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Original Article

Isolation, Identification and Characterization of Extremophilic Bacteria from Cosmetic Foundation

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ABSTRACT

Cosmetic products are prone to microbial contamination especially those that are resistant to preservatives which can survive under extreme pH environments. This paper sought to isolate and characterize alkali-tolerant bacteria in samples of cosmetic foundations and to computationally assess the potential antimicrobial targets. The isolates of bacteria were obtained through the culture of foundation samples using Luria-Bertani agar at pH 10. The isolate was determined to be Pseudomonas aeruginosa, a non-acid-fast, Gram-negative organism, and 99.85% similar to reference strains by 16S rRNA genome sequencing. Biochemical analysis showed a positive catalase, positive oxidase, and positive citrate metabolism characteristic of P. aeruginosa. The isolate survived at pH 10 and this confirms its alkali-tolerant nature. Computational studies were performed on the pyochelin biosynthesis protein F (pchF), with a Ramachandran score of 92.3, an ERRAT score of 94.77, and a VERIFY3D score of 90.48, and the quality of the model is acceptably good. Quercetin-3-glucuronide was found to have a high binding affinity (ΔG = -8.6 kcal/mol) at binding pocket C2. The stability of the pchF-quercetin-3-glucuronide complex during 100 ns was verified by molecular dynamics simulations, and the RMSD (approximately 0.3 nm) was stable, as well as the radius of gyration (1.942.02 nm) and hydrogen bonding (6-10 bonds). These results demonstrate the necessity of greater microbial safety measures in cosmetics and indicate that pchF may be an effective therapeutic target. Quercetin-3-glucuronide is a promising natural antimicrobial agent and should be further proven by experimental studies. Keywords: Pseudomonas aeruginosa, cosmetic contamination, alkali-tolerant bacteria, pyochelin biosynthesis, molecular docking, quercetin-3-glucuronide.

INTRODUCTION

Cosmetic foundations are essential components of daily personal care routines, providing both aesthetic enhancement and psychological well-being (1). However, their frequent use, exposure to environmental conditions, and direct contact with skin make them vulnerable to microbial contamination (2,3). Such contamination compromises product integrity and poses significant health risks, including folliculitis, acne, dermatitis, and opportunistic infections, particularly in immunocompromised individuals (4,5). Despite regulatory limits on acceptable microbial loads in cosmetic products, numerous studies have documented the persistence of pathogenic bacteria in commercially available formulations, indicating failures in current preservation systems (6,7).

Among cosmetic contaminants, Pseudomonas aeruginosa represents a particularly concerning threat due to its intrinsic resistance mechanisms, metabolic versatility, and ability to thrive in diverse environmental conditions (8). This Gram-negative opportunistic pathogen has been repeatedly isolated from cosmetics across multiple geographic regions, demonstrating resistance to conventional preservatives and persistence in product formulations (2,7). The bacterium's capacity to form biofilms,

resist antimicrobial agents through efflux pumps, and adapt to nutrient-limited environments enables prolonged survival in cosmetic matrices (9). Furthermore, P. aeruginosa can tolerate a wide pH range, from acidic to alkaline conditions, allowing it to survive both in cosmetic formulations and on human skin (pH 4.6–5.6), thereby facilitating transmission and infection (10).

While microbial contamination of cosmetics has been extensively documented, critical gaps remain in understanding the survival mechanisms of P. aeruginosa under alkaline conditions typical of aged or improperly formulated foundations. Traditional cosmetic safety testing is conducted at neutral or slightly acidic pH, yet oxidation, air exposure, and formulation aging can shift pH toward alkaline values where conventional preservatives lose efficacy (11). The molecular basis of P. aeruginosa persistence in such environments, particularly the role of iron acquisition systems, remains underexplored. Pyochelin, a siderophore synthesized via the pchEFGHI gene cluster, plays a crucial role in bacterial virulence and environmental adaptation (12,13). The pyochelin biosynthesis protein F (pchF), a non-ribosomal peptide synthetase, represents a potential therapeutic target; however, its structural characteristics and druggability have not been computationally validated for cosmetic-associated strains.

Recent advances in computational biology enable structure-based drug design without requiring experimental protein crystallization. Homology modeling, molecular docking, and molecular dynamics simulations can identify and validate therapeutic targets, predict ligand binding modes, and assess protein-ligand stability under physiological conditions (14,15). Flavonoids, particularly quercetin derivatives, have demonstrated antimicrobial properties and quorum-sensing inhibition in P. aeruginosa, suggesting potential as natural preservative alternatives or therapeutic agents (16,17). However, the interaction between quercetin-3-glucuronide and pchF has not been investigated, nor has the stability of this complex been assessed through molecular dynamics simulation.

