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Applications of Escherichia Coli Esterases for Bioremediation and Treatment of Wastewater Organic Chemical Pollutants

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Abstract: ***This study used computational techniques, including 3D enzyme structural modeling and molecular docking, to gain insight into the bioremediation of organic wastewater contaminants using E. coli esterase enzymes. Furthermore, a total of 24 wastewater organic chemicals belonging to different categories, such as pharmaceuticals, artificial sweeteners, pesticides/herbicides, endocrine disrupting chemicals, and persistent organic pollutants, were identified through the toxicology database. Comparative genetics and reported literature. Furthermore, 3D PDB and AlphaFold structures of 194 esterase enzymes from E. coli were retrieved by first identifying a common domain (Alpha Beta hydrolase domain) using the InterPro database. Molecular docking of esterase enzymes and pollutants was used, resulting in the best binding enzymes to their respective organic wastewater pollutants, including bezafibrate which showed the best binding with all enzymes ranging from -6.33 kcal/mol to -9.87 kcal/mol . Subsequently, the majority of the ligands (organic wastewater pollutants) reacted with enzymes such as the ORFC-like enzymes, which were computationally annotated in this study for the first time, yuaR (strain K12), menH (strain ETI89/UPEC), and menH (strain O157). :H7) has significant binding affinities and consists of a common Alpha Beta hydrolasefold-1 domain. This suggests that esterase enzymes containing the Alpha Beta hydrolasefold-1 domain may be involved in the efficient degradation of organic wastewater pollutants.***

Keywords: Bioremediation, Organic Wastewater Pollutants, E. Coli.

1. INTRODUCTION

Wastewater pollutants are considered an important environmental issue. They come from industrial, agricultural, and domestic sources. When not treated properly, they can harm ecosystems and human health (1). They are mainly categorized into physical, chemical, and

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biological contaminants. Different types of wastewater pollutants are suspended solids, heavy metals, toxins, nutrients (like nitrogen and phosphorus), and pathogens (bacteria, viruses, and parasites) (2) (3).

Organic pollutants that include oils, fats, proteins, and carbohydrates are especially problematic because of their pervasive nature and dangerously harmful environmental impact (4). Organic wastewater pollutants mainly originate from food processing, agricultural runoff, and sewage (5). These pollutants use up oxygen levels in water bodies, causing hypoxic conditions that become a threat to aquatic life (6). In aquatic ecosystems, heavy metals cause the death of aquatic life, algal blooms, and habitat destruction (7). Moreover, the decomposition of organic matter can release harmful by-products, leading to diseases like cholera and dysentery. Wastewater pollutants cause several water-related infections, such as cholera, typhoid fever, and diarrhea (8).

Heavy metals in wastewater have several environmental and health impacts. Humans can be exposed to heavy metals through inhalation, ingestion, and vapourization, resulting in severe effects such as reduced growth and development, cancer, organ damage, nervous system damage, and even death (7). Waterborne pathogens in industrial wastewater produce toxins that can lead to poisoning, immune system damage, and reproductive issues. According to the World Health Organization (WHO), about 80% of diseases are waterborne. Proper physical, chemical, and biological treatments are necessary to address these problems, and wastewater recycling is needed (9).

2. LITERATURE REVIEW

Traditional and long-used wastewater treatment methods include chemical treatment, filtration, and sedimentation. Although they are used all over, they can be expensive and energy-intensive. Besides, they may not properly eliminate organic contaminants. This has stimulated interest in bioremediation as an alternative approach (10).

Many existing treatment techniques can produce secondary pollutants, which are additional contaminants that may appear during the treatment process; this poses further environmental challenges. Traditional methods might not be as effective as newer approaches, like using adsorbents, which have shown higher efficacy in treating wastewater with minimal secondary pollutant production (11–13).

Bioremediation employs microorganisms to degrade environmental pollutants naturally. It includes in-situ methods, which treat contaminants on-site, and ex-situ methods, which involve removing the contaminated material for treatment elsewhere. Both methods result in microbial activity to transform pollutants into less harmful substances (14). Therefore, bioremediation plays a significant role in environmental sustainability by offering an eco-friendly solution to pollution. It reduces hazardous substances, decreasing their environmental impact. Microorganisms, such as bacteria, fungi, and algae, are crucial in this process (15).

Escherichia coli (E. coli) is a microorganism known for its ability to help clean up pollution (16). It produces Esterases, enzymes that break down organic pollutants by splitting ester bonds. This process aids in the degradation of harmful substances in the environment (17). Moreover, Bhatt, P., et al., conducted an in-silico study and identified a novel *Pseudomonas nitroreducens* (strain CW7) that degraded an insecticide, allethrin, effectively in

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wastewater sludge, mineral salt medium, and soil environments and also has the potential to degrade other synthetic pyrethroids (SPs) (18).

Therefore, the aim of this study is to use bioinformatics techniques such as homology modeling, novel enzyme identification, and molecular docking to explore the use of *E. coli* esterases for the bioremediation of organic wastewater pollutants. By focusing on the degradation capabilities of these enzymes.

3. METHODOLOGY AND MATERIALS

3.1. Retrieval of Organic Wastewater Pollutants: Retrieval of organic wastewater contaminants was performed using a combination of the Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/) (19). The Simplified Molecular Entry Line (SMILES) system for organic wastewater contaminants identified by CTD was retrieved using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), (20). The recovered SMILES were converted to 3D structures in pdb format using a custom Python script using the rdkit toolkit (https://www.rdkit.org/). Finally, the recovered organic wastewater contaminants were classified based on their source and function.

3.2. Retrieval of Esterase Enzymes of *E. coli:* A domain common to esterases found in *E. coli* was identified, and used to retrieve enzymes containing the identified domain by searching in InterPro (https://www.ebi.ac.uk/interpro/), (21). UniProt IDs, enzyme names, sequence lengths, and taxonomy names were retrieved from the InterPro database, while UniProt IDs for E. coli enzymes were specifically used to retrieve PDB or AlphaFold structures using a custom Python script. The recovered PDB structures were subjected to UCSF Chimera (22).

3.3. Loop Modeling and Energy Minimization of Enzyme Structures: The cleaned PDB structures were subjected to loop modeling performed using MODELLER (23). FASTA sequences of the enzymes to be modeled were retrieved from the UniProt database, and the structures of the already retrieved enzymes were then imaged as templates. Alignment of query sequences with template sequences was performed using a Python program, giving an "output.pap" file showing similarity within both sequences. Finally, models were predicted using aligned sequences and enzyme structures, giving a total of 10 predicted models, and the best model was selected based on the lowest discrete protein energy (DOPE) score. The enzyme structures (PDB and AlphaFold) were then subjected to energy minimization using OpenMM (24). CHARMM36 introduction with 100 steps was used to reduce enzyme structures and for virtual screening of organic wastewater contaminants.

