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In Silico Comprehensive Study for Finding Potential Anti Biofilm Inhibiting Phyto-Chemical by Homology Modeling, Virtual Screening, Docking and Molecular Dynamics Simulation

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ABSTRACT

Biofilm is an essential requirement of microbes for its propagation and it helps in self-defense against antibiotics and adverse environmental factors. But a boon for bacteria has unfavorable economic and health implications on humans. The sheer scale of biofilm formation makes it very difficult for a prepared industrial inhibitor to be economically feasible. This is where Phyto-chemicals can be used as a potential inhibitor because of its low cost of production and easy availability to be used on such a large scale. In this study, we aim to find a potential Phyto-chemical ligand for a Cellulose synthesizing protein BcsF for *Salmonella typhimurium* (strain LT2 / SGSC1412) which is one of the leading species of microbe that responsible for a biofilm-forming matrix. By screening antibacterial Phyto-chemical against our protein, we found that Procyanidin (Pubchem id 124017) had the least binding energy, which can be taken as a probable anti-biofilm agent for experimental validation.

Keywords- phyto-chemical, simulation, homology modeling, virtual screening, docking.

I. INTRODUCTION

Biofilms are non-motile microbe communities that are stabilized by an extracellular matrix of cross linking proteins, nucleic acids and polysaccharides. Because of their capacity to reduced susceptibility to antimicrobial treatments and their ability to adhere to surfaces, matrix forming bacteria are responsible for a high number of nosocomial infections [1-2]. Therefore a deeper explanation of how biofilm formation occurs and how various bacteria bio synthesize and secrete extracellular polysaccharides are urgently required and capable low-cost molecules to hinder it[3]. Microorganism that attach to a non-motile surface and grow as a biofilm are safeguarded from eradication by antibiotics. In biofilms, slow growth, nutrient deficit, poor antibiotic penetration, prolonged stress, and formation of persister cells are thought to create a multi-layered defense. In the food processing industries, the presence of these Biofilms forming microorganism can cause severe consequences down the line to individuals health due to the risk of increasing food borne complication and outbreak[4-6].

It also increase the loss to the business by decreasing the self life of the food products, increasing the merchandise decay, interfering with the warmth transfer processes, elevating the corrosion rate at the surfaces, and additionally, as a result of product could also be thought of adulterated and subject to recall[7-11]. Presently, several researchers were conducted that was able to identify improved strategies for Biofilms formation minimization[11-18]. Thus, considering Phytochemicals and the fact that these compounds are considered to be an essential part of both animal and human diets thus it becomes essential to study their activity against bacterial biofilms. As previously demonstrated by some researchers, Phytochemicals can act as a natural anti biolim strategy with a significant impact on bacterial extracellular matrix formation and development[19]. The work aims to find the efficiency of Phytochemicals as a Biofilm inhibitor for this In silico study, the protein bcsF of *Salmonella Typhimurium (strain LT2 / SGSC1412 / ATCC 700720) was taken which takes part in the cellulose synthesis pathway.* Cellulose is a linear Polysaccharide polymer, that has a structural and functional role in bacterial biofilms formation by providing a scaffold that safeguards and help in the growth of the biofilm[20-22].

II. MATERIALS AND METHOD

1. Sequence Retrivation and Blast Serch

The primary peptide sequence of our protein was obtained from the UniPort Database (www.uniprot.org) (UniPort ID Q7CPI8) of *Salmonella typhimurium (strain LT2 / SGSC1412)*. The Crystal structure of that protein was not found in any established protein data bank, so blast search[23] was performed against possible homologous sequences with an expected threshold of 100 for homology modeling.

2. Homology Modelling

Homology modeling is a pretty good way of in Silico structure prediction in the absence of experimental X-ray or NMR structure. Hence the protein structure was build using MODELLER9.22 [24] software. Around 100 sequences were generated from which the predicted model with the lowest DOPE (Discrete Optimized Protein Energy) score was taken towards structure validation and Ramachandran plot analysis. But as

BLAST search against Protein Data Bank structures gave a result of only three proteins that also with sequence similarity of less 50% other ways of protein modeling ways were explored. The same sequence structure was predicted by using various online serves, such as the I-TASSER server[25] (structure with the minimum c value was taken), Raptor X[26], Pyre 2[27], and galaxy web basted tool GalaxyTBM[28].