This study aimed to isolate and characterize alkali-tolerant bacteria from cosmetic foundation, identify the species using biochemical and molecular methods, and computationally evaluate the pyochelin biosynthesis protein F as a potential therapeutic target. Specifically, we employed 16S rRNA gene sequencing for definitive species identification, homology modeling to predict pchF structure, molecular docking to assess quercetin-3-glucuronide binding, and molecular dynamics simulation to validate protein-ligand complex stability. The findings contribute to understanding P. aeruginosa persistence mechanisms in cosmetics and identify novel antimicrobial strategies for enhancing product safety.

MATERIALS AND METHODS

This observational study was conducted at the Department of Life Sciences, University of Management and Technology, and the Department of Biological Sciences, The Superior University, Lahore, Pakistan. Three sealed, unexpired cosmetic foundation samples were purchased from retail stores in Lahore, ensuring minimal pre-purchase contamination. These included both liquid and cream formulations from commonly used brands. Sample details (brand, batch number, manufacturing, and expiry date) were recorded, and the samples were transported to the laboratory under sterile conditions, stored at 4°C, and processed within 24 hours. To isolate alkali-tolerant bacteria, one gram of foundation from each sample was aseptically transferred to sterile saline (0.85% NaCl) and homogenized for 10 minutes. Serial dilutions (10^{-1} to 10^{-5}) were prepared, and selective isolation was performed using Luria-Bertani (LB) agar and LB broth adjusted to pH 10.0, followed by incubation at 37°C for 24–48 hours. Bacterial colonies with distinct morphologies were subcultured for purification and preserved at 4°C and -80°C in glycerol stocks.Bacterial isolates were subjected to Gram staining and acid-fast staining for morphological assessment. Biochemical tests were conducted to assess catalase, oxidase, carbohydrate fermentation, urease production, indole production, and citrate utilization using standard protocols from the Manual of Determinative Bacteriology. For molecular identification, genomic DNA was extracted from pure isolates using the GeneJET Genomic DNA Purification Kit, and DNA quality was assessed

spectrophotometrically. The 16S rRNA gene was amplified using universal bacterial primers, and PCR products were visualized on agarose gels. Sanger sequencing was performed, and sequences were compared to the NCBI GenBank database using BLAST for taxonomic identification. Phylogenetic analysis was carried out using the neighbor-joining method in MEGA X software with bootstrap analysis to assess branch support.

Molecular docking was performed to evaluate the binding affinity of quercetin-3-glucuronide with the pchF protein model. Docking was conducted using AutoDock Vina, and binding energies were analyzed for favorable protein-ligand interactions. Molecular dynamics simulations were performed using GROMACS to assess the stability of the protein-ligand complex, with RMSD, RMSF, radius of gyration, and hydrogen bond occupancy analyses used for further evaluation.

Descriptive statistics were applied to summarize biochemical test results and bacterial isolation frequencies. No ethical approval was required as the study involved only commercially available products with no involvement of human or animal subjects.

RESULTS

Bacterial Isolation and Growth at Alkaline pH

Foundation samples were cultured on LB agar and in LB broth adjusted to pH 10, yielding visible bacterial growth after 24 hours at 37°C. Colonies on LB agar (pH 10) were circular, smooth, translucent, and exhibited a greenish pigmentation, typical of Pseudomonas species. In LB broth (pH 10), visible turbidity and a greenish-yellow pigment diffusing into the medium confirmed metabolic activity. These observations confirmed the successful isolation of alkali-tolerant bacteria from the cosmetic foundation.

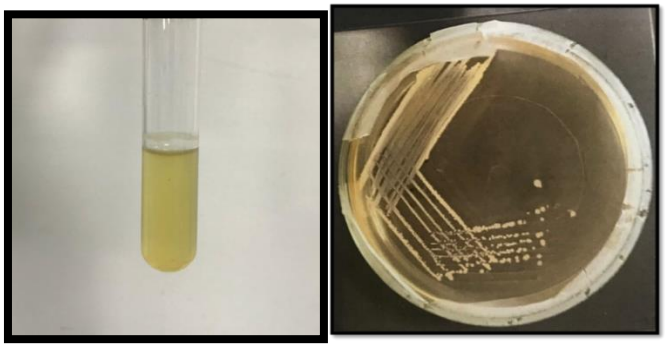


Fig. 1. Growth in LB media at pH 10 (a) LB Agar (b) LB Broth

Biochemical Characterization

The isolate presented a biochemical profile that was in line with that of Pseudomonas aeruginosa. The presence of catalase was ensured by the quick appearance of bubbles with the addition of 3% hydrogen peroxide. The oxidase reagent strip was dark purple, indicating that the activity of oxidase is positive. Fermentation tests on carbohydrates identified the production of acid and gases with glucose, and this indicated that the fermentation was able to occur. The use of citrate was verified by the ability to grow on Simmons citrate agar a change of color to blue. Urease and indole tests were negative, which means that there was no activity in urea hydrolyzing or tryptophan degradation.

