

Bacteriophage as a Novel Antibacterial Agent in Industry and Medicine

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ABSTRACT

Bacteriophages are viruses that infect bacteria, and are distinct from animal and plant viruses that could have either lytic or lysogenic cycle. Lytic phages known as candidates for phage therapy, since they rapidly replicate into their host and lyse them. Theoretically, phages have more than a few benefits over routine antibiotics. They are extremely specific for their targeted hosts and also are safe for human, because they have no activity against eukaryotic cells. Over this time, phages were used to treat various infections. Although, phages have a number of advantages over antibiotics, their industrial production as a commercial product ceased in most of the western European countries after the introduction of antibiotics. These days, by increasing of antibiotics resistance and inefficiency of antibiotics against bacterial biofilms, there is renewed global interest in phage applications as potentially powerful antibacterial agents. Different published paper through the world indicates bacteriophages could be recruited as suitable agent for therapeutic purposes in medicine and food industry. Therefore, here we tried to review most of these ideas about phage application as a rapid review.

Keywords: Bacteriophage; Virus; Antibacterial agent; Industry; Medicine

INTRODUCTION

Bacteriophages are an order of viruses that are able to infect bacteria, resulting usually in propagative lysis (lytic cycle) or lysogenization (lysogenic cycle) of the infected cell. Lytic phages may be candidates for phage therapy, because they replicate fast within their hosts and lyse them. Based on the species of phage and host, phages could be produced between 50 and 250 progeny per infective life cycle (Fig-1). Each daughter infects a host and produce 40,000 progeny at the end of the second cycle. This will result in 8 million progeny at the end of the third cycle and 1.6 billion at the end of the fourth cycle [1]. Theoretically, phages have more than a few benefits over routine antibiotics. They are strongly specific for their targeted bacteria and also are safe for human, since they did not show any activity against eukaryotic cells. Bacteriophage therapy as an

emerging methods to combat with bacterial infections is presented [2]. Phage recruiting as a therapeutic agent was initiated in 1919, short times after its discovery, to the treatment of dysentery and it is continued until the 1940s. Over this time, phages were used to treat various infections. Although, phages have a lot of advantages against antibiotics, their industrial production as a commercial product ceased in most of the western European countries [3, 4]. These days, by raising of antibiotics resistance and inefficiency of antibiotics against bacterial biofilms, there is renewed global attention in phage applications as a potentially powerful antibacterial agents. There are various published paper throughout the world that indicate bacteriophages could be recruited to phage therapy, both in humans and animals, and also in food industry [5- 7] .

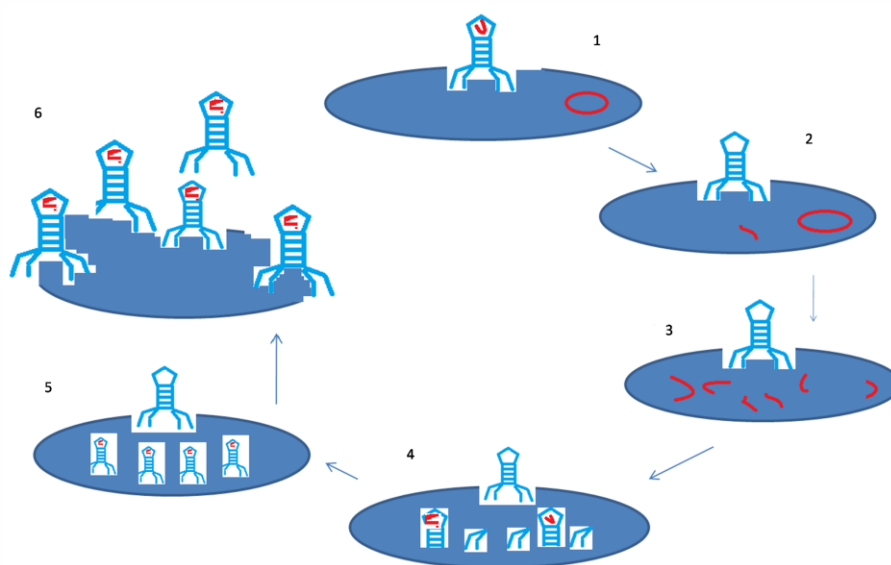


Figure 1. Lytic Cycle. 1) A phage recognizes a specific receptor on host and attaches to its host via that receptor, and 2) injects its DNA. 3) Phage by disrupting the host replication induce the bacterium killing, 4) taking over the bacterial DNA replication and protein synthesis machinery to make its parts. 5) This process ends with the assembly of new phage, and 6) the lysis of the host cell wall to release a hundred new copies of phage. (This image is designed by Our teams in MahanGenePajoh Co.)

