ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Mini Review

# Bacteriophages in Biofilm Control

**Shilpa Deshpande Kaistha***

*Department of Microbiology, Institute of Biosciences and Biotechnology, CSJM University, Kanpur, UP, India*

***Corresponding Author**: Shilpa Deshpande Kaistha, Department of Microbiology, Institute of Biosciences and Biotechnology, CSJM University, Kanpur, UP, India.

**Received:** June 08, 2017; **Published:** July 26, 2017

## Abstract

Biofilms or bacterial growth in the form of surface adhering communities that are protected by an encompassing exopolymeric matrix and which operate via a quorum driven gene regulation system have redefined the study of basic and applied microbiology. Microbial biofilms show genetic and phenotypic heterogeneity that confer on its residents a heightened protection from a variety of environmental stressors including antimicrobial compounds. Such microbial growth forms thus become the cause of recalcitrant acute and chronic infections as well as induce biofouling and biocorrosion in industry and environment alike.

Biological control of biofilms includes the use of intracellular obligate parasites of bacteria known as bacteriophages (phages). Natural and genetically modified bacteriophages have evolved as promising biofilm control agent's due to their high host specificity, non-toxicity and low carbon footprint. This report attempts to review recent advances in the use of phages and their products in the effective control of biofilms.

***Keywords:*** *Bacteriophage; Biofilm; Quorum Sensing; Genetically Engineered Phages*

## Introduction

### Bacterial Biofilms

Biofilms are naturally occurring growth forms of uni or multi species populations of micro-organisms which are characterized by the presence of an encompassing exopolymeric matrix that protect its residents from environmental stressors [1]. Biofilms within nature are highly heterogeneous in terms of its shape, size, exopolymeric matrix composition as well as the internal architecture [2]. Advances in imaging technologies such as confocal and atomic force microscopy have revealed intricate constructions within the biofilm with water channels and pores for the distribution of nutrition as well as removal of excretory products [3,4]. The nature of a biofilm depends highly on the type of exopolymeric substance secreted by its residents and the protection it hence confers on its residents [1,5]. The role of polysaccharide, proteins as well as extranuclear DNA has been reported in the formation of a successful biofilm [1]. Several studies show increased phenotypic and genotypic resistance of biofilm to antimicrobial compounds as well as other stresses in comparison to their planktonic/ free living counterpart [6]. The outermost heterogeneous exopolymeric matrix provides a barrier for the penetration of large molecular weight compounds and predatory cells including the immune response [1,6]. In addition within the biofilm, the close proximity of species ensures successful horizontal gene transfer perpetuating genetic resistance to control agents [6]. The presence of persister or slow growing cells within the biofilm always ensures that a population of biofilm forming cells would be left behind as killing strategies such as antibiotics target fast growing cells [7].

Within the biofilm complex could be present uni or multi species residents that function as a quorum and determine the fate of the community living with chemical signals known as quorum sensing molecules [8].

The nature of the quorum sensing molecules determines the phenotypic as well as genotypic heterogeneity within the community. Quorum cue dependant up-regulation of multiple drug efflux pumps as well as antibiotic resistance enzymes has been reported within biofilms [9].

The role of cyclic di guanosine mono phosphate (cyclic di GMP) as secondary messenger in biofilm formation has been described for several biofilm forming clinical isolates [10]. The bacterial regulatory networks which determine planktonic to biofilm switch have also been reported with species of small non coding RNA (sRNA) [11]. Study of genomics and next generation sequencing methodologies have provided avenues for *in silico* genome wide identification of gene responsible for biofilm formation [12].

## Bacteriophages as natural biofilm biocontrol agents

Phages are natural predators of bacteria causing cell lysis with the release of several virion progeny [13]. Since the hosts are ubiquitous, phages have been isolated from every ecological niche resided by the host. The role of bacteriophages in controlling bacterial population dynamics within biofilms has been extensively reviewed [14-16]. Phages as lytic agents of biofilms form the most direct means of controlling bacterial populations [17].

