Chapter 9
Biological Strategies Against Biofilms

Ganga Sharma and Arun Karnwal

Abstract Biofilms are microbial aggregates which consist of extracellular polymeric substances (EPSs) produced by the microorganism itself that adhere to biological environments such as in rivers, streams, and alimentary canal or living tissues of mammals or nonbiological surfaces like in wastewater treatment plant, tickling beds, indwelling medical devices (IMDs), and industrial or potable water system piping. Constituents of EPS are microorganism originated components of homologous proteins, polysaccharides, lipids, and DNA. The formation of biofilm involves the migration of microbial cells, the interaction between them through cell-to-cell signaling, synthesis of EPS, and in later stages, interaction between cell and EPS.

Biofilms have a unique biochemical profile rendering structural integrity to the microorganisms which the planktonic counterparts lack. This structural stability protects them from various troubles present in their environment such as antibiotics, the host's defense mechanism, harsh nutritive conditions, predators, etc. The survival of microorganisms in biofilms although beneficial to them gives rise to a significant amount of problems in humans in various essential fields including that of medicine and industries like pharmaceutical, food, and marine industries causing adverse health effects as well as economic loses. This resistance of microorganisms, therefore, is a major concern to handle in controlling biofilms. Various traditional strategies to control biofilms of pathogenic/spoilage bacterial species, which are either physical/mechanical removal of biofilms by cleaning, selection of appropriate bactericidal material, preconditioning of surfaces by methods like ultrasonication and plasma treatment, or chemical removal using antimicrobial agents such as disinfectants/sanitizers, are not always successful. In light of the above problems of biofilm control by conventional methods, in recent times, progress has been taking place in the field of fundamental biofilm research discovering novel methods of

G. Sharma
Central Research Institute, Kasauli, Himachal Pradesh, India

A. Karnwal (✉)
Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

J. Singh et al. (eds.), *Microbial Biotechnology: Basic Research and Applications*, Environmental and Microbial Biotechnology,
https://doi.org/10.1007/978-981-15-2817-0_9

controlling biofilms. In the current chapter, we tend to discuss these recent and cutting-edge methods which are much more effective as an antibiofilm strategy focusing mainly on the use of biological components such as enzymes, phages, and antimicrobial molecules (AMPs, QS inhibitors) for the improvisation of areas of healthcare and food safety and in industrial processes.

Keywords Biofilm · Antimicrobial molecules · Quorum sensing inhibitors · Exo-poly Saccharides · Bacteriophage

9.1 Introduction

Biofilms are universal and found in a wide variety of environments, both natural, such as in rivers, streams, and alimentary canal or living tissues of mammals, and man-made like in wastewater treatment plant, tickling beds, indwelling medical devices (IMDs), and industrial or potable water system piping (Donlan 2002). Biofilms can evade host defense mechanisms that include both innate and adaptive immunity (Dunne Jr. 2002). It is the reason why biofilm formation is an increasing cause of concern throughout the world.

Bacterial biofilms not only contribute to hospital-acquired infections, but also are a leading cause of corrosion, fouling of water pipes, and food and pharmaceutical spoilage (Henderson 2010; Kumar and Anand 1998). Some of the health issues associated with biofilms are indirect such as in drinking water distribution system where biofilms corrode water pipes and weaken them and this loss of integrity weakens pipes aside from causing esthetic problems which may lead to a health concern. Microorganisms forming biofilms can cause infection in humans and animals and may be transmitted to each through cross-contamination. Biofilm-associated infection in animals can cause massive economic loss such as in livestock/poultry industry and others in terms of production (Chakraborty et al. 2018). Also, biofilms producing microorganisms contaminate foods and generate damage to the product, equipment, and consumers leading to economic loses.

In the food product manufacturing facilities, biofilm formation leads to deleterious hygiene issues due to adherence of a variety of microbes on food and degradation of equipment (Kabwanga et al. 2018). In the pharmaceutical industry, the development of biofilms and adherence of it into the production equipment and facilities are critical issues that need to be addressed (Kabwanga et al. 2018; Stewart 2015). Although most of the biofilm-forming microbes are harmful in many ways, some of them exhibit beneficial properties which have been put to use in several industrial processes (Morikawa 2006). The infections caused by biofilm-forming microbes are chronic, and for the treatment, antimicrobial agents need to be administered, but biofilms make the microbe resistant to antimicrobial agents compared to their planktonic counterparts (Costerton et al. 1999; Mah and Toole 2001; Stewart and Costerton 2001; Donlan and Costerton 2002).

Therefore, treatment of infections caused by biofilm-forming microbes is not resolved with the sole administration of antibiotics due to the problem of the development of resistance against them. Although highly sterile conditions and practices are fundamental to maintain a strategic distance from biofilm development, for proper resolution, some of the novel antibiofilm compounds should be explored as a potential antibiofilm agent in the near future. Some of them, which are already discovered or tested till date, are active herbal compounds such as essential oils, quorum-sensing inhibitors, antimicrobial peptide alone or in combination with antibiotics, and synthetic or genetically engineered compounds.

Out of these new control strategies which are continually emerging, most of the focus is on antibiofilm agents of biological origin such as enzymes, phages, AMPs, and QSIs. The present review will focus on describing in detail the various biocontrol agents explored till date for the eradication of biofilms from the site of its formation.

9.1.1 Biofilms

Biofilms are defined as the structural community of bacterial cells which are formed by a self-produced polymeric matrix known as exopolysaccharide (EPS), which takes around 85% of the volume of a biofilm. This community of cells adheres either to living or nonliving surfaces (Costerton et al. 1999) (Fig. 9.1).

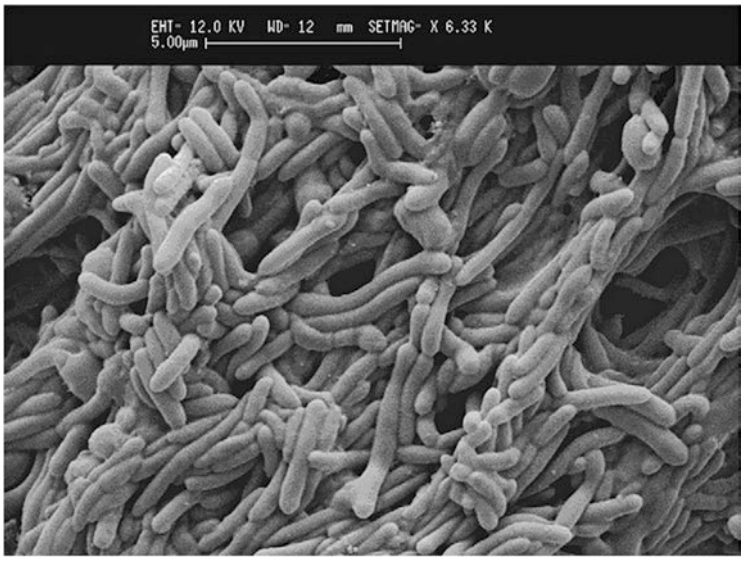


Fig. 9.1 Scanning electron microscopy photomicrograph of a 6 days old *B. cereus* biofilm formed on a stainless steel surface. 6330 magnification; bar ¼ 5 mm (Simões et al. 2010)

The distinct levels in the process of biofilm formation can be divided into various steps (Crouzet et al. 2014). The following are the general stages for biofilm formation, though the precise details of the regulation of biofilm formation vary significantly from species to species.

1. Macromolecules in the liquid where biofilms are forming precondition the surface (living or nonliving) for adhesion.
2. Transportation of bacterial cells to the surface also occurs.
3. The cells transported are adsorbed to the surface.
4. Desorption of reversibly adsorbed cells and retention of irreversibly adsorbed cells occur.
5. Metabolism of the substrate by the biofilm-bound cells and then transportation of the by-products out of the biofilm.
6. The adsorbed cells produce cell-to-cell signaling molecules for monolayer/microcolony formation.
7. Maturation of biofilms occurs through the formation of extracellular matrix (EPS) and other cell materials. It forms a three-dimensional structure of cells known as a microcolony (O' Toole et al. 2000).
8. Detachment or dispersal of bacteria to migrate and then colonize in new areas (Landini et al. 2010).

The main composition of biofilms is the EPS matrix which is formed by retaining water and other bacterially originated substances released by bacterial cells which get embedded in this EPS matrix, and it provides the following advantages to the cells (Crouzet et al. 2014; Donlan and Costerton 2002; Jamal et al. 2018):

(a) Structural stability to the microbe due to aggregation and adhesion of cells to one another.
(b) Transportation of the necessary nutrients becomes easy in closely associated cells.
(c) Acts as an electron donor or receptor.
(d) Storage of most of the energy.
(e) Provides the binding or receptor site to enzymes.
(f) Protects from external factors such as antimicrobials and other environmental changes.
(g) Provides adaptation.

During biofilm formation, several species of bacteria communicate with one another through quorum sensing (Davies et al. 1998; Shirtliff et al. 2002). During biofilm formation, genetic information can be modified by horizontal gene transfer (HGT) within and between bacterial species and increase the adaptation in bacteria for changing environments. Moreover, this kind of higher gene transfer rates was observed more in biofilms than their counterparts. It confers protection and survival in adverse environmental conditions such as antibiotics (Costerton et al. 1999; Mah and Toole 2001), predators (Kadouri et al. 2007), and human immune system (Anderson and O'Toole 2008). This way biofilms enhance the virulence of microbes (Brooks et al. 2005).

HGT in biofilms is beneficial to microbes but are harmful to us because antimicrobial resistance and virulence genes get disseminated or new ones get emarginated, making multiple drug-resistant (MDR) strains which are known as multiresistant "superbugs." Moreover, biofilms' architecture is tuned under a specific environment with the help of different enzymes secreted by bacteria that modify its EPS composition when a change in nutrient availability occurs (Sauer et al. 2004; Ma et al. 2009).

In the natural environment, 99% of bacteria exist in biofilms. As per reports from the National Institutes of Health (NIH), up to 65% and 80% of all microbial and chronic infections, respectively, are related to biofilms which feature their immense clinical impact (Jamal et al. 2018). Biofilms are responsible for more than 65% of nosocomial infections (Böhme et al. 2009) and approximately 61% zoonotic human infections (García and Percival 2011). Not only human infections but most of the infections caused in animals like pneumonia, liver abscesses, enteritis, wound infections, and mastitis are caused by biofilm-forming microbes (Olson et al. 2002; Clutterbuck et al. 2007).