Broad Host Range bacteriophage:

Bacteriophages like other viruses as obligate intracellular parasites must enter an appropriate host to reproduce. Their infection are initiated when the virions interact with receptors on the surface of host cell. Most of bacteriophages are known to be extremely specific for their hosts (because of specific receptors) [8]. In contrast, it is clear that some bacteriophages are called broad host range could infect a range of bacterial species (Fig-2). P1 and Mu are presented as broad host range bacteriophages. P1 is able to produce plaque on several enteric species culture in addition to *E.coli*, while bacteriophage Mu produces progeny virions on different bacterial species because of the the variation of invertible viral G segment region orientation [9]. Based on the origin of virus particles that make up a large amount of the dissolved organic carbon in all ecosystems as well as marine ecosystems, the prevalence of broad-host-range phages would be different [10, 11].

Bacteriophage and biofilm:

Bacteria attach to both surfaces: biotic and abiotic, and form densely and packed bacterial cells embedded in an extracellular

polysaccharide matrix known as a biofilm [12]. One of the most important specificity of these attached cells is up to 1000 times higher resistance to antimicrobial agents, primarily as the result of slow growth and presence of impermeable exopolysaccharides (EPS) on bacterial surface [13].

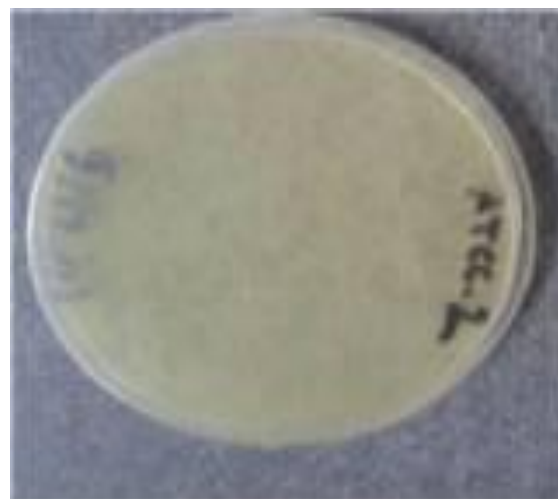


Figure 2. Isolated phage against *P.aeruginosa* (ATCC: PAO) from sewage, which effect against *E.coli*, *P.putida* and *A.baumannii*, Ilam University of Medical Sciences. R.Azizian, Clinical Microbiology Research Center, Ilam University of Medical Sciences

Phages have been examined to a lesser extent as a potential agents to control biofilm [14-17]. Phage application has ability to degrade EPS by enzymes attached to their base plate-polysaccharide depolymerases and lyases, important for penetration into a host cell [16]. Phage enzymes has potency to eliminate or inhibit biofilm formation. Different Multiplicity of Infection (MOI: the ratio of infectious phage to infection host) is needed to bacterial growth and probably phage enzymatic activity inhibit biofilm forming. While primary receptors are located on outer membrane, EPS acts as a secondary receptor for some of phages [18].

Even if a phage cannot attach to primary receptors and infect cells, it can degrade EPS and inhibit biofilm formation [19]. Although phages showed considerable effect on growth inhibition and biofilm formation, their effect on already formed biofilm was very low. It is not unexpected, as phages are usually highly specific for their receptors and slightly different receptors structure on bacterial surface may result in resistance to phage infection [20]. McLaughlin and colleagues experiments illustrate this event by an emergence of mutant species that show resistant to phages (21). Mutants usually spontaneously appear during overnight incubation of bacteria and phages, and it could be explain this phenomenon [22].

Bacteriophage and Quorum-sensing:

Quorum-sensing known as a mean pathway of regulating the interactions between bacteria and its responses to environmental signals and density of population, is presumed to be recruited to regulate antiviral defense strategies.

