

Open Access

Review Article

Using Phages to Exterminate Biofilms

Manal Mohammed Alkhulaifi*

Department of Botany and Microbiology, College of Science, King Saud University, Saudi Arabia

Abstract

Biofilms are thought to be always a major concern within the healthcare field and food industries. The resistance properties of biofilm mediated bacteria confer persistent that is being somewhat challenging to address. Biofilms can be more resistant to antibiotics than individual planktonic cells. For this reason, the use of novel alternative strategies to management biofilm formation is needed. Currently, phages as an anti-biofilm agent are suggested as possible replacements to antibiotics. In this review, some of diverse strategies to the prevention of biofilm formation have been exhibited by a number of studies. Phages use as anti-biofilm agents can involve either phage application prior to biofilm formation, application to biofilms that are already formed, or using phage impact that is found in association with other additional mechanisms that can physically disrupt the biofilm. The development novel methods as an anti-biofilm agent would hopefully add an important dimension to the search for new potent compounds to solve biofilm-associated infections problems.

Keywords: Phages; Biofilm; Bacteria; Antibiotics; Resistance

Introduction

In general, biofilm is an organized multicellular of bacteria, which can be formed either from one or a number of different species and these species live together inside a matrix made of extracellular polymeric substances (EPS) with the capability of attachment to numerous surfaces [1]. EPS mainly include polysaccharides, but other biomolecules are also present among which are nucleic acids, lipids, proteins and nucleic acids, which form a scaffold that help the bacteria to stay attached within the biofilm [2,3]. This matrix displays a modified phenotype and regulation of specific drug resistance genes and virulence factors can be observed in bacterial biofilms. Horizontal genetic transfer may occur easily, and therefore facilitating cross- breeding of resistance genes [4,5]. Biofilm is formed in five different stages, Figure 1 shows those five stages [6].

The complexed composition of the matrices adds an original property to the biofilm which can be the survival ability under extreme conditions, furthermore in addition it enhances the inflow of nutrients, water and signaling molecules which are important accountable for cells communication [7,8]. Furthermore, EPS matrix supplies a barrier between the external environment and the bacteria that prevent antimicrobials from penetration in to the biofilm [9]. Biofilms of Salmonella are more resistant to the triclosan antibiotic than Salmonella's individual planktonic cells [10]. Furthermore, the negative charges of the EPS can prevent the antibiotics to achieving the biofilm [11,12].

Biofilms basically play a fundamental role in infectious diseases. Taking a look at previous literature, it had been proven that 60% to 70% of most nosocomial infections are directly linked to the clear presence of biofilms [13]. The most bacteria that is repeatedly associated with medical devices come in particular *S. epidermidis* and *S. aureus*, followed by *P. aeruginosa* and a boost of other bacteria that opportunistically infect weakened patients [14-16]. Moreover, they can exist as at first glance of medical implants including catheters [17,18].

Bacteria within biofilms demonstrate both antibiotic and the host defences resistance [18], additionally they show a decline in the rate of growth, limitation in diffusion and a growth in efflux and enzymes accountable for antimicrobials degradation [19,20]. Generally, the usage of antibiotics to cope with biofilm-related infections doesn't result in successful cures [11]. Many studies confirmed that for biofilm, the Minimum Inhibitory Concentration (MIC) and the Minimum

Bactericidal Concentration (MBC) were generally higher compared to the planktonic bacterial cells (about from 10-1000 times) [21-23].

As numerous antimicrobials function on actively growing cells which means the antimicrobial function maybe decreased by Clutterbuck in 2007. Once bacteria are embedded within a biofilm then all these factors with the altered gene expression and quorum sensing altogether result in the increased resistance against antibiotics [24]. The treating biofilm is difficult and challenging which explains why scientific attention was drawn towards it [25]. Because of this, it is extremely important to find and develop new antimicrobial agents or some other efficient way to a target and destroy biofilm responsible for infections [26,27].