Microscopic Examination and Staining

Gram staining displayed Gram-negative bacilli (rod-shaped, microscopic appearance), which were pink in color. There was the normal bacillary morphology of Pseudomonas in the cells. Acid-fast stain was negative which verified that there were no mycolic acids in the cell wall eliminating mycobacterial identity.

Molecular Identification and Phylogenetic Analysis

The 16S rRNA gene PCR amplification produced a product of about 1465 bp that was sequenced and matched with the NCBI GenBank database. The isolate was 99.85 percent sequences identity Pseudomonas aeruginosa reference strains, and 95 percent query coverage. Phylogenetic analysis with the neighbor-joining technique revealed that the isolate is placed in a monophyletic clade of Pseudomonas aeruginosa, which confirmed its identification at the species level with a high bootstrap value (95%-100%).

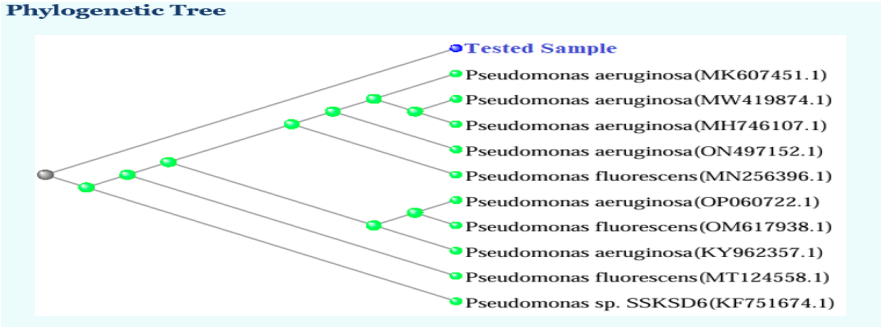
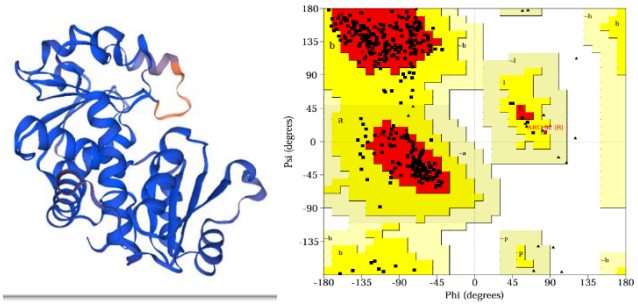


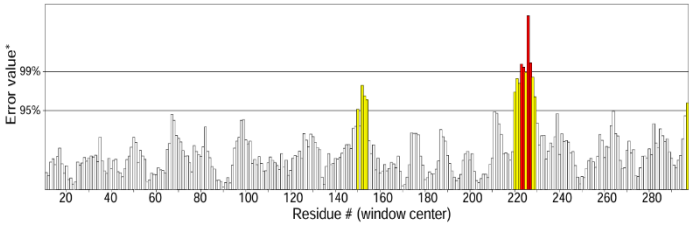
Fig. 2. Phylogenetic diagram of the isolate

Structural Validation of pchF Protein Homology Modeling and Structural Validation of pchF Protein



The pyochelin biosynthesis protein F (pchF) was built up into a homology model in the absence of an experimentally determined crystal structure. Ramachandran analysis was used to test the model, which demonstrated that 92.3% of the residues were in preferred regions, which is more than 90. The quality factor of 94.77% was offered by ERRAT analysis, which shows that the model is very reliable. VERIFY3D was used to verify that 90.48 percent of residues had 3D-1D score 0.2 and above, yet another confirmation of the structural accuracy of molecular docking experiments

Fig. 3. (a) Expected conformation for the pchF (b) Validation through Ramachandran plot



ERRAT analysis yielded an overall quality factor of 94.77%, indicating high reliability of non-bonded atomic interactions within the model (Figure 5).

Fig. 4. Structural validation of the pchF protein model

VERIFY3D analysis demonstrated that 90.48% of residues had a 3D-1D averaged score ≥0.2, confirming compatibility between the predicted three-dimensional structure and the amino acid sequence (Figure 6). Collectively, these validation metrics confirmed that the pchF homology model possessed sufficient structural quality for molecular docking studies.