3. Structure validation

The quality of the best model obtained by Ramachandran plot analysis which was measured by Ram Page using SAVES meta server comprising of PROCHECK [29], VERIFY3D, ERRAT and PROVE server. Image production and structure visualization were carried out by using PyMol (The PyMOL Molecular Graphics System version 1.5.0.4, Schrödinger, LLC).

4. Molecular Dynamics (MD) simulation

The predicted and validated protein model was added with required number of of Na+ and Cl- ions to neutralize the charge on the system. Then 9673 water molecules were added in a cubic box to replicate the native state that a protein is going to exist. Energy Minimization was carried out by steepest Descent Method for 10000 steps. Isothermal and Isochoric simulation was undergone for about 100ps respectively till temperature and pressure stabilized at 300K and o bar respectively. Finally a 10000ps molecular dynamics simulation was carried out. The graphs of RMSD and Radius of Gyration was obtained and was visualized using qt grace software package.

5. Virtual Screening, Docking, Complex refinement

Phytochemicals were downloaded from PubChem, and around 20 compounds with antibacterial properties were selected. PyRx software was selected for virtual screening. The target protein was designated as a macromolecule and other 20 phytochemicals as the ligands. Because of the absence of any literature on the possible binding sites of this protein, blind docking was done. The entire protein was taken inside the grid box. Energy Minimization was performed on the ligands and then proceeded with screening. The top hit compound with the least binding energy was then taken for further studies in AutoDock. The best binding conformation of the three ligand molecules was then put through galaxy web protein complex refinement tool.

III. RESULT AND DISCUSSION

The primary sequence of the protein was obtained from the UniPort Database (Boeckmann et al. 2003) Primary accession number: **Q7CPI8.** Secondary accession number(s): Q7AY57 of *Salmonella typhimurium (strain LT2 /).* The protein is of 63 amino acids in length, and the molecular weight is 7252 Dalton. Blast search result shows E.coli (strain CFT073 / ATCC 700928 / UPEC) bCSF protein to have a E-value: 1.1e-35, Score: 286, Identify.: 81.0%, Positives: 87.3% and amino acid length of also 63 and plays a role in cellulose biosynthesize, which also is essential is biofilm formation. The homology model created using Modeller provided us with a structure with 56 amino acid 91.8% of the residue in the favored region, five amino acid 8.2% in the favored region, and zero amino acid in the disallowed region when checked through Ramachandran plot. Raptor X provided structure had few unmodelled base pairs which could not be proceeded with simulation. I Tasser structure had over more than 10% of the residues in the disallowed region. The best structure was obtained from the Galaxy Web server with 100% of the residue in the favored region; hence, with structure was validated again with saves meta server with over eight evaluation, the structure showed zero errors two warnings and eight passes in different parameters. To validate the stability of our predicted protein and to verify if the protein could be synthesized under normal temperature and pressure Molecular Dynamics (MD) simulation study of the energetically stabilized structure was carried out using GROMACS v18.1 software. By doing Molecular Dynamics Simulation, one can identify the various Newtonian forces acting on the residues and the potential energy between the atoms. The dynamic behavior of our validated protein structure was analyzed in a solvated system with different Gromacs in-build all-atom force fields for 10000ps. The RMSD and Radius of Gyration were obtained and viewed by qt Grace software. The RMSD (root mean square had a peak around 2300ps after that from 2500ps onwards. It showed a steady decline), indicating the protein stability is increasing. The Radius of Gyration of a molecule is a measure of its stability when present in its natural conditions. If a macromolecule is stably folded, it will likely maintain a relatively constant value of *Rg*. If a protein opens up, its *Rg* will change over time. The protein seems to remain folded around 6000ps. Still, after that, the gyration seems to fluctuate, but with a simultaneous decrease in the RMSD value indicating the protein may be having a stable conformational change, Phytochemicals with antibacterial activity were selected and downloaded from PubChem server. All the phytochemicals were loaded as ligands in the Pyrx workspace. Protein was contained in a grid box with coordinates X:40.58 Amstrong Y:57.85 z:39.4. Energy minimization was done on all the ligands to avoid any steric hindrance, and virtual screening was done with the highest hit molecule was Procyanidin (PubC hem ID 124017). Then the ligand and protein docking was verified and analyzed using AutoDock tool, the grid box was generated with as spacing of 0.442 Amstrong X:269.22 Y:169.87 Z:87.919, About ten pose was generated from which the least energy binding state was selected and was visualized by PyMol software to find out the residue binding and the distance between the ligands.