Literature Review

Studies involving biofilm-phage interaction

Bacteriophages or (phages) in general are viruses that infect bacteria (Figure 2). These viruses were created for targeting the within biofilms [28]. They are able to either reside in the bacterial host genome whilst the lysogenic phages do or they can destroy them similar to the lytic phages; which are one of the most suited type for therapeutic model usage. Currently phages are suggested as you are able to alternatives to antibiotics against bacterial infections and are widely explored to minimize the pathogen loads in food products. However, phages may be safer than antibiotics. It is quite simple, simple and fast to isolate them. Their production is inexpensive. Phages are competent against one specific host or host range making them ineffective unlike the natural microflora that exists initially attacked by the biofilm. Phages are green and, until today no serious uncomfortable side effects have been reported [29].

*Corresponding authors: Manal Mohammed Alkhulaifi, Department of Botany and Microbiology, College of Science, King Saud University, Box Office: 55670, 11544 Riyadh, Saudi Arabia, Tel: 9668051685; Fax: +966 8055315; E-mail: manalk@ksu.edu.sa

Received July 27, 2017; Accepted August 16, 2017; Published August 21, 2017

Citation: Alkhulaifi MM (2017) Using Phage's to Exterminate Biofilms. J Med Microb Diagn 6: 259. doi:10.4172/2161-0703.1000259

Copyright: © 2017 Alkhulaifi MM. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



Figure 1: Biofilm is formed in five stages, these stages are 1) initial, reversible attachment, 2) Irreversible binding and growth, 3) EPS production and inter communication through quorum sensing, 4) Mature biofilm, and 5) dispersal; essential stage for biofilm dispersion and life cycle (adapted and modified from Mizan et al. in 2015).



(Adopted and modified from http://www.nwes-medical.net/news/20151202/ Bacteriophage-therapy-an-alternative-to-antibiotics-An-interview-Professor-Clokie.aspx). The promising phage Strategies against biofilm such as for instance.

Figure 2: Bacteriophage targeting bacteria within biofilm.

Biofilms functions as targets of phage predation has become addressed by numerous studies [30,31]. There exists a lengthy history of phage used in roles that are comparable to those of antibiotics, in addition to ongoing use of phage therapy against bacterial infections [32-34]. Biofilms enucleation through phages can involve either phage application ahead of biofilm formation, application to biofilms that are already formed, or using phage impact that's present in association with other additional mechanisms that may physically disrupt the biofilm.

Inhibition of Attachment by Phages

Phages are capable to effect on initial adsorption stage of biofilms (or adhered cells). When employing phages that are lytic, as generally could be the case with anti-biofilm phages, then phage infection results in the killing and lysis of bacteria. This likely both impacts biofilms structurally and releases new phage virions that potentially can reach and then infect adjacent bacteria [35]. The effect is really a cyclical acquisition and then killing of biofilm bacteria [34,36]; reported that single cells followed glass surfaces during 60 minutes, were efficiently inhibited with phage φ S1. The cell removal was fast and efficient that result in a biomass reduction of about 90%.

Inhibition of EPS Matrix by Depolymerizing Enzymes

It's been reported that some phages are well effective at penetrating through the EPS matrix by diffusion or because of the presence of phage associated enzymes. It is an undeniable fact a large range of enzymes are able to destroy the biofilm's EPS matrix. In the case of phages, these enzymes include some which are mainly produced to help in releasing the phages from the host cell and also tailspike proteins that really help in infecting the bacteria within the biofilm, but in general the activity of those enzymes and proteins are strictly localized. However, studies revealed that proteins with activity limited to the virus particle might be released from the lysing cells, and effect the biofilm matrix [37].

Phages can also capable of making depolymerizing enzymes that can degrade the EPS from the genome of the host. Many phage's genomes also include genes that specialize in producing enzymes effective and functional in breaking down the matrix [28,38,39]. In many conditions, these enzymes aim for the wall of the bacterial cell through the release process from the host cell, but similarly, these enzymes are able to degrade the biofilm EPS. Including the T4 and HK620 phages of E. coli have enzymes that exist on the viral tail, and may have a role in degrading the matrix [38,39], and yes it was noted that-polysaccharide depolymerase is really an important part of the phage tail and also thatmany tail spike proteins have endoglycosidase activity, by breaking down their polysaccharide receptors through hydrolyzation [39]. It has been reported that a phage-induced method of earning the matrix of the biofilm more porous, and therefore helping in the infection process by progeny phage, or a quick infected bacteria reaction can seek to encourage moving away from the focus of infection. Although the presence of polysaccharide depolymerase in phages has been reported. The problem in isolating phages possessing EPS degrading enzymes has led to the re- construction of phages, including the T7 [40].