3.4. Virtual Screening with Organic Wastewater Pollutants: Virtual screening of recovered enzymes with organic wastewater contaminants was performed by using the GNINA molecular docking tool, which uses convolutional neural networks (CNNs) (25). DiscoveryStudio Visualizer (Biovia, DS (2019) Discovery Studio Visualizer. San Diego) was used to analyze the 2D interactions between enzyme residues and the ligand molecule.

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Functional Annotation of Uncharacterized Enzyme: The enzyme was best annotated using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov), (26). FASTA sequences were searched by UniProtKB/Swiss-Prot(Swiss-prot) and *E.coli* species (taxid:562). The resulting best-aligned enzyme was selected to annotate the best docked uncharacterized enzyme to its closest functional homologue.

4. RESULTS

4.1. Enzymes and Ligands Structure Retrieval: A literature review conducted showed a common domain, the Alpha/Beta hydrolase domain, among different esterase enzymes, such as *frsA*, *yjfP*, and *ybfF*, found in *E. coli*. This domain was utilized and showed a total of 194 enzymes on the InterPro database containing the Alpha/Beta hydrolase domain. A total of 186 AlphaFold structures, while 8 PDB structures (PDB IDs: 1M33, 1U2E, 2GZS, 3BF7, 4KRX, 4MYD, 5T3D, and 5XB6) were retrieved through the custom Python script. Additionally, the BLAST search of the uncharacterized enzyme resulted in the ORFC enzyme as best-aligned with 100% coverage and 99.65% identity with the sequence length of 286 and the UniProt ID: Q99390, therefore functionally annotated the uncharacterized enzyme to ORFC-like enzyme, its closest homolog. which are mentioned in Table 1. The 2D illustration of the ligands is shown in Figure 1.

4.2. Virtual Screening with Organic Wastewater Pollutants: The virtual screening of the enzymes with the organic wastewater pollutants performed using GNINA resulted in a wide range of binding affinities, ranging from -3.37 kcal/mol to -9.87 kcal/mol. The categorized pollutants resulted in various ranges of binding affinities with the enzyme.

4.3. Pharmaceuticals: The wastewater pollutants from pharmaceutical industries, such as fenofibrate, bezafibrate, clonazepam, medazepam, diclofenac, and clofibric acid, exhibited binding affinities ranging from -6.07 kcal/mol to -9.28 kcal/mol, -6.33 kcal/mol to -9.87 kcal/mol, -5.98 kcal/mol to -9.71 kcal/mol, -5.56 kcal/mol to -8.03 kcal/mol, -4.96 kcal/mol to -8.25 kcal/mol, and from -5.03 kcal/mol to -7.45 kcal/mol, respectively. The fenofibrate, bezafibrate, clonazepam, medazepam, diclofenac, and clofibric acid showed the best affinities with ORFC-like enzyme (E. coli), *yheT* (strain K12), *menH* (strain UTI89 / UPEC), *aes* (strain SMS-3-5 / SECEC), *menH* (strain SMS-3-5 / SECEC), and *yuaR* (strain K12), respectively. The ORFC-like enzyme-fenofibrate, *yheT*-bezafibrate, *menH*-clonazepam, *aes*-medazepam, *menH*-diclofenac, and *yuaR*-clofibric acid complexes are shown in Figure 2.

4.4. Artificial Sweeteners: The artificial sweeteners such as saccharin, acesulfame, and sucralose exhibited binding affinities ranging from -4.25 kcal/mol to -6.65 kcal/mol, -4.83 kcal/mol to -7.57 kcal/mol, and from -5.42 kcal/mol to -8.47 kcal/mol, respectively. The saccharin showed the best affinity with aes (strain SMS-3-5 / SECEC), acesulfame showed the best binding affinity with ORFC-like enzyme (E. coli) enzyme, and sucralose exhibited the best affinity with menH (strain UTI89 / UPEC). The aes-saccharin, menH-sucralose, and ORFC-like enzyme-triclocarban complexes are shown in Figure 2.

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Table 1. The retrieved organic wastewater pollutants, along with their categories

Categories	Pollutants
Pharmaceuticals	Medazepam
	Diclofenac
	Fenofibrate
	Clonazepam
	Clofibric acid
	Bezafibrate
Artificial Sweeteners	Saccharin
	Acesulfame
	Sucralose
Pesticides/Herbicides	Chlorpyrifos
	Diazinon
	Terbutylazine
	Atrazine
	Phorate
	Prometryn
Endocrine Disrupting Chemicals	Bisphenol A (BPA)
	Triclosan
	Triclocarban
	4-Nonylphenol
	4-tert-Octylphenol
	3,3',5-Trichlorobisphenol A
	2,6-Dichloro-4-nonylphenol
Persistent Organic Pollutants	Dieldrin
	Aldrin