The ability of the phage surface protein to recognize specific complementary host receptors determines its host specificity. The large and closely associated cellular populations within the biofilm niche further help in the rapid propagation of the phages. Phage infection may occur during early stages of biofilm formation, or the phage may have to penetrate through the highly defensive exopolymeric matrix to access the residents within or alternatively it may infect the occasional swarming bacteria released from a dispersing biofilm. Many such scenarios allow us to imagine the diverse opportunities and strategies a phage may have evolved in order to survive in nature wherein more than 90% of the micro-organisms are thought to be biofilm residents [15].

Persister cells, a small population of antibiotic sensitive cells that are slow growing or dormant cells and hence remain protected from the action of drugs acting of rapidly growing cells, are observed to be susceptible to lytic phage activity as well lysogenic occupation [18]. Phage induced lysis within the biofilm structure also causes the release of extracellular DNA which can be taken up by neighboring cells via gene transfer mechanisms and propagate horizontal transfer of antibiotic resistance amongst other genes [19]. Lytic phage infections have an disadvantage of sudden release of cellular toxins as well as release of bacterial endotoxins that may induce inflammatory response [20]. Since, lysogenic control or non-lytic phages have also been widely researched for their antimicrobial activity. Phages are thought to typically establish lysogeny when the host numbers fall due to environmental stresses [21]. Establishment of *Tectivirus* lysogeny in *Bacillus thuringiensis* sp has shown to have a negative impact on biofilm formation while enhancing swarming motility [22]. The effect of spontaneous phage induction (SPI) observed in *Bacillus megaterium* lysogens where free phage was observed in culture media even in non-inducing conditions. In contrast, the release of eDNA in *Streptococcus pneumoniae* and *Shewanella oneidensis* MR-1 biofilm matrix following SPI was found to be enhance biofilm formation [23].

In initial stages of phage infections, phage encoded enzymes in its tail and tail fibres help in hydrolyzing capsular antigens, peptidoglycans, lipopolysacharrides as well as outer member proteins for penetrating the outer layers of the bacterial cell to facilitate injection of the phage genome [24]. Pires., *et al.* provide an exhaustive review of bacteriophage derived depolymerase and endolysins, their structure and diversity [25].

The role of phage encoded proteases and polysachharases have been demonstrated in exopolymeric matrix degradation and biofilm detachment and dispersal [26] while it has been recorded that all biofilm diffusing phages need not produce depolymerases [15].

Phage infections have known to influence biofilm genetics and population dynamics [15,21,27]. Phage genomes encoding quorum sensing molecules (agr loci) which influence biofilm formation were first reported in *Clostridium difficile* [28]. Since, *Clostridium tyrobutyricum* phage φCTP1, *Iodobacteria* phage ϕPLPE encoding a predicted acyl hydrolase and *Pseudomonas* phages encoding response regulators associated with the *agr* system have been described [28].

## Genetically Engineered Phages & Products (GEPP)

Conventional phage therapy has several restrictions such as host specificity, inability to target multispecies biofilms and host immune response [20]. Availability of phage whole genome sequences and their gene products has facilitated genetic engineering of these highly moldable entities to overcome many of prevailing challenges. The advances in the development of synthetic phages and *in vitro* reengineering of phages has been since excellently reviewed [25]. Genetically modified phages have revolutionized the biotechnological applications of phages which are being increasingly studied as Nano sized Biosystems [29]. Nano Biosystems are defined as entities less than 100 nm size which are self-fabricating and non-self-replicating [30]. Phages such as M13 show properties such as supra molecular assembly wherein requisite phage density show nematic liquid crystal display properties which allows them to be used as nanoscaffolds or coatings [31]. In recent times, the use of filamentous non lytic phages such as M13, Ike, fd and *Pseudomonas* phage Pf3 with single strand DNA are being manipulated to increase the antibacterial phage activity [32,33]. Such phages are further nanoengineered to express anti-microbial compounds that prevent biofilm forming growths [29,30].

