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PREVENTION AND INHIBITION OF ENVIRONMENTAL BIOFILMS: FUTURE PERSPECTIVES

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ABSTRACT

Biofilm formation is a universal phenomenon pertaining to a microorganism attaching itself to a surface that is in constant contact with water. It occurs widely on the ships hulls, dentures, eyes due to non compatible contact lenses, implanted medical devices, showers tubes, cooling pipes in nuclear reactors, water distribution systems etc. Environmentally and economically viable solutions are increasingly being researched to prevent and control the biofilm formation. Natural and synthetic compounds have been studied extensively to address the issue. This paper reviews the use of various methods being adopted to prevent and inhibit the formation of biofilms and other futuristic approaches.

KEYWORDS: **Biofilms, Environment, Bioinformatics, Inhibition, Bacterial adhesion**

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INTRODUCTION

Biofilms are widespread on substrates which are in continuous contact with water and are a topic of discussion in environmental science, microbiology and medical science. Biofilms are ubiquitous in nature and its formation is part of a strategy that microorganisms use in order to survive in hostile environments. Biofilms are responsible for loss of billions of dollars to the industries, since they cause fouling of ship hulls, water treatment and distribution systems, causing corrosion of pipes and thereby resulting in contamination. Biofilms are also of great interest in medical context since they result in persistent and chronic infections and also contaminate implanted devices. Although biofilms play crucial roles in many processes (biodegradation of environmental pollutants or the microbial balance within the human body), they are often considered as unwanted guests and cause serious problems in the food, biomedical, environmental and industrial fields. Biofilms can lead to increase in the costs of production and equipment maintenance, as well as to public health and environmental concerns and impacts. Biofilms can be broadly classified as environmental or medical health related, depending on the source of occurrence as shown in Figure 1. Environmental biofilms can either occur in natural environments like oceans, seas, lakes, river beds, streams and on hulls of boats, vessels and ships[1] or in controlled environments like aquaculture ponds, shower tubes[2], wastewater treatment plants[3], nuclear reactors[4] and pipelines of water distribution systems[5]. Health related or medical biofilms also occur naturally like dental plaque[6], biliary tract infections[7], diabetic ulcers[8] or on artificial implants which include those formed on the contact lenses[9], catheters[10], medical instruments and equipments, dental biofilms[11,12], and implants[13] in the human body.

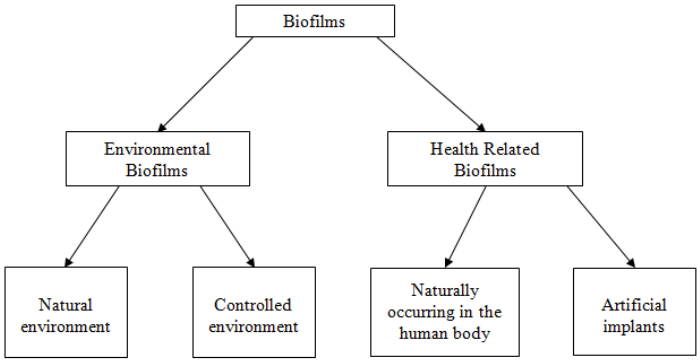

Figure 1
Classification of biofilms based on the source of occurrence

Numerous solutions have also been offered by scientists worldwide, from simple mechanical methods like scraping the fouling organisms off the ship surfaces to use of antifouling paints containing compounds like copper[14] and tributyltin (TBT)[15] etc. However TBT has damaging effects on the marine environment as it is not biodegradable and has recently been banned under the IMO treaty[16]. Several chemicals have been studied to address the biofilm challenge which includes benzalkonium chloride[17], chlorine[18], sodium hypochlorite[19], hydrogen peroxide[20], pyrethroids[21]etc. Natural compounds from plants[22] and other organisms are also being researched because of their eco friendly properties. Recent developments in the field include researching the use of less toxic options for the prevention of the biofilm formation like phytochemicals and marine microbes. Moreover, the use of computational methods has

gained lot of importance in the field of health sciences and they are useful in the early stages of target identification and drug discovery due to the exponentially increasing speed of computers. Bioinformatics provide faster and more efficient tools for the development of new antibiotics and also to satisfy the pressing need for new antibiotics and the economic realities of drug discovery. Comparative modeling is one of the useful tools in bioinformatics and it is an invaluable aid to understand functional properties of protein and also a preliminary step towards establishing a promising drug. Bioinformatics encompasses chemoinformatics approaches like docking, virtual screening of compounds, QSAR studies etc. Bioinformatics tools are also being researched for marine studies, particularly in the inhibition and prevention of biofilms[23]. This paper also reviews the scope of applying bioinformatics to address the biofilm challenge.