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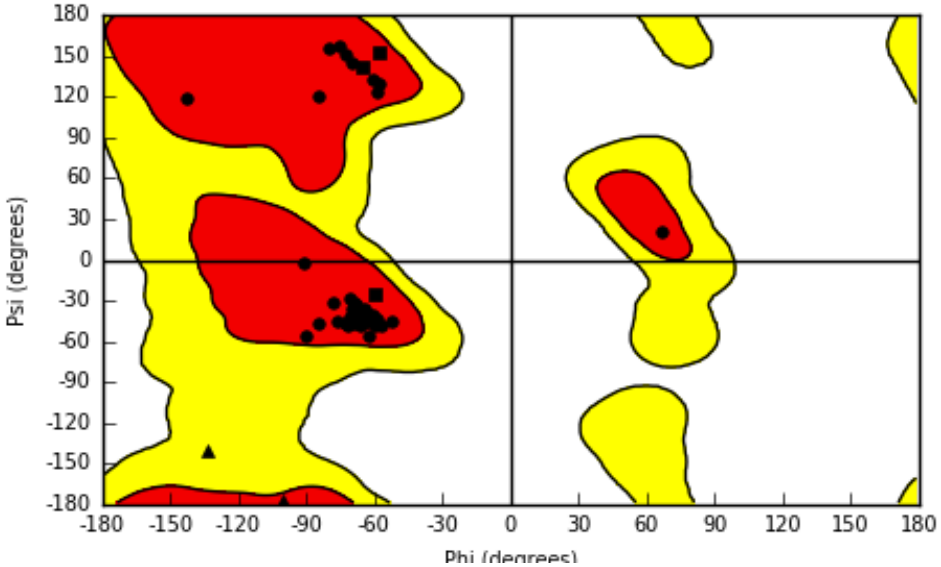
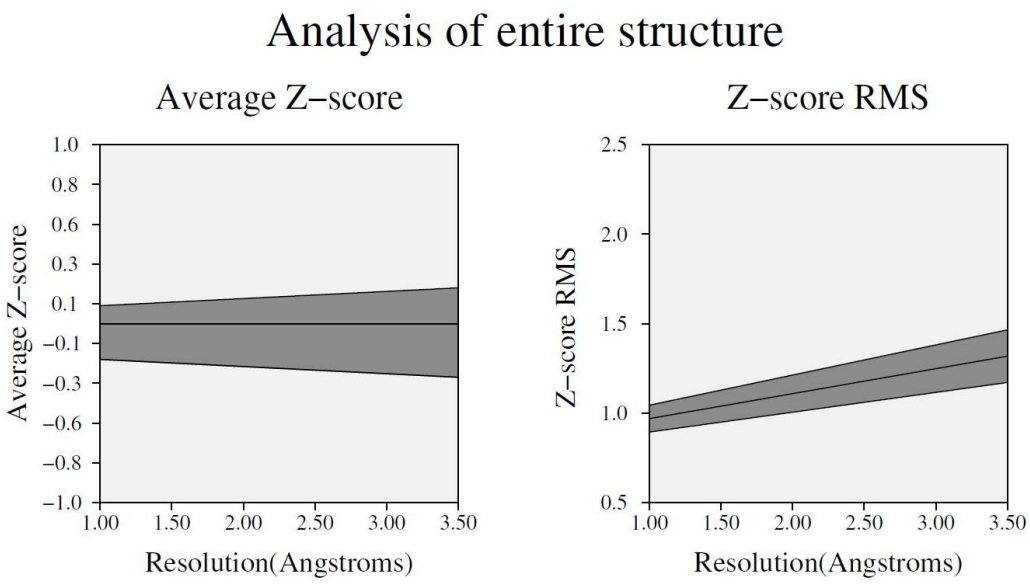
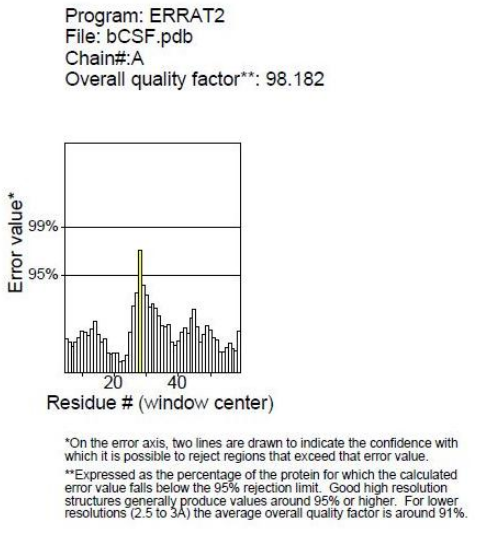


