In silico Analysis for Laccase-mediated Bioremediation of the Emerging Pharmaceutical Pollutants

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Abstract: *Laccases, a copper oxidase enzyme, has been employed for bioremediation of anthropogenic pollutants in the recent past. Laccase has a broad range of substrate specificity which offers the prospect for screening in numerable xenobiotics. The present study was aimed to use protein-ligand docking as a tool for prediction of biodegradation of selected pharmaceutical pollutants. A comparative study was also done to determine the binding efficacy of bacterial and fungal laccase for those selected pollutants. The laccase-pollutant docking was carried out using HEX software. The docking scores of bacterial and fungal laccase for predefined pollutants were comparable to ABTS, a substrate for laccase, which suggested that laccase might be able to degrade emerging pharmaceutical pollutants. The docking analysis approach can be useful in prediction of binding competence of pharmaceutical pollutants with laccase for in situ laccase-mediated bioremediation.*

Keywords: *Laccase, Bioremediation, Docking, Pollutants.*

Introduction

Pharmaceutical pollutants are anthropogenic chemical pollutants which contaminate the environment through discharges from pharmaceutical industries, wastewater treatment plants, medical care units, domestic and irrigation sewage [13]. The pharmaceutical pollutants mostly comprise of antibiotics, stimulants, steroids, antidepressants, analgesics, anti-inflammatories, antipyretics, beta-blockers, lipid regulators, contrast media and diuretics [3]. These pollutants can be transportable and persistent in ecosystems even at low concentrations. The geochemically stable micro pollutants can potentially lead to long-term effects like bio-accumulation, scarcity of drinking water and development of drug-resistant microorganisms, which poses a severe threat to aquatic and terrestrial organisms [8]. Therefore, there is an urgent need for innovative and effective treatments for the eradication of these emerging pharmaceutical pollutants. Bioremediation is one of the most efficient processes to remove chemical pollutants [10]. Several micro-organisms have been employed for bioremediation owing to their capacity for degrading hazardous chemicals. Researchers have exploited several microbial enzymes for the degradation of contaminants. The laccase enzymes, which are obtained from fungi, bacteria and plants, have been extensively studied for the bioremediation [15]. In recent years, these enzymes have gained its application in pulp and paper, textile and food industries and development of biosensors for environmental remediation [14]. In this context, we report *in silico* docking studies of emerging pharmaceutical pollutants as potential targets for bioremediation by bacterial and fungal laccase enzymes.

Methodology

Preparation of target proteins

The crystallographic tertiary structure of the laccase proteins, namely, fungal laccase from *Trametes versicolor* (PDBID: 1GYC) [5] and bacterial endospore coat laccase from *Bacillus subtilis* (PDBID: 3ZDW) [11] were retrieved from the RCSB protein data bank. The obtained tertiary structure of fungal laccase (length: 499 amino acids) and the bacterial laccase (length: 513 amino acids) have been determined using X-ray diffraction at a resolution of 1.9 Å and 2.4 Å respectively. The associated non-protein ligands from both proteins were deleted from the protein-ligand complex using Chimera v1.8 [12]. The Dock Prep tool of Chimera was further used to complete the receptor preparation. The prepared laccase enzymes were further energy minimized in Chimera and saved in .pdb format for docking studies. The Ramachandran plots were generated using RAMPAGE server [9].

Preparation of ligands

Seven emerging pharmaceutical pollutants (roxithromycin, clarithromycin, indomethacin, bezafibrate, metoprolol, celiprolol and iopromide) were selected from U.S. Environmental Protection Agency (EPA) List [2]. 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was used as positive control for docking score analysis [7]. The 2D structures of all the ligands were drawn and converted to 3D structure in ChemDraw Ultra v12.0. All the 3D structures were energy minimized using MM2 force field module of ChemDraw software. The energy minimized ligand structures were saved in .pdb format for docking studies.

Docking studies

The energy optimized ligand structures were docked one by one with fungal and bacterial laccase respectively, using HEX software [4, 15] and docked complexes were visualized in PyMol. The parameters used for the docking of laccases with ligands were as follows: Correlation type: Shape only, FFT Mode: 3D, Calculation device: CPU, Grid Dimension: 0.6, Ligand and Receptor range angle: 180, Step size: 7.5, Number of solutions: 2000.