BIOFILM FORMATION IN THE ENVIRONMENT

Aquatic environments are very dynamic complex systems which host a range of microorganisms and macroorganisms that are able to survive in extreme conditions. Environmental factors like temperature, humidity, pressure changes and salinity of water all influence the existence of the organisms in such environments. In spite of all these changes that happen dynamically in the system the biofilms are formed in the marine environment. Surfaces that are in continuous contact with water are sources of biofilm formation. Preventing the formation of biofilms is a better option than trying to remove or disinfect them from the attached surfaces. The major difference in naturally occurring biofilms in the environment is that they usually take place in large areas which are easily accessible but the biofilms occurring in the controlled environment typically happen in small areas and are mostly inaccessible. In medical biofilms, it is mostly found that there is only one specific bacterial species affecting the region of the human body. The environmental biofilm samples obtained from the aquatic environment usually contain a microbial consortium with more than one bacterial species, thereby forming a complex microbial community. Such complex microbial biofilms are simultaneously fascinating to the microbiologists, yet causing trouble to environment, industry and medicine. Much of the fascination and concern relates to their recalcitrance towards even the most aggressive antimicrobial treatments. The presence of more than one bacterial species makes it difficult to investigate strategies to inhibit the formation of biofilms in both controlled and natural environments.

Biofilm formation is a precursor to the biofouling process which includes both microorganisms and macroorganisms. The attachment of a single bacterial cell to the surface initiates the biofilm formation. The adhesion between the bacterial cells aids in the formation of multiple layers and then subsequently leads to the mature biofilm formation[24] as illustrated in Figure 2. The biofilm thus formed by these microbial populations serves as an energy source for the macroorganisms which then lead to macrofouling.

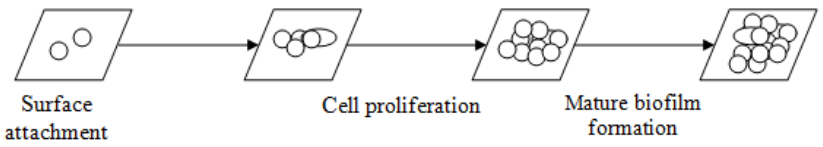

Figure 2
Microbial biofilm formation in the natural environment

BACTERIAL ADHESION

The matrixes of biofilms formed contain nucleic acids, lipids, proteins and exopolysaccharides. These extracellular membrane proteins mediate bacterial attachment. The substratum and the properties of the cell surfaces strongly influence

the formation of biofilms. It is observed that smoother the surface of the substrate the better it is to prevent the formation of biofilms, since the bacteria are less susceptible to the surface adhesion. The membrane adhesive proteins in the microorganisms and macroorganisms promote their attachment to the substrates. The initial attachment of bacteria to the surface is a reversible process and the subsequent adhesion of the macroorganisms is an irreversible process[25]. The bacterial layer initially formed in the biofilms can be cleaned easily. The irreversible adhesion is due to the release of extra cellular polysaccharides (EPS), which is a highly polar mixture that aids in strong attachment of the marine macroorganisms like mussels, barnacles and seaweeds. These macroorganisms are difficult to clean from the attached substratum. Biofouling includes both microfouling and macrofouling. Microfouling is the attachment of microorganisms like bacteria on the surface that is in contact with water. This bacterial layer further serves as a nutrient and energy source to the macroorganisms that attach to the surface and cause macrofouling. There are several marine bacteria reported to contain adhesive proteins like the gram positive bacteria, *Bacillus subtilis*[26], *Staphylococcus aureus*[27], *Bacillus cereus*[28], and gram negative bacteria, *Pseudomonas aeruginosa*[29], *Escherichia coli*[30], *Shewanella oneidensis*[31], *Vibrio cholerae*[32], *Vibrio harveyi*[33], *Vibrio vulnificus*[34]. Various compounds have been researched to prevent the adhesive nature of these bacteria.

