Biofilm Threat for Food Industry: An Approach for Its Elimination Using Herbal Food Components

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Abstract

Biofilm formation is an alliance of microorganisms in which microbial cells constitute an alternative lifestyle and embrace a multicellular behavior that facilitates and/or prolongs survival in adverse environmental niches. In nature, biofilms are present everywhere, and they can be found in places like waste water channels, labs, bathrooms, industrial places, and hospital settings and frequently occur on hard surfaces that are immersed in or exposed to an aqueous solution. It can also be formed as buoyant on surface of liquid; biofilm formation comprises a substitute lifestyle in which microorganisms espouse a multicellular behavior that smooth the way and/or promote prolong survival in diverse environmental niches. Cells in a bacterial biofilm communicate via quorum sensing which is a multistep process that starts with the attachment of cells to a surface and then formation of microcolony that further leads to the formation of three-dimensional structure and finally ending with maturation followed by dispersion or detachment. According to National Institutes of Health (NIH), about 65% of all microbial infections and 80% of all chronic infections are associated with biofilms. Bacterial biofilm is less

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attainable to antibiotics and human immune system and thus gives rise to ultimatum to public health because of its participation in variety of infectious diseases. A greater understanding of steps leading to biofilm formation on surfaces and within eukaryotic cells, pointing several medically important pathogens, for the development of novel, effective control strategies aimed at biofilm prevention and/or elimination.

Keywords

Biofilm · Antibiotic · Immune system and eukaryotic cells

11.1 Introduction

Formation of a biofilm permits single-cell organisms to reckon a short-term multicellular lifestyle, in which "group behavior" enables survival upon living and inanimate surfaces under unfavorable environments. These surfaces may happen to be in numerous forms, for example, including those that are found in soil and aquatic systems, those on indwelling medical devices, and those of living tissues such as tooth enamel, heart valves, or the lungs. The indwelling medical devices that enable the microorganisms to adhere on their surfaces (in most cases) are associated with many bloodstream (circulatory) infections and urinary tract infections. The most common feature of this adhered growth state is that the cells develop a biofilm. Biofilms have great importance for public health, because biofilm-associated microorganisms display substantially decreased susceptibility to antimicrobial agents.

11.2 Biofilm and Its Composition

Biofilm is an association of microorganisms on a living or nonliving surfaces that produce a matrix of extracellular polymeric substance (EPS) which is composed of proteins ($<$1–2%) including some enzymes, DNA ($<$1%), polysaccharides (1–2%), and RNA ($<$1%), and apart from these components, water plays the most important part in theis biofilm formation (up to 97%); it is responsible for the flow of nutrients inside the biofilm matrix (Fux et al. 2005). Antibiotics and human immune system are incapable of accessing the bacterial biofilms. Biofilms-associated microorganisms have embellished probability of counteracting antimicrobial agents that results in tedious treatment. Bacteria within a biofilm trigger some genes due to certain changes like cell density, nutritional, temperature, pH, and osmolarity that activate the expression of gene stress which in turn switch to resistant phenotypes (Hall-Stoodley et al. 2004).

11.3 Development of Biofilm

During the formation of biofilm, bacteria of different species are capable of interacting with one another via particular mechanism called quorum sensing. Development of a biofilm is a multistage procedure, which starts with the binding of microbial cells to a surface (living or nonliving), and then the formation of microcolony which further leads to the formation of three-dimensional structure and eventually finishing with maturation followed by detachment (dispersal) (Fig. 11.1).

11.4 Historical Background of Biofilm

Bacterial cells manifest two types of growing approach, i.e., planktonic cell and sessile or sedentary aggregate which is also known as the biofilm. Bacteria themselves produce a substance on a surface, known as extracellular polymeric substance. Inside this substance, microorganisms come together, and cells stick to each other (Costerton et al. 1999).

Antonie van Leeuwenhoek, a Dutch researcher, abraded the plaque biofilm from his teeth and for the first time observed “animalcule,” by using a simple microscope, and this was considered as microbial biofilm discovery. Characklis, subsequently in 1973, stipulated that biofilms are not only sedulous but reveal higher resistant to disinfectants, e.g., chlorine. In 1978, Costerton invented the term biofilm and notifies the world about the significance of biofilm (Naves et al. 2010).