Fig. 1: Ramachandrana Plot of the protein that is to be taken for Molecular Dynamics Simulation





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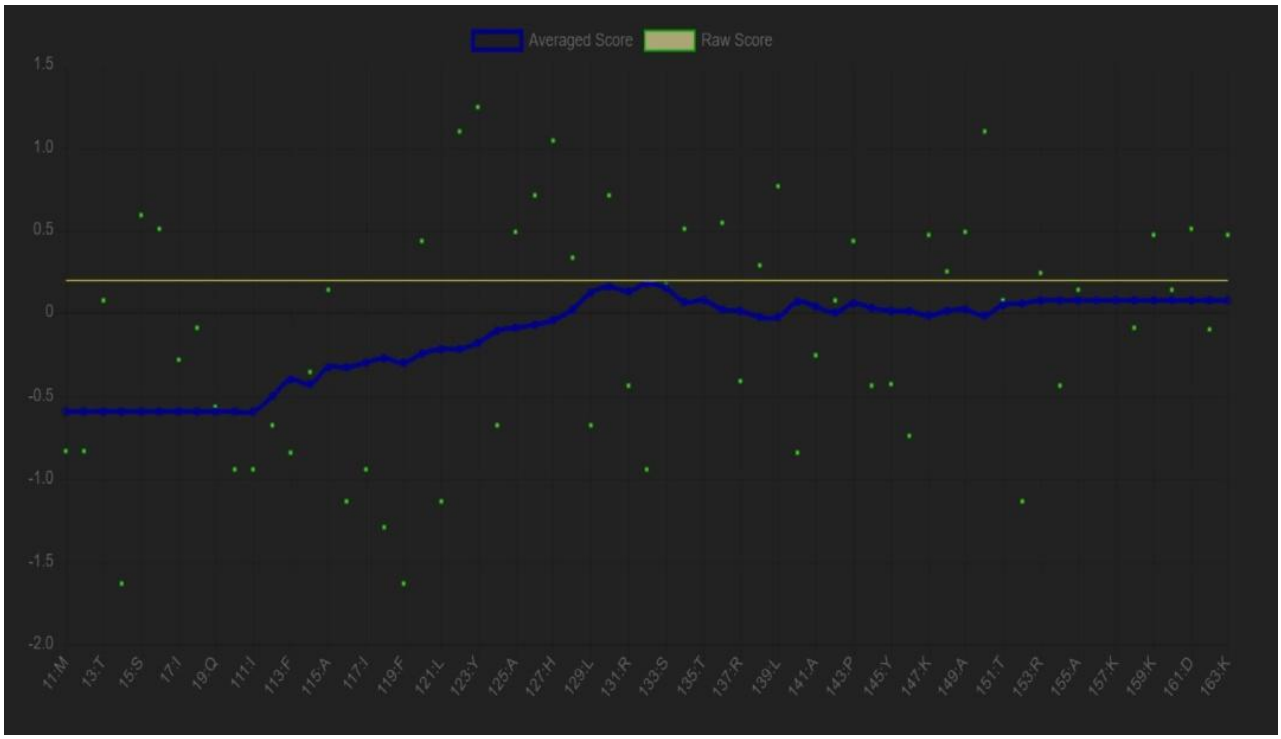