The bacterium related evolution of phage is characterized by altering selection and thus generates bacteria that are more resistant to current phages than to past [23, 24].

Phage defense mechanism that regulated by quorum-sensing signaling pathway could play a main role on the phage evolution in their natural hosts and environments. To explain phage coexistence with their hosts, different mechanisms are presumed that would generate a minority group of host cells that are protected

from infection, this group by stochastic fluctuations in receptor expression, decrease lysis in stationary phase [25, 26].

E. coli has evolved the ability to increase the subpopulation of phage-tolerant receptor-free cells at times by subjecting genes encoding phage receptors (LamB and flagella) to quorum-sensing control [27]. Quorum-sensing-mediated down-regulation of phage susceptibility may well turn out to be an important factor in understanding phage-host coexistence. Albeit, quorum-sensing has been found to up-regulate the generation of all of these minority groups [28, 29]

Bacteriophage in industry

Poultry:

Phages were recruited as a spray and intramuscular injection, to cure *Escherichia coli* infection in chicken [30]. These products experiments shown, when the equal number of phage and bacteria were utilized, no morbidity was observed at all, but 100-fold lower phage titration also conferred significant protection, demonstrate the multiplication of the phage in in-vivo. Phage therapy was effective in most of studies, even at the onset of clinical symptoms [2, 31, 32].

Meat (Calves):

Phage application in calves prevent diarrhea, but it does not efficiency at the initiation of symptoms, although it strongly prevent death [33]. Phage titration in feces is high but it is not detectable in blood and spleen. Although, there is phage-resistant cells in most of the calves, their titration remained low. Mutant cells indicate less competitive effect than their parents through re-inoculation into new calves [32].

In other study, recruited low dose of phage (10^5 p.f.u.) investigated at the onset of diarrhea and calves were sacrificed in a time series. After 40 hours when bacteria were not detectable, phages disappeared, but there appeared a range of bacteria which shown resistance to bacteriophage. Remained bacteria had lost major potency to produce infection [33].

Phages have this potency to survive at room temperature almost 100 days, resistance to phenolic disinfection [34, 35], confirmed that intramuscular injection in calves postponed the presence of the bacterium in the faeces and blood of animal. This sort of phage application improve the life length of the animals [34, 35].

Commercial Products:

Phages exploited in the USA as commercial products. Various products were designed by recruiting proteolytic enzymes of lytic phages in USA. For examples a bandage designed for treating a bacterial infection of skin involves groups of bacteria such as; *Staphylococcus*, *Pseudomonas*, *Streptococcus*. This lytic enzyme is specific and has the ability to digest a cell wall of the bacteria and is coded by the same bacteriophage capable of digesting infected bacteria. The enzyme produced is mixed with a carrier, (Patent's No: 6432444; <http://www.patentstorm.us/patents/6432444.html>) [36].

In addition, a chewing gum was produced that its composition included of mixing an effective amount of proteolytic enzyme produced by group C *streptococcal* bacteria infected with a C1 bacteriophage and a carrier for delivering these enzyme via mouth, throat, or nasal passage, (Patent's No: 6685937; <http://www.google.com/patents/US6685937>) [37].

Phagetherapy

Phage-Antibiotic Synergy (PAS):

André M. Comeau study indicates surprisingly, the phage plaques zone increase with some of β -lactam antibiotics disks where there was a sub-lethal concentration of them. This effect was observed for aztreonam, monobactam, cefixime, and cephalosporin disks, but not for tetracycline and gentamicin [38]. Hence, a sub-lethal dosage of β -lactam antibiotics stimulate phage propagation within host. This synergistic effect called the Phage Antibiotic Synergy (PAS). Recently, it has been indicated that the inactivation of Penicillin Binding Protein III synthesis gene (*ftsI*) by β -

lactam antibiotics also induces the expression of the SOS response system via a two-component signaling pathway. SOS response to β -lactam antibiotics in bacteria leads in filamentation that allows the host to decrease the lethal effects of the antibiotics [39].

Thereby, it seems that the phenotype of filamentation plays an important role in the PAS effect, possibly through various pathways. Filamentation cell altering clearly make phage assembly faster, maybe by inducing alteration in the peptidoglycan layer that this possibly results a more sensitivity to the lysis action of phage (e.g. lysozymes, holins) [38].

