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Larvicidal efficacy of *Bacillus cereus*-derived compounds against *Culex quinquefasciatus* via D7 protein inhibition

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Abstract
Aim: Mosquito-borne diseases pose major global health challenges, and resistance to chemical insecticides highlights the need for ecofriendly alternatives. This study aimed to evaluate the larvicidal potential of metabolites extracted from *Bacillus cereus* against *Culex quinquefasciatus* and investigate their inhibitory effects on the mosquito salivary gland D7 protein using molecular docking.
Methods: *Bacillus cereus* was isolated and identified by culture and 16S rRNA analysis. The bacterial metabolites were extracted, and GC-MS analysis was performed to identify bioactive compounds. Molecular docking simulations were conducted to assess the binding affinities of compounds such as 2-piperidinone, dodecanoic acid, and n-hexadecanoic acid to the D7 protein (PDB ID: 7TVY) of *Culex quinquefasciatus*.
Results: Molecular docking showed strong interactions of these compounds with the D7 protein, with 2-piperidinone exhibiting the highest docking score of -7.0 kcal/mol, followed by n-hexadecanoic acid (-5.4 kcal/mol) and dodecanoic acid (-4.4 kcal/mol). These interactions suggest disruption of the D7 protein's role in mosquito blood feeding and disease transmission.
Conclusion: *Bacillus cereus* metabolites showed strong binding to the mosquito salivary gland D7 protein, indicating potential larvicidal activity and supporting their use as ecofriendly mosquito control agents.

Keywords: *Bacillus cereus*, Mosquito larvicidal metabolites, *Culex quinquefasciatus*, salivary gland D7 protein, Molecular docking, 2-piperidinone

Introduction

Mosquitoes act as vectors for diseases like dengue, chikungunya, filariasis, malaria, Japanese encephalitis, and yellow fever [1], and also trigger allergic reactions in humans, ranging from localized skin irritations to severe systemic responses such as angioedema. Over the past several decades, mosquito-borne illnesses have caused widespread health crises, exerting a profound impact on public health worldwide [2]. Currently, these diseases lead to high rates of illness and death and impose substantial economic burdens—including significant losses in commercial productivity and labor—especially in tropical and subtropical regions [3]. Notably, no region is entirely free from the risks posed by mosquito-borne diseases, underscoring their global importance. Mosquito-borne diseases are responsible for almost 700 million cases and more than a million deaths each year [4].

The control of mosquitoes in India relies primarily on the use of chemical synthetic pesticides as larvicides and adult-repellents. However, misuse and over-reliance on these chemicals have led to significant negative impacts on human health and the environment, as well as contributed to the development of resistance among Indian mosquito species. Commonly used insecticides—such as DDT, permethrin, propoxur, deltamethrin, pyrethrum, organophosphates, and carbamates—have shown reduced effectiveness due to resistance, particularly in species like *Culex quinquefasciatus, Aedes aegypti, and Anopheles mosquitoes*. Consequently, health and environmental authorities now emphasize integrated approaches, including source reduction, environmental management, biological control, and personal protection, to combat the challenges posed by resistant mosquito populations in India [5, 6].

Currently, there is a growing global interest in developing various bioproducts as sustainable

alternatives to conventional chemical insecticides for mosquito control. Numerous microbial species have been investigated for their larvicidal properties. Among bacteria and actinomycetes, several genera—such as Streptomyces, Pseudomonas, Bacillus, and Serratia—have been identified and characterized for their antimicrobial, larvicidal, and pesticidal potentials, making them promising candidates in the search for eco-friendly mosquito control agents [7, 6].

Many researchers have determined that various microbial metabolites are not only larvicidal against mosquitoes, particularly *Culex pipiens*, but are also considered safe for human skin fibroblasts. However, only limited studies have explored the effect of these metabolites on the salivary short D7 protein 3 of *Culex quinquefasciatus* (PDB ID: 7TVY) using molecular docking. Hence, this study aims to evaluate the larvicidal potential of metabolites extracted from Bacillus sp., and metabolites were determined with GCMS analysis. Furthermore, the study investigates their interaction and inhibitory effects on the D7 protein through molecular docking analysis.

Methodology

Microorganisms and media used

Bacillus sp., was collected from the Chromopark research centre, Tamil Nadu, India. All media were obtained from Himedia, India, where nutrient agar medium was used for subculturing, maintenance and production of larvicidal compounds of *Bacillus* sp. isolate.

