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Review

Status of Bacteriophage Genetic Modifications: a Review

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Abstract

Engineering is the branch that covers many areas leading to a discipline termed as synthetic biology. The researchers made possible engineering at genetic level of bacteriophages which are well known for killing bacteria although they develop resistance against bacteriophages resulting into challenges in disease control programs. The genetic alterations give new characters to the phage which may be helpful to bring vital changes in phage science. In this review, we have investigated the genetic modification of bacteriophages with its applications.

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1. Introduction:

The viruses that are categorized as plant viruses, animal viruses, and bacterial viruses have their ecological functions in a nature. The bacterial viruses also called as bacteriophages are enemies of bacteria. However, these can be used to kill pathogenic bacteria and help to control health disasters including pandemics. Nevertheless, inconsistent results and poorly controlled trials were responsible for generation of contrasting opinions among scientists regarding reproducibility as well as efficacy of use of phages against bacterial infections [1,2,3]. Bacteriophages [4,5] are useful as antimicrobials, and this concept is known as phage therapy, which is gaining high interest [6,7,8,9,10,11,12,13,14,15]. The bacteriophages are not affected by drug resistant process since they are orthogonal to antibiotics functionally and are therefore, promising to treat infections [16].

Interestingly, the condition where antibiotic treatment gets failed, the phage therapy works successfully [17,18] showing value of bacteriophages in bacterial disease control operations. The phages of bacteria were used as antimicrobial drugs (natural) for controlling infections caused by bacteria [19]. Phages received importance as antibacterial entities [20,21,22].

The infections by bacteria in humans have been treated by bacteriophages, especially, in Eastern Europe [23,24,25]. The applications of phages as agents that are used against bacteria are increasing [19]. The phage therapy received the first approval by Food and Drugs

Administration (FDA) in 2019 [26]. The phages can be evolved efficiently to target specific bacteria as well as can be used to treat complex infections of bacteria which are drug resistant. The antibacterial drugs that we use are harmful to body and may cause severe illness when consumed in excess or without prescription of medical expert.

Additionally, long term administration of these agents may cause failure of vital organs such as liver and kidney which may be chronic or acute. Such situation, ultimately, reduces life span of people at global level. In turn, the biological agents that control growth and reproduction of pathogenic bacteria can be recommended to be used. Bacteriophages are, however, difficult to isolate and maintain in human supervision, and ultimately, to get rid of bacterial diseases. Phage genetic medication can provide a platform for their study and as a result, new tools required for bacterial manipulation can be developed [27]. In the history, development of bacteriophage mutations was achieved with common mutagenesis with the help of either chemical compounds or UV irradiations which may damage DNA [27]. In this review, we have investigated the status of genetic modification of bacteriophages and its various applications.

2. Present status:

The appearance of the synthetic biology period coupled with the extraordinary phage diversity resulted into potential uses for material sciences, diagnostics and therapeutics [26]. Furthermore, the arrival of new genetic engineering technologies resulted into more accurate and enhanced modification of genome of phage for both engineering and basic sciences [26] although the research of phages was still in its infancy [26]. Unfortunately, engineering of phages included only a less percentage of available phage types [26]. Many methodologies of phage engineering need the capacity to genetically modify bacterial hosts of them, which was challenging regarding several species of bacteria [26].

In turn, new tools of genetic manipulation were required [26]. To continue, introduction of many genetic alterations in the phage genomes are possible with precise location as well as high efficiency [26]. Besides, genetically modified phages may have valuable advantages in the diagnosis and treatment of infections of bacteria, to cure diseases which are nonbacterial and to construct new materials [26]. However, for real world applications, the approval of phages that are genetically modified may differ across various areas of the globe [26]. Engineering of phages is an area which is attracting considerable interest as well as immense potential usefulness even if it has yet to be completely exploited.

Moreover, the fields of genetic engineering along with synthetic biology have been advanced by bacteriophages by providing many tools [28]. As well, new strategies of genome engineering have the capacity to fasten the designing of new phages as tools, diagnostics and therapies [28] which may bring potential changes in phage engineering. The engineering has made possible to kill pathogenic bacteria after detecting it [29]. Besides, bacteriophages as well as components which were functional obtained from genomes of them were potent tools for longer duration allowing the knowledge of biological processes that were basic, and that enlightened the molecular biology field [28].