Enzybiotics is a terminology used for phage encoded bacteriolytic enzymes [34]. The bacteriophage lysin cassette gene which typically comprise of a holin and lysine have been isolated for several host bacteria and further genetically engineered to use as biocontrol agents [32]. The T7 engineered phage was genetically engineered to express the dsp B gene encoding biofilm dispersing enzyme dispersin A from *Acinobacillus actinomycetemcomitans* under T7 select 415 - 110B capsid gene under the control of the T7 φ10 promoter [35]. The engineered T7 phage inhibited biofilm cell counts by 4.5 orders of magnitude after 24h of treatment.

A lactonase *aiiA* from *Bacillus anthracis* genetically engineered into the T7 select 415 - 1 phage vector, which acts as acyl homoserine lactone antagonist, was shown to effectively degrade quorum sensing molecules and inhibit multi-species biofilm forming isolates such as *Pseudomonas aeruginosa* and *E. coli* [36] .

The use of phage endolysins rather than using the entire phage can circumvent some of the potential problems of intact virions [34]. Recombinant endolysins, termed Artilysin® are able to pass though the outer membrane and act on the peptidoglycan showing antibacterial activity against gram positive, gram negative as well as *Mycobacteria* [37]. Synergistic effect of *E. coli* bacteriophage T5 endolysin, a l-alanyl-d-glutamate peptidase, with low levels of various cationic membrane permeabilizing compounds such as polymyxin B, gramicidin D, poly-l-lysine, chlorhexidine and miramistin reduced bacterial load by 4 - 5 orders of magnitude [38]. A chimeric protein CHAPSH3b, which consists of a catalytic domain from the virion-associated peptidoglycan hydrolase of phage vB_SauS-phiIPLA88 (HydH5) and the cell wall binding domain of lysostaphin can control biofilm embedded *S. aureus* [39].

### Limitation of Phage Biofilm Control and their Possible Solutions

One of the advantages as well as drawbacks of phage therapy is a limited host range with high specificity [15]. Control of multispecies biofilms that usually occur in environment can be targeted with the isolation of broad host range phages, genetically engineered phages or the use of phage cocktails [33,40]. Recently, Mapes., *et al.* reported the development of a host range expansion (HRE) protocol, wherein cycle of co-culturing of 16 different *Pseudomonas aeruginosa* with phage mixture developed into a phage cocktail with a predictable broad host range [41].

Phage host ratio (PHR) for the effective reduction of biofilm has received considerable attention as low level phage predation of phages is known to induce biofilms [42]. Effective phage dosing is essential for successful phage therapy. The concept of "Multiplicity of infection" in defining phage dosage has been revisited in the context of biofilm infections. Phage added to host solution may not equate with actual number of cells infected within the biofilm and in terms of phage pharmacodynamics, actuals in numbers and volumes may provide an accurate and reliable information with respect to successful phage therapies [43]. Successful control of acute infections is observed in as low as single phage dose while multiple dosing is advocated in the case of chronic infections [43]. For effective phage therapy in human infections, the rapid clearance of phage from the circulation by the immune response is anticipated and remains a problem with recurrent infections [20]. Here again, phage dosage remains an important issue to be addressed.

Bacterial hosts may develop resistance to phages by several mechanisms including alteration of phage entry receptors and by developing adaptive immunity by acquiring CRISPR (clustered regularly interspaced short palindromic repeats) and CRISPR associated Cas 9 proteins. This prokaryotic small RNA based recognition and elimination defense systems protects host from invasion of foreign DNA such as phages and plasmids [44].

