survival of the bacteria, since the cell wall protects the microorganism against lysis osmotic, which could cause its rupture (GORDON et al, 2000).

It is also worth noting that knowledge of the three-dimensional structure of proteins (mainly enzymes) is of great importance as it constitutes an auxiliary factor to biochemical information in the design of new compounds of pharmacological interest. Such structural information can be obtained experimentally through ray diffraction techniques X and nuclear magnetic resonance, for example (VERLI, 2014).

2. MATERIAL AND METHOD

The amino acid sequence of MurE from the bacterium *Pseudomonas aeruginosa* was obtained . *Seattle Structural Genomics Center for Infectious Disease (SSCGID)* database . With the amino acid sequence of MurE, the *Basic Local Alignment* search tool was used *Search Tool* (BLAST), based on data from the *Protein Data Bank* (PDB). The goal was to find homologous proteins, present in other microorganisms, that had their 3D structures resolved. In total, 19 results were obtained, however, only 4 proteins were selected homologous considering the parameters *"E-value",* "% coverage" and the issue of similarity and difference in structures compared to PaMurE. Thus, homologous proteins with significant structural differences were ruled out. The homologous proteins selected were:

one from *Escherichia coli* (PDB: 1e8c), one from *Mycobacterium tuberculosis* (PDB: 2wtz), one from *Staphylococcus aureus* (PDB: 4c12) and one of *Thermotoga maritima* (PDB: 4bub).

With the help of the SALIGN module (SALI; SHEN, 2006) of the Modeller program and also through the T-Coffee program, it was possible to obtain structural alignments with the proteins homologs and MurE itself from *Pseudomonas aeruginosa* (PaMurE). Thus, it was possible to verify which regions are conserved with respect to amino acids (Figure 1).

With the Modeller program, 500 models were built using the homology modeling technique, through the satisfaction of spatial constraints. After this stage, the constructed models were evaluated for stereochemical quality and folding, considering the secondary, tertiary and quaternary structures. The best model obtained referring to the lowest DOPE score was represented by the number structure 302. With regarding stereochemical quality, Ramachandran graphs were obtained through the PROCHECK program (LASKOWSKI et al., 1993), for all template proteins used and for the selected model of PaMurE.

Using the PyMOL program, three-dimensional structures were represented, in which alpha helices are highlighted in red, loops and turns in green, and beta strands in blue (Figures 2-6). Figure 7 shows the overlay of all structures used in the project. In Ramachandran graph, the regions represented in red are the most favorable, those highlighted in yellow are the favorable regions, those represented in beige are the least favorable and the blank regions are unfavorable. The data collected in relation to these regions are described in Table 1.