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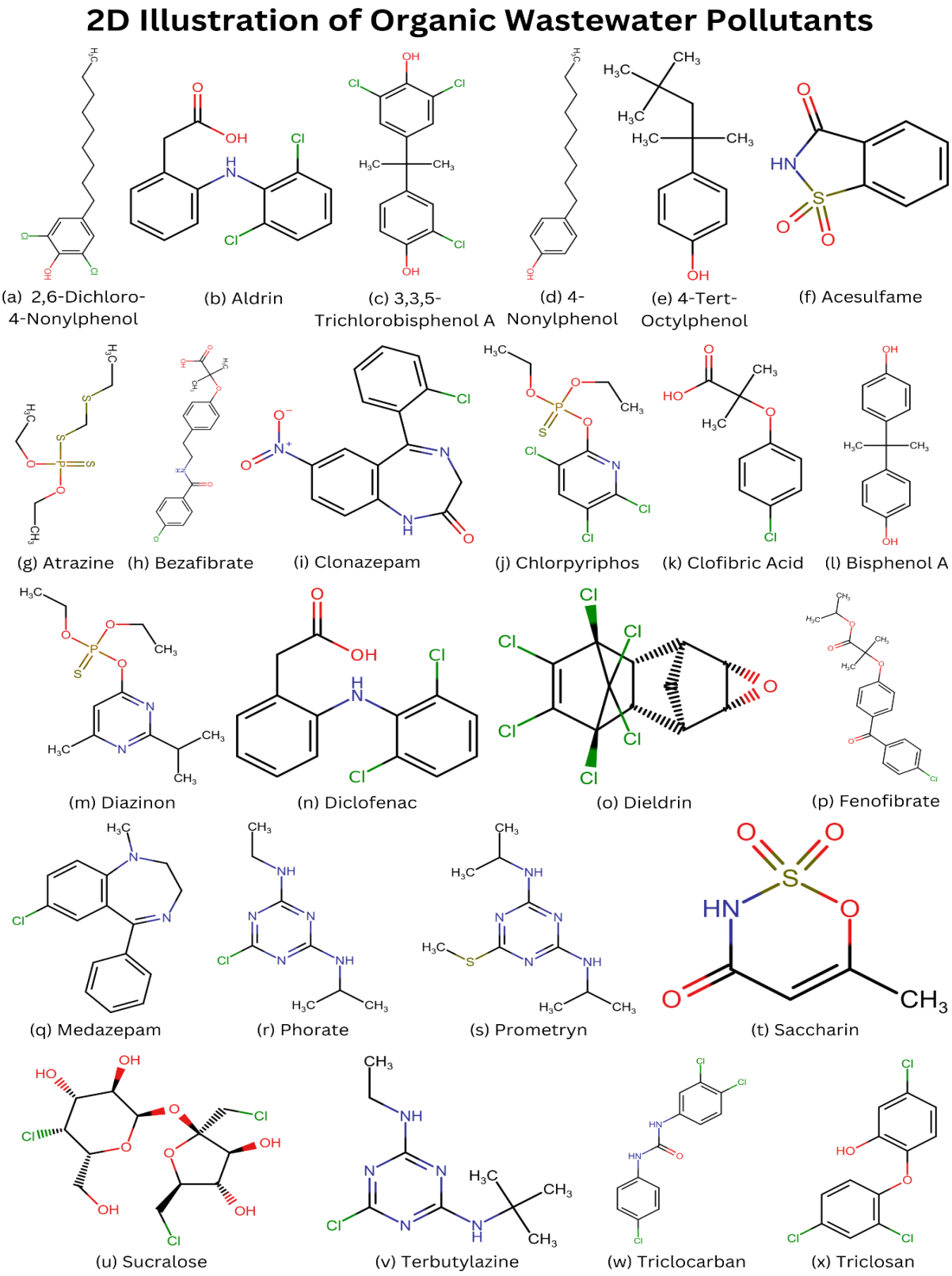

Figure 1. 2D illustration of the organic wastewater pollutants

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4.5. Pesticides/Herbicides: The pesticides/herbicides phorate, chlorpyrifos, atrazine, diazinon, prometryn, and terbutylazine exhibited binding affinities ranging from -4.45 kcal/mol to -6.43 kcal/mol, -4.61 kcal/mol to -6.71 kcal/mol, -3.37 kcal/mol to -5.12 kcal/mol, -4.68 kcal/mol to -7.33 kcal/mol, -4.67 kcal/mol to -6.71 kcal/mol and from -4.62 kcal/mol to -7.09 kcal/mol, respectively. The phorate and atrazine exhibited the best binding affinities with *menH* (E. coli O157:H7), while chlorpyrifos and diazinon showed the best affinities with *menH* (strain UTI89 / UPEC). Subsequently, promethryn and terbutylazine exhibited the best binding affinities with *fes* (strain K12) and *frsA* (strain UTI89 / UPEC), respectively. The *menH*-phorate, *menH*-atrazine, *menH*-chlorpyrifos, *menH*-diazinon, *fes*-prometryn, and *frsA*-terbutylazine complexes are shown in **Figure 2**.

4.6. Endocrine-Disrupting Chemicals: The endocrine-disrupting chemicals, such as bisphenol A, 2,6-dichloro-4-nonylphenol, triclosan, triclocarban, 4-nonylphenol, 4-tert-octylphenol, and 3,3,5-Trichlorobisphenol A exhibited binding affinities ranging from -5.8 kcal/mol to -8.44 kcal/mol, -4.83 kcal/mol to -7.56 kcal/mol, -5.35 kcal/mol to -8.37 kcal/mol, -5.77 kcal/mol to -9.2 kcal/mol, -4.5 kcal/mol to -7.52 kcal/mol, -5.07 kcal/mol to -8 kcal/mol, and from -6.11 kcal/mol to -8.91 kcal/mol, respectively. The best binding affinity enzyme for bisphenol A was *menH* (strain ED1a), while 2,6-dichloro-4-nonylphenol, triclosan, and triclocarban showed the best binding affinity with ORFC-like enzyme (E. coli), 4-nonylphenol and 4-tert-octylphenol showed the best affinity with *yuaR* (strain K12), are shown in Figure 3.

4.7. Persistent Organic Pollutants: The persistent organic pollutants such as aldrin and dieldrin showed binding affinities ranging from -5.32 kcal/mol to -8.36 kcal/mol and from -5.05 kcal/mol to -8.27 kcal/mol, respectively. The aldrin and dieldrin showed the best affinity with *menH* (strain E. coli O157:H7) and *menH* (strain SMS-3-5 / SECEC), respectively. The *menH*-aldrin and *menH*-dieldrin complexes are shown in Figure 3.

Lastly, it was observed that atrazine (pesticides/herbicides) exhibited the weakest affinities with all enzymes (-3.37 kcal/mol to -5.12 kcal/mol), while bezafibrate (Pharmaceutical pollutant) showed the strongest and best affinities with all enzymes ranging from -6.33 kcal/mol to -9.87 kcal/mol. The binding affinity ranges and best binding affinities of the ligands with their respective enzymes, along with their strains, are mentioned in Table 2.