Human phage therapy Efforts:

550 cases of bacterial infections such as empyemas, peritonitis, osteomyelitis, and etc in humans were investigated by a group led by Prof. S. Slopek (Institute of Immunology and Experimental Therapy, branch of Polish Academy of Sciences) in the mid-1980s [40]. Most of these cases were chronic and resistant to routine antibiotics. Pathogenic bacteria were involved *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *E. coli* [40]. Recruited phages by S. Slopek's group have cured approximately 90% of the cases [40]. Cessation of suppuration and, where applicable, complete closure of wounds or fistulae presumed as criteria of the cure. To prevent biohazard of remained bacteria debris, it is preferred to not use oral administration. Patients were received anti-acids and gelatin in order to protect the phages from gastric acid destruction potential [40]. Prof. S. Slopek and his colleagues have been rigorous in matching the phages to the bacterial strain infecting the given patients. They cultured the bacteria during the course of treatment, so that the occurrence of a mutant resisting the phage can be countered by switching to a different phage strain. This group also had panels of multivalent phages available, to use in fulminate infection such as septicemia. The statistics of achievement cure of human cases by this group were approximately 1,300 ones. Overlay, the rate of cure across the pathogens and infection sites was nearby 86% [40].

Table.1. Chronologically table of selected studies

Row	Researcher	Year	Title	Results
1	Jingmin Gu	2011	A Method for Generation Phage Cocktail with Great Therapeutic Potential	Phage cocktail as a more effective agent in bacterial mutation frequency reduction than monophage suggesting that produced cocktail by SBS method has strong therapeutic potency to treat multidrug-resistant infection [45].
2	Steven P.T. Hooton	2011	Application of a bacteriophage cocktail to reduce <i>S.typhimurium</i> U288 contamination on pig skin	Phage cocktail application produced significant reductions of <i>S. typhimurium</i> U288 on experimentally-contaminated pig skin; however this appears to be linked to an MOI in excess of the target bacterium [32].
3	Sabah Abdel Amir Jassim	2011	Novel phage-based bio-processing of pathogenic <i>Escherichia coli</i> and its biofilms	Phage bio-processing of vegetables, egg, and meat was highly successful in eliminating completely <i>E. coli</i> . Moreover, using designed phage master mix was shown to cover all of the tested <i>E. coli</i> bacteria without show signs of the development of bacterial resistance. On the other hand, phage bio-processing was remarkably successful in disintegrating <i>E. coli</i> biofilms [14].
4	Yannick Born	2011	Novel Virulent and Broad-Host-Range <i>Erwinia amylovora</i> Bacteriophages Reveal a High Degree of Mosaicism and a Relationship to <i>Enterobacteriaceae</i> Phages	L1, M7, S6, and Y2 were introduced as bio-control phages of fire blight. All of them are broad host ranges. Analysis of sequencing details provided information to avoid undesired transfer of host genetic material and facilitated complementary phages selection for pooled applications [8].
5	Gee Leng Lau	2012	Characteristics of a phage effective for colibacillosis control in poultry	Phage ØEC1, is be able to reduce infection b y <i>E. coli</i> O78:K80,14. It shown an optimum MOI of 0.1 – 1. This Phage has characteristics can serve as a guideline for selection of effective candidates for phage therapy, in colibacillosis control in chickens [31].
6	Victor Krylov	2012	A Genetic Approach to the Development of New Therapeutic Phages to Fight <i>Pseudomonas aeruginosa</i> in Wound Infections.	Live phages utilizing as a therapeutic agent is valid only in direct contact with the virus and bacteria. Although, the direct usage of highly purified phage in the blood could be very risky [41].
7	Nina Molin Høyland-Kroghsbo	2013	A Quorum-Sensing-Induced Bacteriophage Defense Mechanism	AHL signaling in quorum sensing response leads to downregulation of the number of <i>LamB</i> receptors, which causes increase survival bacteria. Also, this mechanism protect bacteria against broad-host-range phage χ [27].