Fortunately, the current attempts resulted into a long step in engineering of phages with the improved properties presenting completely novel functions along with derivation of areas that were repurposed, for the investigation, detection in addition to treating diseases which were infectious in nature [28]. Interestingly, as our capability of phage engineering by synthesis of genome along with modification continues improvement, we may promote further these evolution products constituting the most frequent factors of biology familiar to man [28].

Besides, the phages have host selectivity and infection which is dependent on its attachment to the receptors of cell surface of the host [30]. However, a cocktail mixture of various phages and studying their immunogenic properties and pharmacodynamics are complicated resulting into limitations in their progress as drugs against microbes [31].

Conversely, bacteria develop resistance against phages. Different approaches have been investigated to help phages to fight against bacterial resistance [32,33,34,35,36,37,38,39,40,41]. Bacteria may develop resistant capacity against phages [42]. The resistance to antibiotics has arrived with constant increasing frequency [43,44,45,46].

Additionally, tail fibers of phage, antibodies are appreciably selective to target antigen of them [47,48]. The engineering of catalytic activity of capsule degradation is possible [49,50]. Moreover, engineered capsids of phage can be used for delivering therapeutic payloads inside the bacteria e.g CRISPR- Cas nucleases [6,8]. The components of viral tails are resolved structurally and also sequenced [51,52,53,54]. By the help of European Directive 2009/41/EC the assessment of risk for applications of bacteriophages that are genetically modified may be studied [19].

To add, it is possible to engineer phages to increase their range of host, tropism of cell or expression of antibacterial toxins in order to control infections caused by bacteria [19]. Additionally, phages that are used in applications in the field of research are generally viruses that are engineered genetically that still infect bacteria [19]. Whenever, phages are manipulated in the laboratories, the potential risk factors associated with it should be assessed [19]. The proper strategies such as enough disinfection as well as inactivation of phages are required to avoid accidental release of manipulated bacteriophages [19].

3. Methods:

3.1. Homologous recombination:

Homologous recombination is a method for engineering of genomes of phages in their hosts (bacterial) that occurs between two DNA sequences which are homologous and are short (23 bp) [55,56]. By using this mechanism, the foreign gene can be incorporated into the genome of a phage [26]. Moreover, the gene which is to be introduced in the genome of phage is cloned in the replicative plasmid and is flanked with two areas of homology with the genome of phage [26].

Likewise, the homology regions help to decide the place where the foreign gene may be incorporated in the genome of phage [57]. The bacteria that possess the plasmid which is donor are infected with the phage in which engineering has to be carried out [57].Between the phage genome and plasmid, the recombination which is homologous takes place that allows the gene (heterologous) to be incorporated in the genome of phage and in turn, packaged within bacteriophage particle [58].

3.2. Bacteriophage Recombineering of Electroporated DNA:

Use of homologous recombination for engineering of recombinant DNA is termed as 'recombioneering' [59,60]. It is a method of phage genome engineering [27,61] which is used to insert, delete, create point mutations and gene replacing in phage genomes [26]. As well, it may be used so as to target the chromosome of bacteria or replicating molecules which are extra chromosomal [27]. Additionally, this method consists coelectroporating of recombineering substrates into bacterial cells that are electro competent carrying the plasmid able to encode proteins that promote homologous recombination at high levels e.g RecE/RecT like proteins [27,61]. In this method, the genetically modified bacteriophages are obtained with increased frequencies (10 to 15%) [61].

3.3. *In Vivo* Recombineering:

In the presented method, the cells of *E.coli* carrying lambda prophage which is defective in addition with *PI* operon are processed through infection with phage which is targeted for engineering with MOI (of 1 to 3) in addition to allowing the process of absorption with 15 minutes duration [26]. The functions of lambda Red combination are induced with heating the bacterial culture in the log phase (mid) to 42°C [26], and this is the point where electroporation of the cells occur with either dsDNA or ssDNA [62,63]. Furthermore, the phase lysate is then checked for successful incorporation of the DNA of interest [63].

This technique may be considerably adjusted to other bacterial species and other bacteriophages through introduction of the labmbda Red system through plasmid or any other recombination machinery in the bacteria

which is host which may be targeted by bacteriophage that has to be passed through the process of engineering [26]. In fact, recombioneering is a robust technique which uses homologous recombination for introducing modifications that are highly targeted, deletions or insertions to loci within the cells [28]. Similarly, a concept of homologous recombination was used to make deletions in gene or recombinant phages [64,65]. However, low frequency of it persists and screening is required to get the desired mutant which is time consuming and tedious task [27].