Extraction of secondary metabolites from bacteria

The isolate was inoculated into nutrient broth, and after 24 hours of incubation, the broth was centrifuged at 10,000 rpm for 10 minutes to collect the cell-free supernatant. Ethyl acetate was added in a 1:1 (v/v) ratio to the cell-free supernatant, and the mixture was shaken vigorously for several minutes. The ethyl acetate mixture was then transferred to separating funnels and allowed to stand until the organic and aqueous phases separated. The organic phase was collected and passed over anhydrous sodium sulfate to remove residual water. This extraction process was repeated three times, and finally, the combined organic phase was evaporated to dryness.

GCMS analysis

The GC-MS analysis was performed using an Agilent system consisting of a GC 8890, MS 5977C, and an Autosampler 7693A. The separation was achieved on a DB-5ms column with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 microns. Helium gas (99.999% purity) was used as the carrier at a flow rate of 1 ml/min. The oven temperature program started at 50 °C for 1 minute, then increased at a rate of 10 °C per minute to 300 °C and held for 1 minute. Compound identification was performed using the NIST20 library, and data analysis was carried out using the MassHunter software package.

Molecular docking study

The molecular docking procedure begins with the preparation of protein and ligand structures. First, the 3D structure of the target protein, such as the D7 protein from *Culex quinquefasciatus* (PDB ID: 7TVY), is downloaded from the Protein Data Bank. Next, the 2D or 3D structures of ligands like Dodecanoic acid, 2-Piperidinone, and n-Hexadecanoic acid are obtained or drawn and converted into the PDBQT format using PyRx's integrated OpenBabel tool. The prepared protein and ligand molecules are then loaded into PyRx via the "Load Molecule" option. The active site region of the protein is defined by setting a grid box centered on crucial amino acid residues involved in ligand binding, and docking parameters such as exhaustiveness are adjusted as needed. Docking simulations are performed using AutoDock Vina within PyRx for each ligand against the target protein, with monitoring until completion. After the docking finishes, results are analyzed by examining the binding affinities (docking scores) and predicted binding poses, and all output files are saved for further study [8]. Finally, the docked complexes are imported into Flare software (Cresset) for detailed protein-ligand interaction analysis, which includes visualization of hydrogen bonds, hydrophobic contacts, key residue involvement, and generation of interaction maps to better understand the binding modes within the 3D protein structure.

Results and Discussion

The procured isolate was confirmed with culture characterization and morphological characterization on nutrient agar media was confirmed as *Bacillus sp*. After routine culture on nutrient agar at 37 °C for 48 h, milky, large, convex, opaque, dry, crude colonies, Gram-positive and could produce spores. Furthermore, 16srRNA sequencing analysis was confirmed that as *Bacillus cereus*. This was closely related to sequence KP717563.1 in the NCBI database.

This aligns with previous research where *Bacillus cereus* has been widely reported as a Gram-positive, spore-forming bacterium commonly isolated from environmental and soil samples [9]. Traditional methods for *Bacillus cereus* identification, including culture on selective media and morphological characterization, are well documented but often require confirmatory molecular techniques such as 16S rRNA gene sequencing for higher specificity [10]. The genetic closeness to KP717563.1 supports the isolate's taxonomic assignment and may indicate functional capabilities consistent with known *Bacillus cereus* strains, such as spore formation and environmental resilience.

The confirmed isolate of *Bacillus cereus* was inoculated into nutrient broth, and its extract was subjected to GC-MS analysis to identify potential bioactive compounds responsible for larvicidal activity. The analysis revealed several key compounds including 2-Piperidinone, Dodecanoic acid methyl ester, 9-Hexadecenoic acid methyl ester, and n-Hexadecanoic acid. The predominant compound was Isopropyl myristate, alongside others like tert-Hexadecanethiol, Octadecanedioic acid, and Boscartol F, highlighting a complex chemical profile (Table.1). Previous studies have similarly reported the larvicidal efficacy of Bacterial isolates against mosquito vectors, linking their activity to bioactive metabolites identified via GC-MS and proteomic approaches [11], n-Hexadecanoic acid and Dodecanoic acid [12] and 2-piperidinone [13] compounds were known for insecticidal properties, likely contribute to the observed larvicidal effects, this was supporting the potential of *Bacillus cereus* as an eco-friendly biocontrol agent in mosquito management programs.

