Farnesol wins the wrestle against 'biofilm associated protein (bap)' of *Staphlyococcus epidermidis*

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Abstract

Background/Objectives: Using simple *in silico* genomic and proteomic techniques, this study is an approach to identify some molecules against the biofilm associated protein (bap) protein of *Staphylococcus epidermidis*. 'bap' is a gene that is associated with biofilm formation in many *Staphylococcus* species, which cause biofilms in venous catheters of indwelling medical devices.
Methods/Statistical analysis: Various genomic and proteomic analyses revealed that 'bap' has a good scope as a target to combat biofilms on indwelling medical devices.
Results: Farnesol, a terpenoid by nature exhibited very good binding with the 'bap' protein compared to other molecules of similar structures, when docked with Hex 4.2.
Conclusion/Application: Further analyses on other associated natural compounds on other genes which promote adherence of biofilms, may reveal their capacity to inhibit the biofilm formation on medical devices, which will be a boon to both doctors and patients alike.

***Keywords*:** biofilm, bap, farnesol, *Staphylococcus epidermidis*, venous catheters, indwelling medical devices.

1. Introduction

Many human microbial nosocomial infections are caused by biofilms on indwelling medical devices like central venous catheters, contact lenses, endotracheal tubes, intrauterine devices, mechanical heart valves, pacemakers, dialysis catheters, prosthetic joints, urinary catheters and the like, which adhere to a surface. These replicating bacteria (both commensals and pathogens) secrete insoluble gelatinous exopolymers which form a three-dimensional polymer matrix known as a biofilm. They escape host defenses and are significantly less susceptible to antibiotics.[1]

Significant morbidity and mortality and a great impact on healthcare delivery has been caused due to biofilms on indwelling medical devices such as central venous catheters. Due to the resistance shown by the microorganisms in biofilms on these devices, routine treatment of patients with catheter-associated bloodstream infections is often ineffective. The factors influencing rate and extent of biofilm formation are adherence, the number and types of cells in the liquid to which the device is exposed besides the rate of flow of liquid through the device and the physicochemical characteristics of the surface. The rate of growth is influenced by flow rate, composition of the medium, antimicrobial-drug concentration and favourable temperature.[2]

Some of the microorganisms most commonly isolated from venous catheter biofilms include *Staphylococcus epidermidis, K. pneumoniae, P. aeruginosa, Enterococcus faecalis, Candida albicans* and *S. aureus*. It is claimed that these microbes originate from the patient's skin microflora, microflora from healthcare providers or contaminated intravenous fluids.[1] It is also found that biofilm formation on venous catheters is universal. The location of biofilm formation and its extent depends on the duration of catheterization.

Biofilm-associated protein (bap gene) is involved in the formation of biofilms in *Staphylococcus epidermidis*. The presence of bap may induce biofilm formation in persistent *S. aureus* infections.[3] Research on bap gene revealed the presence of alternative forms of the bap protein, which contain varying number of repeats in *S. epidermidis* isolates. The presence of anti-bap antibodies in serum samples taken from animals with confirmed *S. epidermidis* infections indicated the production of bap during infection.

2. Materials and Methods

Nucleotide sequence: EU011247.1 (*Staphylococcus epidermidis*)
Gene: Biofilm-Associated Protein Gene
Protein sequence: DQ008306.1
Organism: *Staphylococcus epidermidis* (*Staphylococcus epidermidis*)
Ligand: Farnesol, Molecular Weight: 222.366340g/mol., Molecular Formula: C15H26O

The primary literature search was performed in ScienceDirect, Medline, Pubmed and Patents Forum for published valid information for genomic and proteomic analyses and docking studies on 'bap' gene and protein. The nucleotide sequence of the given gene was retrieved from the GenBank database repository at the National Centre for Biotechnology Information (NCBI). The standard blastn nucleotide-nucleotide BLAST was used for identifying similar sequences and to find local regions of similarity in the non-redundant nucleotide database for the gene of interest.[4] The identification of genomics islands of potential horizontally transferred genes using the variation in G+C percentage and other associated factors in the genome of the organism of interest, *Staphylococcus epidermidis* were determined using IslandPath.

The protein was obtained from the protein database repository from the National Center for Biotechnology Information (NCBI). The standard blastp protein-protein BLAST was used for identifying similar sequences and to find local regions of similarity in the non-redundant protein database for the protein of interest. The conserved domains of distinct functional and/or structural units of the protein of interest were detected using Conserved Domains Search (Fig 2.1) of NCBI.[5] The known and predicted protein interactions were searched in the STRING database (Fig. 2.2) amongst a large number of organisms.[6] The structure for the protein of interest was searched for in the Protein Data Bank (PDB) database which provides a standard representation for macromolecular structure data derived from X-ray diffraction and NMR studies.