One important point would be to realize that different species of bacteria produce different EPS components. And that is way a depolymerase active contrary to the polysaccharides created by one species of bacteria might not digest that created by other bacteria. However, depolymerases will likely have broader activity than their parent phages among closely related bacteria, since the complexity and the variability in the EPS is below that of the host bacteria [41]; observed this by comparing the experience of a phage of S. aureus with this specific of the depolymerase so that it produced. However, neither would affect any bacteria other than Staphylococci, suggesting that multiple depolymerases is likely to be required for targeting mixed biofilms. In which a dynamic depolymerase is liberated, special-haloes may be observed over the phage plaques formed on bacterial cultures, showing the areas where bacterial polysaccharide has been destroyed by Gutiérrez et al. [42]; used this approach to detect such activity in two phages infecting S. epidermidis, both which were then confirmed by sequencing to contain genes for pectin lyases, while Glonti et al. [43]; identified haloes in cultures of a phage infecting P. aeruginosa and purified a depolymerase protein from the phage. Yan has classified Phages polysaccharide depolymerases [39] as endorhamnosidases, alginate lyases, endosialidases and hyaluronidases.

Pretreatment of catheter using phages

Another important challenge studied in medical care to reduce biofilm formation by *S. epidermidis* is pre-treating the surfaces of catheter with phages [44]. The utilization of phages for the treatment of device-related infections has been the focus of attention since the 20th century. It has been discovered that pretreatment of hydrogel-coated catheters by phage caused the inhibition of *S. epidermidis* and *P. aeruginosa* biofilms [44,45].

Quorum Sensing Inhibition (QSI) by phages

One strategy that can be used against biofilm could be the inhibition of Quorum Sensing (QS), that is the cell-to-cell signaling

system, this method is in charge of controlling the expression of genes which can be necessary for adding virulence factor, that is responsible for interactions with the host bacteria and also for the regulating the development of the biofilm [46-52]. The key intent behind this strategy is not to kill pathogens but to disarm them making them oversensitive to the normal antimicrobial treatments. Furthermore, the QS system is not contributing in any way in mechanisms which can be essential for the bacteria survival, but inhibiting this method won't be described as a reason behind producing a firm selective pressure suitable enough to cause resistance development [53,54]; showed that the engineered phage strain T7 that creates the metalloenzymes AiiA lactonase range of action against signalling molecules (acyl homoserine lactones) which are mixed up in bacterial quorum sensing is extremely wide that is and these molecules are important for the development of the biofilm.

Phage Growth within Biofilms

Data collected from experiments indicated that phages do grow well in *P. aeruginosa* biofilms [55], at least in the primary stages of their development. Two-days-old biofilms, [56] Olson et al. reported that out of 17 insensitive strains of *P. aeruginosa* phages (therefore, planktonic bacterial hosts were used), 8 strains encouraged the same phages growth in the biofilm. Although they are capable of blocking antibiotics effect within their beginning stages of formation. This finding will follow that of Gupta et al. [57], who also stated that the antibiotic resistance begins to appear in the first stages of biofilm formation. Thus, bacteria can be destroyed by phages in cases where antibiotics did have no effect on them.

Previous studies which helped in explaining the power of to regulate biofilms Hanlon et al. [58], found that phages effecting P. aeruginosa can terminate bacteria in an adult biofilm and (looking at their sizes) might be diffused through the thickest alginate gel studied. But this activity clearly varied from that of the highly-restricted tailspike proteins. In the research of Sillankorva et al. [28] phages of both P. fluorescens and S. lentus were used and the effect on the reduction of both single species and mixed biofilms with these agents was explained. The phages of both of the two hosts were completely sequenced, and clearly it had been explained that neither of these coded for a polysaccharide depolymerase (though the P. fluorescens phage showed which they did encode an endopeptidase). Similarly, Doolittle et al. [59], reported that the T4 which is E. coli's phage doesn't code for polysaccharide depolymerased except for a restricted tailspike protein, which can only break out from the tail of the phage during the host cell penetration but nevertheless, can spread effectively through a biofilm.