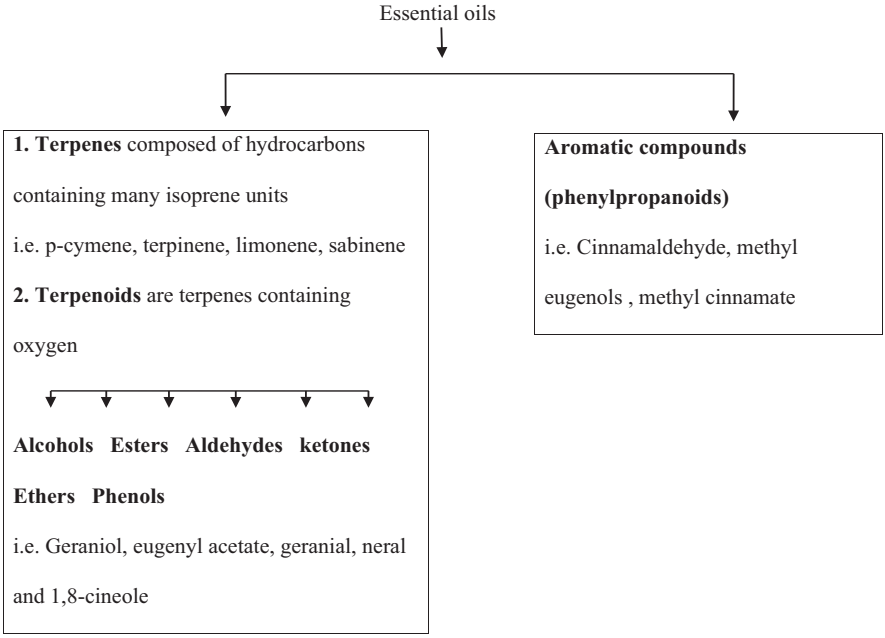
9.2 Biocontrol Agents Against Biofilms

9.2.1 Plant-Derived Essential Oils (EOs)

Essential oils (EOs) are derivatives of the various parts of the plants such as flowers, roots, leaves, seeds, fruits, bark, herbs, twigs, and seeds. They are hydrophobic and aromatic liquids. From ancient times, herbs and spices are commonly used in our homes, as flavoring agents of food, as a food preservative for its long-time storage, or as a medicinal plant product. EOs perform a significant function in the defense of crops from various microorganisms, insects, and animals (Kerekes et al. 2015). They are obtained either traditionally by methods like extraction, steam distillation, cold press/expressing, and enfleurage or by modern techniques employing microwave or ultrasound waves for extraction or pressurized extractions. Among the 3000 EOs known, 300 EOs are commercially explored which comprise more than 60 individual compounds (Van de Braak and Leijten 1999; De Martino et al. 2009; Cowan 1999). The amount of extracted EOs from plants depends upon factors like the part of the plant used for the purpose, its age, and the extraction method used (Lemberkovics et al. 2004; Reyes-Jurado et al. 2015). Essential oils are classified as depicted below (Kerekes et al. 2015).

Their mechanism of action involves the following: they are lipophilic in nature and therefore are permeable in the cell membrane, and they may inhibit ATP production and ATPase activity and bring about the outward flow of ions or other cellular content (Bakkali et al. 2008), disrupting the genetic (de Oliveira et al. 2010, 2012) as well as cellular material of the microorganisms (Perricone et al. 2015). It was found that aldehyde and phenolic EOs are the most effective in fighting against microbes

such as cinnamaldehyde, carvacrol, eugenol, or thymol (Bakkali et al. 2008; Perricone et al. 2015). Gram-positive microorganisms showed more sensitivity to EOs when compared to their Gram-negative counterparts (Burt 2004; Lambert et al. 2001).



Some EOs can also act as quorum-sensing inhibitors (interfering with the communication and regulation of quorum-sensing genes) which leads to the reduced activity of biofilm formation and other virulence-related factors (Nazzaro et al. 2013). Some of the features which make EOs as future therapeutic agents are: they are easily extracted, are nontoxic to the tissue culture cell lines, are rapidly degraded when mixed in water, and have no side effects to health (Fabian et al. 2006; Warnke et al. 2006; Isman 2000). It has been observed that the presence of EOs modifies the antibiotic tolerance ability of the bacterial cell (Yap et al. 2014), and when the two antimicrobials, which target two different components of the bacterial cell, are combined, it changes the tolerance of the microorganism (Rosato et al. 2007; Cox et al. 1998; Langeveld et al. 2014; Longbottom et al. 2004; Cirino et al. 2014) (Table 9.1).

9.2.2 Quorum-Sensing Inhibitors (QSIs)

Quorum sensing (QS) is an interaction strategy in the microbial community that is chemical in nature and is used to regulate various behaviors such as virulence and biofilm formation (Uroz et al. 2009). As soon as the population of bacteria becomes dense, QS compounds start accumulating for the recognition of the population

Table 9.1 Essential oil (EO) associated studies effective against biofilms

Essential oils	Target biofilm organism in the study	Reference
Oregano essential oils, carvacrol, and thymol	*S. aureus*	Nostro et al. (2007)
Cassia, Peru balsam, and red thyme	*Pseudomonas* spp. and *S. aureus*	Kavanaugh and Ribbeck (2012)
5% tea tree oil (TTO)	Coagulase-negative *Staphylococci* (CoNS) 1. Five out of nine of their biofilms are completely eradicated 2. 100% eradication after 1-h treatment to methicillin-susceptible *S. aureus* (MSSA)	Brady et al. (2006)
Pelargonium graveolens essential oil in combination with norfloxacin	Biofilms of two strains of *S. aureus*	Rosato et al. (2007)
Eugenol, cinnamaldehyde, citral, and geraniol	Clinical strains of *Staphylococcus aureus*	Jafri et al. (2014)
Cinnamon (*Cinnamomum zeylanicum*), TTO (*Melaleuca alternifolia*), and palmarosa (*Cymbopogon martini*), combined with ciprofloxacin	*P. aeruginosa* biofilm	Coelho and Pereira (2013)

density to activate a corresponding response. Quorum-sensing inhibitors target the QS molecules to reduce the formation of biofilms, and this disruption reduces the growth, virulence, and dispersion of microorganisms (Papenfort and Bassler 2016).

It was proposed that quorum-sensing inhibitors mainly target the following:

1. The signal generator
2. The quorum-sensing molecule
3. The signal receptor

The QS signal receptor mediates the pharmacological action. One of the modes of action that often facilitates the transformation of biofilm pathogenicity is reducing the biofilm's resistance to conventional antimicrobial treatment. Rasamiravaka et al. (2015) reported several QS-inhibiting compounds, including penicillic acid, solenopsin A, catechin, ellagic acid derivatives, and curcumin. QSIs can be obtained from various sources, but their antibiofilm activity should be explored in future studies.

Most of the plant-derived QSIs have shown to exhibit remarkable antibiofilm activity. Several studies were performed related to QC-mediated inhibition of biofilm formation as shown in Table 9.2. These studies showed that the QSI when used alone or in synergism with various other antimicrobial agents can be used to control biofilms. Christensen et al. (2012) showed that antibiotic tobramycin, when combined with QS compounds including furanone and horseradish juice extract, disrupted the biofilms of *Pseudomonas aeruginosa* in mouse as experimental organism. The synergic effect of QS molecules and availability of QS inhibitors increased the

Table 9.2 QSI associated with biofilm control

QS inhibitor/QSI and antimicrobial agent combination	Synergized antibiotic if any	Target organism	Reference
RNAIII-inhibiting peptide (RIP)	Nil	*Staphylococcus*	Balaban et al. (2007)
Usnic acid (obtained from lichens)	Nil	*S. aureus* and *P. aeruginosa*	Francolini et al. (2004)
Pungent oil of fresh ginger (6-gingerol)	Nil	*P. aeruginosa*	Kim et al. (2015)
Lactonase from *Bacillus* spp. synergize	Ciprofloxacin gentamicin	*P. aeruginosa*	Kiran et al. (2011)
Patulin and penicillic acid obtained from *Penicillium* species	Nil	*P. aeruginosa*	Rasmussen et al. (2005)
Phenyl-DPD (phenyl-4,5-dihydroxy-2,3-pentanedione)	Gentamicin	*P. aeruginosa*	Roy et al. (2013)
Baicalin hydrate, cinnamaldehyde, hamamelitannin	Tobramycin, clindamycin, and vancomycin	*P. aeruginosa and S. aureus*	Brackman et al. (2011)
Chinese medicine baicalein	Nil	*P. aeruginosa*	Zeng et al. (2008)
14-Alpha-lipoyl andrographolide (AL-1) obtained from green chiretta (*Andrographis paniculata*)	Nil	*P. aeruginosa*	Zeng et al. (2011)
LSFE	Tobramycin	*P. aeruginosa*	Jakobsen et al. (2012)
Ajoene synergized	Tobramycin	*P. aeruginosa*	Yang et al. (2006) Christensen et al. (2012)

susceptibility of the *P. aeruginosa* biofilm to tobramycin. Such methods create a less favorable surface for biofilms to reside on, and they reduce biofilm pathogenicity using QS inhibitors, demonstrating a promising and exciting potential avenue for further exploration. However, more work needs to be done to incorporate these ideas into an in vivo environment, particularly in the case of biofilm formation, as in vitro biofilm models may not mimic complex in vivo conditions.

9.2.3 Antimicrobial Peptide (AMP)

Antimicrobial peptides (AMPs) are also known as "host defense peptides." In higher eukaryotic organisms, AMPs are "L"-shaped cationic molecules containing 15–50 amino acids having molecular weights between 1 and 5 KDa and are produced as part of an innate immune defense mechanism by eukaryotes and prokaryotes. They usually contain arginine and lysine residues in excess (Izadpanah and Gallo 2005;

Rossi et al. 2008; de la Fuente-Núñz et al. 2012). They act on a wide variety of organisms like bacteria, yeasts, fungi, viruses, and even cancer cells to directly kill them. They show specific and diverse activities related to normal immune homeostasis, which includes a variety of cytokine and growth factor-like effects. They mainly target cell membranes because the peptides with a positive charge and cell membranes/biofilm surfaces of microbes with a negative charge attract each other, killing the active and slow-growing bacteria in biofilms (Melo et al. 2009; Jorge et al. 2012). However, AMPs at deficient concentrations change their activity from bactericidal to bacteriostatic (Beloin et al. 2014). Cationic peptides induce gene expression in microorganisms by binding to their DNA because they can pass through the cell membrane.