3. RESULTS AND DISCUSSION

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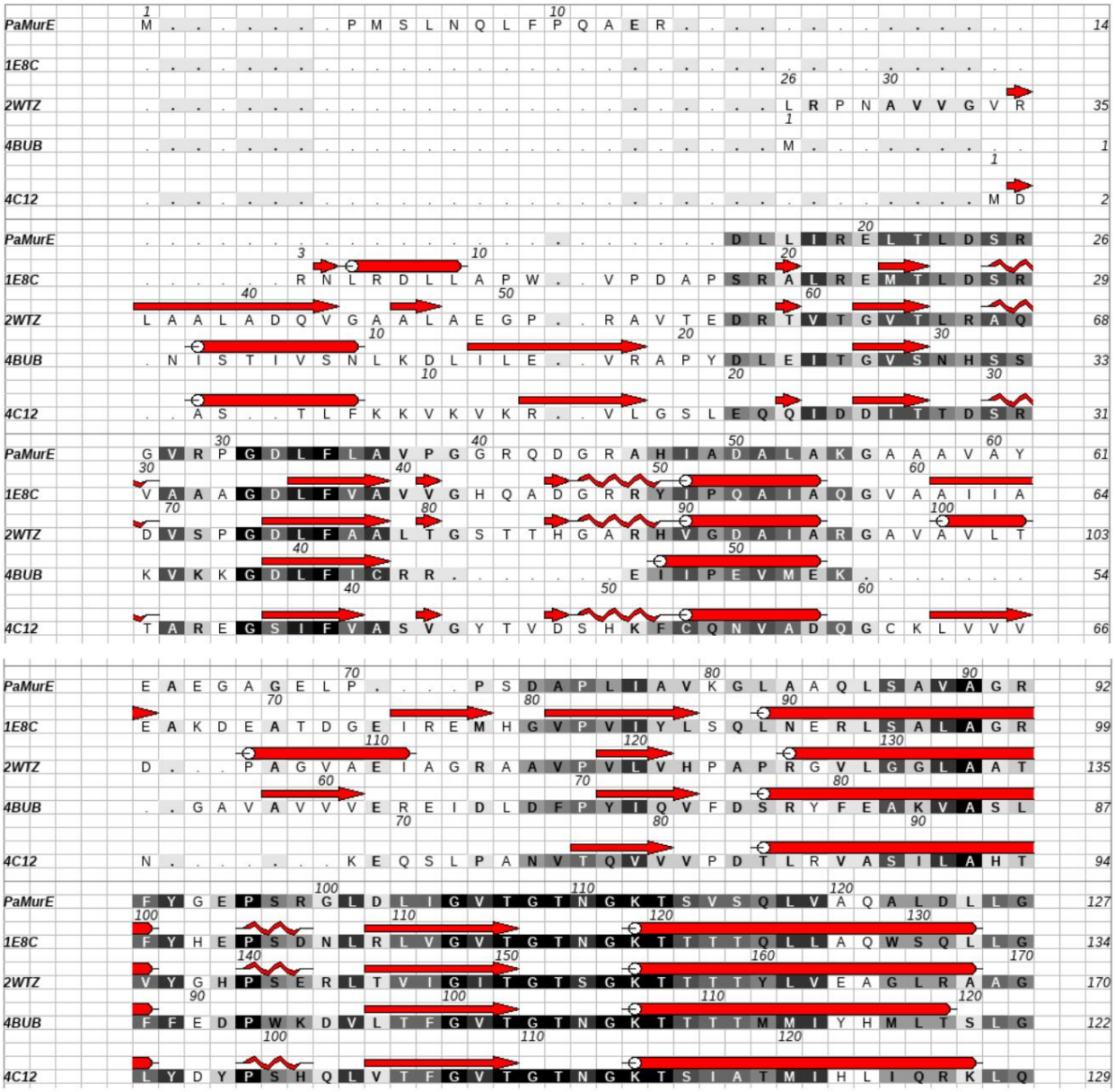