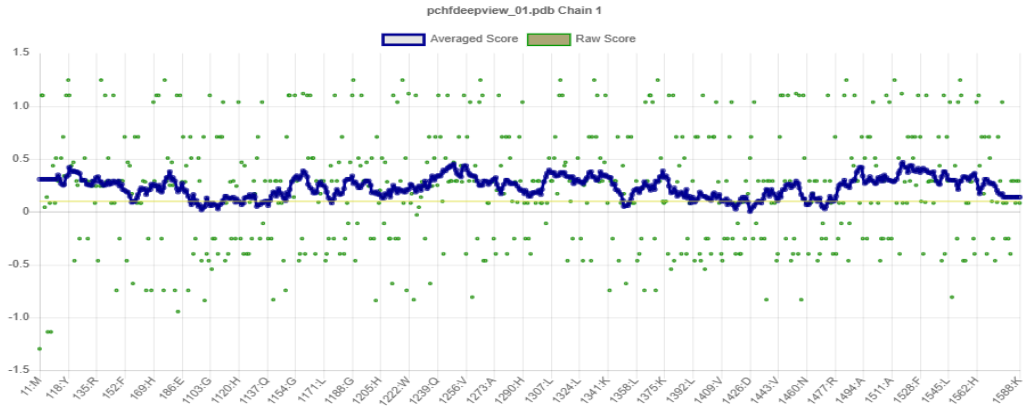


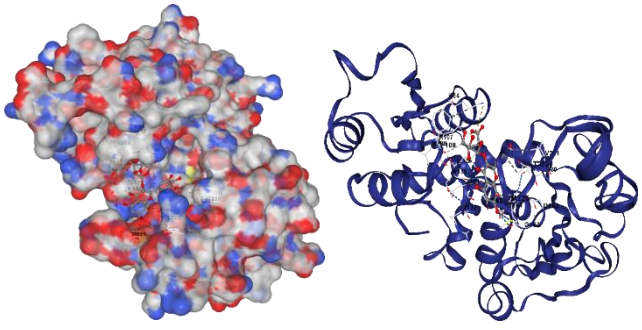
Fig. 5. VERIFY3D validation of pchF protein model

Molecular Docking Analysis

Molecular Docking Analysis Molecular docking of quercetin-3-glucuronide to the pchF protein model revealed 5 different binding pockets, of which pocket C2 had the best binding energy of -8.6 kcal/mol. The binding mode was indicated to be dependent on hydrogen bonds and hydrophobic interactions indicating that quercetin-3-glucuronide might bind to an active site with a functional role in pchF. Pocket C2 was chosen because of its high binding affinity to be analyzed in detail in terms of interaction.

Table. 1. Docking results of quercetin-3-glucuronide with pchF protein pockets

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
◉ C2	-8.6	615	17, 18, 46	22, 22, 22
○ C1	-7.5	647	16, -3, 47	22, 22, 22
○ C3	-6.6	132	11, 34, 56	22, 22, 22
○ C5	-6.4	109	27, 33, 37	22, 22, 22
○ C4	-6.3	112	22, 26, 26	22, 22, 22



Visualization of the pchF-quercetin-3-glucuronide complex at pocket C2 revealed multiple stabilizing interactions (Figure 7).

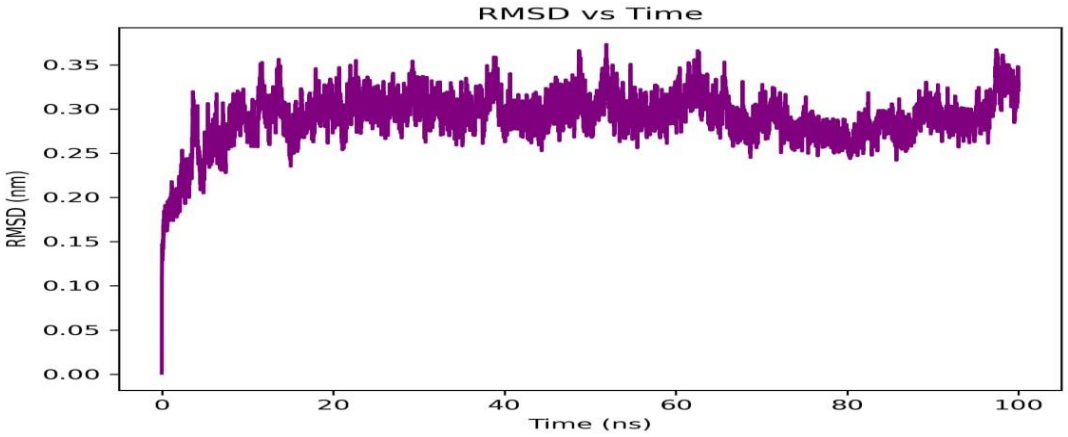
Fig. 6. Docked ligand bound to C2 pocket of pchF represented in cartoon ribbon structure, detailed view showing hydrogen bonds and hydrophobic interactions stabilizing the complex.