As per the literature review done by Yasir et al. (2018), the following mode of actions of *antimicrobial peptides* worked for biofilm removal (Table 9.3):

Various studies (Table 9.4) reported that AMPs are more effective when combined with various conventionally used antibiotics. Also, it was found that by changing the amino acid composition of AMPs, antimicrobial activity can be increased (Ma et al. 2012; Xu et al. 2014; Tiwari et al. 2015). One such example of genetic manipulation is the replacement of functional "defective" sequence RR7 in one of the AMP R-FV-I16 by inserting the antibiofilm sequence FV7 (Xu et al. 2014). Another way in which the manipulation of AMPs can be done is by designing STAMPs (specifically targeted AMPs). The benefit of these STAMPs is that they harm pathogenic bacteria but not nonpathogenic ones (Li et al. 2010; He et al. 2009). These AMPs rupture the cell membrane or act as membrane perturbers (Wimley and Hristova 2011). Genetically engineered peptide such as peptide RN3 (5-17P22-36) of eosinophil granules can also be explored as a potential antibiofilm agent (Venge 1999; Acharya and Ackerman 2014).

Table 9.3 Mode of action of AMP (Yasir et al. 2018)

S. no.	Mode of action	Examples
1.	The membrane potential of cells in biofilms is either disrupted or degraded	Nisin A, lacticin Q, and nukacin ISK-1, an engineered peptide RN3 (5-17P22-36), esculentin (CSA)-13 c
2.	Quorum sensing is interrupted	Human cathelicidin LL-37 and indolicidin
3.	Biofilm EPS matrix is degraded	Peptide PI, AMP derived from *Calliphora vicina*, hepcidin 20, peptide S4(1–16) M4Ka, piscidin-3
4.	Alarmone system is inhibited in both gram-positive and gram-negative bacteria to avoid the bacterial stringent response	Guanosine 50-diphosphate 30diphosphate (ppGpp) (p)ppGpp, 1018, DJK-5, and DJK-6, 1018
5.	Genes which are responsible for biofilm formation are downregulated and transportation of binding proteins is interrupted	Human β-defensin 3 (hBD-3), peptide Nal-P-113

Table 9.4 Antimicrobial peptides associated with biofilm control

Antimicrobial peptides	Synergized antibiotic if any	Targeted organism biofilm	Reference
A 9-amino acid peptide AMP 1037	Nil	*P. aeruginosa* *B. Cenocepacia* *Listeria monocytogenes*	de la Fuente-Núñz et al. (2012)
LL-37	Nil	*P. aeruginosa*	Overhage et al. (2008)
		Group A *Streptococcus* (GAS)	Johansson et al. (2008)
		S. epidermidis	Vuong et al. (2004)
		S. epidermidis ATCC35984	Hell et al. (2010)
Tachyplesin III	Piperacillin-tazobactam (TZP)	*P. aeruginosa*	Hirakura et al. (2002)
Colistin	Ciprofloxacin	*P. aeruginosa*	Herrmann et al. (2010)
Nisin	Daptomycin/ ciprofloxacin	Methicillin-resistant *S. aureus* (MRSA)	Mataraci and Dosler (2012) Dosler and Mataraci (2013)
Indolicidin	Teicoplanin		
Cecropin(1–7)-melittin A(2–9) amide (CAMA)	Ciprofloxacin		
Cathelicidin peptide BMAP-28	Quinupristin/ dalfopristin (Q/D) Linezolid (LZD) Vancomycin	*S. aureus*	Cirioni et al. (2006)
Peptide IB-367 LZD	NIL	*S. aureus*	Ghiselli et al. (2007)
Pal-Lys-LysNH2 Pal-Lys-Lys	Vancomycin	*S. aureus* on vascular grafts	Cirioni et al. (2007)
Peptide 1018	Nil	It blocks or degrades guanosine pentaphosphate [(p)ppGpp], which is essential for biofilm formation. At low concentration, inhibition of biofilm and higher concentration eradication occurred	de la Fuente-Núñez et al. (2014)
D-Enantiomeric	Nil	Study on in vivo and in vitro antibiofilm activity of this newly synthesized broad-spectrum AMP	Low and White (1989)
Nisin A Lacticin Q Nukacin ISK-1	Nil	*S. aureus* (an MRSA strain)	Okuda et al. (2013)

(continued)

Table 9.4 (continued)

Antimicrobial peptides	Synergized antibiotic if any	Targeted organism biofilm	Reference
Esculentin	Nil	*P. aeruginosa* PAO1	Luca et al. (2013)
(CSA)-13 c	Nil	*P. aeruginosa*	Nagant et al. (2013)
LL-37 and indolicidin	Nil	*P. aeruginosa*	Overhage et al. (2008)
Peptide PI	Nil	*Streptococcus mutans*	Ansari et al. (2017)
AMP derived from maggots of the blowfly *Calliphora vicina*	Nil	*Escherichia coli* *Staphylococcus aureus* *Acinetobacter baumannii*	Gordya et al. (2017)
Hepcidin 20 (human liver derived)	Nil	*S. epidermidis*	Brancatisano et al. (2014)
S4(1–16) M4Ka, a derivative of S4	Nil	*P. aeruginosa*	Quilès et al. (2016)
Piscidin-3 (fish derived)	Nil	*P. aeruginosa*	Libardo et al. (2017)
Signaling nucleotides guanosine 50-diphosphate 30-diphosphate (ppGpp) (p)ppGpp	Nil	They can regulate the expression of a plethora of genes	Libardo et al. (2017) Potrykus and Cashel (2008)
1018 DJK-5 DJK-6	Nil	They can block the synthesis and trigger degradation of (p)ppGpp in both Gram-positive and gram-negative bacteria	De la Fuente-Núñez et al. (2014)
		P. aeruginosa	Pletzer et al. (2017)
Human β-defensin 3 (hBD-3)	Nil	*Staphylococcus epidermidis* ATCC 35984	Zhu et al. (2013)
Nal-P-113	Nil	It can inhibit genes controlling the mobility of extrachromosomal elements and transport and binding proteins such as Porphyromon	Wang et al. (2017)

9.2.4 Biofilm-Degrading Enzymes

Primarily, enzymes whose composition are proteins or RNAs are natural catalysts that either accelerate chemical reactions without being consumed or altered or increase reaction rates without changing the chemical equilibrium between the reactants and products. Based on their functional characteristics on the ENZYME database (https://www.expasy.org/enzyme/), there are mainly six classes of enzymes (Shen and Chou 2007) (Table 9.5):

Table 9.5 Different classes of enzymes with their mode of action (Shen and Chou 2007)

S. no	Name of class	Mode of action
1.	Oxidoreductases	Targets the quorum-sensing molecules by acting on peptide bonds, in linkages of acid anhydride
2.	Transferases	Catalyzes reactions of oxidation and reduction by electron transfer producing H_2O_2. This affects the bacterial growth
3.	Hydrolases	Targets the EPS matrix and transfers atoms between compounds
4.	Lyases	Cleavage of C-C, C-O, and C-N bonds in EPS occurs leading to elimination of atoms
5.	Isomerases	Catalyze the formation of a substrate's isomer by transferring the specific functional groups within the molecule
6.	Ligases or synthetases	Catalyzes the joining together of two molecules using energy derived from ATP

The biofilms produce an extracellular polysaccharide substance (EPS). The main composition of EPS are proteins, polysaccharides, and nucleic acids (Low and White 1989, Bayles 2007). EPS adheres to surfaces and protects the associated microorganisms from various antimicrobials and other shearing stress due to its structural stability factors (Cooksey and Wigglesworth-Cooksey 1995; Ramasamy and Zhang 2005). Therefore, disorganization of EPS with certain classes of enzymes will lead to detachment of biofilm (Stewart 2015) and would expose the bacteria to these agents.

Various actions of enzymes involve biochemical breakdown of EPS, inhibition of QS signaling, degradation of the adhesive bonds between cells, and the toxic substance accumulation, the cumulative effect of which leads to lysis of affected cell and deactivation of necessary enzymes needed for cell development (Thallinger et al. 2013). Enzymes such as DNase I-amylase and dispersin B (DspB) minimize the exopolysaccharide layer of the microbe; thus, the number of biofilm cells is reduced (Eckhart et al. 2007; Whitchurch et al. 2002; Kalpana et al. 2012). Moreover, the specificity of enzymes and their activities are interfered or influenced by many environmental factors like availability or nonavailability of activators, cofactors, or inhibitors, temperature, substrate, and pH (Baidamshina et al. 2017). One of the critical characteristics of enzymes is that they are substrate-specific, i.e., they cleave the EPS at a specific site (Bridier et al. 2015).

EPS composition governs a significant role in deciding whether enzymes alone or a blend of enzymes in synergy with other treatment methods, physical (ultrasound, stress) or chemical (chelating agents, buffers, surfactants, and detergents), is required to remove the EPS altogether (Thallinger et al. 2013; Darouiche et al. 2009; Izano et al. 2007). Additionally, significant reduction of biofilm mass is obtained, when the active enzyme is immobilized by entrapping in substances like poly(ethylene-alt-maleic anhydride), ceramics, polycaprolactam, etc. (Regina et al. 2012). The resistance of biofilm-forming pathogens to enzymes is quite uncommon; however, there are few exceptions, like *L. monocytogenes* resistant to lysozyme

(Nguyen and Burrows 2014), *S. aureus* mutant to lysostaphin (Gründling et al. 2006), and *P. aeruginosa* to peroxidase (Lewis 2001).

Though enzymatic therapies also have some limitations, the first one is that they are costly compared to several other antimicrobials. In the natural environment, biofilms are a composition of variably diversified microbial species; therefore, the EPS is also diverse (Jahid and Ha 2014). This diverse biofilm matrix is difficult to treat with substrate-specific enzymes. It is known that wrong selection of enzymes and their combinations sometimes leads to attenuation instead of killing (Baidamshina et al. 2017), or sometimes it does the reverse of increasing virulence factors and biofilm formation, i.e., induction of biofilm formation occurred in *Pseudomonas aeruginosa* and *Enterococcus faecalis* that is generated by a protease enzyme (Ołdak and Trafny 2005; Xu et al. 2014) (Table 9.6).

9.2.5 Bacteriophage

Bacteriophages were discovered by Frederick Twort in 1915 and Félix Bd'Hérelle in 1917 independently. These are viruses, shorter in size, and survive on host prokaryotes (d'Herelle 1917, 1918). Taxonomically, they are divided into Myoviridae, Siphoviridae, and Podoviridae (Ackermann 2009). Bacteriophages are bacterial viruses that exhibit two kinds of life cycles: the first one is lytic and the other is lysogenic. They have the ability to lyse the host bacterial cell or grow generation by generation with bacterial cell (Twort 1936). Bacteriophages have been applied medically to take care of human microbial diseases from the last 80 years in former Soviet Union and European countries (Clark and March 2006).