QUORUM SENSING BACTERIA

Bacteria are able to “talk” to each other and communicate through a complex signaling system called quorum sensing. This involves cell to cell communication of the signals in bacteria which is a chemical process. Quorum sensing is a critical phenomenon in maintaining the biofilm formed intact. Signals are transmitted to the bacterial cells in the biofilm rapidly, thus increasing the resistance of the biofilm to antibiotics. In quorum sensing AHL (N-acyl homoserine lactone) and AIP (autoinducing peptide) are found to be the active signaling molecules in gram-negative organisms and in many gram-positive organisms respectively. The quorum sensing process highly depends on the density of the bacterial cells in the area[35]. The higher the number of bacterial cells the better the signal transduction. Research is currently ongoing to identify inhibitors that can intervene with the signaling pathway and disrupt the formation of biofilms. It is of great importance to study the quorum sensing phenomenon since most of the biofilm forming bacteria are slowly developing tolerance to the antimicrobial inhibitors[36].

IMPACT OF BIOFILMS

The bacterial adhesion which leads to microfouling[37], provides a space for macrofouling which includes attachment of larger organisms like barnacles, mussels[38] etc. This type of biofouling leads to several environmental problems like introduction of invasive species[39], energy related issues[40] and formation of chemical pollution. This not only has an economical impact on the shipping industries but also affects the environment adversely[41]. Billions of dollars have already been spent on this global challenge of removing the biofilms. It has been reported that over one billion dollars have been spent in cleaning the ship hulls which amount for more than the costs associated with the fuel costs[42]. Biofilm formation in water systems affects the water quality thereby affecting the human health by release of pathogens in water[43]. According to the Centers for Disease Control and Prevention, National Institutes of Health, report titled, “Issues in Healthcare Settings: CDC’s Seven Healthcare Safety Challenges”, biofilm based microbial infections make up to 80% of all infections in humans[44].

Not all the biofilms formed are detrimental in nature. Biofilm reactors are used to treat the environmental wastes in the wastewater treatment plants[45], industrial wastewaters and contaminated groundwater. Biofilms are also used for layering in food industry for preservation, which of course, is still a topic of debate [46]. Bioremediation involves coating bacteria which form biofilms for the cleanup process[47].

ROLE OF BIOINFORMATICS

Bioinformatics is being used in health related and medical studies successfully in identifying lead molecules for drugs. Bioinformatics and chemoinformatics tools aid in the simulation and modeling of biological structures like DNA, RNA and proteins. The bioinformatics tools are also applied to compare genomic sequences to understand the evolutionary aspects of various organisms. Chemoinformatics includes *in silico* approaches like virtual screening, docking, quantitative structure activity relationship (QSAR) studies, simulation and visualization of molecules. The chemoinformatics tools can be applied to the environmental studies in the context of biofilms to identify novel inhibitors to prevent the formation of biofilms. Docking tools facilitate in the process of simulating the binding of a ligand to a receptor. The docked complex can then be studied to determine the binding energies and to identify the binding sites and inhibition sites[48]. Virtual screening of compounds *in silico* is an easy and quicker means to get to the target[49]. Virtual screening coupled with docking can lead to the identification of potential inhibitors with fair amount of reliability. The known compounds screened can then be rendered for QSAR studies to quantify their biological responses. New unknown compounds can then be derived from the chemical structures of the known compounds by altering the functional groups and tested for their efficacy. These methods are already being used successfully to identify lead compounds for drugs in the medical and health studies. The same approaches of docking, virtual screening and QSAR studies could be applied to environmental studies as well to identify new inhibitors to prevent biofilm formation.

LIMITATIONS OF BIOINFORMATICS AND FUTURE DIRECTIONS

Marine environments are highly complex and are dynamic in nature. The bacteria in marine environments form a microbial consortium with different bacterial species attaching to the surface of the substrate. The advantage is that there is a synergistic effect of the bacteria that coexist on the biofilm formation. This also leads to the challenge of identifying different inhibitors for each of the bacteria attached to the surface forming the biofilm, since each of the bacterial species might respond differently to different inhibitors. This is not the case in medical or health related studies where the target is just one bacterial species at a given time, where it only depends on varying the concentration of the drug to be given. Even if the same inhibitors are found to be active against various bacterial species in the biofilms, their concentrations would differ and it is difficult for us to identify the concentrations needed to inhibit these biofilm forming bacteria, using the existing bioinformatics tools. Therefore, there is a need to develop tools which can not only simulate the dynamic marine environment by taking into account factors like temperature, pressure, humidity, salinity of water but which also aid in arriving at the right amount of inhibitor concentrations that can actively target the bacteria in the microbial consortia. Also most of the bioinformatics docking softwares like GOLD[50] and GLIDE[51] are expensive which limits their application. The National Center for Biotechnology Information (NCBI) is a repository for information relating to biological and chemical molecules. The Protein Data Bank (PDB) stores the entries deposited for few of the cell adhesive proteins whose structures have been determined. For example, the PDB structure, PDB ID: 3VAC, given in Figure 3, is the crystal structure of the CFA/I Enterotoxigenic *Escherichia coli* adhesion CfaE mutant G168D[52].

