Chapter 5
Anti-biofilm Peptides: A New Class of Quorum Quenchers and Their Prospective Therapeutic Applications

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Abstract Biofilms are the major concerns to the researchers, due to their universal distribution among prokaryotes and involvement in antibiotic drug resistance towards conventional drugs. It led the bacteria to become up to 1000 times resistant towards antibiotics. Therefore, diverse types of anti-biofilm agents are continuously designed to target them namely (phyto) chemicals, peptides, enzymes, biosurfactants, microbial extracts, nanoparticles, and many more. Antibiofilm peptides have demonstrated high potential in targeting biofilm due to their low toxicity, and off-target effects. These peptides are experimentally validated to disrupt most of the biofilms developed on medical devices like catheters, stents, dentures, etc. implicated in nosocomial infections by ESKAPE pathogens. However, one of the important reasons for the peptides, to emerge as a new hope against biofilms, is their wide mode of action against different stages and microbial species. In the present chapter, we are focusing to explore various aspects of this important class of antibiofilm therapeutics.

Keywords Anti-biofilm peptides · nosocomial infections · ESKAPE pathogen · antibiotic resistance

5.1 Introduction

Biofilms are the consortium of microbes encapsulated in the self-secreted cocoon composed mainly of extracellular matrix. Biofilm mode of growth is an alternate lifestyle of microbes, where they mimic multicellular behavior rather than unicellular (Kostakioti et al. 2013). Among the microcolonies, the bacteria exhibits difference in their physiological state as compared to their planktonic form. The colonization process is an adaptive mechanism that arose in response to various

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environmental stresses including nutrient deficiency, introduction to sub-inhibitory concentration of antibiotics, etc. (Flemming and Wingender 2010). It is ubiquitous in nature and is the dweller of numerous living and non-living surfaces. It was firstly discussed in two seminal papers published in 1930s by Arthur Henrici and Claude Zobell, whereas, the term was coined by Bill Costerton in 1978.

In general, there are five developmental stages of biofilms: reversible attachment, irreversible attachment, proliferation, maturation, and dispersal (Kostakioti et al. 2013). At the very first stage of reversible attachment the planktonic bacteria starts attaching to the surface through weak Vander waal forces or their appendages like flagella. In the next stage the microbes convert the reversible connection to irreversible by overcoming the physical repulsive forces. It is accomplished by increasing the hydrophobic forces among bacteria and extracellular matrix due to secretion of extracellular polymeric substances (EPS) like polysaccharides, proteins, DNA, and many more. After attachment the microbes start proliferating within the biofilm to intensify the colonization process *via* cell division and cell recruitment. In the final stage of development, the biofilm undergoes specialization through certain physiological modifications like efflux pumps, oxygen gradients, division of labor, etc (Dang and Lovell 2016). At the end, the fully developed biofilm starts dispersing with the help of various enzymes that degrades matrix like dispersin B, deoxyribonucleases, etc.

The presence of *cell-to-cell* communication inside the biofilm emerged as an important parameter in shaping the biofilms (Toyofuku et al. 2015). The structure of the biofilms depends on the interaction among the species that can be either competitive or cooperative based on the type of microbial species within them (Li and Tian 2012). Moreover, the linking of the quorum sensing and biofilms was termed as "*sociomicrobiology*" (Parsek and Greenberg 2005; Rajput et al. 2015, 2016).

Various anti-biofilm agents are continuously being designed to impede the biofilm growth namely chemicals, phytochemicals, peptides, nanoparticles, biosurfactants, enzymes, etc as catalogued in ***aBiofilm*** resource by our group (Rajput et al. 2018). In this book chapter we will focus to decipher various important aspects of anti-biofilm peptides (ABPs), which emerged as a new hope to the researchers in the era of antibiotic drug resistance. The chapter will cover the detail of biofilms, its composition and consequences, along with the role of ABPs for targeting the biofilms, its source, chemical modifications, targets and antibiotic drug resistance.