*Pseudomonas* lysogenic phage DMS3 encoding CRISPR modulated swarming motility and inhibited biofilm formation [45]. To counteract the antiphage defense mechanisms five different anti CRISPR protein families that inhibit the CRISPR defense system have also been identified from bacteriophages [46]. Genetic introduction of anti CRISPR proteases into promising phage or phage products may circumvent issues regarding host resistance. Similarly, the problem associated with development of phage resistant mutants can also be addressed with the use of phage cocktails [40].

Although several lytic phages for different pathogens and biofouling bacteria have been described, targeting biofilm dwellers has been a considerable challenge in terms of phage dosage and challenges associated with biofilm penetration. The use of biofilm dispersal agents in concert with phage such as the dsp engineering T7 phage [35], sub lethal nitric oxide [47], enzymes [48] and quorum sensing antagonists [49] can radically increase the effectiveness of the phage formulation. In a recent study with *Enterococcus faecalis*, autoinducer mediated dispersal of biofilm and the distribution of virulence factors due to phage release was reported in commensal gut bacteria [19]. The use of phage therapy alone provided externally may not provide consistent and reliable eradication or dispersal of mature biofilms. Concurrent application of phage particularly with a dispersal agent or low levels of antimicrobial agents appears to be a strategy that may prove to be successful in the treatment of multiple drug resistant bacterial growth [50,51].

## Conclusions

"The return of the phages"- a biofilm control strategy that is being revisited in the new light of scientific advances has many promises to keep. The recent patents afforded to genetically manipulated phages and the approval of their use in the food industry provides an optimistic future for this burgeoning treatment in the face of the global emerging problems of resistance to current antimicrobial agents [17,52]. Phages have played a major role in carving the bacterial population dynamics and its ecology (much of which we are yet to understand). It is hoped that man is kind to phage exploitation and prudent in the use of this co-evolving agent.

## Acknowledgement

This material is based on the research supported by 'Minor Research Project Grant' received from Chhatrapati Shahu Ji Maharaj University, Kanpur.

## Bibliography

1. Flemming H-C and Wingender J. "The biofilm matrix". *Nature Reviews Microbiology* 8.9 (2010): 623-633.

2. Wimpenny J., *et al.* "Heterogeneity in biofilms". *FEMS Microbiology Reviews* 24.5 (2000): 661-671.

3. Schlafer S and Meyer RL. "Confocal microscopy imaging of the biofilm matrix". *Journal of Microbiological Methods* (2016).

4. James SA., *et al.* "Atomic Force Microscopy of Biofilms—Imaging, Interactions, and Mechanics". *Microbial Biofilms - Importance and Applications* (2016).

5. Wei Q and Ma LZ. "Biofilm matrix and its regulation in Pseudomonas aeruginosa". *International Journal of Molecular Sciences* 14.10 (2013): 20983-21005.

6. Høiby N., *et al.* "Antibiotic resistance of bacterial biofilms". *International Journal of Antimicrobial Agents* 35.4 (2010): 322-332.

7. Balcázar JL., *et al.* "The role of biofilms as environmental reservoirs of antibiotic resistance". *Frontiers in Microbiology* 6 (2015): 1216.

8. Rutherford ST and Bassler BL. "Bacterial quorum sensing: its role in virulence and possibilities for its control". *Cold Spring Harbor Perspectives in Medicine* 2.11 (2012).

9. Sun J., *et al.* "Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations". *Biochemical and Biophysical Research Communications* 453.2 (2014): 254-267.

10. Valentini M and Filloux A. "Biofilms and Cyclic di-GMP (c-di-GMP) Signaling: Lessons from Pseudomonas aeruginosa and Other Bacteria". *The Journal of Biological Chemistry* 291.24 (2016): 12547-12555.

11. Parker A., *et al.* "Alternative pathways for Escherichia coli biofilm formation revealed by sRNA overproduction". *Molecular Microbiology* 105.2 (2017): 309-325.