The ligand occupied the binding cavity in an extended conformation, forming hydrogen bonds with key amino acid residues in the pocket. Hydrophobic interactions and van der Waals contacts further

stabilized the complex. The binding mode suggested that quercetin-3-glucuronide could potentially occupy a functionally relevant site within the pchF protein structure, though experimental validation would be required to confirm inhibitory activity.Fig. 7. Docked ligand bound to C2 pocket of pchF represented in cartoon ribbon structure, detailed view showing hydrogen bonds and hydrophobic interactions stabilizing the complex.

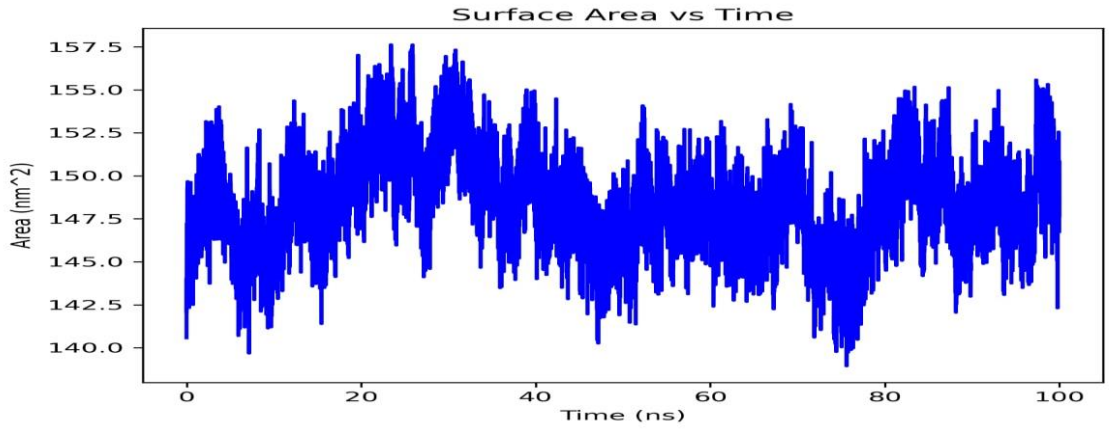
Molecular Dynamics Simulation Analysis

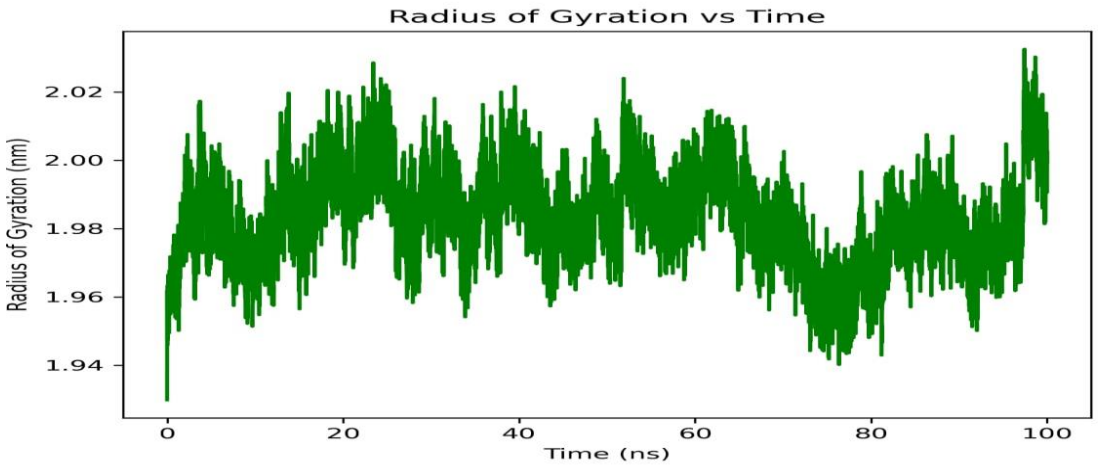
Molecular Dynamics Simulation Analysis The pchF-quercetin-3-glucuronide complex was shown to be stable in a 100 ns molecular dynamics simulation. The RMSD leveled off at 0.30 nm, which means that the protein-ligand complex has obtained a stable conformation. The radius of gyration was kept constant at 1.94-2.02 nm, indicating that the protein had a compact and well-folded structure. Per-residue RMSF analysis showed low fluctuation with exception of surface-exposed loops that displayed high flexibility. The solvent-accessible surface area did not change significantly, and this shows that there were no significant conformational changes. Hydrogen bond analysis of intramolecular hydrogen bonds showed that there was an intact system of 6-10 hydrogen bonds that led to the stability of the complex.