Limitations of Human Bacteriophage Therapy:

Utilizing of live phages in order to phage therapy is valid only in cases that provide direct contact between the virus and bacterial host [41]. However, exploitation of highly purified phage in human fluids like blood (in septicemia case) is very risky [42]. Also, there is an unobvious debate on oral administration of phages because it is presumed on some of literatures that phages might penetrate into the

blood, and then arrive to the urine [43]. But, the phage utilizing for wound, urogenital, intestinal, eye infections and infections of the ear, nose and throat are suitable. Though, a lot of efforts done in animal models, phages are not applicable to achieve Cystic fibrosis cure, due to phages of *P. aeruginosa* are in contact with different phages and there is not any specific direct contact [44].

A particular mixture of phages has not sufficient ability to offer long-term efficiency as

therapeutic agent. Commercial cocktails of phages which manufactured by companies were included with broadest spectrum of lytic activity for their hosts. Despite of the spectrum of lytic activity of phage cocktail, survived bacteria might reveal resistance to cocktail after a short time of cure initiation. Consequently, single mutation in Gram-negative bacteria like *P.aeruginosa*, leading to resistance to attachment, and might inhibit the phage growth within host and lytic activity [43].

CONCLUSION

Based on antibiotics resistant infections emergence throughout the world, seeking for appropriate antimicrobial agents to deal with this issue has become a critical topic in health world. Previous of the antibiotics discovery and widespread usage of them, it was presumed that bacteriophages could be exploited to combat with bacterial infections.

Although there is not global agreement to use bacteriophages for therapeutic purposes specially in human, some of countries believe it could be useful. Bacteriophages are introduced as high specific antibacterial agents which lysis targeted bacteria, and rapidly modify to combat with the appearance of recently bacterial infections.

A lot of published studies suggest that phages as therapeutic agents might be effective in chosen clinical settings, many of them believe there is not any globally approved standards for clinical trials and there is still remained many unanswered questions that must be response before widely usage of lytic phages (Table-1) [46]. For the future phage therapy, most of aspects must be determined by attitude of health authorities that allow to use of phages.

Currently, there is a globally conflict about phage usage, in the West, the US Food and Drug Administration, prefer to use a single well known phage, while in the East, phage therapy lay in phage cocktails. Even individualized

treatments done for each patient by using specific phages which isolated against the agent of infection in surgical settings [45]. Also, Intesti-phage that was a mixture of a group of phages against *E. coli* and other *Enterobacteria* [47] was made in large quantity 80 % in the 1980s. This product were used widely by Soviet army, as prophylactic and without any particular pre-testing in the individual patients [48].

In other hand, phage purification, stabilization and sterilization are time spending and expensive process that need complicated and equipped laboratories base of standardization of Good Manufacturing Practices (GMP) protocols recruiting.

According to large of unanswered questions in phage therapy scope, and success of phage recruiting in industry, it is obvious that phage could be suitable antimicrobial agent in industries. But, to achieve a global agreement about phage therapy, there is need to establish an international institute that prepare a standard to provide clinical approves. Also, based on our rapid review, bacteria improve their manner when they have interaction with phage and shown resistance to phage. According to successful experiments about proteolytic enzymes of phage in commercial products such as toothpaste, chewing gum, and ointment for dermatological treating. It is supposed to use proteolytic enzymes instead of whole virion.

ACKNOWLEDGMENT

We appreciate valuable guidance of Prof. Dr. Andrew M. Kropinski (Program Lead, Host & Pathogen Determinants Laboratory for Food borne Zoonoses Public Health Agency of Canada) and Dr. Marine Henry (PHD, Post-doc, Dep. of Molecular Microbiology, Pasteur Institute de France) and Zack Hobbs Research Associate at The Evergreen State College T4 Bacteriophage Lab, Olympia, Washington.