5.2 Characteristics of Biofilms

Overall architecture of the biofilm comprised of self-secreted matrix of hydrated EPS, which is responsible to adhere on the surface and cohesion within them (Branda et al. 2005; Flemming and Wingender 2010). However, the composition of EPS varies according to the microbial species present in the biofilms. Although, polysaccharides are amongst the major constituents of biofilms followed by water, proteins (or enzymes), extracellular DNA (eDNA) that are hydrophilic molecules (Wingender et al. 2001; Conrad et al. 2003). Moreover, the few hydrophobic molecules like

lipids and biosurfactants were also reported, which help in adherence and initial microcolony formation among few species like *Thiobacillus ferrooxidans*, *Serratia marcescens*, *P. aeruginosa*, etc (Davey et al. 2003; Sand and Gehrke 2006).

The *polysaccharides* secreted by the microbes in the biofilms are homo and/or hetero in nature. They are composed of monosaccharide units, which can be O- or N- acylated. The EPS of the biofilm are generally species-specific e.g. poly-β-1,6-N-acetylglucosamine is secreted by *E. coli*, *S. aureus*, and *A. pleuropneumoniae*; the polymerization of α-D-galactose, β-D-galactose, and β-D-glucose monomers is the main constituent of *E. persicina* biofilms; the *E. coli* and *Salmonella* biofilms are mainly composed of cellulose; *P. aeruginosa* biofilms comprised of alginate (α-L- guluronic acid and α-L-mannuronic acid), etc (Batoni et al. 2016).

Water channels are amongst the main constituent of biofilm and responsible for nutrient transport. The water channels are considered as homologs to the circulating system of the multicellular organisms, therefore the biofilms are often considered as primitive multicellular organisms. They are able to transport the nutrients in and out from the depths of biofilms (Stewart 2003).

The *protein* portion of biofilms comprised of extracellular proteins, cell surface adhesins, subunits of appendages like flagella and pili, and outer vesicle protein covering. It helps in maintaining structure and stability of the biofilms. Moreover, few proteins with enzymatic properties are responsible for catalyzing matrix components like dispersin B hydrolyzes polysaccharides (Kaplan et al. 2003), DNases to disintegrates extracellular nucleic acids (Nijland et al. 2010), and proteases degrades matrix proteins (Fong and Yildiz 2015).

The *extracellular DNA* is considered as an important stabilizer and maintainer of biofilm architecture in bacteria and fungus. The extracellular DNA derives from the lysis of bacterial cell, and is a hot spot for the horizontal gene transfer within the polyspecies in biofilm. Moreover, they are also considered as one of the factor for transfering the antibiotic drug resistance genes. Intriguingly, the eDNA is also the nutrient source, and cation chelator (Montanaro et al. 2011).

Apart from the hydrophilic molecules, the *hydrophobic molecules* like lipids and biosurfactants are also the constituents of biofilms especially of *Rhodococcos* and *Mycobacterium* genus. The lipids are known to assist in cell adhesion, biofilm formation and development. For example, rhamnolipids helps in modulating the biofilm architecture by retaining the water channels accessible during maturation phase of biofilm (Branda et al. 2005).

The universality of the biofilms and antibiotic resistant behavior is the major concern for the researchers worldwide (Koul et al. 2016; Koul and Kalia 2017). According to the Centre for Disease Control and Prevention (CDC), biofilms are the major cause of nosocomial (hospital-acquired) infections (Davey and O'Toole 2000). However, they are also involving in human health threatening infections e.g. lungs, heart, gastrointestinal tract, oral cavity, urogenital tract, and many more (Li and Tian 2012). Therefore, there is an emergent need to target them through various anti-biofilm agents.

5.3 Anti-biofilm Peptides

Diverse biofilm targeting agents are being designed to impede the dreadful effect of bacteria in biofilm mode. These anti-biofilm agents are varied in nature and ranges from chemicals (Gui et al. 2014; Balamurugan et al. 2015), phytochemicals (Bhargava et al. 2015), peptides, phages (Ahiwale et al. 2017), antibody, biosurfactants, nanoparticles, etc (Kalia 2013; Agarwala et al. 2014; Kalia 2014). Among all the anti-biofilm agents, ABPs emerged as a novel and efficient quencher (Pletzer et al. 2016). Most of the ABPs are ribosomally synthesized and post translationally modified. Moreover, they are also possessing low toxicity as they can be broken down upon ingestion. Mostly, ABPs are short with 5–50 amino acids with cationic and amphipathic in nature (Batoni et al. 2016). The peptide structure of some important ABPs was predicted using PEPstrMOD software (Singh et al. 2015) is provided in Table 5.1.