Results and discussion

Laccase belongs to the small group of metallo-proteins called the blue copper oxidase [1]. Fig. 1 shows the X-ray crystallographic structures of the fungal (Fig. 1A) and bacterial laccase (Fig. 1C) obtained after structural refinement and energy minimization using Chimera along with their respective Ramachandran plots, as predicted by RAMPAGE server. The fungal laccase was structurally compared to bacterial laccase using RCSB PDB Protein Comparison Tool. The pair wise sequence alignment revealed that the fungal laccase has ~ 24% identity and ~ 41% similarity with bacterial laccase with highly conserved copper binding motifs (see Appendix). Thus, it was observed that the tertiary structure of the both laccase enzymes differs from each other and this may result in binding efficacy to the same ligand. The Ramachandran plots (Fig. 1B and Fig. 1D), revealed that 97.4% of the amino acid residues of the fungal laccase and 95.6% of the amino acid residues of the bacterial laccase were in the favored region. This finding suggested that the optimized X-ray crystallographic .pdb files for both laccases resulted in good quality receptors for docking operation.

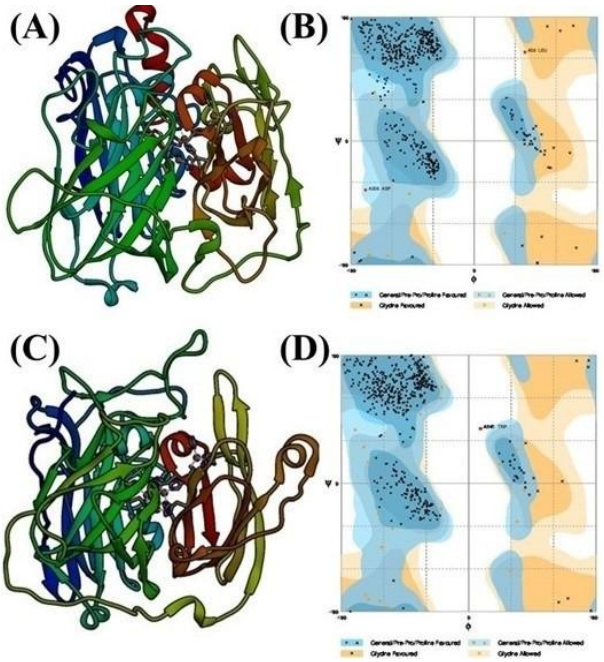

Fig. 1 (A) Tertiary structure of fungal laccase from *Trametes versicolor*;
(B) The Ramachandran Plot for fungal laccase;
(C) Tertiary structure of bacterial laccase from *Bacillus subtilis*;
(D) The Ramachandran Plot for bacterial laccase.

Following this, the selected laccase enzymes and ligand datasets were docked using HEX software. ABTS, a standard substrate for laccase assay, was first docked with both the laccases. The resulting HEX docking scores (E-value) were –328.4 kcal/mol and –313.69 kcal/mol for fungal (Fig. 2A) and bacterial laccase (Fig. 2B) respectively. ABTS was used for comparative check in binding affinity of the pharmaceutical pollutants with laccase protein.

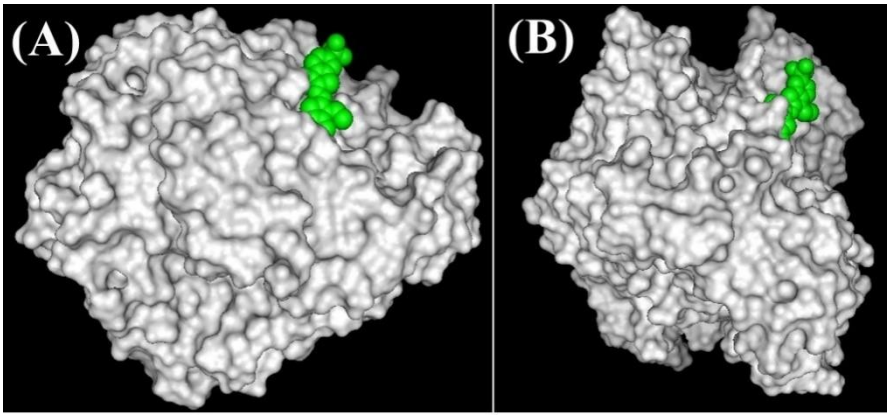

Fig. 2 The interaction of ABTS (green) with (A) fungal laccase and
(B) bacterial laccase (as viewed in PyMol)