12. Kang X-M., *et al.* "Genome-Wide Identification of Genes Necessary for Biofilm Formation by Nosocomial Pathogen Stenotrophomonas maltophilia Reveals that Orphan Response Regulator FsnR Is a Critical Modulator". Kivisaar M, editor. *Applied and Environmental Microbiology* 81.4 (2015): 1200-1209.

13. Sharma S., *et al.* "Bacteriophages and its applications: an overview". *Folia Microbiologica* 62.1 (2017): 17-55.

14. Motlagh AM., *et al.* "Biofilm control with natural and genetically-modified phages". *World Journal of Microbiology and Biotechnology* 32.4 (2016): 67.

15. Harper D., *et al.* "Bacteriophages and Biofilms". *Antibiotics* 3.3 (2014): 270-284.

16. Abedon ST. "Ecology of Anti-Biofilm Agents II: Bacteriophage Exploitation and Biocontrol of Biofilm Bacteria". *Pharmaceuticals* 8.3 (2015): 559-589.

17. Kaistha S and Umrao P. "Bacteriophage for Mitigation of Multiple Drug Resistant Biofilm Forming Pathogens". *Recent Patents on Biotechnology* 10.2 (2016): 184-194.

18. Pearl S., *et al.* "Nongenetic Individuality in the Host–Phage Interaction". Waldor M, editor. *PLOS Biology* 6.5 (2008): e120.

19. Rossmann FS., *et al.* "Phage-mediated dispersal of biofilm and distribution of bacterial virulence genes is induced by quorum sensing". *PLoS Pathogens* 11.2 (2015): e1004653.

20. Loc-Carrillo C and Abedon ST. "Pros and cons of phage therapy". *Bacteriophage* 1.2 (2011): 111-114.

21. Hargreaves KR., *et al.* "Bacteriophage behavioral ecology: How phages alter their bacterial host's habits". *Bacteriophage* 4.3 (2014): e29866.

22. Gillis A and Mahillon J. "Influence of lysogeny of Tectiviruses GIL01 and GIL16 on Bacillus thuringiensis growth, biofilm formation, and swarming motility". *Applied and Environmental Microbiology* 80.24 (2014): 7620-7630.

23. Nanda AM., *et al.* "Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions". *Journal of Bacteriology* 197.3 (2015): 410-419.

24. Latka A., *et al.* "Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process". *Applied Microbiology and Biotechnology* 101.8 (2017): 3103-3119.

25. Pires DP., *et al.* "Genetically Engineered Phages: A Review of Advances over the Last Decade". *Microbiology and Molecular Biology Reviews 80.3 (2016): 523-543.*

26. Chan BK and Abedon ST. "Bacteriophages and their enzymes in biofilm control". *Current Pharmaceutical Design* 21.1 (2015): 85-99.

27. Koskella B and Meaden S. "Understanding bacteriophage specificity in natural microbial communities". *Viruses* 5.3 (2013): 806-823.

28. Hargreaves KR., *et al.* "What Does the Talking? Quorum Sensing Signalling Genes Discovered in a Bacteriophage Genome". Kaufmann GF, editor. *PLoS One* 9.1 (2014): e85131.

29. Sunderland KS., *et al.* "Phage-Enabled Nanomedicine: From Probes to Therapeutics in Precision Medicine HHS Public Access". *Angewandte Chemie International Edition in English* 56.8 (2017): 1964-1992.

30. Cao B., *et al.* "Phage as a Genetically Modifiable Supramacromolecule in Chemistry, Materials and Medicine". *Accounts of Chemical Research* 49.6 (2016): 1111-1120.

31. Dogic Z. "Filamentous Phages as a Model System in Soft Matter Physics". *Frontiers in Microbiology* 7 (2016): 1013.

32. Drulis-Kawa Z., *et al.* "Bacteriophages and phage-derived proteins--application approaches". *Current Medicinal Chemistry* 22.14 (2015): 1757-1773.