Table 2. The affinity ranges of the ligands and their best affinity enzymes with their strains

Ligands	Range of Affinities (kcal/mol)	Best Affinity Enzymes	Enzyme Strains
BisphenolA	-8.69 to -5.8	*menH*	ED1a
saccharin	-6.65 to -4.25	*aes*	SMS-3-5/ SECEC
Medazepam	-8.03 to -5.56		
diclofenac	-8.25 to -4.96	*menH*	SMS-3-5/ SECEC
dieldrin	-8.27 to -5.05		

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Promethryn	-6.71 to -4.67	*fes*	K12
Fenofibrate	-9.28 to -6.07	ORFC-like enzyme	*E. coli*
Triclocarban	-9.2 to -5.77		
2,6-dichloro-4-nonylphenol	-7.56 to -4.83		
acesulfame	-7.57 to -4.83		
triclosan	-8.37 to -5.35		
4-Nonylphenol	-7.52 to -4.5	*yuaR*	K12
4-tert-Octylphenol	-8 to -5.07		
clofibricacid	-7.45 to -5.03		
Bezafibrate	-9.87 to -6.33	*yheT*	K12
Clonazepam	-9.71 to -5.98	*menH*	UTI89 / UPEC
chlorpyrifos	-6.71 to -4.61		
diazinon	-7.33 to -4.68		
sucralose	-8.47 to -5.42		
Terbutylazine	-7.09 to -4.62	*frsA*	UTI89 / UPEC
3,3,5-trichlorobisphenolA	-8.91 to -6.11	*entF*	*E. coli* O157:H7
aldrin	-8.36 to -5.32	*menH*	*E. coli* O157:H7
atrazine	-5.12 to -3.37		
phorate	-6.43 to -4.45		

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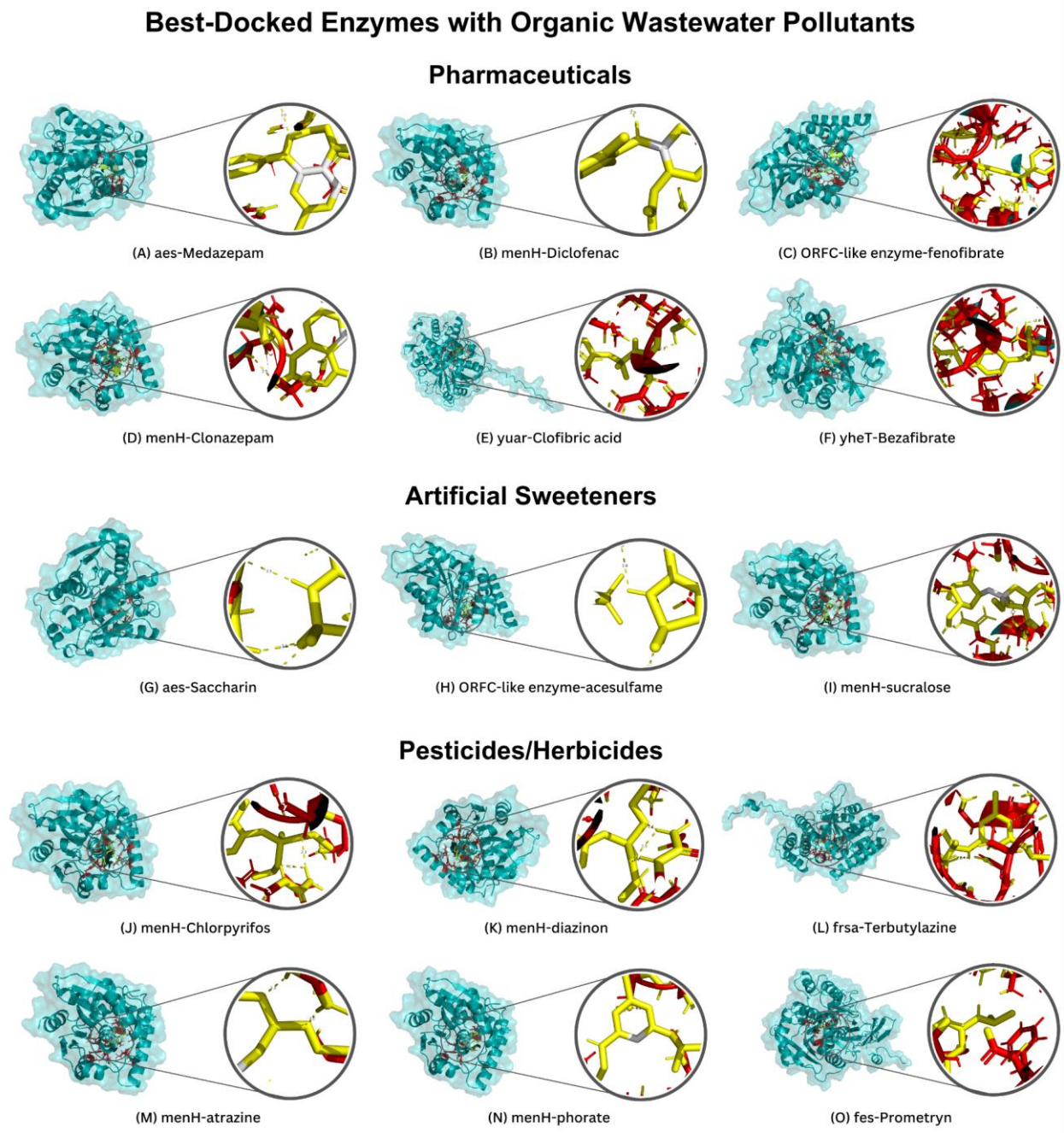

Figure 2. The best-docked enzymes with their respective organic wastewater pollutants, including complexes within the pharmaceuticals, artificial sweeteners, and pesticides/herbicides categories