Roxithromycin, clarithromycin, indomethacin, bezafibrate, metoprolol, celiprolol and iopromide were docked with bacterial and fungal laccase using HEX software and the docking results were tabulated in Table 1. The chemical structures of the docked molecules and the laccase-pollutant complexes have been enlisted in the Supporting Information.

Table 1. Result of the docking analysis of the emerging pharmaceutical pollutants with fungal and bacterial laccase

		Docking scores, (kcal/mole)	
Category	**Molecules**	**Fungal laccase**	**Bacterial laccase**
Control	ABTS	**–328.4**	**–313.69**
Pharmaceutical pollutants			
Antibiotic	Roxithromycin	–396.7	–417.72
Antibiotic	Clarithromycin	–380.5	–421.77
Anti-inflammatory	Indomethacin	–294.0	–290.90
Lipid regulator	Bezafibrate	–304.7	–282.10
Beta blocker	Metoprolol	–263.6	–263.79
Beta blocker	Celiprolol	–299.7	–311.46
Contrast media	Iopromide	–337.7	–359.24

The laccase enzymes of bacterial and fungal origin were docked efficiently with selected (Table 1) molecules and on the basis of energy value (E-value) score it was observed that for both the laccases the E-value scores of Roxithromycin, Clarithromycin and Iopromide were lower than the E-value score of ABTS. A minor variance in docking scores was noted for fungal and bacterial laccase with the same ligand. This was due to the difference in their 3D structure, which leads to alteration of ligand-binding efficacy. Thus, out of the selected seven pharmaceutical pollutants, Roxithromycin, Clarithromycin and Iopromide, may be degraded by both fungal and bacterial laccase. The remaining four pollutants (Indomethacin, Bezafibrate, Metoprolol and Celiprolol) have marginally greater E-value than ABTS molecule, suggesting that the bacterial and fungal laccases may not act as effective bioremediation agents in case of these pollutants. In addition to this, E-value of Roxithromycin, Clarithromycin and Iopromide in case of docking with bacterial laccase were lower than their E-value scores with fungal laccase, suggesting that bacterial laccase may be more effective for *in situ* removal of these pharmaceutical pollutants. In addition to these seven pollutants, analogous docking studies with laccase can also been carried out in order to inspect various enzyme-pollutant systems for bioremediation [16].

Conclusion

The identification of new enzymes is essential for the development of more precise bioremediation of emerging pharmaceutical contaminants. The aim of the study was to identify the potential of laccase enzyme as the bioremediation tool against emerging pharmaceutical pollutants. Docking results indicated that these emerging pollutants can bind to fungal and bacterial laccase enzymes. In preview of *in silico* docking analysis, pre-treatment of effluents from drug industries could be carried out for efficient bioremediation using laccase enzyme.

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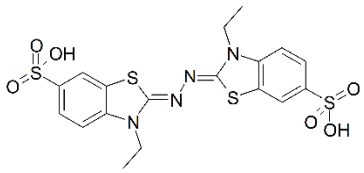
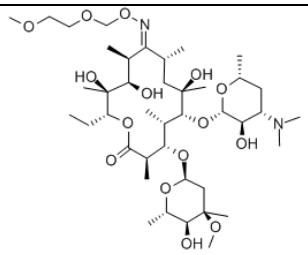
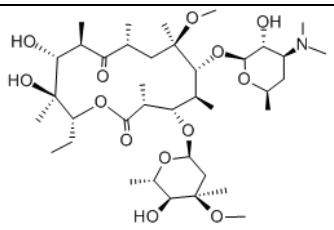
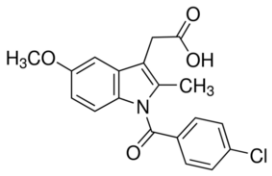
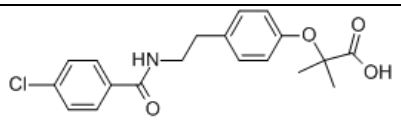
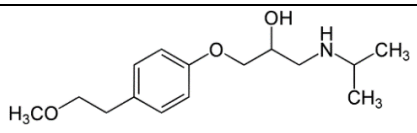
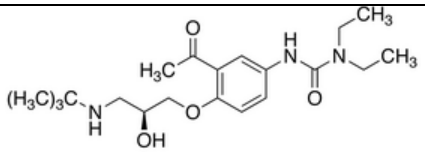
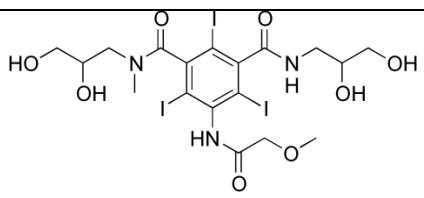
APPENDIX