REFERENCES

1. Richard MC. Phage therapy: Past history and future prospects. *Arch Immunol Ther Exp*. 1999;47:267-74.
2. Barrow PA SJ. Bacteriophage therapy and prophylaxis: Rediscovery and renewed assessment of potential. *Trends Microbiol*. 1997;5:268-71.
3. WC S. Bacteriophage therapy. *Annu Rev Microbiol* 2001;55:437-51.
4. Merrill C SD, Adhya SL. The prospect for bacteriophage therapy in Western medicine. *Nat Rev Drug Discov* 2003;2:489-97.
5. Bruttin A BH. Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob Agents Chemother*. 2005;49:2874-8.
6. Capparelli R PM, Borriello G, Salvatore P, Iannelli D. Experimental phage therapy against *Staphylococcus aureus* in mice. *Antimicrob Agents Chemother*. 2007;51:2765-73.
7. Heo Y-J LY-R, Jung H-H, Lee J, Ko G, Cho Y-H:r. Antibacterial Efficacy of Phages against *Pseudomonas aeruginosa* Infections in Mice and *Drosophila melanogaster*. *Antimicrob Agents Chemother*. 2009;53:2469-74.
8. Born Y, Fieseler L, Marazzi J, Lurz R, Duffy B, Loessner MJ. Novel virulent and broad-host-range *Erwinia amylovora* bacteriophages reveal a high degree of mosaicism and a relationship to *Enterobacteriaceae* phages. *Applied and environmental microbiology*. 2011;77(17):5945-54.
9. Van de Putte P, S. Cramer, and M. Giphart-Gassler. . Invertible DNA determines host specificity of bacteriophage Mu. *Nature*. 1980;286.
10. Torrella F, and R. Y. Morita. Evidence by electron micrographs for a high incidence of bacteriophage particles in the waters of Yaquina Bay, Oregon: ecological and taxonomical implications. *Appl Environ Microbiol*. 1979;37:774-8.
11. E.C. JENSEN HSS, B. RIELAND, T. L. THOMPSON, KIT W. LEE, K. W. NICKERSON, T. A. KOKJOHN. Prevalence of Broad-Host-Range Lytic Bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*. 1998;64(2):575-80.
12. O'Toole G, Kaplan, H.B., Kolter, R. Biofilm formation as microbial development. *Annu Rev Microbiol*. 2000;54:49-79.
13. Gilbert P, Das, J., Folez, I. Biofilm susceptibility to antimicrobials. *Adv Dent Res* 1997;11:160-7.
14. Jassim SAA, Abdulmir AS, Abu Bakar F. Novel phage-based bio-processing of pathogenic *Escherichia coli* and its biofilms. *World Journal of Microbiology & Biotechnology*. 2012 Jan;28(1):47-60.
15. Hanlon GW, Denyer, S.P., Olliff, C.J., Ibrahim, L.J.,. Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through *Pseudomonas aeruginosa* biofilms. *Appl Environ Microbiol*. 2001;67(6):2746-53.
16. Sutherland IW, Hughes, K.A. , Skillman, L.C., Tait, K.,. The interaction of phage and biofilm. *FEMS Microbiol Lett*. 2004;232:1-6.
17. Hanlon GW, Denyer, S.P., Olliff, C.J., Ibrahim, L.J.,. Bacteriophages: an appraisal of their role in the treatment of bacterial infections. *Int J Antimicrob Agents* 2007;30:118-28.
18. Hughes KA, Sutherland, I.W., Jones, M.V.,. Biofilm susceptibility to bacteriophage attack: the role of phage-borne polysaccharide depolymerase. *Microbiology*. 1998;144:3039 -47.
19. Azizian. R AJF, Askari. H, Naser. A, Karimi. S, Sadeghifard. N, Mosavi Nasab. S.D, Ahmadi. N. Bacteriophage as a new candidate for the prevention and removal of biofilms. *Journal of Ilam University of Medical Sciences* 2013;20(5):84-93.
20. Welkos S, Schreiber, M., Baer, H.,. Identification of *Salmonella* with the O-1bacteriophage. *Appl Microbiol*. 1974;28(4):618-22.
21. McLaughlin MR. Simple colorimetric microplate test of phage lysis in *Salmonella enterica*. *J Microbiol Methods* 2007;69(2):394-8.
22. Fridholm H, Everitt, E., . Rapid reproducible infectivity end-point titration of

virulent phage in a microplate system. *J Virol Methods*. 2005;128:67-71.

23. Gómez P BA. Bacteria-phage antagonistic coevolution in soil. *Science*. 2011;322:106-9.

24. Hall AR SP, Morgan AD, Buckling A. . Host-parasite coevolutionary arms races give way to fluctuating selection. *Ecol Lett*. 2011;14:635–42.