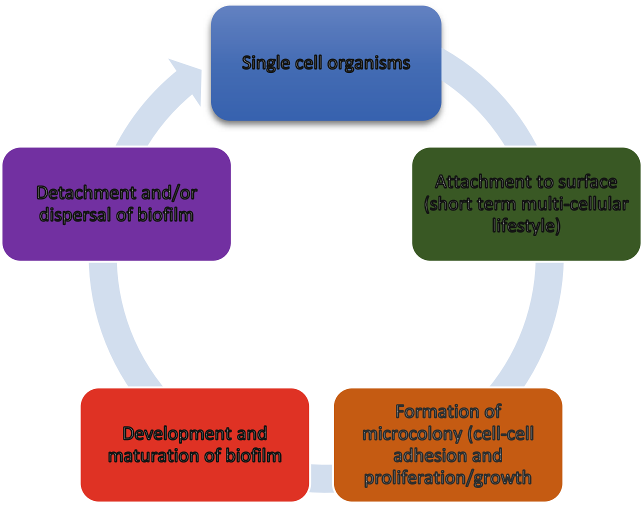


Fig. 11.1 Representation of biofilm life cycle

11.5 Biofilm-Forming Bacteria

Microorganisms are capable of producing extracellular polymeric substance (EPS) and form a biofilm, whenever they bind to a surface. The biofilm formed by these microorganisms poses a great threat for public health due to its resistant nature to antibiotics and disease linked with indwelling medical devices (Sekhar et al. 2009). A vast variety of microorganisms nearly 99.9% have the ability to form biofilm on a wide range of surfaces, i.e., inert and biological surfaces. In a study it was observed that *H. influenza* has the ability to form biofilm in human body, and not only this, but they can even escape from human immune system (Dethlefsen et al. 2008). A few biofilm-forming bacterial species have been enlisted below (Table 11.1).

11.6 Biofilm Resistance to Antimicrobial Agents

Typically antibiotics are either bacteriostatic (that prevents the bacterial cell division or inhibits bacterial growth) or bactericidal (that kills the cells). Antibiotics have been proven critical in abolishing bacterial pathogens, enormous evidence stipulate that they damage the host microbiota considerably and generate an environment where opportunistic pathogens can reign, and they increase the selective pressure toward antibiotic resistance (Nickel et al. 1985).

Significantly, sessile bacterial cells, with respect to medicine, can withstand host immune responses, and they are very less vulnerable to antibiotics than their nonattached individual planktonic counterparts (Stewart 1998). It is probably because the biofilms avoid antimicrobial challenges by multiple mechanisms.

One of the main mechanisms of biofilm resistance toward antimicrobial agents is the lack of success of an agent to pierce the full depth of the biofilm. Diffusion of antibiotics is retarded by the polymeric substances (acting as a physical barrier) that are known to make up the matrix of a biofilm and generally solutes diffuse at slower rates within biofilms than they do in water (Hogan and Kolter 2002).

Several other mechanisms which have been explored that are contemplated to be an important factors in high-resistance nature of biofilms toward antibiotics are:

Table 11.1 List of common biofilm-forming bacterial species (Khan et al. 2014)

S.no.	Biofilm-associated bacterial species
1.	*Escherichia coli*
2.	*Pseudomonas aeruginosa*
3.	*Staphylococcus epidermidis*
4.	*Staphylococcus aureus*
5.	*Staphylococcus epidermidis*
6.	*Enterobacter cloacae*
7.	*Klebsiella pneumonia*
8.	*Actinomyces israelii*
9.	*Haemophilus influenzae*
10.	*Burkholderia cepacia*

1. Limited diffusion
2. Neutralization caused by enzymes
3. Heterogenous functions
4. Slow growth rate
5. Presence of nondividing (persistent) cell
6. Biofilm phenotype such adaptive mechanisms, e.g., efflux pump and membrane alteration (Poole 2002; Böttcher et al. 2013)

11.7 Natural Agents for the Elimination of Biofilm

Biofilms are protective shields that microbes use to protect themselves from the host's immune system. The traditional approach to eliminate biofilm infection would be to administer prescription antibiotics. Research has shown that this approach is not very effective. As a matter of fact, prescription antibiotics have often been isolated as a benefactor to biofilm formation (Hammer et al. 1999).

Hereunder, research has highlighted some natural alternatives that are able to address biofilm gently without creating deeper issues. Essential oils are known to be the natural antibiotic formulations with broad-spectrum activities against virus, fungi, and bacteria (Kavanaugh and Ribbeck 2012). *Cassia*, *Peru balsam*, and *red thyme* (their essential oils) when compared to ofloxacin and gentamicin have high biofilm-eradicating effect against biofilms of *Pseudomonas* and *S. aureus* (Nostro et al. 2007a). Oregano essential oils, *carvacrol* and *thymol*, were used against *S. aureus* for the inhibition of biofilm formation (Kwiecinski et al. 2009).

An essential oil (tea tree oil) extracted from the leaves of *Melaleuca alternifolia* has been found to eradicate biofilm in *S. aureus*, including MRSA by damaging ECM (extracellular matrix) and subsequent removal of the biofilm from the surface (Artini et al. 2012).

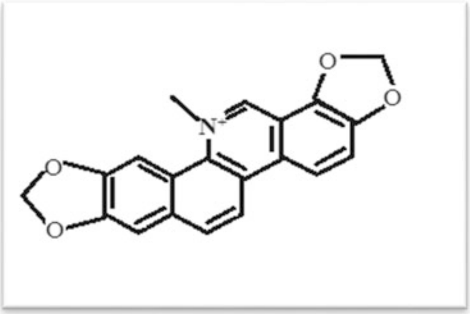
Four compounds are yielded from the extracts of *Karmeria*, *Aesculus hippocastanum*, and *Chelidonium majus*. The compounds are sanguinarine, chelerythrine, dihydroxybenzofuran, and proanthocyanidin (Fig. 11.2). These compounds have been reported for exerting inhibition of biofilm formation in *S. aureus* (Zorofchian Moghadamtousi et al. 2014).