Source of Anti-biofilm Peptides ABPs are anti-microbial peptides (AMPs) with biofilm targeting activity and are of natural, semi-synthetic and synthetic in nature. Naturally occurring peptides are secreted from humans, plants, animals, and microbes for example Magainin-II, Defensins, Histatins, LL-37, etc (Pletzer and Hancock 2016). While semi-synthetic or synthetic are derivative from natural ABPs or through peptide synthesis methods IDR-1018; C16G2; L-K6; F2,5,12W; R-FV-I16; etc (Batoni et al. 2016).

Chemical Modifications in Anti-biofilm Peptides The chemical modifications in the ABPs make them highly efficient towards the target and increase their half-life. The modifications include post-translational modifications (amidation, carboxylations, etc) (Zhou et al. 2016), physicochemical modifications (deletion and/or substitution of amino acids), sequence truncations (Nagant et al. 2012), designing of retro-inverso peptides (D-enantiomers) (de la Fuente-Nunez et al. 2015), cyclization, hybrids construction (Gopal et al. 2014), and many more (de la Fuente-Nunez et al. 2016).

5.4 Mode of Action of Anti-biofilm Peptides

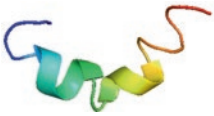
ABPs target the biofilms *via* different mechanisms both at molecular and physiological level. Several ways by which the ABPs target the biofilms are: degradation of signals within biofilms, permeabilize within cytoplasmic membrane/EPS, modulating EPS production, downregulating the biofilm associated genes, disrupt the adhesion of biofilm, killing of metabolically active cells, and interferes with the motility. The diagrammatic details of mode of action of the ABPs are shown on Fig. 5.1.

Table 5.1 Table showing tertiary structure of important anti-biofilm peptides

Anti-biofilm peptides	Peptide sequence	Structure
1018	VRLIVAVRIWRR-NH2	
Human α-defensin 1	DCYCRIPACIAGEKKYGT CIYQGKLWAFCC	
Amphotericin B	KKVVFWVKFK-NH2	
Competence Stimulating Peptide	SGSLSTFFRLFNRSFTQALGK	
DJK5	VQWRAIRVRVIR	

(continued)

Table 5.1 (continued)

Anti-biofilm peptides	Peptide sequence	Structure
G H12	GLLWHLLHHLLH-NH2	
Histatin 5	DSHAKRHHGYKRKFHEKHHSHRGY	
Lactoferricin B	FKCRRWQWRMK KLGAPSITCVRRAF	
Magainin II	GIGKFLHSAGKFGKAFVGEIMKS	
Pleurocidin	GWGSFFKKAAHVGKHVGKAALTHYL-NH2	
Tachyplesin I	KWCFRVCYRGICYRKCR-NH2	

Degradation of Signals Within Biofilms (QS) The ABPs are known to interfere with the binding and causes the degradation of QS signals and/or secondary messengers (ppGpp) that are important for biofilm formation and maintenance for example, DJK5, DJK6 (de la Fuente-Nunez et al. 2015).

Permeabilize Within Cytoplasmic Membrane The peptides are able to permeabilise and/or form pores inside the cytoplasmic membranes like LL-37, LL-31 (Kanthawong et al. 2012). Moreover, the ABPs are also responsible for the pore formation ability within the lipid component of EPS e.g. pleurocidin (Choi and Lee 2012).

