Review Article

Green pharmacy to combat pathogenic bacterial biofilms: An overview

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Abstract

Pathogenic bacterial biofilms are emerging threat to the mankind. The frequency of human bacterial infections associated with biofilms has been reported to be greater than 65%. These miniature microbial communities could establish chronic and hard to treat infections thereby triggering therapeutic failure. The biofilm scientists over the world, in their endeavor to combat the hazardous biofilms, have attempted to devise anti-biofilm tools such as acid shock treatment, genetic engineered phages and matrix-degrading enzymes (NucB). However, the commercially validated master formula record and relevant safety data sheet for antibiofilm agents is hardly reported. Moreover, the need for state of art facility and superb expertise limit their market viability. Thus, an appropriate qualified remedial solution is still awaited. Having anticipated that the nature has the requisite potential to deliver promising tool to counter the challenge posed by biofilm world, the scientists are thriving to explore the green wealth as biofilm combat weapon. A vast green wealth including *Caatinga*, *Coffea canephora, C. longa,* formulated garlic ointment, manuka honey have shown promising anti biofilm properties and further insight into plant biomolecules as quorum sensing molecules like Nisin, for breaking biofilm, could address another innovative approach to biofilm control . Thus, a qualitative and mechanistic approach to screen the green wealth could evolve commercially viable antibiofilm biomolecules with validated process and product parameters to control and disperse pathogenic bacterial biofilms. In general practices, the proven natural remedies could curb the menace of pathogenic biofilms, encourage alternative medicine, and curtail indiscriminate use of synthetic antibacterial drugs.

***Key words*:** Biofilms, green solution,green wealth**,** bacterial pathogens, biomolecules

Introduction

Bacterial biofilms adhering to biotic or abiotic surfaces exploit their engineering and architectural skills to build safe societies for themselves (Sawhney and Berry, 2009). They predominate numerically and metabolically in virtually all nutrient sufficient ecosystems viz. environmental, industrial and medical niches and processes of interest to the microbiologists (Costerton et al., 1995). More than 65% of all the human bacterial infections are associated with biofilms and that the antibiotic resistance in biofilm bacteria is a thousand times higher than their planktonic counterparts (Alasil et al., 2013). Such a modification could trigger therapeutic failure and establish chronic infections. Voluminous research to tackle these hazardous microbial formations is being carried out. However, control and dispersal of pathogenic biofilms still occupies "Aims and Objectives" column of any relevant research being pursued. This write up highlights the key efforts made by the researchers to resolve this problem and the current prospective to explore earth's green natural resources to evolve a validated commercially viable healthcare outcome.

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Bacterial Biofilms: A health hazard

Biofilms could be thought of as an important virulence factor. Biofilm formation has been established in number of infections viz. dental caries (Alam et al., 2007; Webster et al., 2006), cystic fibrosis (Jabra-Rizk et al., 2006; Suci et al., 1994). osteonecrosis, urinary tract infection, prosthetic infections (Faruque et al.,2006), burn patients (Evans et al., 1990), endocarditis (Chavez de Paz et al., 2008) , otitis media, corneal infections through contact lens (Leroy et al., 2007) and infectious diseases of head and neck region (Akyildiz et al., 2013). Recently, bacterial biofilms among infected and hypertrophied tonsils has highlighted the

importance of early detection and prevention of biofilms in therapeutic management of biofilm related infections (Alasil et al., 2013). Many food borne pathogens such as *E.coli, Salmonella, Yersinia enterocolitica, Listeria, Campylobacter* form biofilms on the surface of food or the storage equipments. The potentially pathogenic bacteria viz. *Staphylococcus aureus, Enterococcus faecalis, Streptococcus, E.coli, Klebsiella, Proteus* and *Pseudomonas* have been found to associate with medical devices such as catheters, artificial joints, mechanical heart valves etc. (Jacobsen et al., 2008; Litzler et al., 2007; Donlan and Costerton, 2002). The biofilm development by oral microflora on orthodontic ligatures, attributed to poor oral hygiene, form a focus of infection in the oral cavity. The oral streptococci might participate in the process that could lead to implant failure and that biofilm formation by oral streptococci on different implant surfaces is species dependent (Nakazato et al., 1983; Pedro Paulo et al., 2015).