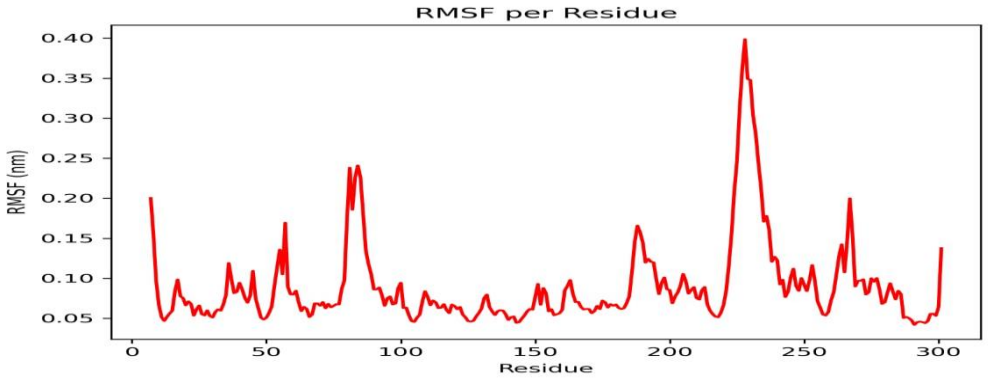
Root Mean Square Deviation (RMSD): The RMSD of protein backbone Cα atoms relative to the starting structure showed an initial equilibration phase during the first 10 ns, followed by stabilization around 0.30 nm for the remainder of the simulation (Figure 11). The plateau in RMSD values indicated that the protein-ligand complex achieved a stable conformation with minimal large-scale structural rearrangements after equilibration.

Radius of Gyration (Rg): The radius of gyration remained stable throughout the 100 ns trajectory, fluctuating between 1.94 and 2.02 nm (Figure 9). The narrow range of Rg values indicated that the protein maintained a compact, well-folded structure without significant unfolding or aggregation events during the simulation period.

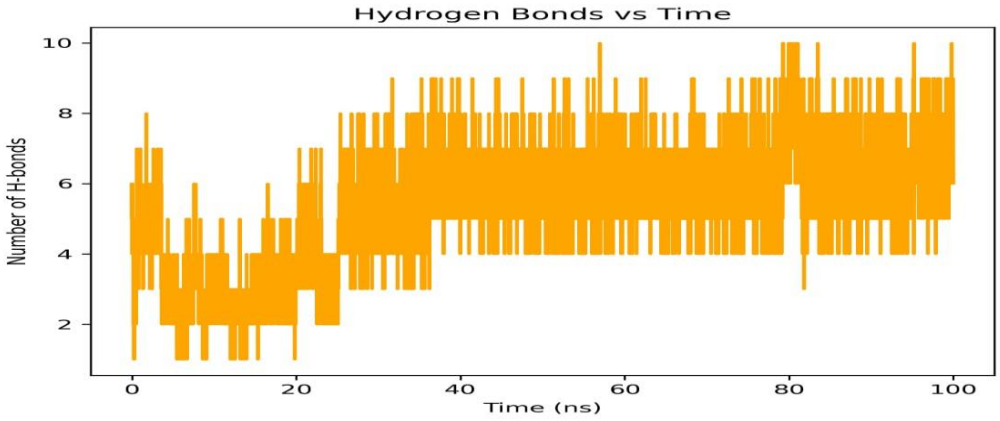




Root Mean Square Fluctuation (RMSF): Per-residue RMSF analysis revealed that most regions of the protein exhibited low fluctuations (<0.15 nm), consistent with a rigid core structure (Figure 10). Notable exceptions included residues 240–260, which demonstrated elevated flexibility (RMSF ~0.20–0.25 nm). These regions likely correspond to surface-exposed loops or linker regions that retain conformational mobility while maintaining overall structural integrity.



Solvent-Accessible Surface Area (SASA): The SASA fluctuated within a consistent range of 140–157 nm² throughout the simulation (Figure 8). The stability of SASA values indicated that the protein did not undergo major conformational transitions that would significantly alter its exposure to the solvent environment.



Hydrogen Bond Analysis: Intramolecular hydrogen bond analysis revealed a consistent network of 6–10 hydrogen bonds maintained throughout the 100 ns trajectory (Figure 12). This stable hydrogen bonding pattern contributed to the internal cohesion and structural stability of the pchF protein during the simulation.

Collectively, the molecular dynamics simulation results confirmed that the pchF protein model exhibited stable structural dynamics, maintained compact conformation, and preserved key stabilizing interactions over an extended simulation period, supporting the reliability of the homology model and the stability of the protein-ligand complex.