Table A1. Chemical structure of the pharmaceutical pollutants used in this study

Serial No.	Molecules	Chemical structure (image source: www.wikipedia.org)
1.	ABTS	
2.	Roxithromycin	
3.	Clarithromycin	
4.	Indomethacin	
5.	Bezafibrate	
6.	Metoprolol	
7.	Celiprolol	
8.	Iopromide	

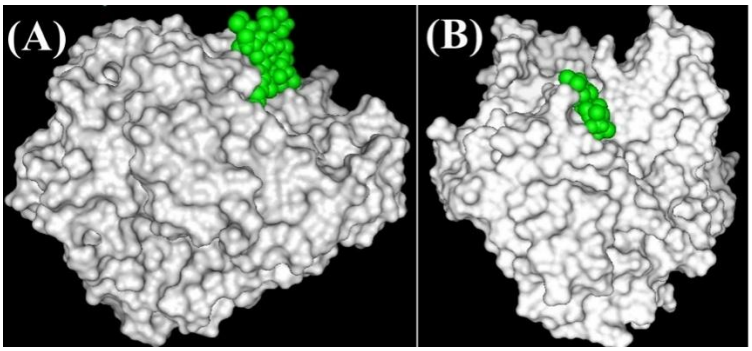


Fig. A1 Roxithromycin (green) docked with (A) fungal laccase and (B) bacterial laccase

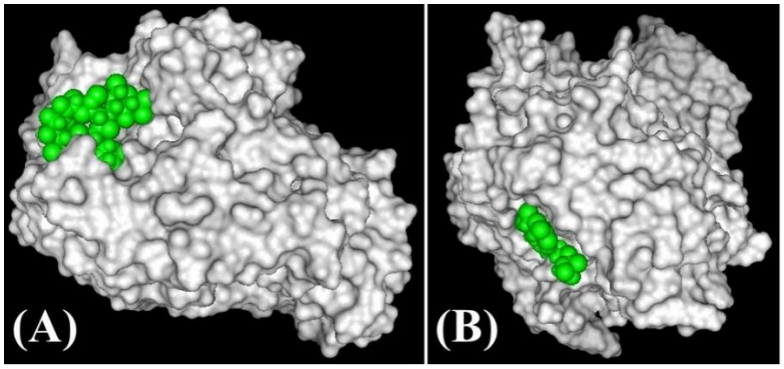


Fig. A2 Clarithromycin (green) docked with (A) fungal laccase and (B) bacterial laccase

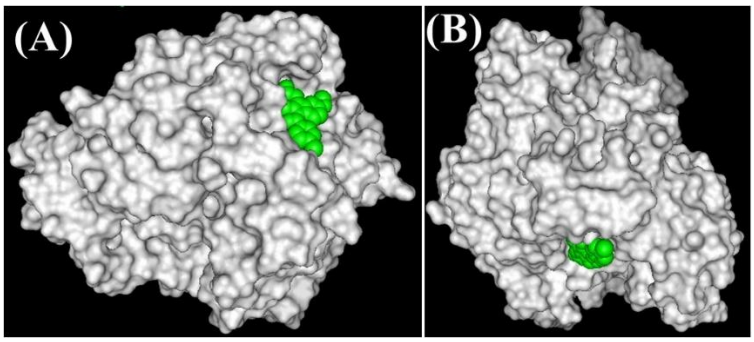


Fig. A3 Indomethacin (green) docked with (A) fungal laccase and (B) bacterial laccase