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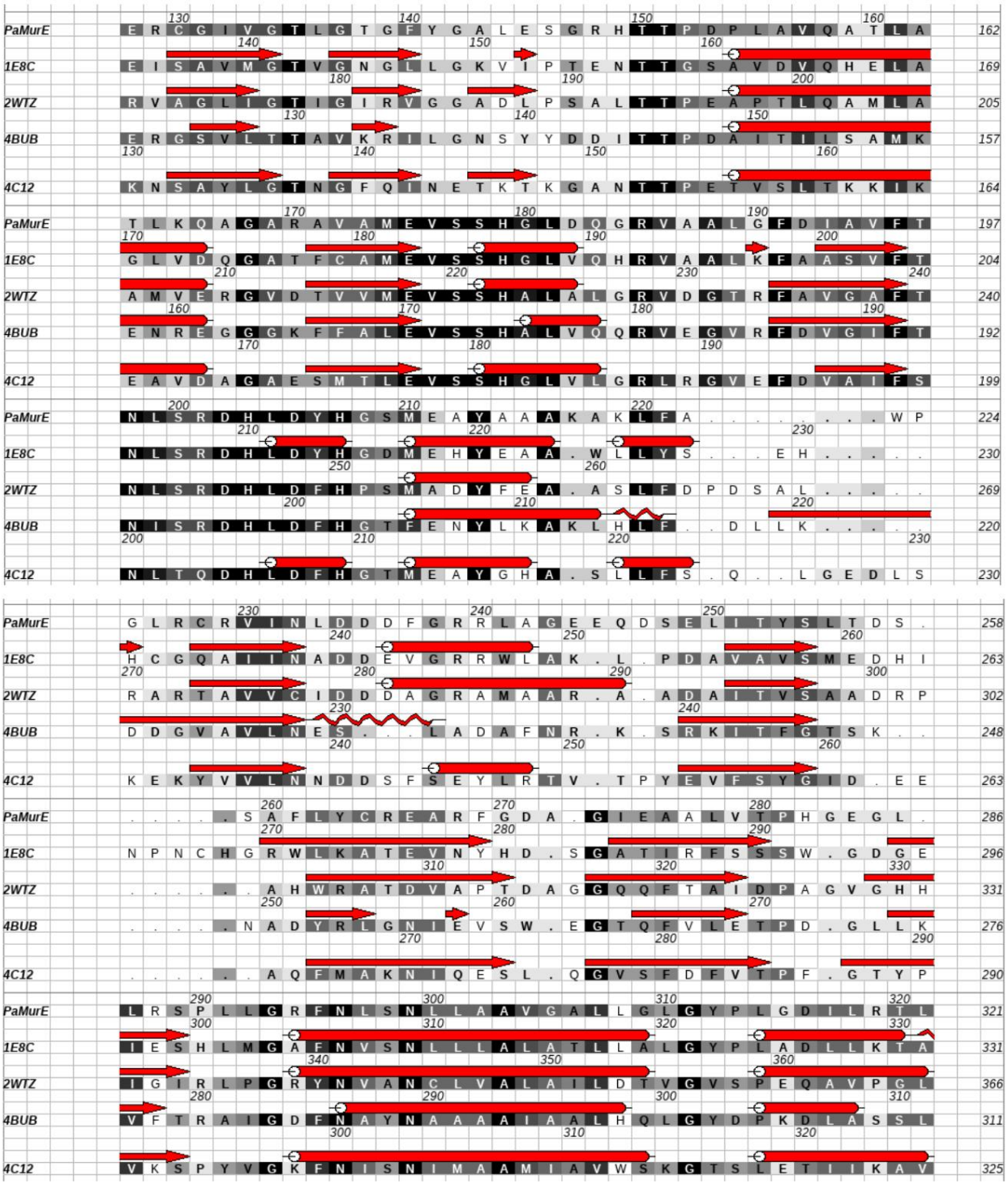