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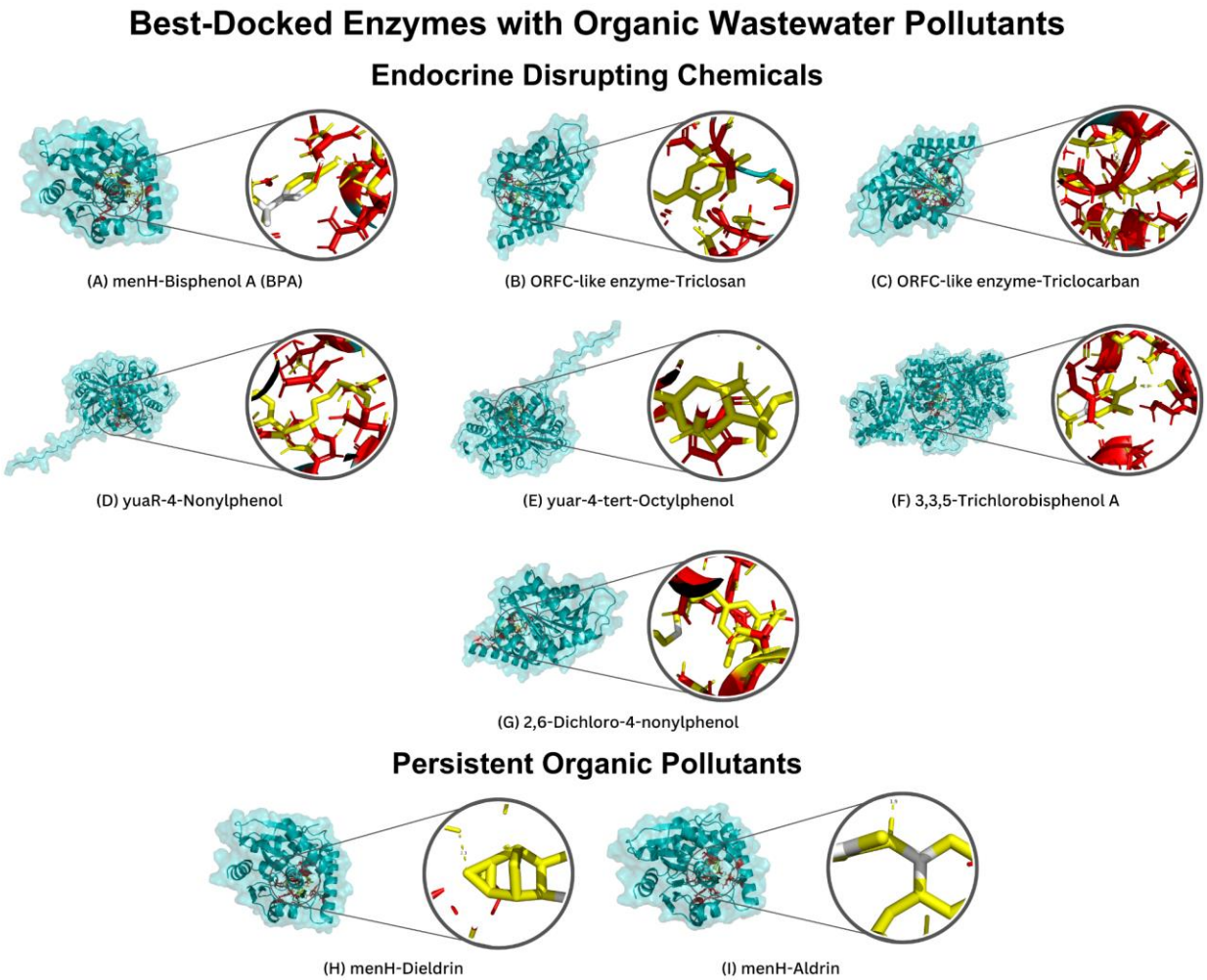

Figure 3. The best-docked enzymes with their respective organic wastewater pollutants, including complexes within the endocrine-disrupting chemicals and persistent organic pollutants categories

4.8. Domain Analysis of Best Affinity Enzymes: The domain analysis of the best affinity enzymes indicated that most enzymes showed Alpha/beta hydrolase fold-1 domain within their structures. The enzymes *menH* (strain ED1a), *menH* (strain SMS-3-5 / SECEC), ORFC-like enzyme (*E. coli*), *yuaR* (strain K12), *yheT* (strain K12), *menH* (strain UTI89 / UPEC), and *menH* (*E. coli* O157:H7) showed the Alpha/beta hydrolase fold-1 domain. This domain ranged within 17-243 amino acids for *menH* of strains ED1a, SMS-3-5 / SECEC, UTI89 / UPEC, and *E.coli* O157:H7, while ranged within 26-268, 96-462, and 73-313 amino acids for ORFC-like enzyme (E. coli), *yuaR* (strain K12), and *yheT* (strain K12) enzymes, respectively. The domains of the best-docked enzymes and their regions are mentioned in Table 3. The domains of the best-docked enzymes are shown in Figure 4.

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Table 3. The domains and their respective regions of the best-docked enzymes

Enzymes	Strains	Domains	Length
menH	ED1a	Alpha/beta hydrolase fold-1	17-243
aes	SMS-3-5 / SECEC	Alpha/beta hydrolase fold-3	87-295
menH	SMS-3-5 / SECEC	Alpha/beta hydrolase fold-1	17-243
fes	K12	Enterochelin esterase, N-terminal	29-155
ORFC-like enzyme	E. coli	Alpha/beta hydrolase fold-1	26-268
yuaR	K12	Alpha/beta hydrolase fold-1	96-462
yheT	K12	Alpha/beta hydrolase fold-1	73-313
menH	UTI89/ UPEC	Alpha/beta hydrolase fold-1	17-243
frsA	UTI89 / UPEC	No Domain, Homologous Superfamily (Alpha/Beta hydrolase fold)	16-414
entF	*E. coli* O157:H7	AMP-dependent synthetase/ligase domain	462-814
		Thioesterase	1067-1282
		Condensation domain	2-442
		Phosphopantetheine binding ACP domain	971-1046
		Amino acid adenylation domain	482-886
		Polyketide synthase, thioesterase domain	1069-1245
		Polyketide synthase, phosphopantetheine-binding domain	977-1046
menH	*E. coli* O157:H7	Alpha/beta hydrolase fold-1	17-243

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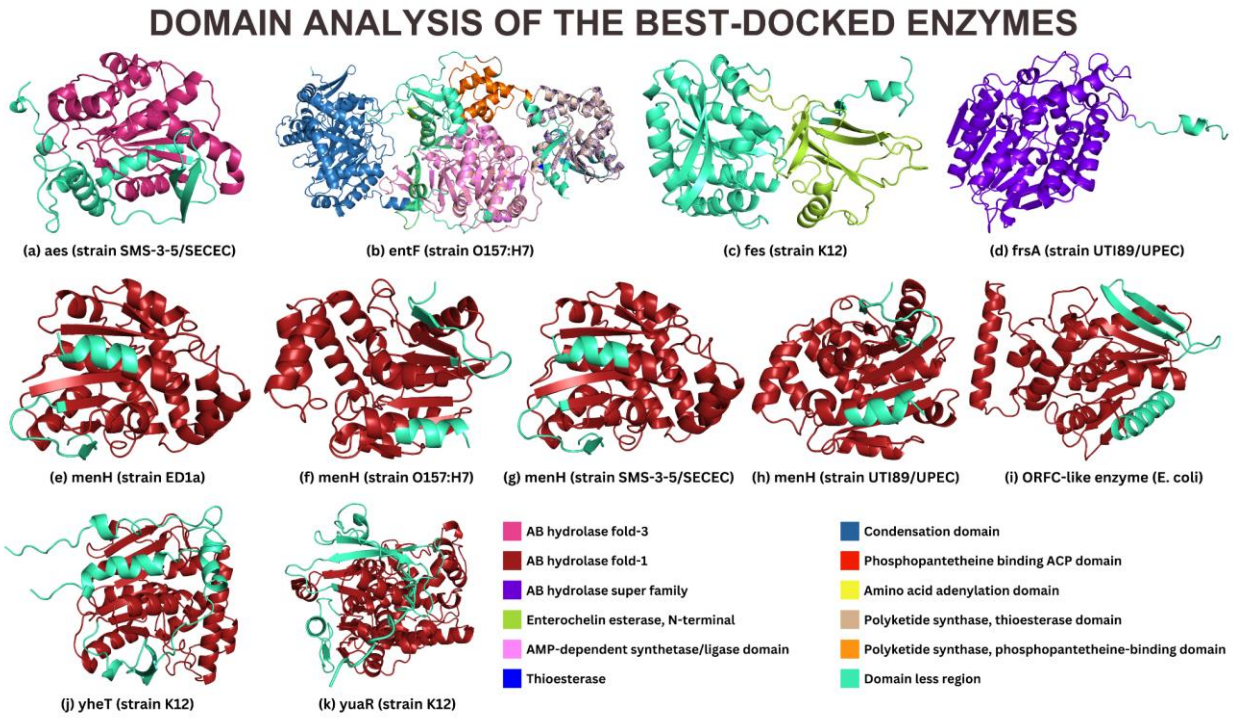