Bacteriophages penetrate biofilms (Pires et al. 2011; Vilas Boas et al. 2016); therefore, phages are active against both planktonic and biofilm form of bacteria (Kim et al. 2011; Gutiérrez et al. 2016). Antiphage refuges are formed in bacteria in biofilms, which establishes bacteria phage coexistence (Heilmann et al. 2012). The phage takes advantage of high cell density in biofilm and spreads rapidly; this weakens the biofilm structural integrity of bacterial cells and causes its lysis. Phages and antibiofilm substances can be applied together to target host bacteria for complete removal of biofilms (Uppuluri and Lopez-Ribot 2016). Alternatively, another method to enhance the broad host range of bacteriophages is that they can be genetically engineered. Dispersin B from *Aggregatibacter actinomycetemcomitans* is a biofilm-degrading enzyme expressed from engineered phages (Lu and Collins 2007).

The phage therapy has many advantages over conventional antibiotic therapy (Matsuzaki et al. 2005): it attacks the targeted microbe and does not affect the healthy microbial flora, is effective against MDR and phage-resistant bacterial mutants, is cheaper compared to antibiotics, and has minimum/rare side effects (Matsuzaki et al. 2003). One of the critical factors determining the efficacy of phage therapy is attaining high phage "killing titers" (Abedon and Thomas-Abedon 2010). However, Defence mechanisms and other host-mediated responses should be considered before adapting any conventional therapeutics methods in mammals.

Table 9.6 Enzymes associated with biofilm control

Enzymes	Source of enzyme	Synergized antimicrobial agent if any	Targeted organism biofilm	Reference
Amylase	*Bacillus subtilis* S8-18	Nil	Methicillin-resistant *S. aureus* (MRSA) *V. cholerae* *P. aeruginosa* ATCC10145	Kalpana et al. (2012)
	B. subtilis-derived	Nil	*S. aureus* *P. aeruginosa*	Craigen et al. (2011)
			P. aeruginosa	Singh et al. (2015)
	Bacillus subtilis S8-18	10% human plasma	Methicillin-sensitive strain MRSA strains	Watters et al. (2016)
DNase I	NA	Nil	*L. monocytogenes*	Nguyen and Burrows (2014)
		Nil	*E. faecalis*	Abedon and Thomas-Abedon (2010)
		Nil	*C. jejuni* *Campylobacter coli*	Kim et al. (2017)
DNase I derivative (DNase1L2)	Human stratum corneum	Nil	*P. aeruginosa* *S. aureus*	Eckhart et al. (2007)
	NA	Chlorhexidine gluconate povidone iodine	*S. aureus biofilm*	Kaplan et al. (2012)
Recombinant human DNase I (rhDNase I)	NA	Triclosan	*S. aureus*	Darouiche et al. (2009)
	Staphylococcus simulans	Clarithromycin	MRSA	Aguinaga et al. (2011)
Enzyme complex (amylase, cellulase, protease)	*Penicillium janthinellum mutant* EU2D-21	Nil	*Escherichia coli* *Salmonella enteric* *Pseudomonas aeruginosa* *Staphylococcus aureus*	Nagraj and Gokhale (2018)

Lysozyme	Microorganisms Plant extracts Animals	Nil	*S. aureus* *P. aeruginosa* strains 10,145 and 3989	Hukić et al. (2017)
Alginate lyase	NA	Glutaraldehyde	*P. aeruginosa*	Meshram et al. (2016)
Cellulase	NA	Nil	*P. aeruginosa* *S. aureus*	Fleming et al. (2017)
Ficin	NA	Nil	*S. aureus* *S. epidermidis*	Baidamshina et al. (2017)
Subtilisin	Strains of *Bacillus* sp.	Nil	*Pseudoalteromonas* sp. *Serratia marcescens*	Leroy et al. (2008)
		Immobilization of enzyme on poly(ethylene-alt-maleic anhydride), in ceramics, and on polycaprolactam	*P. fluorescence* *S. aureus* *E. coli* *B. subtilis* *S. typhimurium* *Staphylococcus xylosus*	Regina et al. (2012)
Proteinase K broad-spectrum serine protease	*Tritirachium album*	Nil	*Lactobacillus plantarum* *Staphylococcus lentus* *Staphylococcus cohnii* *Staphylococcus saprophyticus*	Fagerlund et al. (2016)
Trypsin	*Rhodococcus ruber* strain C208	Nil	*P. aeruginosa*	Banar et al. (2016)
Serratiopeptidase (Spep)	*S. marcescens* Spep	Nil	*Staphylococcus* spp.	Artini et al. (2013)
Papain Bromelain	Present in living organism	Nil	*L. monocytogenes* pathogen *Klebsiella pneumoniae* *Acinetobacter* sp. *S. aureus*	Mohamed et al. (2018) Nguyen and Burrows (2014)

(continued)

Table 9.6 (continued)

Enzymes	Source of enzyme	Synergized antimicrobial agent if any	Targeted organism biofilm	Reference
Endolysins peptidoglycan hydrolases	Bacteriophage lysins	Nil	Gram-positive pathogens	Gutierrez et al. (2016)
Extracellular enzyme complex	*Penicillium janthinellum* EU2D-2	Nil	*Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa* *Salmonella enterica*	Nagraj and Gokhale (2018)
Endolysin SAL-2	NA	Nil	*Staphylococcus* sp.	Son et al. (2010)
Endolysin LysH5	NA	Nil	*S. aureus*	Gutierrez et al. (2015)
Artilysins	Engineered endolysin based	Nil	*MDR P. aeruginosa*	Briers et al. (2014)
Exopolysaccharide depolymerase, Dpo7	NA	Nil	*S. epidermidis* *S. aureus*	Gutierrez et al. (2015)
Glucose oxidase (Gox)	NA	Nil	*Acinetobacter calcoaceticum* *Hansenula polymorpha* *Corynebacterium aquaticum*	Thallinger et al. (2013)
Cellobiose dehydrogenase (Cdh)	NA	Nil	*S. aureus* on polydimethylsiloxane (PDMS)-based catheter surface	Thallinger et al. (2016)
Naturally derived lipases	NA	Nil	*Listeria* sp. *Serratia* sp. *B. cereus*	Seghal Kiran et al. (2014)
Serine Cysteine Metalloproteases	NA	Nil	*E. coli* *P. fluorescens* *Vibrio parahaemolyticus*	Hou et al. (2017) Kumar (2008) Watters et al. (2016)

The phage therapeutics should be developed to have active, harmless, safe, and long-term treatment options (Szczaurska-Nowak et al. 2009). Phages also modulate the immune system. One of the primary example is respiratory burst induced by bacterial cell wall that is inhibited by phagocytes in human blood (Levin and Bull 2004). Another essential feature observed about phages is the normalization of cytokine production by blood cells isolated from patients (Weber-Dabrowska et al. 2000). All these studies showed that mammal–phage interactions should be explored in detail for their further use as a treatment option either alone or in synergism with antibiotics.

Many phage combinations can be applied to obtain broader activity, i.e., cocktails of phages (Chan et al. 2013). Alternatively, an excellent strategy to fight against older biofilms is the use of combinations of both bacteriophages and antibiotics. The combination of a bacteriophage with amoxicillin was much more effective in reducing a mature biofilm of *Klebsiella pneumoniae* B5055 than each of the agents alone. The advantage of using phage–antibiotic combinations are decreased with the emergence of resistant cells that would appear upon using phages or antibiotica lone (Chhibber et al. 2009a, b). The recent multidrug-resistant (MDR) strains found in clinical isolates of bacteria are emerging day by day, and it has become difficult to treat these infections causing endemics (Alisky et al. 1998; Carlton 1999).

The principal downside of the use of therapeutic phages in medical treatment is the introduction of resistance against phages by pathogenic bacteria. The resistance of bacteria to phage may be developed due to inactivation of phage by the immune system of the host, and it may occur when virulence genes get incorporated into the host bacterial genome (Dolan 2009). The bacteriophage therapy has limitations of specificity towards the host which limits the phage to have a narrow range of host bacteria except for some exceptions, e.g., *Staphylococcal* phage K, Sb-1, and Stau2 (Curtin and Donlan 2006; Sharma et al. 2005). Cross-infections in closely related species, for example, of *Staphylococcus* by polyvalent phage K, SK311, U16, ϕ131, and ϕ812 are also one of the problems while using phage therapy (Pantůček et al. 1998). However, if a phage uses a bacterial virulence factor as a receptor, it should target the "virulent" subpopulation only (Bedi et al. 2009). Some of the obstacles which come across in the commercial production of phage as therapeutic agents are their complex manufacturing and testing methodology, current regulations, patenting and efficacy problems, and costly clinical trials (Debarbieux et al. 2016; Vandenheuvel et al. 2015). Despite these limitations, it can be summarized that phages are quite safe and effective as a future antibiofilm agent. Table 9.7 summarizes some of the phage-associated biofilm control studies done in the past years.