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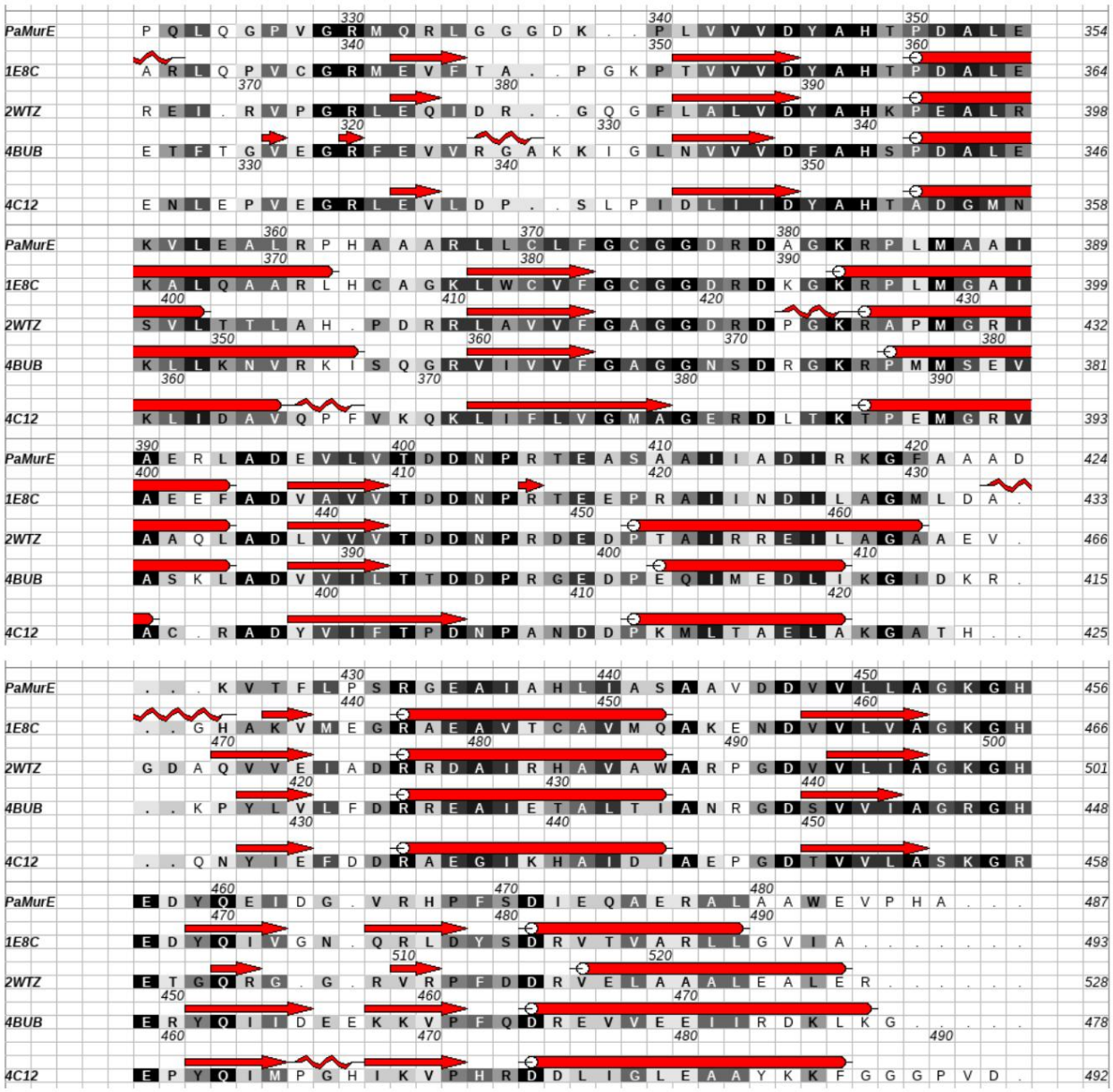

Figure 1. The ALSCRIPT Calcon tool in the Aline program allows you to identify the most and least conserved regions. Conserved regions are highlighted in black, while less conserved regions are colored light gray. The secondary structure of each protein is represented by cylinders (alpha helix) and arrows (beta strand), and helix 310 can also be seen.