Fig. 2: Quality maps obtained from Saves meta server

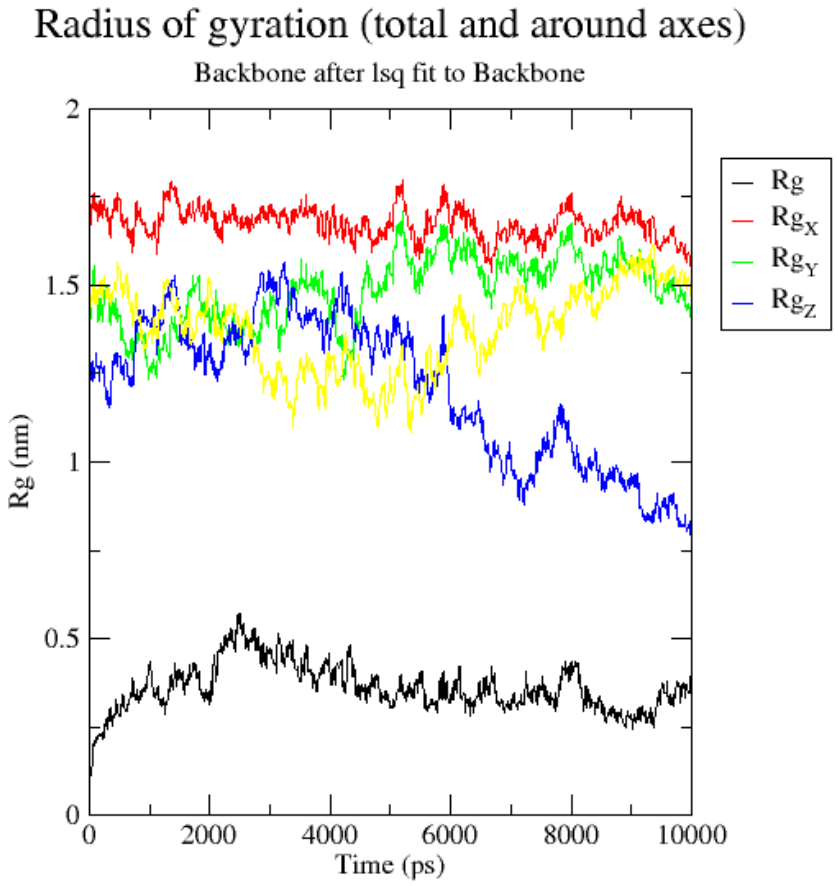

Fig. 3: Molecular dynamic simulation analysis. Root Mean Square Deviation of protein bCSf(black line)and radius of gyration of the protein in different axis(color lines).