DISCUSSION

The isolation of alkali-tolerant Pseudomonas aeruginosa from cosmetic foundation demonstrates the capacity of this opportunistic pathogen to survive in hostile environments typically unfavorable to most cosmetic-associated microbes. The isolate's viability at pH 10 reveals significant limitations in current preservation strategies applied to cosmetic products. This pH tolerance likely reflects adaptive mechanisms including Na^+/H^+ antiport systems, outer membrane lipopolysaccharide modifications, and alkaline protease synthesis, though experimental validation of these mechanisms in cosmetic-associated strains remains necessary.

The identification of P. aeruginosa as the predominant alkali-tolerant organism in this study contrasts with previous reports that documented diverse microbial communities in cosmetics. Alshehrei (15) isolated Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and fungal species including Aspergillus and Penicillium from Saudi Arabian cosmetics. The absence of fungal isolates in our study likely reflects the selective pressure imposed by alkaline pH, which favors alkaliphilic bacteria while suppressing fungal growth. This finding underscores the importance of pH-specific microbial safety testing rather than relying solely on neutral-pH protocols.

The global prevalence of P. aeruginosa in cosmetic products has been documented across multiple studies. Bashir and Lambert (2) reported microbial contamination in nearly 90% of used cosmetics in the United Kingdom, with Pseudomonas species among the isolated organisms. Pakistani salon cosmetics have similarly yielded multidrug-resistant P. aeruginosa exceeding regulatory microbial limits (26). Our findings contribute molecular confirmation through 16S rRNA sequencing (99.85% identity), providing definitive species-level identification that strengthens the evidence base for P. aeruginosa as a persistent cosmetic contaminant.

The computational modeling of the pyochelin biosynthesis protein F (pchF) represents a novel contribution to understanding potential therapeutic targets for cosmetic-associated pathogens. The homology model achieved validation metrics indicating acceptable structural quality (Ramachandran >90%, ERRAT 94.77%, VERIFY3D 90.48%), sufficient for preliminary drug discovery applications. Molecular docking identified quercetin-3-glucuronide as a high-affinity ligand (ΔG = –8.6 kcal/mol),

suggesting potential for natural compound-based antimicrobial interventions. The 100 ns molecular dynamics simulation confirmed structural stability, though experimental validation through MIC testing, enzyme inhibition assays, and in vitro antimicrobial efficacy studies is essential before clinical translation.

Targeting siderophore biosynthesis offers conceptual advantages over broad-spectrum antibiotics by potentially disrupting iron acquisition without eliminating beneficial skin microbiota. However, several limitations constrain interpretation of these findings. First, the study characterized a single isolate, limiting generalizability regarding P. aeruginosa diversity in cosmetic contamination. Second, the number of foundation samples tested was not explicitly reported, preventing assessment of contamination prevalence. Third, computational predictions require experimental validation including binding affinity measurements, enzymatic activity assays, and antimicrobial susceptibility testing with quercetin-3-glucuronide. Fourth, the relationship between alkaline tolerance (pH 10 survival) and cosmetic persistence under normal storage conditions (typically pH 6–8) remains unclear. Fifth, the study did not assess preservative resistance mechanisms or biofilm formation capacity, both relevant to cosmetic contamination persistence.

The implications for cosmetic safety standards are significant. Current microbiological testing typically employs neutral pH conditions, potentially underestimating the survival capacity of alkaliphilic organisms as products age and pH shifts occur. Enhanced monitoring protocols should incorporate pH-variant testing and molecular identification methods to complement culture-based approaches. The presence of preservative-resistant P. aeruginosa in commercially available products indicates need for reformulation strategies, particularly in natural or preservative-free cosmetic lines where antimicrobial defense may be compromised.

CONCLUSION

This study isolated and characterized alkali-tolerant Pseudomonas aeruginosa from cosmetic foundation, confirming bacterial viability at pH 10 through culture-based methods and definitive species identification via 16S rRNA gene sequencing (99.85% identity, GenBank accession MK607451.1). Computational modeling of the pyochelin biosynthesis protein F yielded a structurally validated homology model (Ramachandran 92.3%, ERRAT 94.77%, VERIFY3D 90.48%) suitable for structure-based drug design. Molecular docking identified quercetin-3-glucuronide as a high-affinity ligand (ΔG = –8.6 kcal/mol), and molecular dynamics simulation confirmed stable protein-ligand interaction over 100 ns (RMSD ~0.3 nm, Rg 1.94–2.02 nm). These findings highlight inadequacies in current cosmetic preservation systems and identify pchF as a potential therapeutic target for developing natural antimicrobial agents against opportunistic pathogens. Experimental validation of computational predictions through enzyme inhibition assays and antimicrobial susceptibility testing is warranted to advance translational applications.