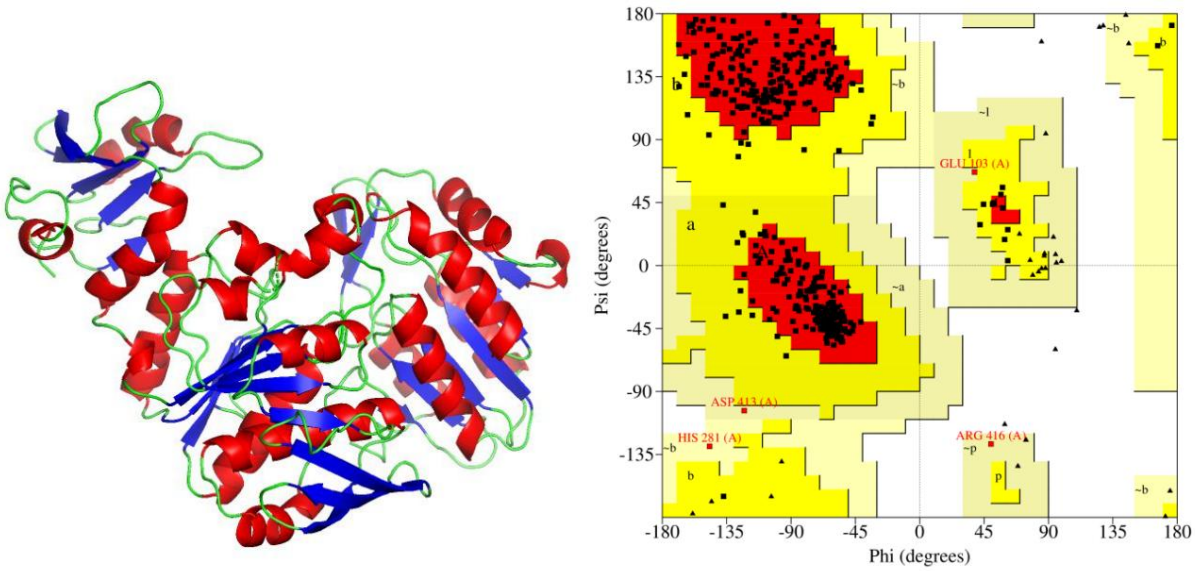

Figure 2. Three-dimensional structure of MurE from *E. coli* (PDB: 1e8c) obtained by the PyMOL program and its respective Ramachandran plot.

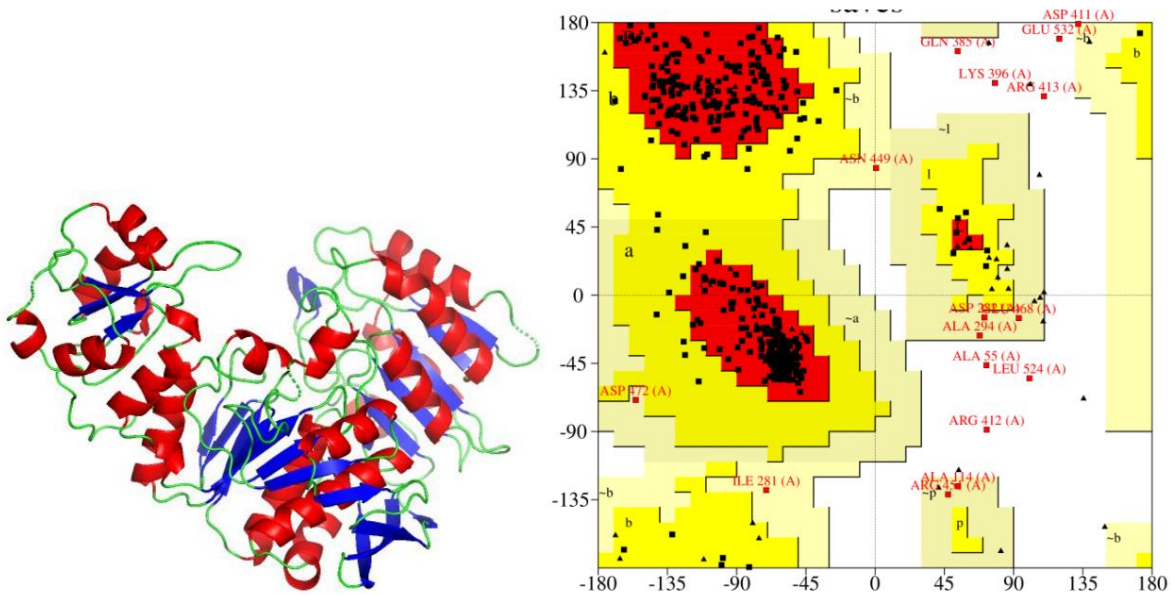

Figure 3. Three-dimensional structure of MurE from *M. tuberculosis* (PDB: 2wtz) obtained by the PyMOL program and its respective Ramachandran plot.