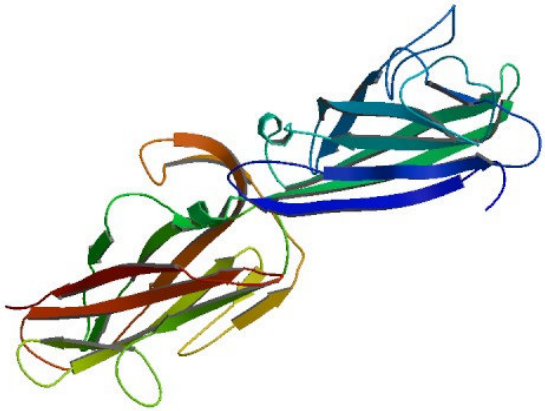

Figure 3
Crystal structure of the CFA/I Enterotoxigenic Escherichia coli adhesion CfaE mutant G168D (PDB ID: 3VAC)

The major limitation in applying the chemoinformatics approaches to tackle the biofilm issue is that the availability of the crystal structures of these cell adhesive proteins in marine bacteria is inadequate. Few of the protein sequences are available in NCBI which can be modeled to a structure and tried with different inhibitors. The docking data need to be validated with known inhibitors from in vitro studies and protocols can be designed to identify novel compounds by altering the functional groups.

PREVENTION AND INHIBITION OF BIOFILM FORMATION

The prevention of biofilm formation is an age old research problem and a number of solutions have been reported to tackle the global challenge. Traditional methods like scraping, applying animal fat to the boats to organic synthetic compounds[53] have been used traditionally as anti fouling agents. The use of ultra violet radiation, ozone, reverse osmosis technique, low energy surface acoustic waves[54] and lasers have proved successful to a certain extent. Nanomaterials are also increasingly being researched to control biofouling[55,56]. Organotin compounds like tributyltin (TBT) and triphenyltin have been banned since they have adverse effects on the environment. Phytochemicals and other marine microbes[57] are being researched as a non toxic or a less toxic option to synthetic compounds. Marine sponges have also shown potential antimicrobial activity[58]. The antibacterial effect of few of the natural compounds is reported in Table 1 and few of the synthetic chemical compounds are reported in Table 2.

Table 1
Naturally occurring compounds reported to have antibacterial activity

Compound	Plant	Bacterial species	Reference
Macelignan	*Myristica fragrans Houtt*	*Streptococcus mutans, Streptococcus sanguis* and *Actinomyces viscosus*	Yanti et al., 2008[59].
Xanthorrhizol	Javanese Turmeric (*Curcuma xanthorrhiza Roxb.*)	*Candida albicans*	Rukayadi and Hwang, 2012[60].
Phenylethanoid glycosides and Iridoid glycosides	*Penstemon centranthifolius*	*Helicobacter pylori, Propionibacterium acnes* and *Staphylococcus aureus*	Ye et al., 2010[61].
Labdane type-diterpenes and Sesquiterpenes	*Copaifera langsdorffii* Desf	*Streptococcus salivarius, S. sobrinus, S. mutans, S. mitis, S. sanguinis* and *Lactobacillus casei*	Souza et al., 2011[62].
Ellagitannins	*Acalypha wilkesiana var. macafeana hort.*	*Bacillus cereus* (ATCC 11778)*, Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 11632) *and Methicillin-resistant Staphylococcus aureus* (MRSA) clinical strain	Din et al., 2012[63].
	Cranberry extracts	*Escherichia coli* and *Staphylococcus species*	LaPlante et al., 2012[64].
	Mentha piperita and *Rosmarinus officinalis* essential oils and chlorhexidine	*Streptococcus mutans and Streptococcus pyogenes*	Shayegh et al., 2008[65].
	Rose, Geranium, Lavender and Rosemary essential oils	*Chromobacterium violaceum CV026 and N-acyl homoserine lactone (AHL)* producing *Escherichia coli ATTC 31298* and the grapevine colonizing *Ezf 10-17 strains*	Szabo et al., 2010[66].
	Secondary metabolites isolated from the species *Hypericum densiflorum, H.ellipticum, H.prolificum*, and *H.punctatum*	*Staphylococcus epidermidis, Staphylococcus aureus,* clinical *methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Escherichia coli,* and *Acinetobacter baumannii.*	Sarkisian et al., 2012[67].
	Green tea	*Helicobacter pylori, Propionibacterium acnes* and *Staphylococcus aureus*	Lee et al., 2009[68].
	Streptococcus spp	*Potentilla recta*	Tomczyk et al., 2011[69].