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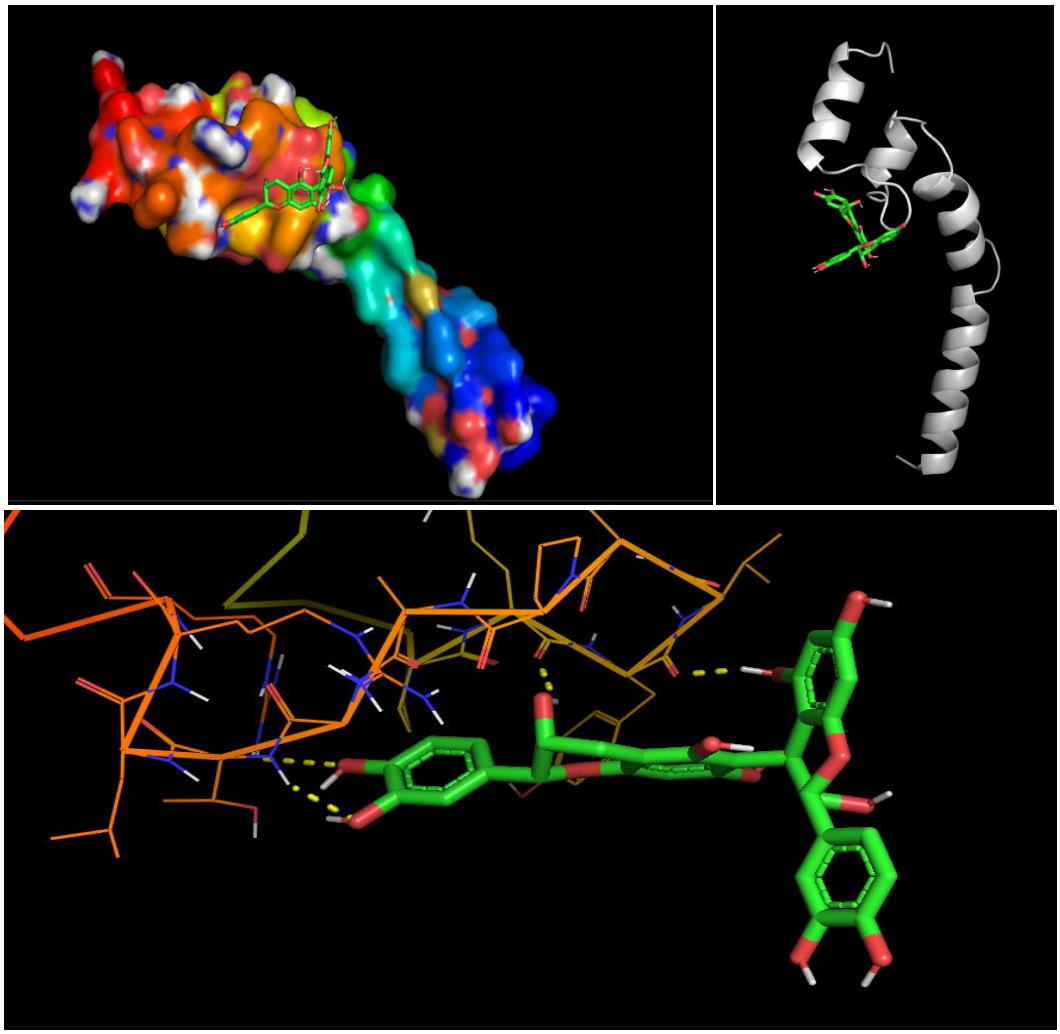

Fig. 4: Interaction of bcsf protein docked with Procyanidin shows stable binding and cnformation.

Table 1

RESIDUE NAME	RESIDUE NUMBER	INTER ATOMIC DISTANCE IN AMSTRONG
Tyrosine	**45**	**1.8**
Arginine	**54**	**2.8**
Threonine	**51**	**2.1**
Arginine	**44**	**2.0**

REFERCENCES

[1] Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon. 2018 Dec 1;4(12):e01067.

[2] Raetz CR, Reynolds CM, Trent MS, Bishop RE. Lipid A modification systems in gram-negative bacteria. Annu. Rev. Biochem.. 2007 Jul 7;76:295-329.

[3] Wang Y, Guo W, Wu X, Zhang Y, Mannion C, Brouchkov A, Man YG, Chen T. Oncolytic Bacteria and their potential role in bacterium-mediated tumour therapy: a conceptual analysis. Journal of Cancer. 2019;10(19):4442.

[4] Kurtz JR, Goggins JA, McLachlan JB. Salmonella infection: Interplay between the bacteria and host immune system. Immunology letters. 2017 Oct 1;190:42-50.

[5] Lynd LR, Weimer PJ, Van Zyl WH, Pretorius IS. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev.. 2002 Sep 1;66(3):506-77.

[6] Roberts K. Structures at the plant cell surface. Current opinion in cell biology. 1990 Oct 1;2(5):920-8.