Table 1: GCMS analysis of extract of *Bacillus cereus*

RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T
10.1599	2-Piperidinone	675-20-7	C5H9NO	1,357,528	91.9	5.94
14.1364	Dodecanoic acid, methyl ester	111-82-0	C13H26O2	219,514	85.4	0.96
18.4116	9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	C17H32O2	487,970	94.5	2.13
18.9687	n-Hexadecanoic acid	57-10-3	C16H32O2	429,153	78.1	1.88
19.6132	Hexadecanoic acid, 14-methyl-, methyl ester	2490-49-5	C18H36O2	193,935	79.5	0.85
20.3852	11-Octadecenoic acid, methyl ester	52380-33-3	C19H36O2	1,805,888	98.1	7.9
21.6853	tert-Hexadecanethiol	25360-09-2	C16H34S	351,226	76.7	1.54
23.5679	Octadecanedioic acid	871-70-5	C18H34O4	138,758	63.9	0.61
26.7105	Boscartol F	1486443-17-7	C20H28O2	1,714,367	75.2	7.5

Many authors have investigated the anti-mosquito activity of bacteria using *in vivo* methods; however, only a few have explored the inhibitory mechanisms of bacterial metabolites through molecular docking studies. Several mechanisms are involved in the repellent activity against mosquitoes. Plant and microbial extracts have been shown to suppress various target proteins to inhibit insects. Among these targets, the D7 protein is essential for mosquito blood feeding and indirectly impacts mosquito-borne disease transmission through its modulator effects. D7 proteins function by binding and sequestering biogenic amines such as serotonin and histamine, as well as eicosanoids like leukotrienes and thromboxanes—host molecules involved in blood clotting and inflammation at the bite site. By inhibiting these host responses, D7 proteins facilitate efficient blood meal acquisition and help mosquitoes evade host detection and immune reactions. Therefore, if this protein is suppressed by any agents, it may result in mosquito death. In the present study, the inhibitory mechanisms of n-hexadecanoic acid and dodecanoic acid against the salivary gland D7 protein (PDB ID: 7TVY) of *Culex quinquefasciatus* were examined through molecular docking analysis.

Molecular docking of the selected ligands—dodecanoic acid, 2-piperidinone, and n-hexadecanoic acid—against the D7 protein (PDB ID: 7TVY) from *Culex quinquefasciatus* yielded specific binding scores and detailed residue interactions. 2-Piperidinone demonstrated the strongest binding affinity with a docking score of -7.0 kcal/mol, interacting primarily with the active site residues LYS 65, TYR 88, ASP 89, MET 92, and PRO 97. Dodecanoic acid showed a moderate binding affinity of -4.4 kcal/mol and was predicted to interact with PHE 63, MET 92, TYR 88, TYR 95, and LYS 116. N-Hexadecanoic acid had a docking score of -5.4 kcal/mol, binding within a pocket formed by LEU 5, ASP 106, GLU 37, ILE 36, LEU 105, and LYS 103 (Table.2,).

Table 2: Docking Scores and Active Sites of *Bacillus cereus* Metabolites with 7TVY D7 Protein

Ligand	Active site region	Score
7TVY_Dodecanoic acid.	PHE 63; MET92; TYR 88; TYR 95; LYS 116	-4.4 kcal/mol
7TVY_2-Piperidinone	LYS 65; TYR 88; ASP 89; MET 92; PRO 97	-7.0 kcal/mol
7TVY_n-Hexadecanoic acid	LEU 5; ASP 106; GLU 37; ILE 36; LEU 105; LYS 103;	-5.4 kcal/mol

These findings suggest that 2-piperidinone forms the most stable complex with the D7 protein, as indicated by its more negative docking score, which generally correlates with stronger predicted binding. The interaction profiles for each ligand highlight the involvement of key amino acids in the D7 protein's binding site, offering insights into the molecular basis for their affinity and supporting further investigation into their biological activity. This is in agreement with earlier research demonstrating that low docking scores typically correlate with stronger and more favourable ligand-protein interactions, which are critical for inhibitory activity against target proteins [14]. Based on the review of literature, there is no direct evidence for the inhibition of the salivary gland of *Culex quinquefasciatus* by fatty acids. However, Hassan and Jebanesan (2022) reported the larvicidal activity of dodecanoic acid against *Anopheles stephensi* [12]. In 2023, Mansour *et al*. 2023 [6] identified beneficial compounds from various bacterial isolates and evaluated their insecticidal activity against *Culex pipiens* through acetylcholinesterase inhibition, which was further confirmed by molecular docking analysis.