Recombioneering is a method that used recombinations encoded by bacteriophages in order to accelerate homologous recombination frequency that permits knock out chromosomal gene construction, insertions, deletions, *in-vivo* cloning, artificial chromosomes mutagenesis of bacteria, libraries of genome as well as phasmids [59,60,66,67,68,69,70,71,72,73,74].The whole genome sequencing has reached at its greatest landmarks. Since bacterial genome sequences are available in increased amount, the application of recombioneering has been facilitated as recombioneering of organism may be possible only when its sequence is known [27].The recombination system regulated expression of bacteriophage is characteristically demanded by recombioneering [27].

One easy approach for engineering of phage genome is use of dsDNA recombioneering for modifying the prophage which is integrated, sufficiently treating it as additional (any) locus of chromosome [27]. Further, mycobacterial recombioneering may be used with the intention of manipulation of prophage that is incorporated into the chromosome of host in a way that is alike to that mentioned for the lambda manipulation [27]. Fortunately, recombioneering of bacteriophages is appreciably effective with the purpose of construction of deletions of gene, gene replacements, insertions as well as point mutations in the lytic phages [61,63]. Growing attention in the use of proteins of phage which can support functions of recombination was especially driven through the number of available sequences of genome of bacteriophage [75].

To add, this technique may allow the performance of studies on broad functional genomic investigations of bacteriophages, substantially showing new as well as exciting characters of the varied population of phages [27]. Phages of *E.coli* and mycobacteriophages can be modified, and a bacteriophage which is isolated newly may be a promising source to get new recombination proteins [27]. To achieve this goal, genetic level manipulation of phages might be very useful as well as recombioneering may become the perfect way for the same [27]. In this way, the bacteriophage recombioneering will facilitate the investigation of genes that are orphan and offer vision into biology of bacteriophages along with assistance to reveal novel and valuable instruments required to manipulate the naive bacteria genetically [27].

3.4. Genome Engineering:

Phage derived technologies and enzymes resulted into the engineering techniques of genome level are important for the tailoring of specific applications [28].

3.5. Antimicrobial Phages:

Some engineering attempts were made towards addition of functions or improvements in existing phages [28]. Bacterial populations may emerge with mechanisms against phages to decrease their adsorption [42] which are solved by application of phage cocktails [76]. The engineered phagemids were constructed for encoding the addiction toxins Gef as well as ChpBK to bring out devastation of cells which are targets [28]. Hagens and Bläsi [77] used this toxic play load theme by means of M13 for delivering genes that encode the restriction enzymes either BglII or the λ *S* holin for killing *E.coli* that acted as target with help of double stranded breaks introduction in chromosome or the generation of lesions of cytoplasmic membrane, respectively.

As a matter of fact, biotechnology companies used methods of recombinant bacteriophage viruses which deliver genes that encode small, protein which was soluble in acid in order to create toxicity for targeting cells by the attachment which was not specific to DNA [78]. The detectors based on engineered bacteriophages have the

benefit of fast readout, high sensitivity along with specificity as well as detection of live cells [79]. Designs of sensor includes genetically engineered phage which show a product responsible for nucleation of ice [80], or which incorporate tags to link detectable elements e.g quantum dots [81].

3.6. New phage engineering strategies:

Genomes of many bacteriophages have relatively large size and inherent toxicity of them to bacterial hosts confused the application of techniques of conventional molecular biology for engineering [28]. In contrast, tools of the current synthetic biology have revived the potential to bring rational modifications or additions to the bacteriophage genomes [28]. Further, in performance with *in vivo* as well as *in vitro* recombination, the current technology of DNA synthesis, there is a permission of de novo chemical synthesis of genomes of bacteriophage [28].

For example, Smith et al. [82] used this concept in order to synthesize, clone along with production of infectious particles of the phage ΦX174 with the genome size 5386 bp. Similar scaled-up concept got credit of creating the first cell of bacteria having 1.1 Mb synthetic genome [83]. Synthetic biology has ability to promote accurate studies of underlying biology of bacteriophage by making them genetically accessible and also it inspires synthesis of novel therapeutic agents [28].