Figure 3: Phage mediated prevention biofilm strategies used within the last few 20 years.

It is proven by some studies that phages are able to penetrate biofilms even if they are not able to produce polysaccharide depolymerases, but within biofilm, effective infection haven't been shown in most studies, also some researchers still believe that the existence of EPS-degrading enzymes are extremely important for applications of biofilm [37]. A study carried out by Tait et al. [60]; revealed that using a variety of three phages can entirely destroy a biofilm that's created from single species, nevertheless in the presence of other bacterial species which were insensitive, this technique didn't have much effect. A study by Kay et al. [61] also demonstrated that the phages efficiency can be worn off in the clear presence of mixed biofilms. In spite of this, it was reported by Sillankorva et al, the efficiency could be high in model biofilms even in cases like if an individual bacterial species in the biofilm is targeted by the phage, explaining that phages have the ability of killing a specific type of bacterial host even when it dwells in a mixed organization. In addition, they reported that phages can target an adult biofilm effectively [28].

Combining Phage with Other Agents

Using phages as mixtures or coupled with antibiotics can completely prevent the development of phage resistance [62,63]; recorded that mature biofilms can become more adaptable to antibiotics if lytic phages are used, which fits and will abide by findings that were currently reported from some clinical trials concerning phage activity [64,65]. According to this, using phages and antibiotics in a combined or sequential manner has been seen to have the potential for therapeutic applications. To supporting this Yilmaz et al. [66]; indicates that whenever phages coupled with antibiotics were utilized on biofilms of S. aureus these were clearly effected. Other study suggested that using a polysaccharide lyase and of DNase enzymes for destroying the matrix, ought to be placed into action alongside with phages. Abedon et al. and Sharp et al. [33,55] also discussed this, although differential diffusion of phages and co-administered enzymes is regarded as being an issue. The use of phage can also be joined with physical wounds cleaning [67]; used a rabbit ear model to find that removing damaged tissue or foreign objects from the wound and using phage treatment each of them separately didn't have any effect in this technique, nevertheless when combining both the result was visible. But, phages could have similar function on biocides and sanitizers used today, but should be applied after the primary cleaning processes, to destroy particular bacterium on the remaining biofilms. Likewise, Ganegama et al. [68]; revealed that using a variety of three different phages could clear Listeria monocytogenes biofilms effectively from steel surfaces. Thus, it should be put in consideration that for treating biofilms temporary by phages, it would be required that the biofilm cells surface be exposed to some disruption prior to phage application. Other combinations are also possible exactly like in the case of biological systems Liao et al. [69]; noticed that combining phages with commensal bacteria had synergistic effects in preventing biofilm formation on silicone catheter segments while Zhang and Hu [70]; observed when using phages coupled with biocide like (chlorine) the effects on filters is increased. However, further studies have to target on exploring phage activity in the multispecies context, animal models, and in conjunction with other antimicrobials [71]. Figure 3, shows the strategies that were used to destroy biofilms within the last few 20 years.

Conclusion

Studies which involved interaction between phage and biofilm indicated that phages contain some unique properties and seems promising in biofilms control Different phages have already been used to infect a number of bacterial biofilms. The treatment of biofilms using phages is a complicated process and only strictly lytic phages ought to be used. Like in phage infection of planktonic cells, there are numerous essential steps that require to occur. Phage adsorption to the receptors on the targeted bacteria is the leading part of infection. It is also evident that phages express enzymes which have the ability to of disrupt biofilms. To be able to allow it to be hard to spot, these types of enzymes are induced from the host genome. However, these kinds of applications remain progressing. Thus, at this time to spot the utmost effective strategies of destroying biofilm, they should be speculative in nature. By the time other results are available, new and better strategies will come to light.