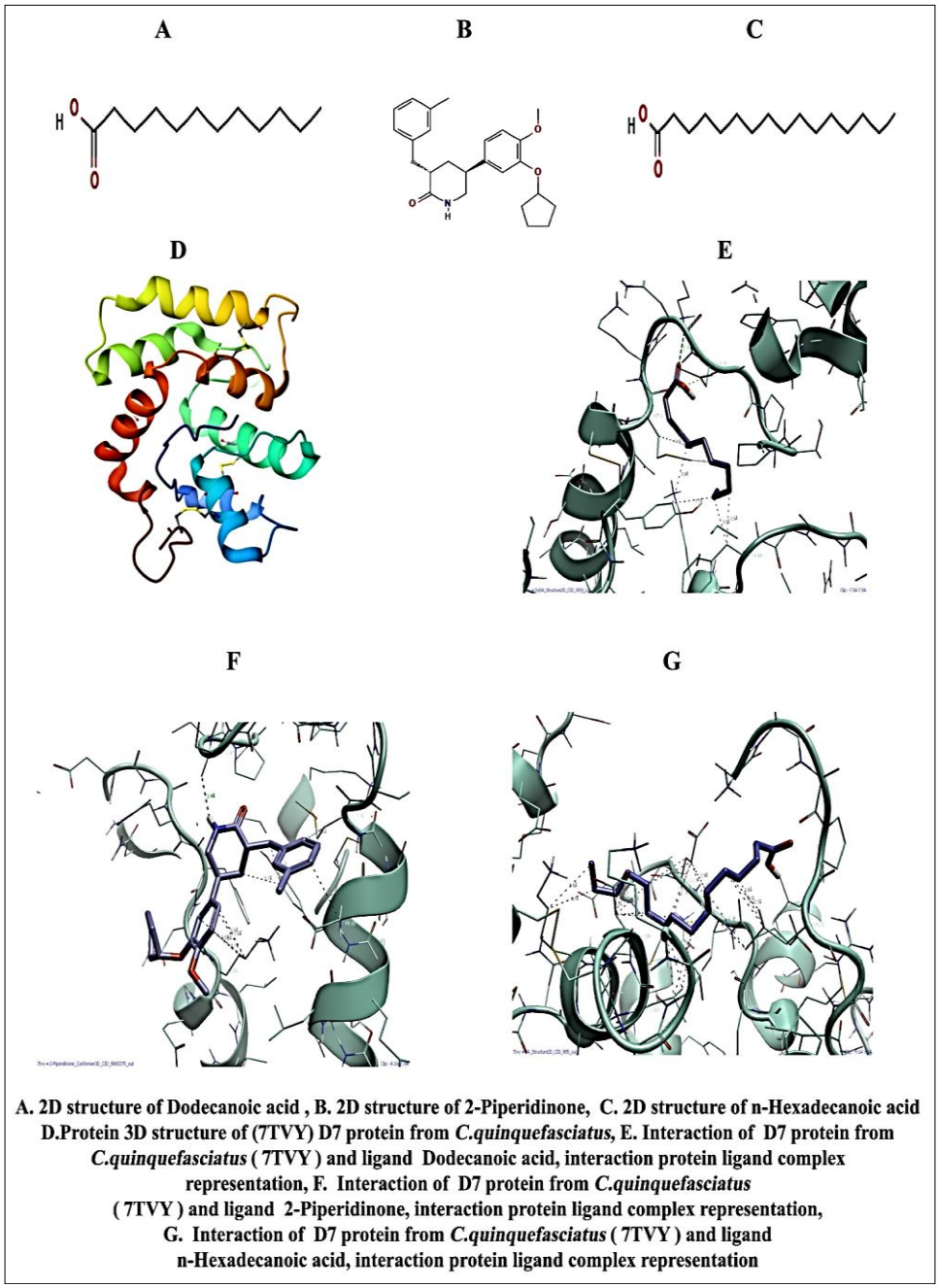

Fig 1: Structural Representations and Molecular Interactions of *Bacillus cereus* Metabolites with Culex quinquefasciatus D7 Protein (7TVY)

Conclusion

The study confirmed the isolate as *Bacillus cereus*, identified through culture and 16S rRNA analysis, consistent with known characteristics of this bacterium. GC-MS analysis revealed bioactive compounds, including 2-piperidinone, dodecanoic acid, and n-hexadecanoic acid, with insecticidal properties likely responsible for larvicidal effects. Molecular docking showed these compounds, particularly 2-piperidinone, strongly bind to the mosquito salivary gland D7 protein, disrupting a key protein involved in blood feeding and disease transmission. These findings support *Bacillus cereus* metabolites as promising ecofriendly biocontrol agents for mosquito management. Overall, the study highlights sustainable alternatives to chemical insecticides for effective vector control.