REFERENCES

1. Jamka K, Wróblewska-Łuczka P, Adamczuk P, Zawadzki P, Bojar H, Raszewski G. Methodology for preparing a cosmetic sample for the development of Microorganism Detection System (SDM) software and artificial intelligence learning to recognize specific microbial species. Ann Agric Environ Med. 2021;28(4):681-5.

2. Bashir A, Lambert P. Microbiological study of used cosmetic products: highlighting possible impact on consumer health. J Appl Microbiol. 2020;128(2):598-605.

3. Jairoun AA, Al-Hemyari SS, Abdulla AM, El-Dahiyat F. Microbiological quality and safety of cosmetic products in the United Arab Emirates. J Pharm Policy Pract. 2020;13:1-10.

4. Tchonkouang RD, Ngangom BL, Nguenang GM, Toghueo RMK. Microbial contamination of cosmetics: a review of global data and hygiene standards. Microorganisms. 2023;11(4):982.

5. Dadashi L, Dehghanzadeh R. Investigating incidence of bacterial and fungal contamination in shared cosmetic kits available in the women beauty salons. Health Promot Perspect. 2016;6(3):159-63.

6. Orús P, Leranoz S, de la Fuente M. Increasing antibiotic resistance in preservative-tolerant bacterial strains isolated from cosmetic products. Int Microbiol. 2015;18(1):51-9.

7. Tazeen SR, Abbas R, Shahid SA, Shinwari ZK, Ali M. Isolation and detection of bacterial strains from cosmetics products available in Pakistan. Proc Pak Acad Sci B Life Environ Sci. 2023;60(SP1):83-92.

8. Ghssein G, Ezzeddine Z. A review of Pseudomonas aeruginosa metallophores: pyoverdine, pyochelin and pseudopaline. Biology (Basel). 2022;11(12):1711.

9. Ma X, Li W, Xiao W, Cheng J, Lin J. Construction and phenotypic characterization of fur-deleted mutant of Pseudomonas aeruginosa. Acta Microbiol Sin. 2023;64:917-24.

10. Llamas MA, Sanchez-Jimenez A. Iron homeostasis in Pseudomonas aeruginosa: targeting iron acquisition and storage as an antimicrobial strategy. In: Pseudomonas aeruginosa: Biology, Pathogenesis and Control Strategies. 2022. p. 29-68.

11. Sánchez-Jiménez A, Marcos-Torres FJ. Mechanisms of iron homeostasis in Pseudomonas aeruginosa and emerging therapeutics directed to disrupt this vital process. Microb Biotechnol. 2023;16(7):1475-91.

12. Almusawi WN. Are cosmetic products harboring bacterial growth? Int J Curr Res. 2016;18(3):28563-5.

13. Skowron K, Budzyńska A, Kaczmarek A, Gospodarek-Kurkowska E. Microbiological quality of cosmetics: the risk of infection. Postepy Mikrobiol. 2017;56(4):391-8.

14. Ravita T, Sharma R, Bhatia A, Singh B, Khar RK. Microbial load of different foundation creams and their preservative efficacy. Indian J Microbiol. 2009;49(2):193-6.

15. Alshehrei FM. Isolation and identification of microorganisms associated with high-quality and low-quality cosmetics from different brands in Mecca region - Saudi Arabia. Saudi J Biol Sci. 2023;30(11):103852.

16. Fatima S, Khan A, Javed S. Microbiological assessment of local and imported cosmetics in Pakistan. J Cosmet Sci. 2024;75(1):12-24.

17. Abdelaziz AA, Ashour MS, El-Tayeb O. Survival of Pseudomonas aeruginosa in cosmetic products. J Appl Bacteriol. 1989;67(3):241-6.

18. Noor R, Ahmed T, Alam M, Munshi SK, Chowdhury IA. Microbiological quality control of various cosmetic products available in local markets of Bangladesh. J Appl Pharm Sci. 2020;10(5):78-84.

19. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547-9.

20. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30(4):772-80.

21. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 2018;46(W1):W296-303.

22. Moradali MF, Ghods S, Rehm BH. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol. 2017;7:39.

23. Orús P, Leranoz S, de la Fuente M. Increasing antibiotic resistance in preservative-tolerant bacterial strains isolated from cosmetic products. Int Microbiol. 2015;18(1):51-9. doi: 10.2436/20.1501.01.234.

24. Padan E, Bibi E, Ito M, Krulwich TA. Alkaline pH homeostasis in bacteria: new insights. Biochim Biophys Acta. 2005;1717(2):67-88.

25. Wretlind B, Pavlovskis OR. Genetic mapping and characterization of Pseudomonas aeruginosa mutants defective in the formation of extracellular proteins. J Bacteriol. 1980;143(1):190-5.