25. Heilmann S SK, Krishna S. . Sustainability of virulence in a phage-bacterial ecosystem. *J Virol*. 2010;84(3016-3022).

26. Chapman-McQuiston E WX. Stochastic receptor expression allows sensitive bacteria to evade phage attack. Part I Experiments *Bio-phys J*. 2008;94:4525-2536.

27. Nina Molin Høyland-Kroghsbo RBM, Sine Lo Svenningsen. A Quorum-Sensing-Induced Bacteriophage Defense Mechanism. *mbio*. 2013;4(1):362-70.

28. Solomon JM LB, Grossman AD. Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in *Bacillus subtilis*. *Genes Dev*. 1996;10:2014 –24.

29. Leung V LC. A stress-inducible quorum-sensing peptide mediates the formation of noninherited multidrug tolerance. *J Bacteriol*. 2012;194:2265–74.

30. Huff WE, Huff, G. R., Rath, N. C., Balog, J. M. & Donoghue, A. M. Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray. *Poult Sci*. 2002;81:1486–91.

31. Lau GL, Sieo CC, Tan WS, Ho YW. Characteristics of a phage effective for colibacillosis control in poultry. *Journal of the Science of Food and Agriculture*. 2012 Oct;92(13):2657-63.

32. Hooton SPT, Atterbury RJ, Connerton IF. Application of a bacteriophage cocktail to reduce *Salmonella Typhimurium* U288 contamination on pig skin. *International Journal of Food Microbiology*. 2011 Dec;151(2):157-63.

33. Smith HW, Huggins, M. B. . Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *J Gen Microbiol*. 1983;129:2659–75.

34. Smith HW, Huggins, M. B. & Shaw, K. M. The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *J Gen Microbiol* 1987;133:1111-26.

35. Barrow P, Lovell, M. & Berchieri, A., Jr. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. *Clin Diagn Lab Immunol*. 1998 5:294–8.

36. Fischetti et al. Use of bacterial phage associated lysing enzymes for treating dermatological. US patent Aug13, 2002.

37. Fischetti.V LL. Chewing gum containing phage associated lytic enzymes for treating streptococcal infections. US patent; Feb3,2004.

38. Andre´ M. Comeau. Phage-Antibiotic Synergy (PAS): b -Lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. *Plos one*. 2007;8:799-803.

39. Miller C TL, Gaggero C, Mosseri R, Ingmer H, et al. SOS response induction by b-lactams and bacterial defense against antibiotic lethality. *Science*. 2004;305(1629-1631).

40. S´ Lopek S. K-KA, Weber-DA, Brownska B. and DA, Brownski M. . Results of bacteriophage treatment of suppurative bacterial infections VI. Analysis of treatment of suppurative staphylococcal infections. *Arch Immunol Ther Exp*. 1985;33:261-73.

41. Krylov V, Shaburova O, Krylov S, Pleteneva E. A Genetic Approach to the Development of New Therapeutic Phages to Fight *Pseudomonas Aeruginosa* in Wound Infections. *Viruses-Basel*. 2013 Jan;5(1):15-53.

42. Stone R. Bacteriophage therapy: Stalin's forgotten cure. *Science*. 2002;298:728-31.

43. Weber-Dabrowska BD, M.; Slopek, S. . Studies on bacteriophage penetration in patients subjected to phage therapy. *Arch Immunol Ther Exp (Warsz)*. 1987;5:563-8.

44. Morello ES, E.; Maura, D.; Huerre, M.; Touqui, L.; Debarbieux, L. . Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards

treatment and prevention. *PLoS One*. 2011;6.

45. Gu JM, Liu XH, Li Y, Han WY, Lei LC, Yang YJ, et al. A Method for Generation Phage

Cocktail with Great Therapeutic Potential. Plos One. 2012 Mar;7(3).

46. Sulakvelidze. A AZ, Glenn. J, Morris Jr. Bacteriophage Therapy. Antimicrob Agents Chemother. 2001;45(3):649-59.

47. Sulakvelidze AK, E. Bacteriophage therapy in humans. In Bacteriophages – Biology and Application. FL: CRC Press. 2005:381-436.

48. Brussow H. Phage therapy: the Escherichia coli experience. Microbiology 2005;151:2133–40.