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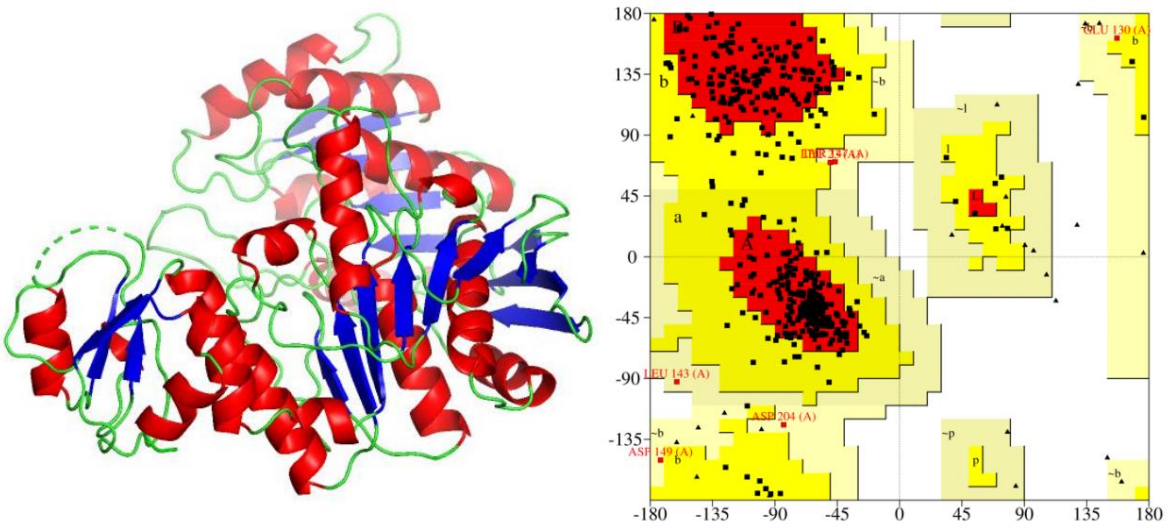

Figure 4. Three-dimensional structure of MurE from *T. maritima* (PDB: 4bub) obtained by the PyMOL program and its respective Ramachandran plot.

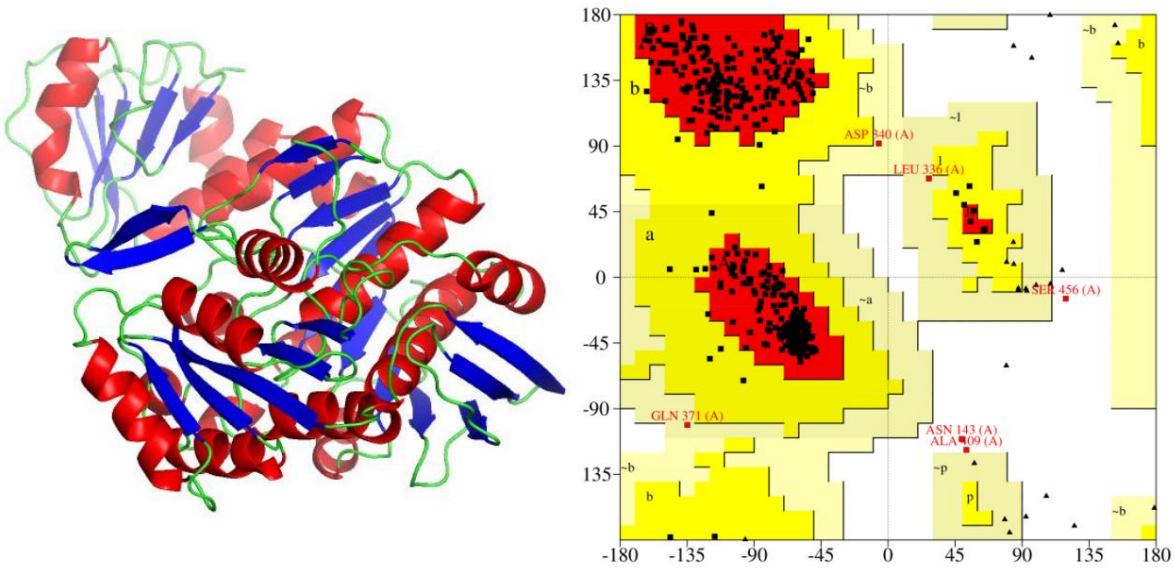

Figure 5. Three-dimensional structure of MurE from *S. aureus* (PDB: 4c12) obtained by the PyMOL program and its respective Ramachandran plot.