[7] Roberts K, Grief C, Hills GJ, Shaw PJ. Cell wall glycoproteins: structure and function. J Cell Sci. 1985 Jan 1;1985(Supplement 2):105-27.
[8] Wingender J, Neu TR, Flemming HC. What are bacterial extracellular polymeric substances?. InMicrobial extracellular polymeric substances 1999 (pp. 1-19). Springer, Berlin, Heidelberg.
[9] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews microbiology. 2004 Feb;2(2):95.
[10] Park SC, Park Y, Hahm KS. The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. International journal of molecular sciences. 2011 Sep;12(9):5971-92.
[11] Pavithra D, Doble M. Biofilm formation, bacterial adhesion and host response on polymeric implants—issues and prevention. Biomedical Materials. 2008 Aug 8;3(3):034003.
[12] Møretrø T, Langsrud S. Listeria monocytogenes: biofilm formation and persistence in food-processing environments. Biofilms. 2004 Apr;1(2):107-21.
[13] Das S, Dash HR, Chakraborty J. Genetic basis and importance of metal resistant genes in bacteria for bioremediation of contaminated environments with toxic metal pollutants. Applied microbiology and biotechnology. 2016 Apr 1;100(7):2967-84.
[14] Simoes M, Bennett RN, Rosa EA. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. Natural product reports. 2009;26(6):746-57.
[15] Schultz G, Bjarnsholt T, James GA, Leaper DJ, McBain AJ, Malone M, Stoodley P, Swanson T, Tachi M, Wolcott RD, Global Wound Biofilm Expert Panel. Consensus guidelines for the identification and treatment of biofilms in chronic nonhealing wounds. Wound Repair and Regeneration. 2017 Sep;25(5):744-57.
[16] Arciola CR, Montanaro L, Costerton JW. New trends in diagnosis and control strategies for implant infections.
[17] Seviour T, Derlon N, Dueholm MS, Flemming HC, Girbal-Neuhauser E, Horn H, Kjelleberg S, van Loosdrecht MC, Lotti T, Nerenberg R, Neu TR. Extracellular polymeric substances of biofilms: Suffering from an identity crisis. Water research. 2018 Nov 28.
[18] Field D, Gaudin N, Lyons F, O'Connor PM, Cotter PD, Hill C, Ross RP. A bioengineered nisin derivative to control biofilms of Staphylococcus pseudintermedius. PLoS One. 2015 Mar 19;10(3):e0119684.
[19] Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews. 1999 Oct 1;12(4):564-82.
[20] Solano C, García B, Valle J, Berasain C, Ghigo JM, Gamazo C, Lasa I. Genetic analysis of Salmonella enteritidis biofilm formation: critical role of cellulose. Molecular microbiology. 2002 Feb;43(3):793-808.
[21] Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ. Bacterial biofilms in nature and disease. Annual Reviews in Microbiology. 1987 Oct;41(1):435-64.
[22] Ude S, Arnold DL, Moon CD, Timms-Wilson T, Spiers AJ. Biofilm formation and cellulose expression among diverse environmental Pseudomonas isolates. Environmental Microbiology. 2006 Nov;8(11):1997-2011.
[23] Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research. 1997 Sep 1;25(17):3389-402.
[24] Sali A, Webb B. MODELLER (Version 9.22).
[25] Zhang Y. I-TASSER server for protein 3D structure prediction. BMC bioinformatics. 2008 Dec;9(1):40.
[26] Källberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, Xu J. Template-based protein structure modeling using the RaptorX web server. Nature protocols. 2012 Aug;7(8):1511.
[27] Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nature protocols. 2015 Jun;10(6):845.
[28] Shin WH, Lee GR, Heo L, Lee H, Seok C. Prediction of protein structure and interaction by GALAXY protein modeling programs. Bio Design. 2014;2(1):1-1.
[29] Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of applied crystallography. 1993 Apr 1;26(2):283-91.
[30] Jorgensen WL, Tirado-Rives J. The OPLS [optimized potentials for liquid simulations] potential functions for proteins, energy minimizations for crystals of cyclic peptides and crambin. Journal of the American Chemical Society. 1988 Mar 1;110(6):1657-66.