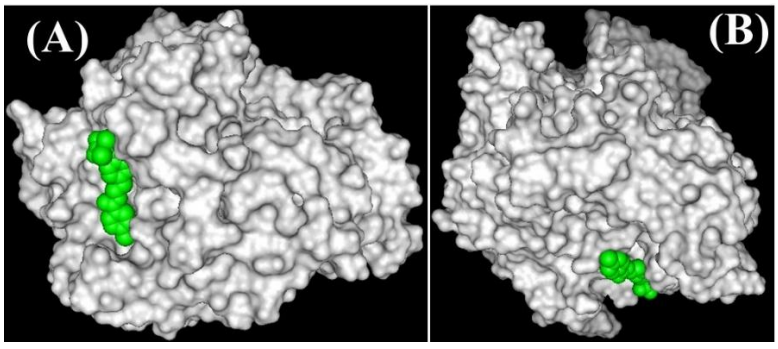


Fig. A4 Bezafibrate (green) docked with (A) fungal laccase and (B) bacterial laccase

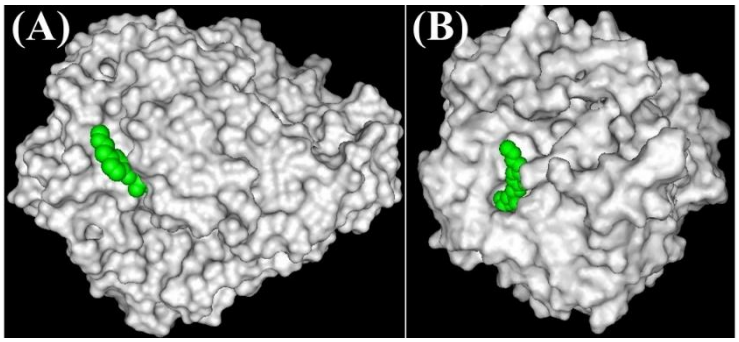


Fig. A5 Metoprolol (green) docked with (A) fungal laccase and (B) bacterial laccase

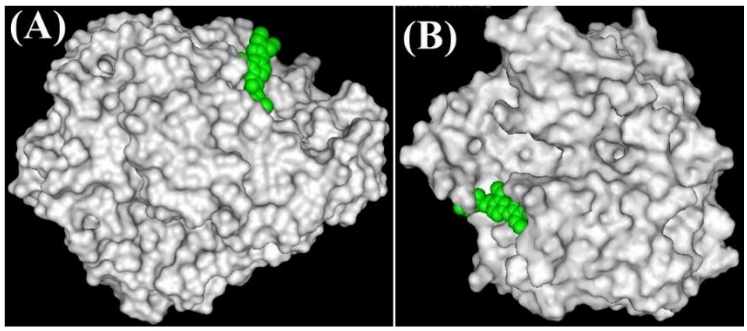


Fig. A6 Celiprolol (green) docked with (A) fungal laccase and (B) bacterial laccase

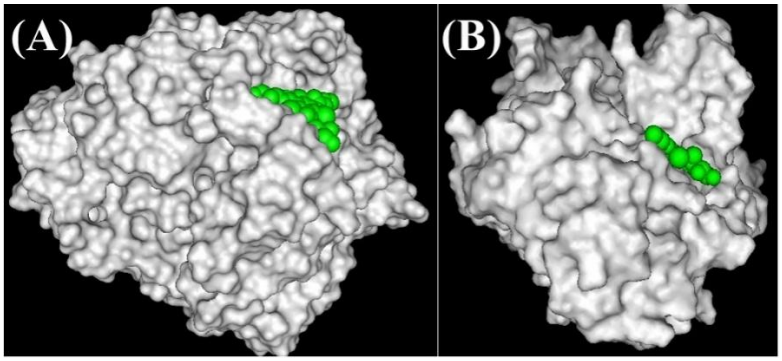


Fig. A7 Iopromide (green) docked with (A) fungal laccase and (B) bacterial laccase