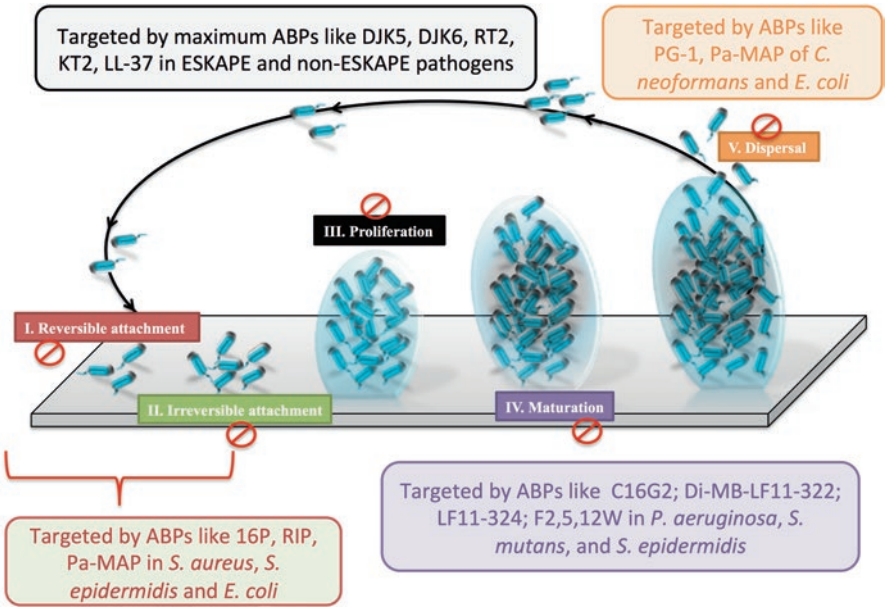


Fig. 5.1 Diagrammatic representation of the mode of action of Anti-biofilm peptides against biofilms

Modulating EPS Production The major components of biofilms are the EPS secreted *via* microbes, which provides a protective sheath and maximally are negatively charged due to presence of eDNA. The ABPs like CSPs used to regulate EPS production in *C. albicans* (Jack et al. 2015).

Downregulating the Biofilm Associated Genes Some of the essential biofilm associated genes were also being targeted with ABPs that are involved in cell adhesion, extracellular matrix hyphal growth, etc like ZAP1, CSH1, ALS3. Whereas, hLF1-11 was used against *C. albicans* biofilm to impede the growth by down regulating various genes (Morici et al. 2016).

Disrupt the Adhesion of Biofilm The initial and important stage of biofilms is adhesion. The ABPs are validated to reduce the adhesion of biofilms with the surface like catheters, stents, dentures, etc. For example, Magainin I, Histatin 5, etc (Pusateri et al. 2009).

Killing of Metabolically Active Cells Within Biofilms The cationic nature of ABPs is also known to target the metabolically active cells (most active and marginally active) located at the centre of the biofilms. Most of the drugs are ineffective against the metabolically active cells as compared to inactive ones due to the modifications in their Lipopolysachharides (LPS). The Cationic Antimicrobial peptides (CAMPs) like GL13K, GH12, hLF1- 11 (Hirt and Gorr 2013) are experimentally validated to target the same.

Interferes with Motility The motility is one of the important parameter for biofilm functionality. It is pre-adhesion stage, when the bacteria started coming to the adherent surface *via* twitching and/or swarming motility under the influence of any chemo-attractant. It is also being interfered through ABPs like LL-37, modified 1037 (Overhage et al. 2008; de la Fuente-Nunez et al. 2012) impede the motility of bacteria.

5.5 Target of Anti-biofilm Peptides

Various ABPs were tested against numerous important pathogens i.e. ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) and non-ESKAPE ones. We have extracted the details of various ABPs targeting important pathogens as described below:

5.5.1 Enterococcus faecium

It is a Gram-positive and commensal bacterium of human intestine, responsible to cause nosocomial bacteremia. It is a multidrug resistant ESKAPE pathogen causes biofilm–associated infection through medical devices. Though, various anti-biofilm peptides emerged as a hope to target it, despite being antibiotic drug resistant. For example, ABPs like Siamycin I, Magainin-I, Coprisin, Pleurocidin, etc are reported to inhibit the biofilm of *E. faecium* (Hwang et al. 2013; Winfred et al. 2014).

5.5.2 Staphylococcus aureus

It is a Gram-positive and facultative anaerobic bacterium. Some of the strains developed antibiotic resistance against commonly used antibiotics. They are amongst the most frequent dwellers of medical instruments, and are also called as opportunistic pathogens. According to Nosocomial Infections Surveillance System, *S. aureus* are

amongst the frequently occurring nosocomial pathogens isolated from intensive care unit (ICU). Various ABPs are designed to target its stubborn biofilm like Human β-defensin 3, HE12, Myxinidin, etc (de la Fuente-Nunez et al. 2014; Li et al. 2015).