9.3 Conclusion

The infections caused by biofilms are chronic, recurrent, and resistant to antibiotics. Also, the contamination caused by them in industrial systems is challenging to eradicate. As a result of strengthening antimicrobial drug resistance, conventionally

Table 9.7 Bacteriophage associated with biofilm control

Phages	Target organism biofilm	Reference
T4	*E. coli*	Corbin et al. (2001)
2307-B1	*L. monocytogenes*	Hibma et al. (1997)
53b SF153b	*E. agglomerans*	Hughes et al. (1998)
F116	*P. aeruginosa*	Hanlon et al. (2001)
11229, φEnt, φ1.15	*E. cloacae*	Tait et al. (2002)
φS1	*P. fluorescens*	Sillankorva et al. (2004)
KH1	*E. coli O157*	Sharma et al. (2005)
456	*S. epidermidis*	Curtin and Donlan (2006)
φ11, φ12	*S. aureus*	Sass and Bierbaum (2006)
K	*S. epidermidis*	Cerca et al. (2007)
TG1 T7	*E. coli*	Lu and Collins (2007)
C2	*S. maltophilia*	Briandet et al. (2008)
φS1	*P. fluorescens*	Sillankorva et al. (2008)
B5055 phage synergizes with antibiotic	*K. pneumoniae*	Bedi et al. (2009)
SAP-2	*S. aureus*	Son et al. (2010)
P100	*L. monocytogenes*	Soni and Nannapaneni (2010)
IBB-PF7A, IBB-SL58B	*P. fluorescens, S. lentus*	Sillankorva et al. (2010)
M4	*P. aeruginosa*	Fu et al. (2010)
Bacteriophage, from the Myoviridae family T4-like phage	NA	Yoon et al. (2010)
phiIBB-PAP21, phiIBB-PAA	*P. aeruginosa*	Pires et al. (2011)
Aab01, Aab01-1	*Aggregatibacter actinomycetemcomitans*	Castillo-Ruiz et al. (2011)
BVPaP-3	*P. aeruginosa*	Ahiwale et al. (2011)
λW60, PB-1	*E. coli, P. aeruginosa*	Kay et al. (2011)
CP8, CP30	*C. jejuni*	Siringan et al. (2011)
phi 15	*P. putida*	Cornelissen et al. (2011)

used antibiotic therapy alone is not sufficient to control biofilm-related infections. Hence, another category of molecules/remedies to treat biofilm-associated threats is an appealing area and still has to be explored by researchers. Each new novel molecule has some advantages and limitations. Although in AMPs, enzymes, and bacteriophages, QSIs have broad-spectrum antibacterial function and tend to be protected from the occurrence of microbial resistance and could work synergistically with antibiotics, extensive research is needed such as chemical studies of the EPS matrix of various microbes and complex immunomodulatory activities inside the host cells which can reduce/enhance their efficacy. However, the effectiveness of biological control strategies might be affected through a range of physical and chemical factors. These factors include temperature or time applied in the biocontrol method, treatment of single species or multiple species biofilm, development strategy used by an organism to develop a biofilm, and composition of the surface matrix. Therefore, strategically defined control methods or validation studies of new

emerging biocontrol assays against microbial biofilms need to be done before the commercialization of these products.

References

Abedon ST, Thomas-Abedon C (2010) Phage therapy pharmacology. Curr Pharm Biotechnol 11:28–47

Acharya KR, Ackerman SJ (2014) Eosinophil granule proteins: form and function. J Biol Chem 289(25):17406–17415

Ackermann HW (2009) Phage classification and characterization. Methods Mol Biol 501:127–140

Aguinaga A, Francés ML, Del Pozo JL, Alonso M, Serrera A, Lasa I, Leiva J (2011) Lysostaphin and clarithromycin: a promising combination for the eradication of *Staphylococcus aureus* biofilms. Int J Antimicrob Agents 37:585–587

Ahiwale S, Tamboli N, Thorat K, Kulkarni R, Ackermann HW, Kapadnis B (2011) In vitro management of hospital *Pseudomonas aeruginosa* biofilm using indigenous T7-like lytic phage. Curr Microbiol 62:335–340

Alisky J, Iczkowski K, Rapoport A, Troitsky N (1998) Bacteriophages show promise as antimicrobial agents. J Infect 36(1):5–15

Anderson GG, O'Toole GA (2008) Innate and induced resistance mechanisms of bacterial biofilms. Curr Top Microbiol Immunol 322:85–105

Ansari JM, Abraham NM, Massaro J, Murphy K, Smith-Carpenter J, Fikrig E (2017) Anti-biofilm activity of a self-aggregating peptide against *Streptococcus mutans*. Front Microb 8:488

Artini M, Papa R, Scoarughi GL, Galano E, Barbato G, Pucci P, Selan L (2013) Comparison of the action of different proteases on virulence properties related to the staphylococcal surface. J Appl Microbiol 114(1):266–277

Baidamshina DR, Trizna EY, Holyavka MG, Bogachev MI, Artyukhov VG, Akhatova Kayumov AR (2017) Targeting microbial biofilms using Ficin, a nonspecific plant protease. Sci Rep 7:46068

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils - a review. Food Chem Toxicol 46:446–475

Balaban N, Cirioni O, Giacometti A, Ghiselli R, Braunstein JB, Silvestri C, Mocchegiani F, Saba V, Scalise G (2007) Treatment of Staphylococcus aureus biofilm infection by the quorum-sensing inhibitor RIP. Antimicrob Agents Chemother 51:2226–2229

Banar M, Emaneini M, Satarzadeh M, Abdellahi N, Beigverdi R, van Leeuwen WB, Jabalameli F (2016) Evaluation of mannosidase and trypsin enzymes effects on biofilm production of *Pseudomonas aeruginosa* isolated from burn wound infections. PLoS One 11(10):e0164622

Bayles KW (2007) The biological role of death and lysis in biofilm development. Nat Rev Microbiol 5:721–726

Bedi MS, Verma V, Chibber S (2009) Amoxicillin and specific bacteriophage can be used together for eradication of biofilm of *Klebsiella pneumoniae* B5055. World J Microb Biotechnol 25:1145–1151

Beloin C, Renard S, Ghigo JM, Lebeaux D (2014) Novel approaches to combat bacterial biofilms. Curr Opin Pharmacol 18:61–68

Böhme A, Risse-Buhl U, Küsel K (2009) Protists with different feeding modes change biofilm morphology. FEMS Microbiol Ecol 69(2):158–169

Brackman G, Cos P, Maes L, Nelis HJ, Coenye T (2011) Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. Antimicrob Agents Chemother 55:2655–2661

Brady A, Loughlin R, Gilpin D, Kearney P, Tunney M (2006) In vitro activity of tea tree oil against clinical skin isolates of methicillin-resistant and -sensitive *Staphylococcus aureus* and coagulase-negative staphylococci growing planktonically and as biofilms. J Med Microbiol 55:1375–1380

Brancatisano FL, Maisetta G, Di Luca M, Esin S, Bottai D, Bizzarri R, Campa M, Batoni G (2014) Inhibitory effect of the human liver-derived antimicrobial peptide hepcidin 20 on biofilms of polysaccharide intercellular adhesin (PIA)-positive and PIA-negative strains of *Staphylococcus epidermidis*. Biofouling 30:435–446

Briandet R, Lacroix-Gueu P, Renault M, Lecart S, Meylheuc T, Bidnenko E, Steenkeste K, Bellon-Fontaine MN, Fontaine-Aupart MP (2008) Fluorescence correlation spectroscopy to study diffusion and reaction of bacteriophages inside biofilms. Appl Environ Microbiol 74:2135–2143

Bridier A, Sanchez-Vizuete P, Guilbaud M, Piard JC, Naïtali M, Briandet R (2015) Biofilm-associated persistence of food-borne pathogens. Food Microbiol 45:167–178

Briers Y, Walmagh M, Van Puyenbroeck V, Cornelissen A, Cenens W, Aertsen A, Oliveira H, Azeredo J, Verween G, Pirnay JP, Miller S (2014) Engineered endolysin-based "Artilysins" to combat multidrug-resistant gram-negative pathogens. MBio 5(4):e01379–e01314

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA (2005) Non-O157 Shiga toxin producing *Escherichia coli* infections in the United States,1983–2002. J Infect Dis 192:1422–1429

Burt SA (2004) Essential oils: their antibacterial properties and potential applications in foods: a review. Int J Food Microbiol 94:223–253

Carlton RM (1999) Phage therapy: past history and future prospects. Arch Immunol Ther Exp 47(5):267–274

Castillo-Ruiz M, Vinés ED, Montt C, Fernández J, Delgado JM, Hormazábal JC, Bittner M (2011) Isolation of a novel *Aggregatibacter actinomycetemcomitans* serotype b bacteriophage capable of lysing bacteria within a biofilm. Appl Environ Microbiol 77(9):3157–3159

Cerca N, Oliveira R, Azeredo J (2007) Susceptibility of *Staphylococcus epidermidis* planktonic cells and biofilms to the lytic action of staphylococcus bacteriophage K. Lett Appl Microbiol 45:313–317

Chakraborty S, Duttal TK, De A, Das M, Ghosh S (2018) Impact of bacterial biofilm in veterinary medicine: an overview. Int J Curr Microbiol App Sci 7(4):3228–3239

Chan BK, Abedon ST, Loc-Carrillo C (2013) Phage cocktails and the future of phage therapy. Future Microbiol 8(6):769–783

Chhibber S, Bedi MS, Verma V (2009a) Amoxicillin and specific bacteriophage can be used together for eradication of biofilm of *Klebsiella pneumoniae* B5055. World J Microbiol Biotechnol 25:1145–1151

Chhibber S, Verma V, Harjai K (2009b) Restricting ciprofloxacin-induced resistant variant formation in biofilm of *Klebsiella pneumoniae* B5055 by complementary bacteriophage treatment. J Antimicrob Chemother 64:1212–1218

Christensen LD, van Gennip M, Jakobsen TH, Alhede M, Hougen HP, Høiby N, Bjarnsholt T, Givskov M (2012) Synergistic antibacterial efficacy of early combination treatment with tobramycin and quorum-sensing inhibitors against *Pseudomonas aeruginosa* in an intraperitoneal foreign-body infection mouse model. J Antimicrob Chemother 67:1198–1206

Cirino IC, Menezes-Silva SM, Silva HT, de Souza EL, Siqueira-Júnior JP (2014) The essential oil from *Origanum vulgare* L. and its individual constituents carvacrol and thymol enhance the effect of tetracycline against *Staphylococcus aureus*. Chemotherapy 60:290–293

Cirioni O, Giacometti A, Ghiselli R, Bergnach C, Orlando F, Mocchegiani F, Silvestri C, Licci A, Skerlavaj B, Zanetti M, Saba V, Scalise G (2006) Pre-treatment of central venous catheters with the cathelicidin BMAP-28 enhances the efficacy of antistaphylococcal agents in the treatment of experimental catheter-related infection. Peptides 27:2104–2110

Cirioni O, Giacometti A, Ghiselli R, Kamysz W, Silvestri C, Orlando F, Mocchegiani F, Vittoria AD, Kamysz E, Saba V, Scalise G (2007) The lipopeptides Pal-Lys-Lys-NH2 and Pal-Lys-Lys soaking alone and in combination with intraperitoneal vancomycin prevent vascular graft biofilm in a subcutaneous rat pouch model of staphylococcal infection. Peptides 28:1299–1303

Clark JR, March JB (2006) Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. Trends Biotechnol 24(5):212–218

Clutterbuck AL, Woods EJ, Knottenbelt DC, Clegg PD, Cochrane CA, Percival SL (2007) Biofilms and their relevance to veterinary medicine. Vet Microbiol 121(1–2):1–17

Coelho FA, Pereira MO (2013) Exploring new treatment strategies for *Pseudomonas aeruginosa* biofilm infections based on plant essential oils. In: Méndez-Vilas A (ed) Microbial pathogens and strategies for combating them: science, technology and education, vol 1. Formatex Research Center, Badajoz, pp 83–89