Figure 2.1. Conserved domains in 'bap'

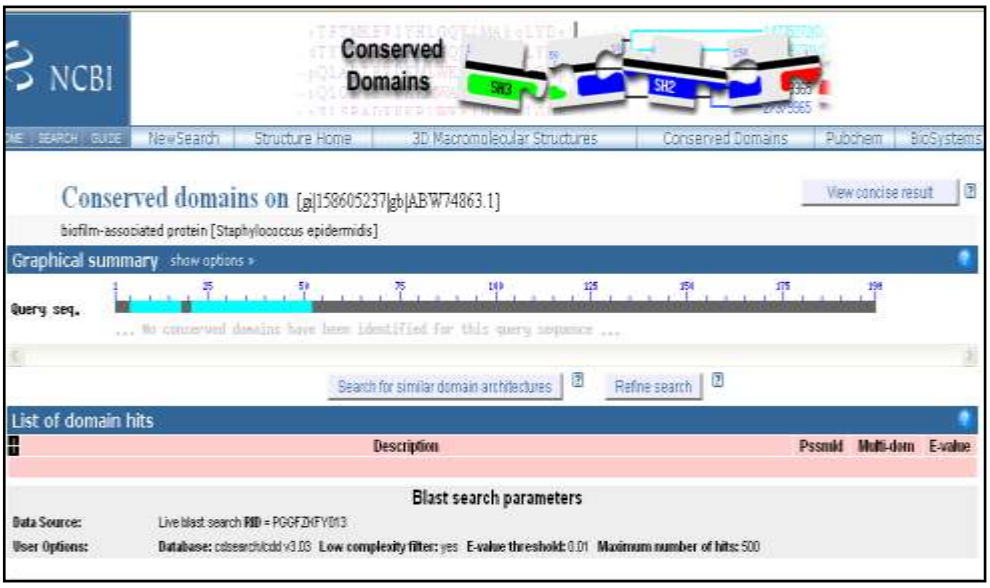
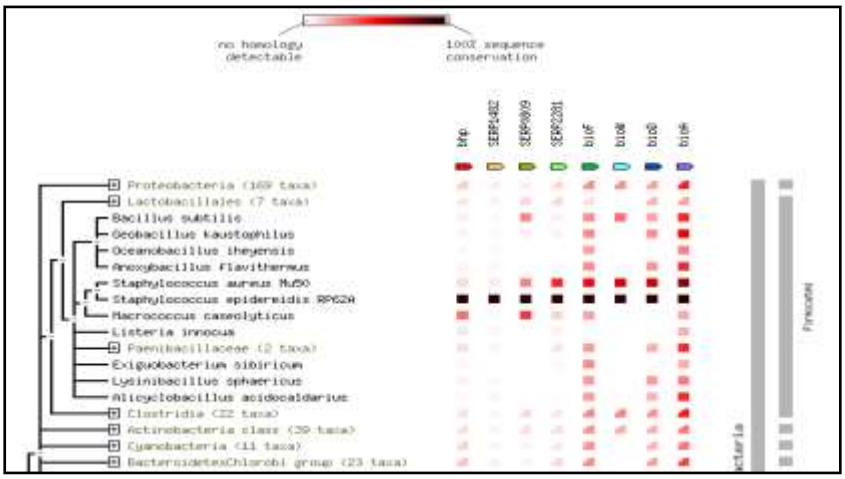


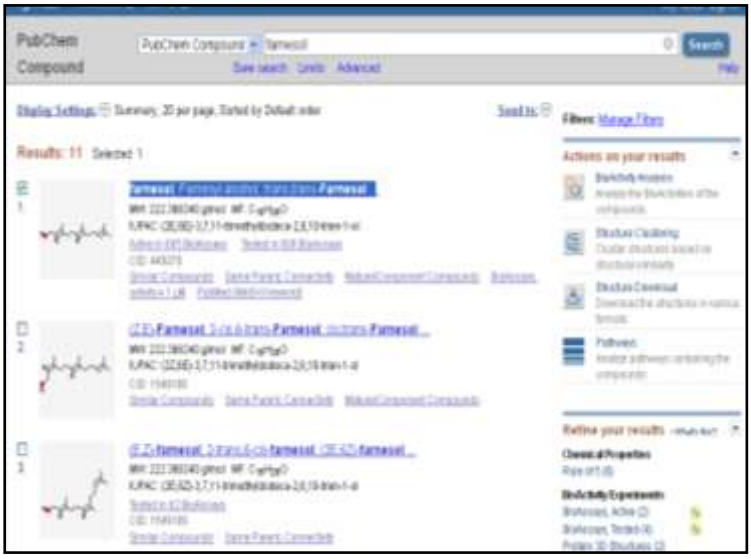
Figure 2.2. Homology of 'bap' using Strings 3.0