A number of physiological, biochemical and genetical mechanisms underlay biofilm formation as elaborated in our earlier review on bacterial biofilms (Sawhney and Berry, 2009). Recently, a study pointed to sub-inhibitory concentrations of certain antibiotics to trigger biofilm formation as evidenced by increased biofilm biomass of NT-Hi (Non-typeable *Haemophilus influenza*) bacteria exposed to beta-lactam antibiotics owing to increased glycogen synthesis by treated bacteria and subsequent up and down regulation of genes involved in variety of metabolic processes such as pyramidine metabolism, cell wall biosynthesis etc. (Wu et al., 2014). Beta-lactam-stimulated NT-Hi biofilms are thought to protect embedded bacteria from subsequent high concentrations of cefuroxime. Undoubtedly, with the advent of newer techniques and know how, scientists are unraveling the mechanisms leading to pathogenic biofilm formation; but then the control of these harmful formations still remains the key objective.

Control of Biofilms: The basic research

Multilayered defense strategies viz. poor antibiotic penetration, nutrient limitation, slow growth, adaptive stress responses, and formation of persister cells have been documented to build up biofilms and breaching these defenses could lower the biofilm resistance making the available antibiotics responsive to eliminate biofilm based infections which are otherwise ineffective (Stewert, 2002). It is well stated that the advancement in understanding of antibiotic resistance and biofilm might be exploited in the development of new strategies to prevent and treat *S. aureus* infections (McCarthy, 2015).

Efforts have been made to explore promising tools to combat biofilms. Our earlier review on bacterial biofilms quoted acid shock treatment on proteins expression in *Streptococcus mutans*, use of catheter lock solutions to block staphylococcal biofilm formation on abiotic surfaces, synergistic activity of dispersin B and cefamandolenafate in inhibition of staphylococcal biofilm, use of bacteriophage ϕIBB-PF7A, and even genetic engineered phages as a important biological agents to outrage biofilm forming pathogens like *Pseudomonas* (Sawhney and Berry, 2009). The studies on *Staphylococcus* biofilms have demonstrated *agr* (accessory gene regulator) detached cells and the role of extracellular protease activity in dispersal mechanism (Boles and Horswill, 2008). The removal of *Escherichia coli, Bacillus subtilis or Micrococcus luteus* biofilms associated with CRS by treatment with matrix-degrading extracellular bacterial deoxyribonuclease (NucB) through eDNA degradation has also been observed which might offer a valuable therapeutic target for CRS sufferers and improvement of post-surgical outcomes of FESS (Shields et al., 2013). A recent study reported the role of Repressor of toxins (Rot) in biofilm formation in *Staphylococcus aureus* and the failure to form biofilms in rot deficient mutants (Mootz et al., 2015). Another study on *Staphylococcus aureus* revealed a novel role for staphylokinase-induced plasminogen activation that prevented *S. aureus* biofilm formation and induced detachment of existing biofilms through proteolytic cleavage of biofilm matrix components (Kwiecinski et al., 2015).

Current Strategies: Green Pharmacy

To date researchers have attempted to define the pathogenic biofilms with special reference their architectural skills, quoram sensing mechanism and adhesion to medical devices with special reference to various infections and their therapeutic outcomes. The emerging threat from pathogenic bacterial biofilm, their enhanced virulence and subsequent therapeutic failures necessitate a search for suitable antibiofilm biomolecules. Thus, a convenient, user compatible, commercially viable remedial technological package is the need of the hour. Having realized this, the scientists over the world have turned to explore the green remedial tech-pack to control pathogenic biofilm formation.

The recent past has seen surge in scientific reports on anti-biofilm potential of a few medicinal plants and associated phytochemicals (Stefano et al., 2014). Polyphenols in tea have been shown to reduce caries development in animals due to decrease in the cell surface hydrophobicity of *S. mutans* and the ability of the organism to synthesize adherent water-insoluble glucan from sucrose (Otake et al., 1991; Ooshima et al., 1993; Ooshima et al., 1994; Ooshima et al., 1998; Hamilton-Miller, 2001). The extract from *Lentinus edodes*, an edible mushroom, was studied in rats

and found to have an inhibitory effect on water-insoluble glucan formation by GTF (Shouji, 2003). The same inhibitory effects have been shown by apple procyanidins (Yanagida et al., 2000). High molecular weight components of hop bract were found to inhibit adherence of water-insoluble glucan synthesis by *S. mutans* (Nogueira et al., 2000).