5.5.3 Klebsiella pneumoniae

It is a Gram-negative ESKAPE pathogen, and often considered as "superbug" due to its efficiency to cause range of diseases. It is related to extreme drug resistance (Vuotto et al. 2017), as it is resistant to most of the antibiotics including carbapenems, and a typical example of nosocomial pathogen. Recently, some ABPs were designed to target *K. pneumoniae namely* Pa-MAP, Bac8C, etc (Ding et al. 2014; Cardoso et al. 2016).

5.5.4 Acinetobacter baumanii

It is a Gram-negative, opportunistic and nosocomial pathogen that possess the ability to survive even in unfavorable condition on the hospital instruments. The important factor that led the cocoon of *A. baumanii* so protective is the pili which are expressed from *csuA/BABCDE* operon followed by QS signals (Rajput and Kumar 2017a, b) i.e. Acyl homoserine lactone secreted by *abaI* gene. Now, a days the multidrug resistant (MDR) problem of *A. baumanii* is being targeted by the use of ABPs like LL-37, DJK5, IDR-1018 (Feng et al. 2013; de la Fuente-Nunez et al. 2015).

5.5.5 Pseudomonas aeruginosa

It is a well-known opportunistic pathogen and majorly responsible to cause lung infection in cystic fibrosis patients through biofilms. However, it is also known to cause infections in both plants and animals. Its MDR strains had been proved to be more dreadful and cause nosocomial infections like sepsis and ventilator-associated pneumoniae. The MDR problem of *P. aeruginosa* are treated with various ABPs like Cathelicidin, Indolicidin, Melittin, RIP, etc (Overhage et al. 2008; Gopal et al. 2013).

5.5.6 Escherichia coli

It is a Gram-negative and non-ESKAPE bacteria that is also responsible for nosocomial infection e.g. prostatitis, and urinary tract infections. It forms a thick biofilm with matrix enriched in polysaccharides, and varies according to environmental conditions. The presence of large number of polysaccharides is the main factor for

its MDR behavior. However, the ABPs like AGE-RK1, KT2, RT2, Lactoferricin B (Anunthawan et al. 2015) are also being used to target its biofilms.

5.5.7 Candida albicans

It is a fungus that normally found inside the human body, but cause serious illness in immunocompromised patients. It is popularly known to form biofilm on medical instruments like pacemakers, catheters, prosthetic joints, dentures and thus responsible for nosocomial infections. The *C. albicans* biofilms are resistant to conventional anti-fungal drugs. It is responsible to form highly structured biofilms with multiple cells e.g. budding, round, oval pseudohyphal, yeast-form, cylindrical, elongated hyphal cells. Along with the anti-fungal drugs various ABPs like BMAP-28, OSIP108, Amphotericin B are being tested against various srains of *C. albicans* (Theberge et al. 2013; Delattin et al. 2014; Scarsini et al. 2015).

Some other important non-ESKAPE pathogens that are experimentally validated to be targeted with ABPs are *P. putida* targets Putisolvin I and II (Dubern et al. 2006); *S. epidermidis* biofilm is known to be targetted with R- Thanatin, Dalbavancin, Hepcidin 20 (Brancatisano et al. 2014; Knafl et al. 2017) etc. Moreover, the examples of major microbes targeted with ABPs, extracted from the literature are enlisted in Table 5.2.

5.6 Role of Anti-biofilm Peptides in Antibiotic Drug Resistance

Antibiotic drug resistance is a colossal problem due to the microbes residing within biofilms. As compared to the conventional drugs the ABPs are the efficient tool to target biofilm due to their pervasive mode of action (Maisetta et al. 2006) namely killing of multispecies bacteria within biofilm; showing synergistic effect with antibiotics; penetration ability within biofilms, etc. The *S. aureus* (MRSA) can be inhibited with LL-37 peptides (Haisma et al. 2014). The 1018 peptide acts synergistically with antibiotics like ceftazidime, tobramycin, and ciprofloxacin to target the biofilms of ESKAPE pathogens (Reffuveille et al. 2014). The human β-defensins are efficient against various Gram-positive and Gram-negative bacteria involved in nosocomial infections (Maisetta et al. 2006). The DLK-5 and 6 are known to eradicate *P. aeruginosa* infections (Kanthawong et al. 2010). RNAIII-inhibiting peptides (RIP) abolish the *S. aureus* biofilms by interfering their adhesion (Kiran et al. 2008). Despite the presence of more than 600 AMPs at various stages of clinical trials (Preclinical, Phase 1, 2, 3), the status of ABPs in clinical trials is lagging. Notwithstanding, the prevalent mode of action and efficiency to tackle antibiotics drug resistance, the research in the field of ABPs need to be enhanced.