Figure 4. The domain analysis of the best-docked enzymes

4.9. PLIP and 2D Interaction Analysis of the Best-Docked Complexes

The best-docked complexes showed various hydrogen bonds between the enzyme and the ligand molecules. It was observed that five complexes exhibited more than 3 hydrogen bond interactions, including *frsA*-Terbutylazine, *menH*-Clonazepam, *menH*-Sucralose, *yheT*-Bezafibrate, and *aes*-Saccharin complexes. Moreover, 18 complexes showed ≤ 3 hydrogen bond interactions including *menH*-Aldrin, *menH*-Atrazine, *menH*-Phorate, *menH*-chlorpyrifos, *menH*-Diazinon, ORFC-like enzyme-2,6-Dichloro-4-Nonylphenol, ORFC-like enzyme-Triclosan, ORFC-like enzyme-Triclocarban, ORFC-like enzyme-Fenofibrate, ORFC-like enzyme-Acesulfame, *yuaR*-4-Nonylphenol, *yuaR*-4-Tert-Octylphenol, *yuaR*-Clofibric acid, *menH*-BisphenolA, *fes*-Promethryn, *menH*-Diclofenac, *menH*-Dieldrin, and *entF*-3,3,5-TrichlorobisphenolA complexes. Lastly, it was observed that the *aes*-Medazepam complex did not show any hydrogen bond interaction, are mentioned in Table 4.

Table 4. The interacting enzyme residues with their positions and distances between hydrogen and acceptor atoms and between donor and acceptor atoms

Complexes	Position	Residues	Distance H-A	Distance D-A
menH-Aldrin	86	SER	1.95	2.99
menH-Atrazine	90	ARG	2.33	3.28
	90	ARG	1.97	2.98

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menH-Phorate	86	SER	2.89	3.3
	90	ARG	2.38	3.35
	90	ARG	2.84	3.85
frsA-Terbutylazine	39	LEU	2.35	3.07
	52	ASN	2.84	3.41
	59	ARG	2.7	3.32
	209	ASP	3.11	3.52
	209	ASP	3.2	3.89
menH-chlorpyrifos	90	ARG	2.27	3.27
	90	ARG	3	3.56
menH-Diazinon	86	SER	2.4	3.24
	90	ARG	2.34	3.06
	90	ARG	2.15	2.93
menH-Clonazepam	23	PHE	2.87	3.77
	86	SER	3.27	4.01
	87	LEU	2.83	3.58
	90	ARG	3.42	3.94
	128	ASP	2.26	3.23
	128	ASP	2.22	3.06
menH-Sucralose	124	ARG	2.78	3.15
	128	ASP	2.25	3.01
	128	ASP	2.2	3.07
	185	LEU	2.33	3.1

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	212	LYS	2.5	3.16
ORFC-like enzyme-2,6-Dichloro-4-Nonylphenol	178	SER	2.36	3.04
	178	SER	2.35	3.04
ORFC-like enzyme-Triclosan	107	SER	2.09	2.96
	235	ASN	2.42	3.26
ORFC-like enzyme-Triclocarban	34	PHE	2.04	2.8
	262	HIS	2.85	3.65
	262	HIS	3.1	3.88
ORFC-like enzyme-Fenofibrate	34	PHE	2.41	3.01
	34	PHE	2.34	3.26
	182	SER	2.75	3.08
ORFC-like enzyme-Acesulfame	107	SER	2.76	3.3
	108	LEU	3.2	3.69
	235	ASN	2.24	3.21
yheT-Bezafibrate	81	LEU	2.24	2.84
	125	SER	2.43	3.15
	153	SER	3.23	3.55
	239	ASP	2.59	3.03
	255	TYR	2.12	2.82
yuaR-4-Nonylphenol	102	GLY	2.19	2.88
	207	SER	2.28	2.77
	208	TYR	2.08	3.18
yuaR-4-Tert-Octylphenol	234	ASP	2.24	3.41

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	234	ASP	2.95	3.7
	440	ASN	2.84	3.3
yuaR-Clofibric acid	207	SER	1.77	2.76
	208	TYR	2.12	3.21
	248	GLN	3.22	3.77
aes-Saccharin	198	TYR	3.3	3.62
	199	GLY	2.48	3.28
	199	GLY	2.21	3.15
	200	LEU	2.95	3.64
	240	TYR	2.9	3.66
	240	TYR	3.18	3.92
menH-Bisphenol A	222	ARG	2.55	3.11
	331	TRP	3.07	4.01
fes-Promethryn	240	ARG	3.11	3.56
	240	ARG	3.48	3.95
menH-Diclofenac	212	LYS	2.25	2.84
menH-Dieldrin	212	LYS	2.26	2.85
entF-3,3,5-Trichlorobisphenol A	863	ARG	3.22	3.81
	1006	SER	1.96	2.72
	1006	SER	2.13	2.72