The template structures for the protein structure modelling were also obtained from PDB.[7] A BLAST program was performed against the Protein Data Bank (PDB) database to search for templates to perform the protein modeling. PDB structure 1Ndb A (Chain A, crystal structure of carnitine acetyltransferases) was found to be the hit with highest similarity to biofilms adherence protein (bap) sequence. The protein of interest did not have a 3D structure and hence was modelled computationally using SWISS-MODEL which is a fully automated online protein structure homology-modelling software, available at the ExPASy web server.[8]

The validated chemical depiction information to describe substances was obtained from PubChem database [9] (Fig 2.3) . Using Open Babel, a chemical toolbox the molecule of interest was searched, analysed and converted into the required file format (SDF to PDB) used in molecular modelling and computational chemistry. [10] Ramachandran Plot was used to check the quality of the receptor (range above 80%). The structure was changed into Pdb format for docking studies. The molecule of interest (ligand) was docked using Hex 4.2, an interactive molecular graphics program used for calculating and displaying feasible docking modes of pairs of protein molecules. The tool was also used to calculate ligand/protein docking.[11]

Figure 2.3. Identification of Farnesol Compound in PubChem Database



3. Results and Discussion

There were no observed horizontal gene transfers found for the gene of interest. The known and predicted protein interactions were searched in the STRING database amongst a large number of organisms. In Swiss Model, the modeled structure retrieved showed 96.6% of structure similarity to the receptor.

Biofilms are as omnipresent as microorganisms in this world. Their importance in the field of medicine is gaining rapid importance owing to reasons like antibiotic resistance and incredible adaptation mechanisms undertaken by the microbes in response to all human efforts taken against them. This study is a small attempt to avoid the formation of biofilms on indwelling medical devices, (venous catheters) using genomic and proteomic analyses. This was done targetting the genes and proteins associated to adherence in the organism of interest. An in-depth literature mining showed the prospect of having good targets which were required for the study.

Staphylococcus epidermidis is one of the main species of staphylococci and other microbes which form biofilms in venous lines. Genes like bap, recN, icaA, icaC, mecA, aap, aae, ygs sarA, sarZ and rsbU were shown to be associated to the adherence of biofilms. The 'bap' gene was selected for study.[12] Using various analyses like similarity search (BLAST), domain search (CD SEARCH), homology search (STRING), motif search (PROSITE), protein structure and template search (PDB) and horizontal gene transfer search, (ISLANDPATH) promising results were derived from the study.

'bap' gene was identified in *Staphylococcus epidermidis.* The homologues of this gene were present in most of the species of *Staphylococcus* (BLASTn). The interacting proteins of this protein (gene product of 'bap') were found in many species of the Enterobacteriaceae family (STRING).[13] A ten letter amino acid motif was found in the 'bap' protein which plays an important role in the adherence property of the organism according to literature. The 'bap' protein did not have a 3D structure; however, its homologues had structures which were used as templates in modeling the structure of 'bap' (PDB). The 3D structure of 'bap' which was obtained using Swiss-Model was found be of good quality (Ramachandran Plot). Many molecules were screened for activity against the target (bap). Of all these molecules, Farnesol seemed to have a good activity profile (PubChem). The receptor and ligand docking (Fig. 3.1 and 3.2) gave a well docked structure which is a good result (Hex 4.2).