Cooksey KE, Wigglesworth-Cooksey B (1995) Adhesion of bacteria and diatoms to surfaces in the sea: a review. Aquat Microb Ecol 9:87–96

Corbin BD, McLean RJC, Aron GM (2001) Bacteriophage T4 multiplication in a glucose-limited *Escherichia coli* biofilm. Can J Microbiol 47:680–684

Cornelissen A, Ceyssens PJ, T'Syen J, Van Praet H, Noben JP, Shaburova OV, Krylov VN, Volckaert G, Lavigne R (2011) The T7-related *Pseudomonas putida* phage phi 15 displays virion-associated biofilm degradation properties. PLoS One 6:e18597

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12:564–582

Cox SD, Gustafson JE, Mann CM, Markham JL, Liew YC, Hartland RP, Bell HC, Warmington JR, Wyllie SG (1998) Tea tree oil causes K leakage and inhibits respiration in *Escherichia coli*. Lett Appl Microbiol 26:335–358

Craigen B, Dashiff A, Kadouri DE (2011) The use of commercially available alpha-amylase compounds to inhibit and remove *Staphylococcus aureus* biofilms. Open Microbiol J 5:21–31

Crouzet M, Le Senechal C, Brözel VS, Costaglioli P, Barthe C, Bonneu M, Vilain S (2014) Exploring early steps in biofilm formation: set-up of an experimental system for molecular studies. BMC Microbiol 14:253

Curtin JJ, Donlan RM (2006) Using bacteriophages to reduce formation of catheter associated biofilms by Staphylococcus epidermidis. Antimicrob Agents Chemother 50:1268–1275

d'Herelle F (1917) Sur un microbe invisible antagoniste des bacilles dysentériques. Comptes Rendus de l'Académie des Sciences Paris 165:373–375

d'Herelle F (1918) Technique de la recherche du microbe filtrant bactériophage (*Bacteriophagum intestinale*). Comptes Rendus des Seances de la Societe de Biologie Paris 81:1060–1062

Darouiche RO, Mansouri MD, Gawande PV, Madhyastha S (2009) Antimicrobial and antibiofilm efficacy of triclosan and Dispersin B combination. J Antimicrob Chemother 64:88–93

Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberrg EP (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280(5361):295–298

de la Fuente-Núñez C, Reffuveille F, Haney EF, Straus SK, Hancock REW (2014) Broad-spectrum anti-biofilm peptide that targets a cellular stress response. PLoS Pathol 10:e1004152

de la Fuente-Núñz C, Korolik V, Bains M, Nguyen U, Breidenstein EBM, Horsman S, Lewenza S, Burrows L, Hancock RE (2012) Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob Agents Chemother 56:2696–2704

De Martino L, De Feo V, Formisano C, Mignola E, Senatore F (2009) Chemical composition and antimicrobial activity of the essential oils from three chemotypes of *Origanum vulgare* L. ssp. hirtum (link) Ietswaart growing wild in Campania (Southern Italy). Molecules 14:2735–2746

de Oliveira MMM, Brugnera DF, Cardoso MDG, Alves E, Piccoli RH (2010) Disinfectant action of *Cymbopogon* sp. essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. Food Control 21:549–553

de Oliveira MMM, Brugnera DF, Do Nascimento JA, Batista NN, Piccoli RH (2012) Cinnamon essential oil and cinnamaldehyde in the control of bacterial biofilms formed on stainless steel surfaces. Eur Food Res Technol 234:821–832

Debarbieux L, Pirnay JP, Verbeken G, De Vos D, Merabishvili M, Huys I, Patey O, Schoonjans D, Vaneechoutte M, Zizi M, Rohde C (2016) A bacteriophage journey at the European medicines agency. FEMS Microbiol Lett 363(2):fnv225. https://doi.org/10.1093/femsle/fnv225

Dolan RM (2009) Preventing biofilms of clinically relevant organisms using bacteriophage. Trends Microbiol 17:66–72

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890

Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15(2):167–193

Dosler S, Mataraci E (2013) In vitro pharmacokinetics of antimicrobial cationic peptides alone and in combination with antibiotics against methicillin resistant *Staphylococcus aureus* biofilms. Peptides 49:53–58

Dunne WM Jr (2002) Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev 15(2):155–166

Eckhart L, Fischer H, Barken KB, Tolker-Nielsen T, Tschachler E (2007) DNase1L2 suppresses biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Br J Dermatol 156:1342–1345

Fabian D, Sabol M, Domaracka K, Bujnakova D (2006) Essential oils–their antimicrobial activity against Escherichia coli and effect on intestinal cell viability. Toxicol In Vitro 20:1435–1445

Fagerlund A, Langsrud S, Schirmer BC, Moretro T, Heir E (2016) Genome analysis of Listeria monocytogenes sequence type 8 strains persisting in salmon and poultry processing environments and comparison with related strains. PLoS One 11(3):e0151117

Fleming D, Chahin L, Rumbaugh K (2017) Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. Antimicrob Agents Chemother 61(2):e01998–e01916

Francolini I, Norris P, Piozzi A, Donelli G, Stoodley P (2004) Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. Antimicrob Agents Chemother 48:4360–4365

Fu W, Forster T, Mayer O, Curtin JJ, Lehman SM, Donlan RM (2010) Bacteriophage cocktail for the prevention of biofilm formation by Pseudomonas aeruginosa on catheters in an in vitro model system. Antimicrob Agents Chemother 54(1):397–404

García AB, Percival SL (2011) Zoonotic infections: the role of biofilms. In: Biofilms and veterinary medicine. Springer, Berlin, pp 69–110

Ghiselli R, Giacometti A, Cirioni O, Mocchegiani F, Silvestri C, Orlando F, Kamysz W, Licci A, Nadolski P, Della Vittoria A, Łukasiak J, Scalise G, Saba V (2007) Pretreatment with the protegrin IB-367 affects gram positive biofilm and enhances the therapeutic efficacy of linezolid in animal models of central venous catheter infection. J Parenter Enter Nutr 31:463–468

Gordya N, Yakovlev A, Kruglikova A, Tulin D, Potolitsina E, Suborova T, Bordo D, Rosano C, Chernysh S (2017) Natural antimicrobial peptide complexes in the fighting of antibiotic resistant biofilms: Calliphora vicina medicinal maggots. PLoS One 12:e0173559

Gründling A, Missiakas DM, Schneewind O (2006) Staphylococcus aureus mutants with increased lysostaphin resistance. J Bacteriol 188:6286–6297

Gutierrez D, Briers Y, Rodríguez-Rubio L, Martínez B, Rodríguez A, Lavigne R, García P (2015) Role of the pre-neck appendage protein (Dpo7) from phage vB SepiS-phiIPLA7 as an antibiofilm agent in staphylococcal species. Front Microbiol 6:1315

Gutiérrez D, Rodríguez-Rubio L, Martínez B, Rodríguez A, García P (2016) Bacteriophages as weapons against bacterial biofilms in the food industry. Front Microbiol 7:825

Hanlon GW, Denyer SP, Olliff SJ, Ibrahim LJ (2001) Reduction of exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 67:2746–2753

He J, Anderson MH, Shi W, Eckert R (2009) Design and activity of a 'dual-targeted' antimicrobial peptide. Int J Antimicrob Agents 33:532–537

Heilmann S, Sneppen K, Krishna S (2012) Coexistence of phage and bacteria on the boundary of self-organized refuges. Proc Natl Acad Sci U S A 109:12828–12833

Hell E, Giske CG, Nelson A, Römling U, Marchini G (2010) Human cathelicidin peptide LL37 inhibits both attachment capability and biofilm formation of *Staphylococcus epidermidis*. Lett Appl Microbiol 50(2):211–215

Henderson P (2010) Fouling and anti-fouling in other industries –power stations, desalination plants-drinking water supplies and sensors. In: Biofouling. Wiley-Blackwell, Chichester, pp 288–305

Herrmann G, Yang L, Wu H, Song Z, Wang H, Høiby N, Ulrich M, Molin S, Riethmüller J, Döring G (2010) Colistin-tobramycin combinations are superior to monotherapy concerning the killing of biofilm Pseudomonas aeruginosa. J Infect Dis 202:1585–1592

Hibma AM, Jassim SA, Griffiths MW (1997) Infection and removal of L-forms of listeria monocytogenes with bred bacteriophage. Int J Food Microbiol 34:197–207
Hirakura Y, Kobayashi S, Matsuzaki K (2002) Specific interactions of the antimicrobial peptides cyclic-sheet tachyplesin I with lipopolysaccharides. Biochim Biophys Acta 1562:32–36
Hou HM, Zhu YL, Wang JY, Jiang F, Qu WY, Zhang GL, Hao HS (2017) Characteristics of N-acylhomoserine lactones produced by *Hafnia alvei* H4 isolated from spoiled instant sea cucumber. Sensors 17(4):772
Hughes KA, Sutherland IW, Clark J, Jones MV (1998) Bacteriophage and associated polysaccharide depolymerases- novel tools for study of bacterial biofilms. J Appl Microbiol 85:583–590
Hukić M, Seljmo D, Ramovic A, Ibrišimović MA, Dogan S, Hukic J, Feric Bojic E (2017) The effect of lysozyme on reducing biofilms by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Gardnerella vaginalis*: an in vitro examination. Microb Drug Resist 24(4):353–358
Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608
Izadpanah A, Gallo RL (2005) Antimicrobial peptides. J Am Acad Dermatol 52(3):381–390
Izano EA, Wang H, Ragunath C, Ramasubbu N, Kaplan JB (2007) Detachment and killing of *Aggregatibacter actinomycetemcomitans* biofilms by dispersin B and SDS. J Dent Res 86:618–622
Jafri H, Husain FM, Ahmad I (2014) Antibacterial and antibiofilm activity of some essential oils and compounds against clinical strains of *Staphylococcus aureus*. J Biomed Ther Sci 1:65–71
Jahid IK, Ha SD (2014) Inactivation kinetics of various chemical disinfectants on *Aeromonas hydrophila* planktonic cells and biofilms. Foodborne Pathog Dis 11:346–353
Jakobsen TH, van Gennip M, Phipps RK, Shanmugham MS, Christensen LD, Alhede M, Skindersoe ME, Rasmussen TB, Friedrich K, Uthe F, Jensen PØ, Moser C, Nielsen KF, Eberl L, Larsen TO, Tanner D, Høiby N, Bjarnsholt T, Givskov M (2012) Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. Antimicrob Agents Chemother 56:2314–2325
Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, Kamil MA (2018) Bacterial biofilm and associated infections. J Chin Med Assoc 81(1):7–11
Johansson L, Thulin P, Sendi P, Hertzén E, Linder A, Åkesson P, Low DE, Agerberth B, Norrby-Teglund A (2008) Cathelicidin LL-37 in severe *Streptococcus pyogenes* soft tissue infections in humans. Infect Immun 76(8):3399–3404
Jorge P, Lourenco A, Pereira MO (2012) New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. Biofouling 28:1033–1061
Kabwanga IT, Yetişemiyen A, Nankya S (2018) Dairy industrial hygiene: a review on biofilm challenges and control. Int J Res Granthaalayah 6(2):268–273. https://doi.org/10.5281/zenodo.1194694
Kadouri D, Venzon NC, O' Toole GA (2007) Vulnerability of pathogenic biofilms to *Micavibrio aeruginosavorus*. Appl Environ Microbiol 73(2):605–614
Kalpana BJ, Aarthy S, Pandian SK (2012) Antibiofilm activity of amylase from Bacillus subtilis S8-18 against biofilm forming human bacterial pathogens. Appl Biochem Biotechnol 167:1778–1794
Kaplan JB, LoVetri K, Cardona ST, Madhyastha S, Sadovskaya I, Jabbouri S, Izano EA (2012) Recombinant human DNase I decreases biofilm and increases antimicrobial susceptibility in staphylococci. J Antibiot 65:73–77
Kavanaugh NL, Ribbeck K (2012) Selected antimicrobial essential oils eradicate *Pseudomonas* spp. and *Staphylococcus aureus* biofilms. Appl Environ Microbiol 78:4057–4061
Kay MK, Erwin TC, McLean RJ, Aron GM (2011) Bacteriophage ecology in *Escherichia coli* and *Pseudomonas aeruginosa* mixed-biofilm communities. Appl Environ Microbiol 77(3):821–829
Kerekes EB, Vidács A, Török Jenei J, Gömöri C, Takó M, Chandrasekaran M, Kadaikunnan S, Alharbi NS, Krisch J, Vágvölgyi C (2015) Essential oils against bacterial biofilm formation and quorum sensing of food-borne pathogens and spoilage microorganisms. In: The Battle against microbial pathogens: basic science, technological advances and educational programs. Microbiology book series, vol 5 (5). Formatex Research Center, Badajoz, pp 429–437