References

- Hurlow J, Couch K, Laforet K, Bolton L, Metcalf D, et al. (2015) Clinical biofilms: A challenging frontier in wound care. Adv Wound Care 4: 295-301.
- Cortes ME, Consuegra J, Sinisterra RD (2011) Biofilm formation, control and novel strategies for eradication. Sci Against Microbial Pathog Commun Curr Res Technol Adv 2: 896-905.
- Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8: 623-633.
- Anderson GG, O'Toole GA (2008) Innate and induced resistance mechanisms of bacterial biofilms. Bacterial Biofilms 85-105.
- Donlan RM, Costerton W (2002) Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microb Rev 15: 167-193.
- Stowe SD, Richards JJ, Tucker AT, Thompson R, Melander C, et al. (2011) Anti-biofilm compounds derived from marine sponges. Mar Drugs 9: 2010-2035.
- 7. Watnik P, Kolter R (2000) Biofilm, city of microbes. J Bacteriol 182: 2675-2679.
- 8. Tarver T (2009) Biofilms- A threat to food safety. J Food Technol 63: 47.
- Mah TF, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9: 34-39.
- Tabak M, Scher K, Hartog E, Romling U, Matthews KR, et al. (2007) Effect of triclosan on Salmonella typhimurium at different growth stages and in biofilms. FEMS Microbiol Lett 267: 200-206.
- Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C (2003) The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest 112: 1466-1477.
- 12. Qu Y, Daley AJ, Istivan TS, Garland SM, Deighton MA (2010) Antibiotic susceptibility of coagulase-negative staphylococci isolated from very low birth weight babies: Comprehensive comparisons of bacteria at different stages of biofilm formation. Ann Clin Microbiol Antimicrob 9: 16.
- Percival SL, Hill KE, Williams DW, Hooper SJ, Thomas DW, et al. (2012) A review of the scientific evidence for biofilms in wounds. Wound Repair Regen 20: 647-657.
- Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: A common cause of persistent infections. Science 284:1318-1322.
- Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: From the natural environment to infectious diseases. Nat Rev Microbiol 2: 95-108.
- Romling U, Balsalobre C (2012) Biofilm infections, their resilience to therapy and innovative treatment strategies. J Intern Med 272: 541-561.
- Maric S, Vranes J (2007) Characteristics and significance of microbial biofilm formation. PeriodicumBiologorum 109: 1-7.
- Vasudevan R (2014) Biofilms: Microbial cities of scientific significance. J Microbiol Exp 1.
- Hall-Stoodley L, Stoodley P (2009) Evolving concepts in biofilm infections. Cell Microbiol 11:1034-1043.
- Kumar A, Schweizer HP (2005) Bacterial resistance to antibiotics: Active efflux and reduced uptake. Adv Drug Deliver Rev 57: 1486-1513.
- Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35: 322-332.
- J Med Microb Diagn, an open access journal ISSN: 2161-0703

- Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N (2011) Pharmacokinetics/ pharmacodynamics of colistin and imipenem on mucoid and nonmucoid *P. aeruginosa* biofilms. Antimicrob Agents Chemother 55: 4469-4474.
- Hengzhuang W, Wu H, Ciofu O, Song Z, Høiby N (2012) *In vivo* pharmacokinetics/ pharmacodynamics of colistin and imipenem in *P. aeruginosa* biofilm infection. Antimicrob Agents Chemother 56: 2683-2690.
- Clutterbuck AL, Woods EJ, Knottenbelt DC, Clegg PD, Cochrane CA, et al. (2007) Biofilms and their relevance to veterinary medicine. Vet Microbiol 121:1-17.
- 25. Wu K, Fang Z, Guo R, Pan B, Shi W, et al. (2015) Pectin enhances biocontrol efficacy by inducing colonization and secretion of secondary metabolites by *Bacillus amyloliquefaciens* SQY 162 in the rhizosphere of tobacco. PLoS ONE 10: e0127418.
- Yarwood JM, Paquette KM, Tikh IB, Volper EM, Greenberg EP (2007) Generation of virulence factor variants in *S. aureus* biofilms. J Bacterol 189: 7961-7967.
- 27. Issam I, Xiang F, Xavier M, Ying J, Robert S, et al. (2008) The role of chelators in preventing biofilm formation and catheter-related bloodstream infections. Curr Opin Infect Dis 21: 385-392.
- Sillankorva S, Oliveira R, Vieira MJ, Sutherland IW, Azeredo J (2004) Bacteriophage phiS1 infection of *Pseudomonas fluorescens* planktonic cells versus biofilms. Biofouling 20: 133-138.
- Olszowska-Zaremba N, Borysowski J, Dabrowska J, Górski (2012) A. Phage translocation, safety, and immunomodulation. In Bacteriophages in Health and Disease; Hyman P, Abedon ST (eds); CABI Press: Wallingford, UK. pp. 168-184.
- Brussow H (2013) Bacteriophage-host interaction: From splendid isolation into a messy reality. Curr Opin Microbiol 16: 500-506.
- Harper DR, Parracho HM, Walker J, Sharp R, Hughes G, et al. (2014) Bacteriophages biofilms. Antibiotics 3: 270-284.
- Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, et al. (2010) Phage therapy in clinical practice: Treatment of human infections. Curr Pharm Biotechnol 11: 69-86.
- Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM (2011) Phage treatment of human infections. Bacteriophage 1: 66-85.
- Abedon ST (2015) Ecology of anti-biofilm agents ii: Bacteriophage exploitation and biocontrol of Biofilm Bacteria. Pharm Basel Switz 8: 559-589.
- Abedon ST (2012) Spatial vulnerability: Bacterial arrangements, microcolonies, and biofilms as responses to low rather than high phage densities. Viruses 4: 663-687.
- Sillankorva S, Neubauer P, Azeredo J (2008) P. fluorescens biofilms subjected to phage philBB-PF7A. BMC Biotechnol 8: 79.
- Cornelissen A, Ceyssens PJ, T'Syen J, Van Praet H, Noben JP, et al. (2011) The T7-related *P. putida* phage phi15 displays virion associated biofilm degradation properties. PLoS ONE 6: e18597.
- Leiman PG, Chipman PR, Kostyuchenko VA, Mesyanzhinov VV, Rossmann MG (2004) Three-dimensional rearrangement of proteins in the tail of bacteriophage T4 on infection of its host. Cell 118: 419-429.
- Yan Y, Su S, Meng X, Ji X, Qu Y, et al. (2013) Determination of sRNA expressions by RNA-seq in *Yersinia pestis* grown *in vitro* and during infection. PLoS ONE 8: e74495.
- Lu TK, Collins JJ (2007) Dispersing biofilms with engineered enzymatic bacteriophage. P Natl Acad Sci USA 104: 11197-11202.
- 41. Son JS, Lee SJ, Jun SY, Yoon SJ, Kang SH, et al. (2010) Antibacterial and biofilm removal activity of a *podoviridae S. aureus* bacteriophage SAP-2 and a derived recombinant cell-wall-degrading enzyme. Appl Microbiol Biotechnol 86: 1439-1449.
- Gutiérrez D, Martínez B, Rodríguez A, García P (2012) Genomic characterization of two Staphylococcus epidermidis bacteriophages with anti-biofilm potential. BMC Genomics 13: 228.
- Glonti T, Chanishvili N, Taylor PW (2010) Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of *P. aeruginosa*. J Appl Microbiol 108: 695-702.
- Curtin JJ, Donlan RM (2006) Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis*. Antimicrob Agents Chemother 50: 1268-1275.