Figure 3.1. Receptor and ligand in Hex 4.2

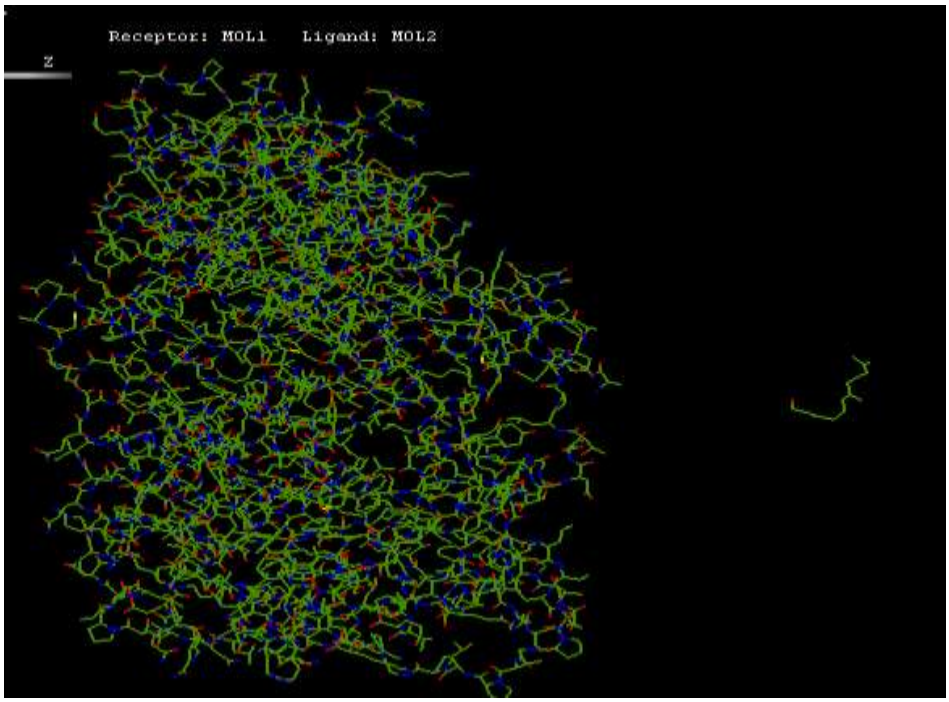
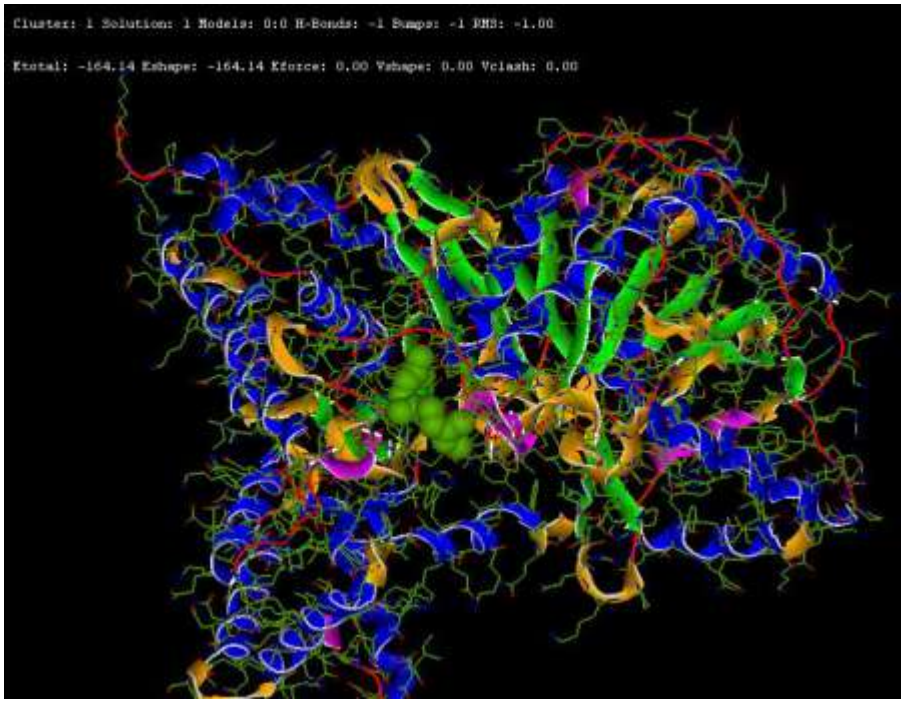


Figure 3.2. Bound Receptor and Ligand in Hex 4.2 after Docking



4. Conclusion

The obtained results suggest that the 'bap' gene in *Staphylococcus epidermidis* may be targeted to avoid the formation of biofilms in venous catheters, the reason being that since the targeted gene is responsible for adherence; the organism may not be able to attach itself to the surface of the catheters. The lead obtained from this study is that even if such biofilms were to form on the catheters, compounds like farnesol may be used against them. However, this aspect of the study needs more evidence from wet-lab techniques in future.

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