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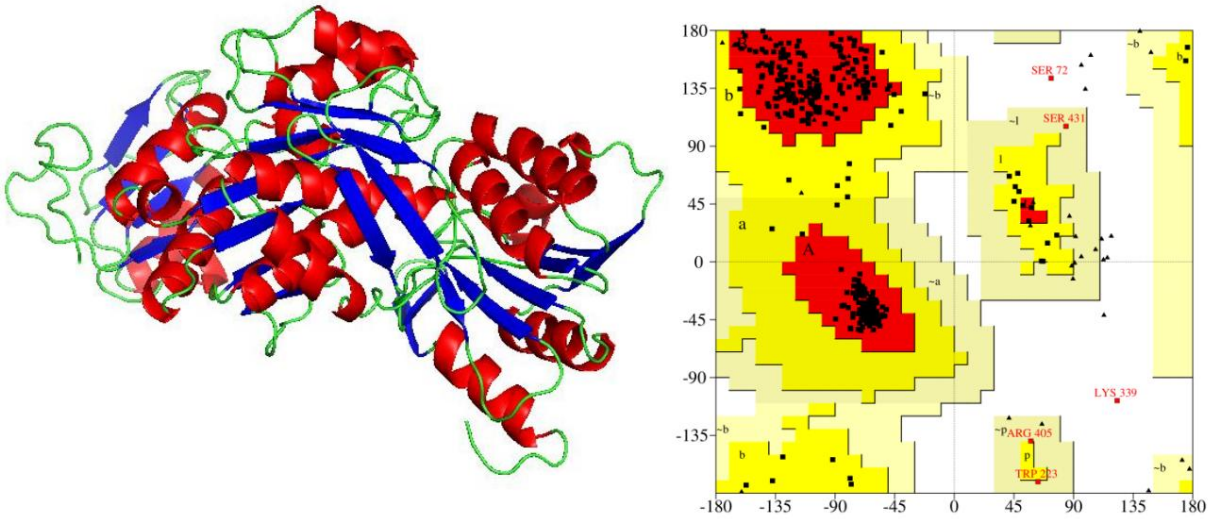

Figure 6. Three-dimensional structure of the best theoretical model obtained from PaMurE under study, obtained by the PyMOL program, and its respective Ramachandran graph.