Table 5.2 List of the anti-biofilm peptides used against important pathogens along with the information of peptide sequence, concentration, % inhibition, mode of action, stage of biofilm targeted, and references

Anti-biofilm Peptides	Peptide sequence	Organism	Concentration	Biofilm inhibition activity (%)	Quantification assay	QQA mode of action against biofilm-target	Stage of biofilm targeted	References
16P	YKPVTNF-ST-YKPVTNF-CONH2	*Staphylococcus aureus* ATCC29213	50 μg/mL	58	Crystal violet staining assay	Not Specified	Adhesion and Formation	Zhou et al. (2016)
2C-4	RWWRWF	*Streptococcus mutans* UA159	25 μg/ml	12	OD at 600 nm	Killing of bacterial cells	Formation	He et al. (2010)
6-MO-LF11-322	PFWRIRIRR	*Pseudomonas aeruginosa* PAO1	64 μg/ml	38	MTT assay	Dysregulation of genes related to biofilm formation	Maturation	Sanchez-Gomez et al. (2015)
C16-33	TRRRLFNRSFTQALGKSGGGFKK FWKWFRRF	*Streptococcus mutans* UA159	2.5 ± 2.1 μM	53	Not Specified	Targeted killing of S. mutans	Formation	Eckert et al. (2006)
C16-33	TRRRLFNRSFTQALGKSGGGFK KFWKWFRRF	*Streptococcus sanguinis* NY101	13.3 ± 5.8 μM	43	Not Specified	Targeted killing of S. mutans	Formation	Eckert et al. (2006)
C16G2	TFFRLFNRSFTQALGKGGGKNL RIIRKGIHIIKKY	*Streptococcus mutans* UA140	100 μM	95	Not Specified	Not specified	Maturation	Sullivan et al. (2011)
Coprisin	VTCDVLSFEAKGIAVNHSACALHC IALRKKGGSCQNGVCVCRN-NH2	*Pseudomonas aeruginosa* ATCC 27853	16 μg/mL	92	Crystal violet staining assay	Not Specified	Formation	Hwang et al. (2013)

(continued)

Table 5.2 (continued)

Anti-biofilm Peptides	Peptide sequence	Organism	Concentration	Biofilm inhibition activity (%)	Quantification assay	QQA mode of action against biofilm-target	Stage of biofilm targeted	References
Coprisin	VTCDVLSFEAKGIAVNHSACALH CIALRKKGGSCQNGVCVCRN-NH2	*Escherichia coli* O-157 ATCC 43895	8 μg/mL	85	Crystal violet staining assay	Not Specified	Formation	Hwang et al. (2013)
Coprisin	VTCDVLSFEAKGIAVNHSACALH CIALRKKGGSCQNGVCVCRN-NH2	*Streptococcus mutans* KCTC 3065	8 μg/mL	74	Crystal violet staining assay	Not Specified	Formation	Hwang et al. (2013)
Coprisin	VTCDVLSFEAKGIAVNHSACALH CIALRKKGGSCQNGVCVCRN-NH2	*Staphylococcus aureus* ATCC 25923	16 μg/mL	45	Crystal violet staining assay	Not Specified	Formation	Hwang et al. (2013)
Coprisin	VTCDVLSFEAKGIAVNHSACALH CIALRKKGGSCQNGVCVCRN-NH2	*Enterococcus faecium* ATCC 19434	8 μg/mL	43	Crystal violet staining assay	Not Specified	Formation	Hwang et al. (2013)
Di-MB-LF11-322	PFWRIRIRR	*Pseudomonas aeruginosa* PAO1	32 μg/ml	80	MTT assay	Dysregulation of genes related to biofilm formation	Maturation	Sanchez-Gomez et al. (2015)
DJK5	VQWRAIRVRVIR	*Pseudomonas aeruginosa* PA14	1 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)