References

1. Reegan AD, Ceasar SA, Paulraj MG, Ignacimuthu S, Al-Dhabi NA. Current status of genome editing in vector mosquitoes: a review. Biosci Trends. 2016;10(6):424-432.
2. Rascalou G, Pontier D, Menu F, Gourbiere S. Emergence and prevalence of human vector-borne diseases in sink vector population. PLoS One. 2012;7(5):e36858. DOI:10.1371/journal.pone.0036858.
3. Reegan AD, Kumar PS, Asharaja AC, Devi C, Jameela S, Balakrishna K, *et al*. Larvicidal and ovicidal activities of phenyl acetic acid isolated from *Streptomyces collinus* against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae). Exp Parasitol. 2021;226-227:108120. DOI:10.1016/j.exppara.2021.108120.
4. Chilakam N, Lakshminarayanan V, Keremutt S, Rajendran A, Thunga G, *et al*. Economic burden of mosquito-borne diseases in low- and middle-income countries: protocol for a systematic review. JMIR Res Protoc. 2023;12:e50985.

DOI:10.2196/50985. PMID:38079215; PMCID:PMC10750235.
5. Farouk SA, Barahim N, Hamzah SN. The detoxification enzymes activity profile in susceptible Aedes and Culex mosquitoes. IOP Conf Ser Earth Environ Sci. 2021;711:012014.
6. Mansour T, Radwan WH, Mansour M, Gomaa M, Farouk F, Shepl M, *et al*. Larvicidal potential, toxicological assessment, and molecular docking studies of four Egyptian bacterial strains against *Culex pipiens* L. (Diptera: Culicidae). Sci Rep. 2023;13:17230.
7. Sardari S. Bioactive compound produced from actinomycetes-*Streptomyces*. Novel Appro Drug Des Dev. 2017;1:1507-15023.
8. Agu PC, Afiukwa CA, Orji OU, *et al*. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in disease management. Sci Rep. 2023;13:13398. DOI:10.1038/s41598-023-40160-2.
9. Ramarao N, Tran SL, Marin M, Vidic J. Advanced methods for detection of *Bacillus cereus* and its pathogenic factors. Sensors (Basel). 2020;20:2667. DOI:10.3390/s20092667.
10. Bagcıoglu M, Fricker M, Johler S, Ehling-Schulz M. Detection and identification of *Bacillus cereus*, *Bacillus cytotoxicus*, *Bacillus thuringiensis*, *Bacillus mycoides* and *Bacillus weihenstephanensis* via machine learning based FTIR spectroscopy. Front Microbiol. 2019;10:902. DOI:10.3389/fmicb.2019.00902.
11. Balakrishnan S, Santhanam P, Srinivasan M. Larvicidal potency of marine actinobacteria isolated from mangrove environment against *Aedes aegypti* and *Anopheles stephensi*. J Parasit Dis. 2017;41(2):387-94. DOI:10.1007/s12639-016-0812-3.
12. Hassan J, Jebanesan A. Bio-efficacy of dodecanoic acid on larvicidal, ovicidal, pupicidal, and repellent activities against malarial vector *Anopheles stephensi* (Liston) (Diptera: Culicidae). Int J Entomol Res. 2022;7(3):84-90.
13. Arif IA, Ahamed A, Kumar RS, Idhayadhulla A, Manilal A. Cytotoxic, larvicidal, nematicidal, and antifeedant activities of piperidin-connected 2-thioxoimidazolidin-4-one derivatives. Saudi J Biol Sci. 2019;26(4):673-80. DOI:10.1016/j.sjbs.2017.12.007.
14. Gaddaguti V, Mounika SJ, Sowjanya K, Rao T, Chakravarthy MSKR, Rao AP. GC-MS analysis and in silico molecular docking studies of mosquito repellent compounds from *Hyptis suaveolens* L. Int J Bioassays. 2012;1(9):36-41.