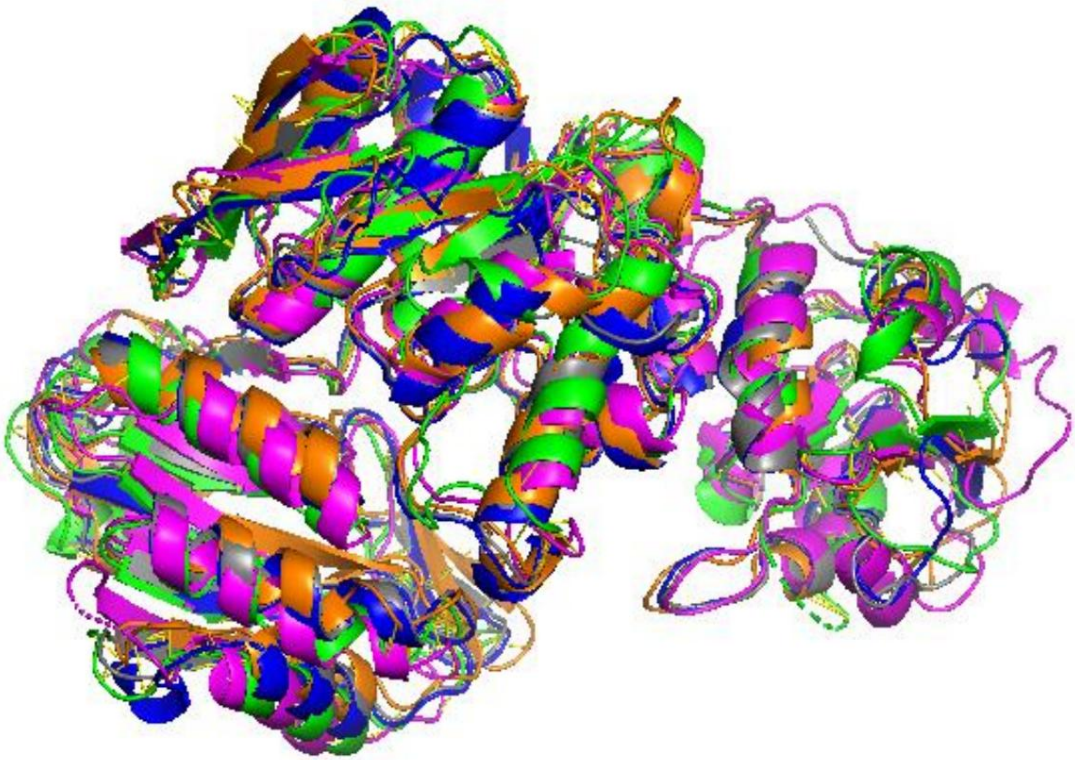

Figure 7. Overlay of all structures involved in the study (blue: *E. coli;* magenta: *M. tuberculosis;* green: *T. maritima;* orange: *S. aureus;* gray: *P. aeruginosa* (theoretical model)). It is observed that the overlap indicates distinct regions between the proteins, which is expected, since in certain regions the amino acids are different, providing different hydrophilic and hydrophobic interactions, causing specific twists in the structures.

Table 1. Ramachandran graph. Percentage values refer to each of the homologs included in the study in addition to PaMurE.

Graph region of Ramachandran / %	Percentage of waste				
	PaMurE	*E. coli*	*S. aureus*	*T. maritima*	*M. tuberculosis*
More favorable	90.9	90.9	94	79.8	86.7
Additionally permitted	7.8	8.2	4.6	18.8	9.4
Generously allowed	0.7	0.9	0.7	1.4	2.2
Not allowed	0.5	0	0.7	0	1.7

Using the Ramachandran graph, it can be seen that more than 90% of the residues are correctly positioned in the PaMurE model, which shows good reliability of the best model obtained in the alignment.

According to Ruane *et al.* (2013), the active binding site of UMT, the final product of the reaction mediated by the MurE enzyme, is represented by residues R383, D406 and E460 in *S. aureus* and by residues R389, D413, N414, R416 and E468 in *E. coli.* On the other hand, Gordon *et al.* (2001) cite the following residues responsible for the active site of the same compound in *E. coli:* S28, H43, Q44, A45, N156, Q190, R192, S184, T157, T158, R389, D413, N414, R416 and E468 and state that binding to UMT makes many polar bonds with the protein.

According to the study by Paradis-Bleau *et al.* (2009), most of the amino acid residues that form the active site of MurE in both *P. aeruginosa* and *E. coli* are conserved, with the exception of a loop at positions 42-47 in MurE from *E. coli.* This region interacts with the pyrophosphate of the product of the compound UDP-MurNAc-Ala-Glu-2-diaminopimelic acid. Furthermore, N156 is replaced, in *P. aeruginosa,* by H150, that is, a change from a neutral polar amino acid to a basic polar one.

FINAL CONSIDERATIONS

The structural information gained from the model will be useful, particularly regarding the amino acids that interact with the substrates, during subsequent steps related to the selection of ligands for the molecular *docking* stage . This will yield a better set of responses regarding more effective interactions that ensure adequate enzyme inactivation. The aim is to provide a basis for subsequent studies regarding the development of new antimicrobials targeting the MurE enzyme, thus providing more effective alternatives for combating hospital-acquired infections caused by *P. aeruginosa.*

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