Caatinga plant species extracts from Brazilian semi-arid region, with active phytochemicals such as polyphenols, coumarins, steroids and terpenes on in-vitro screening were found to be effective in preventing biofilm formation (Trentin Dda et al., 2011). The essential oil of *C. longa* with major components, α-turmerone (35.59%), germacrone (19.02%), α-zingiberene (8.74%), αr-turmerone (6.31%), trans-β-elemenone (5.65%), curlone (5.45%), and β-sesquiphellandrene (4.73%) inhibited the formation of *S. mutans* biofilms at concentrations higher than 0.5 mg/ml, suggesting its possible role in curbing cariogenic properties of *S. mutans* (Lee et al., 2011).

In another study, a small library of cinchona alkaloids, a synthetic derivative, 11-triphenylsilyl-10,11-dihydrocinchonidine (11-TPSCD), was found to be effective against biofilm formation by *Staphylococcus aureus* ATCC 25923 at low micromole concentrations, However, higher concentrations were required to eradicate mature biofilms (Skogman et al., 2012). Benzalkonium chloride has proven its clinical utility as biofilm inhibiting surface coating agent when used with a surfactant solution (Jaramillo et al., 2012). Another compound Diallylsulphide was found to destroy the EPS structure of the *C. jejuni* biofilm, after which the sessile cells were killed in a similar manner as planktonic cells (Lu et al., 2012).

Coffea canephora extract reduced the microbial count in oral biofilm (Antonio et al., 2012). Formulated Garlic ointment (GarO) has been explored as prophylactic therapy to prevent formation of wound biofilms caused by both Gram-negative and Gram-positive bacteria and as a possible potential therapy for disrupting established staphylococcal biofilms. This ointment has been found to prevent biofilm development by *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Acineto bacterbaum annii* and *Klebsiella pneumoniae,* and cause a 2-5 log reduction of the bioburden within *Enterococcus faecalis* biofilms and partial disruption of developing biofilms of *S. aureus, S. epidermidis* and *A. baumannii* (Nidadavolu et al., 2012).

A study documented potential of manuka honey in the topical treatment of wounds containing *S. pyogenes* (Maddocks et al., 2012). Manuka honey permeated 24 h established biofilms of *S. pyogenes*, resulting in significant cell death and dissociation of cells from the biofilm. Sub lethal concentrations of manuka honey effectively prevented the binding of *S. pyogenes* to the human tissue protein fibronectin, but did not inhibit binding to fibrinogen. The observed inhibition of fibronectin binding was confirmed by a reduction in the expression of genes encoding two major fibronectin-binding streptococcal surface proteins, Sof and SfbI.

Inhibitory effect of Iranian plant extracts (*Glycyrrhizaglabra, Quercusinfectoria*) with known alpha-glucosidase activity against biofilm formation by *Pseudomonas aeruginosa* (Mansouri et al., 2013) and the antibiofilm effect of chitosan (polymer) coated plant extracts of *Azadirachta indica, Vitex negundu, Tridax procumbens, Ocimum tenuiflorumi* against *E.coli* have also been reported (Namasivayam and Allen Roy, 2013). A study highlighted that an extract of *Alnus japonica* repressed *S. aureus* biofilm by > 70% and that the transcriptional studies exhibited the repression of intercellular adhesion genes icaA and icaD. The antibiofilm activity of *A. japonicum* was attributed to quercetin and tannic acid, the major antibiofilm compounds in its extract (Lee et al., 2013). Recently, an interesting study on *Pseudomonas aeruginosa* biofilms showed that 6-Gingerol reduced its biofilm formation and virulence via quorum sensing inhibition (Kim Et al., 2015).

With the emerging interest on exploring green belt to combat the undesired biofilms and subsequent encouraging results in hand, it could be anticipated that the nature has the requisite potential to deliver promising tools to counter the challenge posed by biofilm world. However, like Nisin, a quoram sensing molecule, the plant biomolecules might be screened thoroughly based on biochemical, metabolic and genetical aspects as appropriate plant biomolecules for breaking biofilm; as the molecules with potential to regulate or suppress the matrix formation or as molecules that could increase the permeability of matrix where the planktonic cells lay embedded to form large communities. Thus a search for new, diverse, healthcare friendly, economically viable, environmentally feasible molecules having dual benefit of being antibiofilm and antibacterial agents could be a gateway to combat pathogenic bacterial biofilms using green wealth and evolving green pharmacy.

Moreover, in general practices, the thorough insight and review of available natural remedies with proven anti infectious abilities in humans could curb the menace of pathogenic biofilms thereby lowering incidence and emergence of resistant infections, and curtail the indiscriminate use of synthetic antibacterial drugs.

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