Here are some common natural herbal drugs that are prominent in the elimination of biofilm.

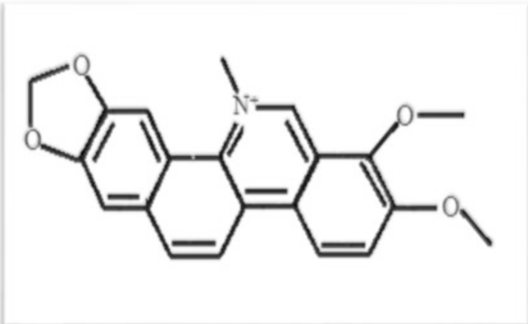
1. *Curcumin*: Curcumin is the primary active constituent of turmeric and is one of the most diversely beneficial natural compounds currently known. In 2014, a reviewed study acknowledged curcumin as a potential antifungal, antiviral, antibacterial, as well as antiparasitic agent. On top of all these properties, curcumin has considered remarkably effective at disrupting biofilm (Magesh et al. 2013).

   Another study carried out in 2013 found out that out of 35 different compounds observed, curcumin alight itself as one of the top six biofilm disrupting-agent (Bjarnsholt et al. 2015).

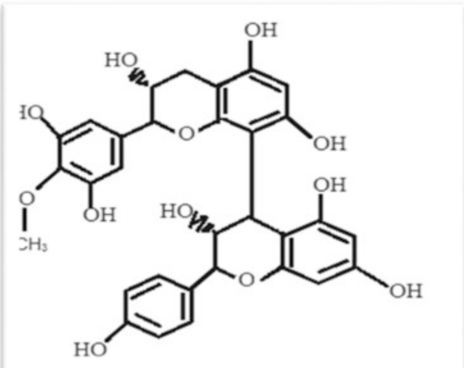
Fig. 11.2 (**a**) Sanguinarine, (**b**) chelerythrine, and (**c**) proanthocyanidin



Sanguinarine



Chelerythrine



2. *Apple cider vinegar*: Apple cider vinegar is one of the ancient tonic, used as a biofilm disruptor. Apple cider vinegar contains acetic acid, which has shown to kill unwanted bacteria and is also capable of cutting through mature biofilms in chronic infections (Nostro et al. 2007b).
3. *Oregano*: Oregano's active constituent carvacrol has been known in terms of pathogen eradication. Oregano (oregano oil especially) has been used for a long time to naturally eliminate unwanted pathogens from the gastrointestinal tract (GIT). Carvacrol has been known to inhibit antibiotic-resistant bacteria, viruses, parasites, and fungi. Moreover, this powerful compound also inhibits the release of harmful toxins released by these pathogens including biofilm (Friedman 2014; Rutherford and Bassler 2012).
4. *Garlic*: Garlic's active constituent allicin is capable of destroying antibacterial resistant bacteria and has also reported to inhibit biofilm formation as well. Garlic is known to be a powerful broad-spectrum anti-pathogenic food. Bacteria form biofilm in order to survive the adverse environments; the formation of biofilm takes place with the help of bacterial communication called quorum sensing (Ta and Arnason 2016). For bacteria, this is an important survival mechanism. Fortunately, allicin in garlic has been shown to disrupt this bacterial communication process and biofilm growth (Kumar et al. 2015).
5. *Berberine*: Berberine is isolated from Oregon grape, goldenseal, and a few other herbs; this compound has an impressive list of benefits. Berberine is a potential antimicrobial agent with few side effects, other than this berberine is also found to consists an antidiabetic and anticancer properties (Kumar et al. 2015).

11.8 Conclusion

Biofilm formation is the strategy that is adopted by the microorganisms for their survival in the harsh/unfavorable environment, which makes their eradication particularly difficult. The age/maturation and composition of a biofilm are the major factors that influence the susceptibility of the colonized microorganism within the biofilm. Metabolism and growth rate majorly get affected as the biofilm matures and accumulation of EPS combined with oxygen and nutrient gradient; this leads to reduced penetration of antimicrobial agents which further results in antimicrobial-resistant pathogens. Novel strategies have been designed and adopted for the elimination of biofilms such as use of natural herbal drugs and anti-adhesion agents, uses of bacteriophages, and many more. Although these natural agents were effective and showed enormous potential in the treatment of biofilm-associated infections, their mechanisms of action remain unclear. The molecular pathways and animal model studies of these potential agents could provide a clearer view on the pathways affected. Another approach is to look into the synergistic effect of combinations of these agents and antibiotics to eliminate the infections associated with biofilm formation. Among these strategies natural herbal drugs have shown a promising result in eliminating biofilms.

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