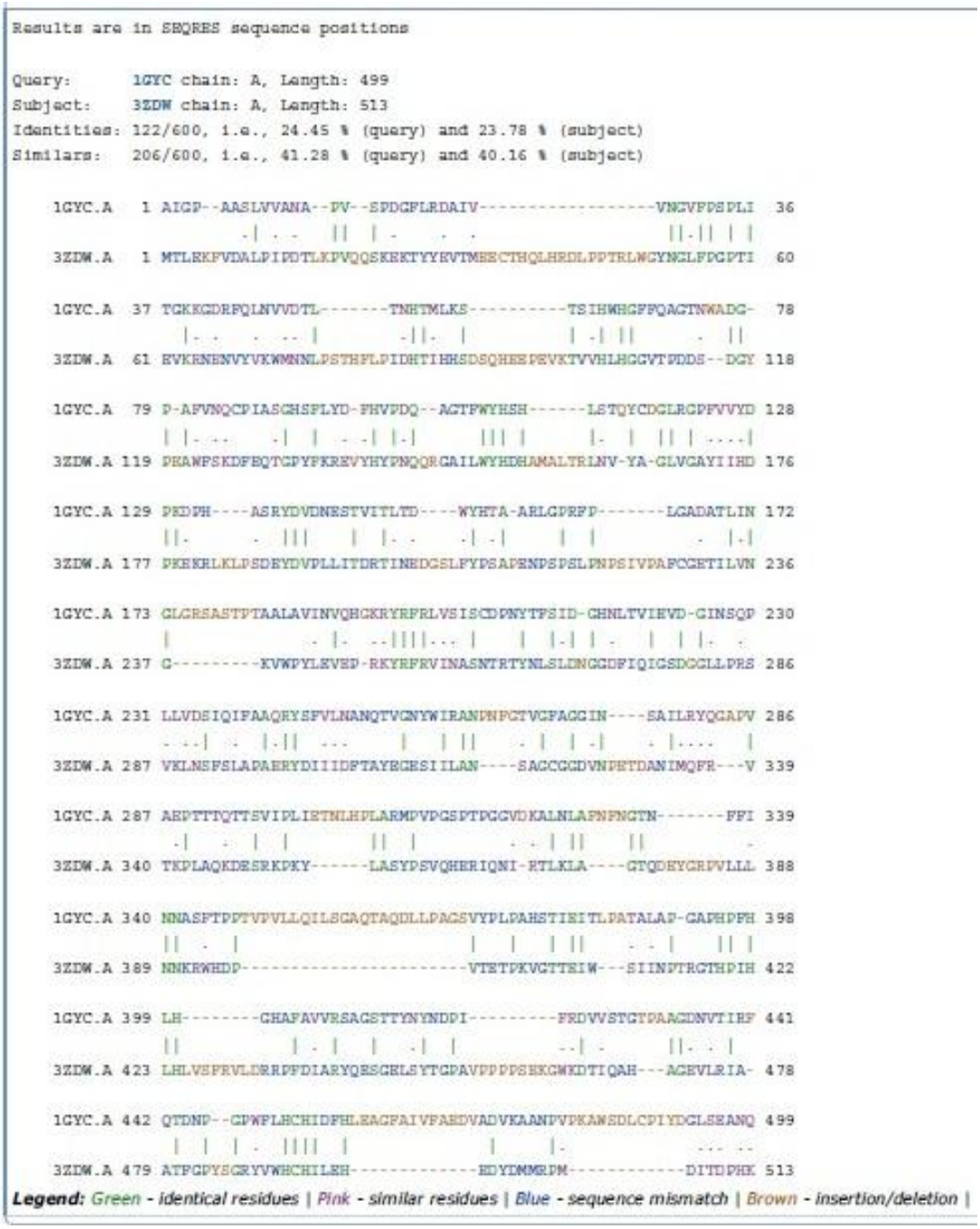


Fig. A8 The fungal laccase was structurally compared to bacterial laccase using RCSB PDB Protein Comparison Tool. The pairwise sequence alignment revealed that the fungal laccase has ~ 24% identity and ~ 41% similarity with bacterial laccase.

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