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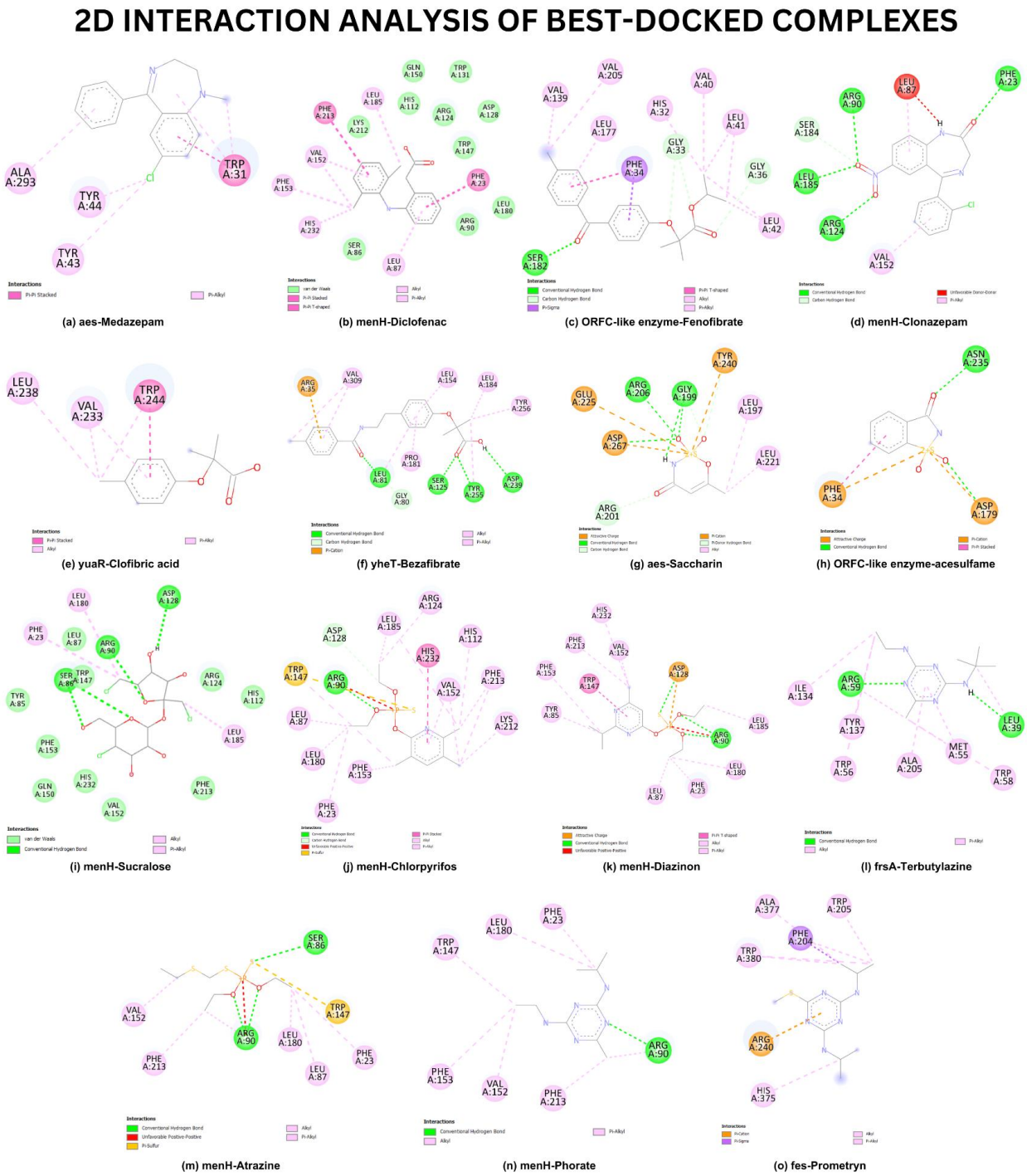

Figure 5. The 2D interaction analysis of the best-docked complexes showing the enzyme residues interacting with organic wastewater pollutants belonging to (a-f) pharmaceuticals, (g-i) artificial sweeteners, and (j-o) pesticides/herbicides.

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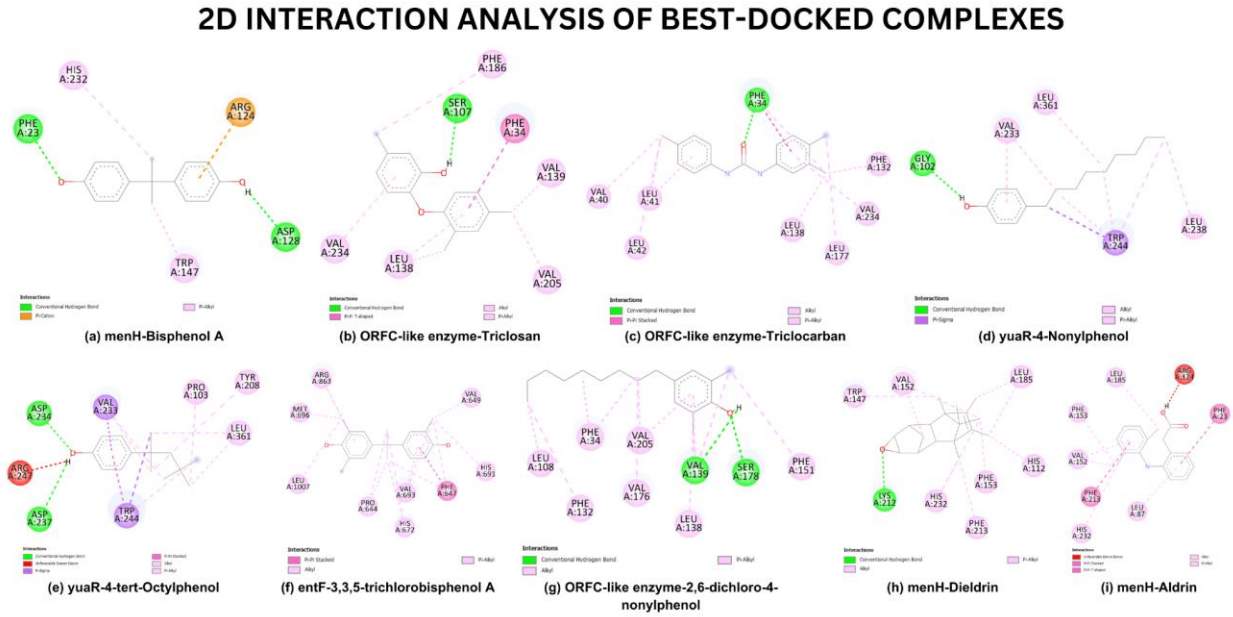

Figure 6. The 2D interaction analysis of the best-docked complexes showing the enzyme residues interacting with organic wastewater pollutants belonging to (a-g) endocrine-disrupting chemicals and (h-i) persistent organic pollutants.