33. Motlagh AM., *et al.* "Biofilm control with natural and genetically-modified phages". *World Journal of Microbiology and Biotechnology* 32.4 (2016): 67.

34. Keary R., *et al.* "Bacteriophages and their endolysins for control of pathogenic bacteria". In: Mendez-Vilas A, editor. *Microbial pathogens and strategies for combating them: science, technology and education*, Formatex 2 (2013): 1028-1040.

35. Lu TK and Collins JJ. "Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy". *Proceedings of the National Academy of Science U S A* 106.12 (2009): 4629-4634.

36. Pei R and Lamas-Samanamud GR. "Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes". *Applied and Environmental Microbiology* 80.17 (2014): 5340-5348.

37. Gerstmans H., *et al.* "From endolysins to Artilysin(R)s: novel enzyme-based approaches to kill drug-resistant bacteria". *Biochemical Society Transactions* 44.1 (2016): 123-128.

38. Shavrina MS., *et al.* "*In vitro* study of the antibacterial effect of the bacteriophage T5 thermostable endolysin on Escherichia coli cells". *Journal of Applied Microbiology* 121.5 (2016): 1282-1290.

39. Fernández L., *et al.* "Downregulation of Autolysin-Encoding Genes by Phage-Derived Lytic Proteins Inhibits Biofilm Formation in Staphylococcus aureus". *Antimicrobial Agents and Chemotherapy* 61.5 (2017): e02724-16.

40. Chan BK., *et al.* "Phage cocktails and the future of phage therapy". *Future Microbiology* 8.6 (2013): 769-783.

41. Mapes AC., *et al.* "Development of expanded host range phage active on biofilms of multi-drug resistant Pseudomonas aeruginosa". *Bacteriophage* 6.1 (2016): e1096995.

42. Fernández L., *et al.* "Low-level predation by lytic phage phiIPLA-RODI promotes biofilm formation and triggers the stringent response in Staphylococcus aureus". *Scientific Reports* 7 (2017): 40965.

43. Abedon ST. "Phage therapy dosing: The problem(s) with multiplicity of infection (MOI)". *Bacteriophage* 6.3 (2016): e1220348.

44. Garneau JE., *et al.* "The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA". *Nature* 468.7320 (2010): 67-71.

45. Palmer KL and Whiteley M. "DMS3-42: the secret to CRISPR-dependent biofilm inhibition in Pseudomonas aeruginosa". *Journal of Bacteriology* 193.14 (2011): 3431-3432.

46. Maxwell KL., *et al.* "Phages Fight Back: Inactivation of the CRISPR-Cas Bacterial Immune System by Anti-CRISPR Proteins". Dutch RE, editor. *PLOS Pathogens* 12.1 (2016): e1005282.

47. Barraud N., *et al.* "Nitric oxide: a key mediator of biofilm dispersal with applications in infectious diseases". *Current Pharmaceutical Design* 21.1 (2015): 31-42.

48. Chai Z., *et al.* "Application of bacteriophage-borne enzyme combined with chlorine dioxide on controlling bacterial biofilm". *LWT-Food Science and Technology* 59.2 (2014): 1159-1165.

49. Li Y-H and Tian X. "Quorum Sensing and Bacterial Social Interactions in Biofilms". *Sensors* 12.3 (2012): 2519-2538.

50. Torres-Barceló C and Hochberg ME. "Evolutionary Rationale for Phages as Complements of Antibiotics". *Trends in Microbiology* 24.4 (2016): 249-256.

51. Sagar SS., *et al.* "Efficacy of Phage and Ciprofloxacin Co-therapy on the Formation and Eradication of Pseudomonas Aeruginosa Biofilms". *Arabian Journal for Science and Engineering* 42.1 (2017): 95-103.

52. Abedon ST. "Bacteriophage exploitation of bacterial biofilms: phage preference for less mature targets?". *FEMS Microbiology Letters* 363.3 (2016).

**Volume 10 Issue 2 July 2017**