DJK5	VQWRAIRVRVIR	*Escherichia coli* O157	0.8 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)
DJK5	VQWRAIRVRVIR	*Klebsiella pneumoniae* ATTC 13883	1.6 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)
DJK5	VQWRAIRVRVIR	*Salmonella enterica* Serovar Typhimurium isolate 14028S	0.8 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)

(continued)

Table 5.2 (continued)

Anti-biofilm Peptides	Peptide sequence	Organism	Concentration	Biofilm inhibition activity (%)	Quantification assay	QQA mode of action against biofilm-target	Stage of biofilm targeted	References
DJK6	VQWRRIRVWVIR	*Pseudomonas aeruginosa* PA14	0.5 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)
DJK6	VQWRRIRVWVIR	*Escherichia coli* O157	8 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)
DJK6	VQWRRIRVWVIR	*Klebsiella pneumoniae* ATTC 13883	2 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)

DJK6	VQWRRIRVWVIR	*Salmonella enterica* Serovar Typhimurium isolate 14028S	1 μg/ml	50	Crystal violet staining assay	Binds to and promote degradation of the signal for biofilm formation and maintenance i.e. (p)ppGpp	Formation	de la Fuente-Nunez et al. (2015)
F2,5,12W	RWGRWLRKIRRWRPK	*Staphylococcus epidermidis* BM185	40 μM	83	Crystal violet staining assay	Eradication of formed biofilm by dettachment from surface	Maturation	Molhoek et al. (2011)
IDR-1018	VRLIVAV- RIWRR-NH2	*Pseudomonas aeruginosa* PA01	10 μg/ml	100	Crystal violet staining assay, SYT0 9-staining/ Propidium iodide-staining	Inhibition and dispersal of biofilm formation	Formation	de la Fuente-Nunez et al. (2014b)
IDR-1018	VRLIVAV- RIWRR-NH2	*Escherichia coli* O157	10 μg/ml	100	Crystal violet staining assay, SYT0 9-staining/ Propidium iodide-staining	Inhibition and dispersal of biofilm formation	Formation	de la Fuente-Nunez et al. (2014b)

(continued)

Table 5.2 (continued)

Anti-biofilm Peptides	Peptide sequence	Organism	Concentration	Biofilm inhibition activity (%)	Quantification assay	QQA mode of action against biofilm-target	Stage of biofilm targeted	References
IDR-1018	VRLIVAV- RIWRR-NH2	*Acinetobacter baumannii* SENTRY C8	10 μg/ml	100	Crystal violet staining assay, SYT0 9-staining/ Propidium iodide-staining	Inhibition and dispersal of biofilm formation	Formation	de la Fuente-Nunez et al. (2014b)
IDR-1018	VRLIVAV- RIWRR-NH2	*Klebsiella pneumoniae* ATTC13883	2 μg/ml	100	Crystal violet staining assay, SYT0 9-staining/ Propidium iodide-staining	Inhibition and dispersal of biofilm formation	Formation	de la Fuente-Nunez et al. (2014b)
IDR-1018	VRLIVAV- RIWRR-NH2	*Salmonella enterica* Serovar Typhimurium 14028S	10 μg/ml	100	Crystal violet staining assay, SYT0 9-staining/ Propidium iodide-staining	Inhibition and dispersal of biofilm formation	Formation	de la Fuente-Nunez et al. (2014b)
IDR-1018	VRLIVAV- RIWRR-NH2	*Staphylococcus aureus* MRSA #SAP0017	2.5 μg/ml	100	Crystal violet staining assay, SYT0 9-staining/ Propidium iodide-staining	Inhibition and dispersal of biofilm formation	Formation	de la Fuente-Nunez et al. (2014b)