Furthermore, the 2D interaction analysis showed various interactions within the complexes. It was observed that medazepam, prometryn, clofibric acid, 3,3,5-trichlorobisphenol A, aldrin, and diclofenac did not show hydrogen bonds with their respective enzymes. On the other hand, saccharin, dieldrin, bisphenol A, clonazepam, 2,6-dichloro-4-nonylphenol, acesulfame, fenofibrate, triclocarban, triclosan, 4-nonylphenol, 4-tert-octylphenol, chlorpyrifos, diazinon, sucralose, atrazine, and phorate showed hydrogen bonds within the domain regions of their respective enzymes.

The interacting enzyme residues exhibiting hydrogen bonds were ARG-206 and GLY-199 in the *aes*-Saccharin complex, LYS-212 in the *menH*-Dieldrin complex, PHE-23 and ASP-128 in the *menH*-Bisphenol A complex, PHE-23, ARG-90, LEU-185, and ARG-124 in the *menH*-Clonazepam complex, VAL-139 and SER-178 in the ORFC-like enzyme-2,6-dichloro-4-nonylphenol complex, ASN-235 in the ORFC-like enzyme-Acesulfame complex, SER-182 in the ORFC-like enzyme-Fenofibrate complex, PHE-34 in the ORFC-like enzyme-Triclocarban complex, SER-107 in the ORFC-like enzyme-Triclosan complex, GLY-102 in the *yuaR*-4-Nonylphenol complex, ASP-234 and ASP-237 in the *yuaR*-4-tert-octylphenol complex, LEU-81, SER-125, TYR-255, and ASP-239 in the *yheT*-Bezofibrate complex, ARG-90 in the *menH*-chlorpyrifos complex, ARG-90 in the *menH*-Diazinon complex, ASP-128, ARG-90, and SER-86 in the *menH*-Sucralose complex, ARG-59 and LEU-39 in the *frsA*-Terbutylazine complex, SER-86 and ARG-90 complex; lastly, ARG-90 in the *menH*-Phorate complex. The 2D interaction analysis of the best-docked complexes is shown in Figures 5 and 6.

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5. DISCUSSION

The organic wastewater pollutants pose a significant environmental threat, eventually deteriorating the biosphere (27). Moreover, conventional treatment methods for wastewater pollutants have limitations due to incomplete removal, high costs, and the generation of secondary pollutants (28,29). Consequently, there is a dire need to harness the biological systems for the bioremediation of wastewater pollutants (30). Therefore, this study utilized multiple esterase enzymes from various strains of *E. coli* and conducted the virtual screening of the organic wastewater pollutants with the esterases to get insights into the potential enzymes or esterases capable of degrading the wastewater pollutants effectively.

Moreover, pharmaceuticals such as diclofenac, medazepam, fenofibrate, clonazepam, clofibric acid, and bezafibrate are implicated in cardiovascular issues, liver damage, and dependency on benzodiazepines (31). Furthermore, artificial sweeteners, including saccharin, acesulfame, and sucralose, pose potential health risks, including metabolic effects, vascular-related diseases, and bladder cancer in rats (32,33). Subsequently, pesticides and herbicides such as chlorpyrifos, diazinon, terbutylazine, atrazine, phorate, and prometryn are associated with neurotoxicity and potential carcinogenicity in humans (34).

Additionally, endocrine-disrupting chemicals such as bisphenol A, triclosan, triclocarban, 4-nonylphenol, 4-tert-octylphenol, 3,3',5-trichlorobisphenol A, and 2,6-dichloro-4-nonylphenol are associated with health issues including developmental disorders and cancer (35,36). Lastly, persistent organic pollutants such as aldrin affect human health, including muscle twitching, vomiting, nausea, irritability, hyperexcitation, dizziness, seizures, hypoxia, headache, etc. (35), while dieldrin is reported to be immunogenic to humans, leading to dopaminergic neurodegeneration, causing chemically immunohemolytic anemia or give rise to Parkinson's disease (36).

These wastewater pollutants are harmful to both human health and the environment; hence, their screening with the esterase enzymes of *E. coli* resulted in various binding affinity ranges, indicating their degradation based on affinity and interactions. The enzymes that exhibited the lowest (best) affinities and interactions with their respective ligands (wastewater pollutants) indicated the potential of the enzymes to degrade the wastewater pollutants rapidly and effectively.

Additionally, it was observed that prometryn interacted outside the domain region of its respective docked enzyme, while medazepam did not show any hydrogen bond interactions with *aes* enzyme, while all other ligands interacted within the domains of their respective enzymes. The "Alpha/beta hydrolase fold-1" and "Alpha/beta hydrolase fold-3" domains are implicated in the cleavage reactions (37,38).

Lastly, it was observed that the majority of the ligands interacted with ORFC-like enzymes, *yuaR* (strain K12), *menH* (strain ETI89 / UPEC), and *menH* (strain O157:H7) enzymes, all of which comprised the same domain, Alpha Beta hydrolase fold-1. This suggests that the esterases comprising this domain have a higher probability of binding efficiently with the organic wastewater pollutants and degrading them.

Moreover, several studies have reported the degradation of pharmaceuticals and pesticides to decontaminate the environment for better living conditions, where bioremediation of chlorpyrifos by *Pseudomonas plecoglossicida*, while diclofenac by bacterium *Streptomyces*

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rochei, *Enterobacter hormaechei* D15, *Enterobacter cloacea* D16, and the fungi *Phanerochaete chrysosporium* and *Trametes versicolor* has been reported (40). Furthermore, *E. coli* has been reported to be utilized for the bioremediation of heavy metals and insecticides such as methomyl (41). E. coli has been reported to be used for the bioremediation of diclofenac sodium but not specifically diclofenac (42). Another study reported the biodegradation of chlorpyrifos using *E. coli* (43). Finally, the esterase enzymes in *E. coli* have not been reported specifically in the bioremediation of the organic wastewater pollutants utilized in this study except chlorpyrifos, suggesting a novel approach for the bioremediation of these pollutants.

6. CONCLUSION

This study provides insights into the potential of the esterase enzymes of *E. coli* for the bioremediation of organic wastewater pollutants. Subsequently, this study concluded several esterase enzymes [(ORFC-like enzyme (*E. coli*), *yuaR* (strain K12), *menH* (strain ETI89 / UPEC), and *menH* (strain O157:H7)] which might effectively degrade the organic wastewater pollutants belonging to pharmaceuticals, artificial sweeteners, pesticides/herbicides, endocrine-disrupting chemicals, and persistent organic pollutants.

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