Page 4 of 5

- 45. Fu W, Forster T, Mayer O, Curtin JJ, Lehman SM, et al. (2010) Bacteriophage cocktail for the prevention of biofilm formation by *P. aeruginosa* on catheters in an *in vitro* model system. Antimicrob Agents Ch 54: 397-404.
- Cotar AI, Chifiriuc MC, Banu O, Lazar V (2013) Molecular characterization of virulence patterns in *P. aeruginosa* strains isolated from respiratory and wound samples. Biointerface Res Appl Chem 3: 551-555.
- 47. Mellbye B, Schuster M (2011) More than just a quorum: integration of stress and other environmental cues in acyl-homoserine lactone signalling. In: Bacterial stress responses, Storz G, Hengge R (eds). Washington, DC: ASM Press, USA. pp. 349-363.
- Mizan MFR, Jahid IK, Ha SD (2015) Microbial biofilms in seafood: A foodhygiene challenge. Food Microbiol 49: 41-55.
- Atkinson S, Williams P (2009) Quorum sensing and social networking in the microbial world. J R Soc Interface 6: 959-978.
- Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: Its role in virulence and possibilities for its control. Cold Spring Harbor Perspect Med 2: a012427.
- Waters CM, Bassler BL (2005) Quorum sensing: Cell-to-cell communication in bacteria. Annu Rev Cell Dev Bi 21: 319-346.
- Watnik P, Kolter R (1999) Steps in the development of a Vibrio cholerae EI T or biofilm. Mol Microbiol 34: 586-595.
- Defoirdt T, Boon N, Bossier P (2010) Can bacteria evolve resistance to quorum sensing disruption? PLoS Pathog 6: e1000989.
- Pei R, Lamas-Samanamud GR (2014) Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes. Appl Environ Microbiol 80: 5340 -5348.
- 55. Sharp R, Hughes G, Hart A, Walker JT (2006) Bacteriophage for the treatment of bacterial biofilms. U.S. Patent 7758856 B2.
- Olson JC, Fraylick JE, McGuffie EM, Dolan KM, Yahr TL, et al. (1999) Interruption of multiple cellular processes in HT-29 epithelial cells by *P. aeruginosa* exoenzype S. Infect Immun 67: 2847-2854.
- 57. Gupta K, Marques CNH, Petrova OE, Sauer K (2013) Antimicrobial tolerance of *P. aeruginosa* biofilms is activated during an early developmental stage and requires the two-component hybrid SagS. J Bacteriol 195: 4975-4981.
- Hanlon GW, Denyer SP, Olliff CJ, Ibrahim LJ (2001) Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *P. aeruginosa* biofilms. Appl Environ Microbiol 67: 2746-2753.

- Doolittle MM, Cooney JJ, Caldwell DE (1996) Tracing the interaction of bacteriophage with bacterial biofilms using fluorescent and chromogenic probes. J Ind Microbiol 16: 331-341.
- 60. Tait K, Skillman LC, Sutherland IW (2002) The efficacy of bacteriophage as a method of biofilm eradication. Biofouling 18: 305e311.
- Kay MK, Erwin TC, McLean RJ, Aron GM (2011) Bacteriophage ecology in *E. coli* and *P. aeruginosa* mixed-biofilm communities. Appl Environ Microbiol 77: 821-829.
- Ho K (2001) Bacteriophage therapy for bacterial infections: Rekindling a memory. Perspect Biol Med 44: 1-16.
- Verma V, Harjai K, Chhibber S (2010) Structural changes induced by a lytic bacteriophage make ciprofloxacin effective against older biofilm of *Klebsiella* pneumoniae. Biofouling 26: 729-737.
- Soothill JS, Hawkins C, Harper DR (2011) Bacteriophage-containing therapeutic agents. U.S. Patent 8105579 B2.
- 65. Harper DR (2013) Beneficial effects of bacteriophage treatment. U.S. Patent 8475787 B2.
- Yilmaz C, Colak M, Yilmaz BC, Ersoz G, Kutateladze M, et al. (2013) Bacteriophage therapy in implant-related infections: An experimental study. J Bone Joint Surg Am 95: 117-125.
- Seth AK, Geringer MR, Nguyen KT, Agnew SP, Dumanian Z, et al. (2013) Bacteriophage therapy for *S. aureus* biofilm-infected wounds: A new approach to chronic wound care. Plast Reconstr Surg 131: 225-234.
- 68. Ganegama Arachchi GJ, Cridge AG, Dias-Wanigasekera BM, Cruz CD, McIntyre L, et al. (2013) Effectiveness of phages in the decontamination of *Listeria monocytogenes* adhered to clean stainless steel, stainless steel coated with fish protein, and as a biofilm. J Ind Microbiol Biotechnol 40: 1105-1116.
- Liao KS, Lehman SM, Tweardy DJ, Donlan RM, Trautner BW (2012) Bacteriophages are synergistic with bacterial interference for the prevention of *P. aeruginosa* biofilm formation on urinary catheters. J Appl Microbiol 113: 1530-1539.
- Zhang Y, Hu Z (2013) Combined treatment of *P. aeruginosa* biofilms with bacteriophages and chlorine. Biotechnol Bioeng 110: 286-295.
- 71. Szafranski SP, Winkel A, Stiesch M (2017) The use of bacteriophages to biocontrol oral biofilms. J Biotechnol 250: 29-44.