Kim M, Park J-M, Um H-J, Lee K-H, Kim H, Min J, Kim Y-H (2011) The antifouling potentiality of galactosamine characterized from *Vibrio vulnificus* exopolysaccharide. Biofouling 27:851–857

Kim HS, Lee SH, Byun Y, Park HD (2015) 6-Gingerol reduces Pseudomonas aeruginosa biofilm formation and virulence via quorum sensing inhibition. Sci Rep 5:8656

Kim SH, Park C, Lee EJ, Bang WS, Kim YJ, Kim JS (2017) Biofilm formation of campylobacter strains isolated from raw chickens and its reduction with DNase I treatment. Food Control 71:94–100

Kiran S, Sharma P, Harjai K, Capalash N (2011) Enzymatic quorum quenching increases antibiotic susceptibility of multidrug resistant *Pseudomonas aeruginosa*. Iran J Microbiol 3:1–12

Kumar JK (2008) Lysostaphin: an antistaphylococcal agent. Appl Microbiol Biotechnol 80(4):555–561

Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42:9–27

Lambert RJW, Skandamis PN, Coote PJ, Nychas G-JE (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 91:453–462

Landini P, Antoniani D, Burgess JG, Nijland R (2010) Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. Appl Microbiol Biotechnol 86(3):813–823

Langeveld WT, Veldhuizen EJA, Burt SA (2014) Synergy between essential oil components and antibiotics: a review. Crit Rev Microbiol 40:76–94

Lemberkovics E, Kéry A, Simándi B, Kakasy A, Balázs A, Héthelyi E, Szoke E (2004) Influence of extraction methods on the composition of essential oils. Acta Pharm Hung 74(3):166–170

Leroy C, Delbarre C, Ghillebaert F, Compere C, Combes D (2008) Influence of subtilisin on the adhesion of a marine bacterium which produces mainly proteins as extracellular polymers. J Appl Microbiol 105(3):791–799

Levin BR, Bull JJ (2004) Population and evolutionary dynamics of phage therapy. Nat Rev Microbiol 2(2):166–173

Lewis K (2001) Riddle of biofilm resistance. Antimicrob Agents Chemother 45:999–1007

Li L, Guo L, Lux R, Eckert R, Yarbrough D, He J, Anderson M, Shi WY (2010) Targeted antimicrobial therapy against Streptococcus mutans establishes protective non-cariogenic oral biofilms and reduces subsequent infection. Int J Oral Sci 2:66–73

Libardo MD, Bahar AA, Ma B, Fu R, McCormick LE, Zhao J, McCallum SA, Nussinov R, Ren D, Angeles-Boza AM, Cotten ML (2017) Nuclease activity gives an edge to host-defense peptide piscidin 3 over piscidin 1, rendering it more effective against persisters and biofilms. FEBS J 284(21):3662–3683

Longbottom CJ, Carson CF, Hammer KA, Mee BJ, Riley TV (2004) Tolerance of Pseudomonas aeruginosa to Melaleuca alternifolia (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. J Antimicrob Chemother 54:386–392

Low CS, White DC (1989) Regulation of external polymer production in benthic microbial communities. In: Cohen Y, Rosenberg E (eds) Microbial mats: physiological ecology of benthic microbial communities, pp 228–238

Lu TK, Collins JJ (2007) Dispersing biofilms with engineered enzymatic bacteriophage. Proc Natl Acad Sci 104:11197–11202

Luca V, Stringaro A, Colone M, Pini A, Mangoni ML (2013) Esculentin (1-21), an amphibian skin membrane-active peptide with potent activity on both planktonic and biofilm cells of the bacterial pathogen *Pseudomonas aeruginosa*. Cell Mol Life Sci 70(15):2773–2786

Ma L, Conover M, Lu H, Parsek MR, Bayles K, Wozniak DJ (2009) Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. PLoS Pathog 5(3):e1000354

Ma L, Liu X, Liang H, Che Y, Chen C, Dai H, Yu K, Liu M, Ma L, Yang CH, Song F (2012) Effects of 14-alpha-lipoyl andrographolide on quorum sensing in Pseudomonas aeruginosa. Antimicrob Agents Chemother 56(12):6088–6094

Mah TFC, Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9(1):34–39

Mataraci E, Dosler S (2012) In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin resistant *Staphylococcus aureus* biofilms. Antimicrob Agents Chemother 56:6366–6371

Matsuzaki S, Yasuda M, Nishikawa H, Kuroda M, Ujihara T, Shuin T, Shen Y, Jin Z, Fujimoto S, Nasimuzzaman MD, Wakiguchi H (2003) Experimental protection of mice against lethal Staphylococcus aureus infection by novel bacteriophage ϕMR11. J Infect Dis 187(4):613–624

Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, Ikeuchi M, Tani T, Fujieda M, Wakiguchi H, Imai S (2005) Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother 11(5):211–219

Melo MN, Ferre R, Castanho MA (2009) Antimicrobial peptides: linking partition, activity and high membrane bound concentrations. Nat Rev Microbiol 7:245–250

Meshram P, Dave R, Joshi H, Dharani G, Kirubagaran R, Venugopalan VP (2016) Biofouling control on ultrafiltration membrane through immobilization of polysaccharide-degrading enzyme: optimization of parameters. Desalin Water Treat 57(55):26861–26870

Mohamed SH, Mohamed MS, Khalil MS, Azmy M, Mabrouk MI (2018) Combination of essential oil and ciprofloxacin to inhibit/eradicate biofilms in multidrug-resistant Klebsiella pneumoniae. J Appl Microbiol 125(1):84–95

Morikawa M (2006) Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. J Biosci Bioeng 101(1):1–8

Nagant C, Pitts B, Stewart PS, Feng Y, Savage PB, Dehaye JP (2013) Study of the effect of antimicrobial peptide mimic, CSA-13, on an established biofilm formed by *Pseudomonas aeruginosa*. Microbiol Open 2(2):318–325

Nagraj AK, Gokhale D (2018) Bacterial biofilm degradation using extracellular enzymes produced by *Penicillium janthinellum* EU2D-21 under submerged fermentation. Adv Microbiol 8(09):687

Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V (2013) Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6:1451–1474

Nguyen UT, Burrows LL (2014) DNase I and proteinase K impair *Listeria monocytogenes* biofilm formation and induce dispersal of pre-existing biofilms. Int J Food Microbiol 187:26–32

Nostro A, Roccaro AS, Bisignano G, Marino A, Cannatelli MA, Pizzimenti FC, Cioni PL, Procopio F, Blanco AR (2007) Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. J Med Microbiol 56:519–523

O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Annu Rev Microbiol 54:49–79

Okuda KI, Zendo T, Sugimoto S, Iwase T, Tajima A, Yamada S, Sonomoto K, Mizunoe Y (2013) Effects of bacteriocins on methicillin-resistant *Staphylococcus aureus* biofilm. Antimicrob Agents Chemother 57(11):5572–5579

Ołdak E, Trafny EA (2005) Secretion of proteases by Pseudomonas aeruginosa biofilms exposed to ciprofloxacin. Antimicrob Agents Chemother 49:3281–3288

Olson ME, Ceri H, Morck DW, Buret AG, Read RR (2002) Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can J Vet Res 66(2):86

Overhage J, Campisano A, Bains M, Torfs ECW, Rehm BHA, Hancock REW (2008) Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 76:4176–4182

Pantucek R, Rosypalová A, Doškař J, Kailerová J, Růžičková V, Borecká P, Snopková Š, Horváth R, GoÈtz F, Rosypal S (1998) The polyvalent staphylococcal phage φ812: its host-range mutants and related phages. Virology 246(2):241–252

Papenfort K, Bassler BL (2016) Quorum sensing signal–response systems in gram-negative bacteria. Nat Rev Microbiol 14(9):576–588

Perricone M, Arace E, Corbo MR, Sinigaglia M, Bevilacqua A (2015) Bioactivity of essential oils: a review on their interaction with food components. Front Microbiol 6:76

Pires D, Sillankorva S, Faustino A, Azeredo J (2011) Use of newly isolated phages for control of Pseudomonas aeruginosa PAO1 and ATCC 10145 biofilms. Res Microbiol 162(8):798–806

Pletzer D, Wolfmeier H, Bains M, Hancock RE (2017) Synthetic peptides to target stringent response-controlled virulence in a Pseudomonas aeruginosa murine cutaneous infection model. Front Microbiol 8:1867