IDR-1018	VRLIVAV- RIWRR-NH_2	*Burkholderia cenocepacia* IIIa 4813	10 µg/ml	100	Crystal violet staining assay, SYT0 9-staining/ Propidium iodide-staining	Inhibition and dispersal of biofilm formation	Formation	de la Fuente-Nunez et al. (2014b)
LF11-324	PFFWRIRIRR	*Pseudomonas aeruginosa* PAO1	8 µg/ml	50	MTT assay	Dysregulation of genes related to biofilm formation	Maturation	Sanchez-Gomez et al. (2015)
LL7-37	RKSKEKIGKEFKRIVQRIKDFLRNL VPRTES	*Pseudomonas aeruginosa* PAO1	10 µM	60	Crystal violet staining assay	Inhibition of biofilm formation	Formation	Nagant et al. (2012)
M8-33	TFFRLFNRSGGGFKKFWKWFRRF	*Streptococcus mutans* UA159	2.5 ± 2.0 µM	64	Not Specified	Targeted killing of S. mutans	Formation	Eckert et al. (2006)
M8-33	TFFRLFNRSGGGFKKFWKWFRRF	*Streptococcus sanguinis* NY101	20 ± 2.0 µM	34	Not Specified	Targeted killing of S. mutans	Formation	Eckert et al. (2006)
M8G2	TFFRLFNRGGGKNLRIIRKGIHIIKKY	*Streptococcus mutans* UA159	25 µM	96	Not Specified	Targeted killing of S. mutans	Formation	Eckert et al. (2006)
M8G2	TFFRLFNRGGGKNLRIIRKGIHIIKKY	*Streptococcus sanguinis* NY101	25 µM	21	Not Specified	Targeted killing of S. mutans	Formation	Eckert et al. (2006)

(continued)

Table 5.2 (continued)

Anti-biofilm Peptides	Peptide sequence	Organism	Concentration	Biofilm inhibition activity (%)	Quantification assay	QQA mode of action against biofilm-target	Stage of biofilm targeted	References
Myxinidin3	RIRWILRYWRWS	*Pseudomonas aeruginosa* ATCC 27853	4 μM	92	Crystal violet staining assay	Through membrane disruption and/or cell penetration.	Formation	Han et al. (2016)
Myxinidin3	RIRWILRYWRWS	*Staphylococcus aureus* ATCC 25923	8 μM	84	Crystal violet staining assay	Through membrane disruption and/or cell penetration.	Formation	Han et al. (2016)
Myxinidin3	RIRWILRYWRWS	*Listeria monocytogenes* KCTC 3710	16 μM	84	Crystal violet staining assay	Through membrane disruption and/or cell penetration.	Formation	Han et al. (2016)
PG-1	RGGRLCYCRRRFCVCVGR	*Cryptococcus neoformans* B3501	8 μM	41	XTT assay	Not specified	Pre-formed	Martinez and Casadevall (2006)
RK-31	RKSKEKIGKEFKRIVQRIK DFLRNLVPRTES	*Burkholderia pseudomallei* 1026b	100 μM	93	Not Specified	Permeabilize/ form pores within cytoplasmic membrane	Formation	Kanthawong et al. (2012)

RNAIII-inhibiting Peptide	YKPVTNF-CONH2	*Staphylococcus epidermidis* XJ75284 (MRSE)	50 µg/mL	60	Crystal violet staining assay	Not Specified	Adhesion and Formation	Zhou et al. (2016)
RNAIII-inhibiting Peptide	YKPVTNF-CONH2	*Staphylococcus aureus* ATCC29213	50 µg/mL	45	Crystal violet staining assay	Not Specified	Adhesion and Formation	Zhou et al. (2016)
RNAIII-inhibiting Peptide	YKPLTNF-CONH2	*Staphylococcus epidermidis* XJ75284 (MRSE)	50 µg/mL	40	Crystal violet staining assay	Not Specified	Adhesion and Formation	Zhou et al. (2016)
SMAP-29	RGLRRLGRKIAHGVKK YGPTVLRIIRIAG	*Burkholderia thailandensis* E264	30 µg/ml	70	Crystal violet staining assay	Not specified	Formation	Blower et al. (2015)

5.7 Conclusion

This book chapter is focused on the new class of therapeutics named ABPs. The ABPs are recently emerged as important and efficient anti-biofilm agents to target MDR biofilms. They are introduced alone and/or in combination with antibiotics. They are proved to be more efficient in inhibiting biofilms involved in nosocomial infections (Pletzer and Hancock 2016). Their importance is gradually increasing due to their broad ranged specificity towards stages and components of biofilms. Moreover, they are proved as a new ray of hope to the world struggling with the problem of antibiotic drug resistance.

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