Table 2
Synthetic chemical compounds reported to have antibacterial activity

Compound	Bacterial species	Reference
Cetrimide	*Enterococcus faecalis strains, E. faecalis ATCC 29212, E. faecalis EF-D1, E. faecalis U-1765 and Enterococcus durans strain*	Arias-Moliz et al., 2012[70].
Sodium hypochlorite	*Enterococcus faecalis, Staphylococcus aureus, Candida albicans, Prevotella intermedia, Porphyromonas gingivalis, Porphyromonas endodontalis and Fusobacterium nucleatum*	Sena et al., 2006[71]
Chlorhexidine	*Staphylococcus aureus, Candida albicans, Enterococcus faecalis, Porphyromonas endodontalis, Porphyromonas gingivalis and Prevotella intermedia*	Vianna et al., 2004[72]
Ethylenediaminetetraacetic acid	*Enterococcus faecalis*	Ozdemir et al., 2010[73]
Chlorhexidine digluconate (CHG)	*Methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli and Candida albicans*	Hendry et al., 2012[74]
2-Aminobenzimidazole Derivatives	*Pseudomonas aeruginosa*	Frei et al., 2012[75]
7-Fluoroindole	*Pseudomonas aeruginosa*	Lee et al., 2012[76]
3,4,5,3',5'-Pentabromo-2-(2'-hydroxybenzoyl) pyrrole	*Staphylococcus epidermidis and Staphylococcus aureus*	Schillaci et al., 2005[77]

DISCUSSION

Emergence of resistant bacteria to conventional antimicrobials clearly shows that new biofilm control strategies are required. Current research is ongoing to explore novel compounds from plants, seaweeds, marine microbes and sponges etc, to inhibit and prevent the biofilm formation. There is a lot of emphasis on screening biological organisms and phytochemicals as anti fouling agents since they are less toxic to the environment. Role of bioinformatics in marine biotechnology is needed for modeling proteins and assessing their functions. Docking can aid in the analysis of inhibitor activities of the compounds, which has proven to be a faster and a fairly reliable technique in medical and health sciences. Since this is an *in silico* approach and bacteria are freely available, the bioethical issues to be addressed as compared to clinical trials are minimal.

CONCLUSION

Biofilm formation is a natural phenomenon which is widespread and has severe economical and environmental impact. Numerous solutions are being researched to address the biofilm challenge. Ideally, preventing biofilm formation would be a more logical option than treating it. However, there is presently no known technique that is able to successfully prevent or control the formation of unwanted biofilms without causing adverse side effects. Bioinformatics tools could play a major role to solve the global and detrimental challenge of biofouling by screening compounds that can inhibit the biofilm formation. Chemoinformatics and *in silico* tools are to be used to validate the identified novel compounds. Approaches like docking are useful in recognizing the inhibition sites and binding sites of the molecules. These tools are already being used in drug lead optimization and identification of potential drugs, in medical and health related studies and need to be extrapolated to environmental studies as well. Bioinformatics in environmental sciences research can be easy and less time consuming to arrive at results than the conventional methods. But the results need to be tested and the protocols need to be validated by in vitro methods. It is also evident that there is an urgent need for a database repository for the marine proteins from marine microbes and other marine macroorganisms. New tools need to be developed to simulate the dynamic complex environmental system. Eco friendly natural compounds from plants and other marine organisms are to be studied to prevent and inhibit the biofilm issue. The development of new biofilm control strategies, following the specifications need to be used in various environmental conditions and based on the use of bioinformatics approach with high antimicrobial activity and specificity may seem to be a step ahead in overcoming the biofilm problems.

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