Potrykus K, Cashel M (2008) (p) ppGpp: still magical? Annu Rev Microbiol 62:35–51
Quilès F, Saadi S, Francius G, Bacharouche J, Humbert F (2016) In situ and real time investigation of the evolution of a *Pseudomonas fluorescens* nascent biofilm in the presence of an antimicrobial peptide. Biochim Biophys Acta Biomembr 1858(1):75–84
Ramasamy P, Zhang X (2005) Effects of shear stress on the secretion of extracellular polymeric substances in biofilms. Water Sci Technol 52:217–223
Rasamiravaka T, Labtani Q, Duez P, El Jaziri M (2015) The formation of biofilms by *Pseudomonas aeruginosa*: a review of the natural and synthetic compounds interfering with control mechanisms. Biomed Res Int 2015:1–17. https://doi.org/10.1155/2015/759348
Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, Andersen JB, Koch B, Larsen TO, Hentzer M, Eberl L, Hoiby N, Givskov M (2005) Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. Microbiology 151:1325–1340
Regina VR, Søhoel H, Lokanathan AR, Bischoff C, Kingshott P, Revsbech NP, Meyer RL (2012) Entrapment of subtilisin in ceramic sol–gel coating for antifouling applications. ACS Appl Mater Interfaces 4(11):5915–5921
Reyes-Jurado F, Franco-Vega A, Ramirez-Corona N, Palou E, López-Malo A (2015) Essential oils: antimicrobial activities, extraction methods, and their modeling. Food Eng Rev 7(3):275–297
Rosato A, Vitali C, De Laurentis N, Armenise D, Milillo MA (2007) Antibacterial effect of some essential oils administered alone or in combination with norfloxacin. Phytomedicine 14:727–732
Rossi LM, Rangasamy P, Zhang J, Qiu X-Q, Wu GY (2008) Research advances in the development of peptide antibiotics. J Pharm Sci 97:1060–1070
Roy V, Meyer MT, Smith JA, Gamby S, Sintim HO, Ghodssi R, Bentley WE (2013) AI-2 analogs and antibiotics: a synergistic approach to reduce bacterial biofilms. Appl Microbiol Biotechnol 97:2627–2638
Sass P, Bierbaum G (2006) Lytic activity of recombinant bacteriophage phi11 and phi12 endolysins on whole cells and biofilms of *Staphylococcus aureus*. Appl Environ Microbiol 73:347–352
Sauer K, Cullen MC, Rickard AH, Davies DG, Gilbert P (2004) Characterization of nutrient – induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. J Bacteriol 186(21):7312–7326
Seghal Kiran G, Nishanth Lipton A, Kennedy J, Dobson AD, Selvin J (2014) A halotolerant thermostable lipase from the marine bacterium *Oceanobacillus* sp. PUMB02 with an ability to disrupt bacterial biofilms. Bioengineered 5(5):305–318
Sharma M, Ryu JH, Beuchat LR (2005) Inactivation of *Escherichia coli* O157:H7 in biofilm on stainless steel by treatment with an alkaline cleaner and a bacteriophage. J Appl Microbiol 99:449–459
Shen HB, Chou KC (2007) EzyPred: a top-down approach for predicting enzyme functional classes and subclasses. Biochem Biophys Res Commun 364:53–59
Shirtliff ME, Mader JT, Camper AK (2002) Molecular interactions in biofilms. Chem Biol 9:859–871
Sillankorva S, Oliveira R, Vieira MJ, Sutherland I, Azeredo J (2004) Bacteriophage Φ S1 infection of *Pseudomonas fluorescens* planktonic cells versus biofilms. Biofouling 20(3):133–138
Sillankorva S, Oliveira R, Vieira MJ, Azeredo J (2008) Real-time quantification of *Pseudomonas fluorescens* cell removal from glass surfaces due to bacteriophage ϕS1 application. J Appl Microbiol 105(1):196–202
Sillankorva S, Neubauer P, Azeredo J (2010) Phage control of dual species biofilms of Pseudomonas fluorescens and Staphylococcus lentus. Biofouling 26(5):567–575
Simões M, Simões LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. LWT-Food Sci Technol 43(4):573–583
Singh V, Verma N, Banerjee B, Vibha K, Haque S, Tripathi CK (2015) Enzymatic degradation of bacterial biofilms using *Aspergillus clavatus* MTCC 1323. Microbiology 84:59–64
Siringan P, Connerton PL, Payne RJ, Connerton IF (2011) Bacteriophage-mediated dispersal of Campylobacter jejuni biofilms. Appl Environ Microbiol 77(10):3320–3326

Son JS, Lee SJ, Jun SY, Yoon SJ, Kang SH, Paik HR, Kang JO, Choi YJ (2010) Antibacterial and biofilm removal activity of a podoviridae *Staphylococcus aureus* bacteriophage SAP-2 and a derived recombinant cell-wall-degrading enzyme. Appl Microbiol Biotechnol 86(5):1439–1449

Soni KA, Nannapaneni R (2010) Removal of Listeria monocytogenes biofilms with bacteriophage P100. J Food Prot 73:1519–1524

Stewart PS (2015) Prospects for anti-biofilm pharmaceuticals. Pharmaceuticals 8(3):504–511

Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358:135–138

Szczaurska-Nowak K, Dąbrowska K, Celka M, Kurzępa A, Nevozhay D, Wietrzyk J, Świtala-Jeleń KI, Syper D, Poźniak G, Opolski A, Gorski A (2009) Antitumor effect of combined treatment of mice with cytostatic agents and bacteriophage T4. Anticancer Res 29(6):2361–2370

Tait K, Skillman LC, Sutherland IW (2002) The efficacy of bacteriophage as a method of biofilm eradication. Biofouling 18:305–311

Thallinger B, Prasetyo EN, Nyanhongo GS, Guebitz GM (2013) Antimicrobial enzymes: an emerging strategy to fight microbes and microbial biofilms. Biotechnol J 8(1):97–109

Thallinger B, Brandauer M, Burger P, Sygmund C, Ludwig R, Ivanova K, Kun J, Scaini D, Burnet M, Tzanov T, Nyanhongo GS (2016) Cellobiose dehydrogenase functionalized urinary catheter as novel antibiofilm system. J Biomed Mater Res B Appl Biomater 104(7):1448–1456

Tiwari SK, Noll KS, Cavera VL, Chikindas ML (2015) Improved antimicrobial activities of synthetic-hybrid bacteriocins designed from enterocin E50-52 and pediocin PA-1. Appl Environ Microbiol 81:1661–1667

Twort TW (1936) Further investigations on the nature of ultra-microscopic viruses and their cultivation. J Hyg 36:204–235

Uppuluri P, Lopez-Ribot JL (2016) Go forth and colonize: dispersal from clinically important microbial biofilms. PLoS Pathol 12(2):e1005397

Uroz S, Dessaux Y, Oger P (2009) Quorum sensing and quorum quenching: the yin and yang of bacterial communication. Chembiochem 10:205–216

Van de Braak SAAJ, Leijten GCJJ (1999) Essential oils and oleoresins: a survey in the Netherlands and other major markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, p 116

Vandenheuvel D, Lavigne R, Brüssow H (2015) Bacteriophage therapy: advances in formulation strategies and human clinical trials. Ann Rev Virol 2:599–618

Venge P (1999) Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. Clin Exp Allergy 29:1172–1186

Vilas Boas D, Almeida C, Sillankorva S, Nicolau A, Azeredo J, Azevedo NF (2016) Discrimination of bacteriophage infected cells using locked nucleic acid fluorescent in situ hybridization (LNA-FISH). Biofouling 32:179–190

Vuong C, Voyich JM, Fischer ER, Braughton KR, Whitney AR, DeLeo FR, Otto M (2004) Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. Cell Microbiol 6(3):269–275

Wang HY, Lin L, Tan LS, Yu HY, Cheng JW, Pan YP (2017) Molecular pathways underlying inhibitory effect of antimicrobial peptide Nal-P-113 on bacteria biofilms formation of Porphyromonas gingivalis W83 by DNA microarray. BMC Microbiol 17(1):37

Warnke PH, Sherry E, Russo PA, Acil Y, Wiltfang J, Sivananthan S, Sprengel M, Roldan JC, Schubert S, Bredee JP, Springer IN (2006) Antibacterial essential oils in malodorous cancer patients: clinical observations in 30 patients. Phytomedicine 13(7):463–467

Watters CM, Burton T, Kirui DK, Millenbaugh NJ (2016) Enzymatic degradation of in vitro Staphylococcus aureus biofilms supplemented with human plasma. Infect and Drug Resist 9:71

Weber-Dabrowska B, Zimecki M, Mulczyk M (2000) Effective phage therapy is associated with normalization of cytokine production by blood cell cultures. Arch Immunol Ther Exp 48(1):31–37

Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS (2002) Extracellular DNA required for bacterial biofilm formation. Science 295:1487

Wimley W, Hristova K (2011) Antimicrobial peptides: successes, challenges and unanswered questions. J Membr Biol 239:27–34

Xu W, Zhu X, Tan T, Li W, Shan A (2014) Design of embedded-hybrid antimicrobial peptides with enhanced cell selectivity and anti-biofilm activity. PLoS One 9:e98935

Yang JY, Della-Fera MA, Nelson-Dooley C, Baile CA (2006) Molecular mechanisms of apoptosis induced by Ajoene in 3T3-L1 adipocytes. Obesity 14:388–397

Yap PSX, Yiap BC, Ping HC, Lim SHE (2014) Essential oils, a new horizon in combating bacterial antibiotic resistance. Open Microbiol J 8:6–14

Yasir M, Willcox M, Dutta D (2018) Action of antimicrobial peptides against bacterial biofilms. Materials 11(12):2468

Yoon S, Choi Y, Lee SJ, Son J, Jun S, Kang S (2010) Bacteriophage or lytic protein derived from the bacteriophage which effective for treatment of *Staphylococcus aureus* biofilm. US 2010/0254950-A1

Zeng Z, Qian L, Cao L, Tan H, Huang Y, Xue X, Shen Y, Zhou S (2008) Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 79:119–126

Zeng X, Liu X, Bian J, Pei G, Dai H, Polyak SW, Song F, Ma L, Wang Y, Zhang L (2011) Synergistic effect of 14-alpha-lipoyl andrographolide and various antibiotics on the formation of biofilms and production of exopolysaccharide and pyocyanin by *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 55:3015–3017

Zhu C, Tan H, Cheng T, Shen H, Shao J, Guo Y, Shi S, Zhang X (2013) Human β-defensin 3 inhibits antibiotic-resistant Staphylococcus biofilm formation. J Surg Res 183(1):204–213