3.7. Engineering of genome brought by CRISPER-Cas:

The CRISPER-Cas systems have two components viz. the Cas proteins (act as the catalytic core that cuts DNA) and CRISPER locus (genetic memory directing catalytic activity against the foreign DNA) [85]. Kiro et al. [86] developed a methodology to accelerate the phage T7 genome engineering with help of the type I-E CRISPER-Cas system. Furthermore, in a bacterial host, the T7 phage was propagated harboring a plasmid that carries homology regions to both the upstream as well as downstream areas of phage gene 1.7 in a way that the gene is deleted by recombination [26].

3.8. *In Vitro* Phage Genome Rebuilding:

The genomes of phage may be altered manually as well as edited in lab prior to their introduction into their bacterial hosts [26]. Chan et al. [87] redesigned T7 phage genome through eliminating overlaps between the segments (73 grouped into 6 sections) of gene in the process what is called as refactoring. Additionally, with the help of molecular cloning, the genomes that were altered were assembled in vitro from these sections [26]. Chan et al. [87] preferred to build 3 chimeric T7 genomes of phage which were made up from various engineered sections after which, viable phages got generated out of these refracted genomes with the help of transferring the bacterial as a host with the engineered phage genomes.

3.9. Synthesis of complete genome along with pack from oligonucleotides which are synthetic:

In vitro, the whole phage genomes may be gathered from the man-made oligonucleotides [26]. With the help of polymerase cycling assembly (PCA), the oligonucleotides that are synthesized were purified using gel, phosphorylated, annealed and assembled in vitro [26]. Furthermore, with the help of PCR, the amplification of full length was carried out, digested with help of restriction enzyme, purified using gel, and circularized by ligation [26]. Then, the ϕX174 genome which was assembled was electroporated in *E. coli* which was followed with plating in order to verify the plaques of bacteriophages [26].

4. Assembly of genomes of bacteriophage using yeast:

Saccharomyces cerevisiae is used as an intermediary host to achieve the manual genetic changes [26]. The genomes of phages are assembled, modified as well as propagated in the yeast and can be isolated and afterwards, introduced in the bacteria in order to generate the phage particles which are functional [88].

4.1. Transcription and translation system without cell:

The genomes of phage may be engineered in a manner that doesn't result into toxicity to host in the case where yeast based or *in vitro* genome modification occurs [26]. There is a bottleneck in both efficiency and throughput of yeast based and *in vitro* systems for the genetic engineering of phage [26].

4.2. Engineering of bacteriophages for controlling disease causing agents:

4.2.1. Bacteriophage dependent natural antimicrobials:

The capacity of lytic bacteriophages of acting against microorganisms that exist in nature that include multiple drug resistant bacteria was investigated in both in vitro [89,90,91,92] as well as in vivo [93,94,95,96,97,98,99]. Different bacteriophages exist in the nature. Their diversity is obtained by genome rearrangements, point mutations as well as the genetic material exchange with other bacteria or phage particles [100]. Additionally, the modified phages which overcome the resistance mechanisms in bacteria can be developed by engineering, mutagenizing, and screening new phages in high-throughput way [26]. In fact, Phago burn is looking for therapies that involve engineered phages [26].

4.2.2. Changing bacteriophages to achieve increased activity against bacteria:

It is possible to engineer the phages for their applications in association with other antimicrobial strategies [26]. For instance, modification of phage can be performed to improve bactericidal activities of antibiotics [101]. Moreover, in order to increase the activity of antibiotics, phages were being engineered as agents that were used against microbes which were extra efficient than related natural bacteriophages for fighting against infections by bacterial [26]. In order to inhibit *C. trachomatis,* the phage under study was engineered for easy endocytosis by eukaryotic cells [26].

4.3. Engineering of bacteriophages with altered hosts:

Yoichi et al. [102] altered the T2 bacteriophage genetically with gene swapping of fiber of tail (long) (*gp*37 and *gp*38) from PP01 bacteriophage that particularly targets *E.coli* O157:H7. In fact, *E. coli* gets infected by the coli phage fd generally but it was passed through the process engineering to detect *Vibrio cholera* [103]. Phage cocktails (synthetic) made up from the bacteriophages having the same scaffold, however, variant components of tail may be applied for targeting mixed populations of bacteria and in order to selectively eliminate particular bacterial species from them [104].

4.4. Engineered bacteriophages accompanying less influence on mammals:

Treatment with the help of lytic phages has potential to cause higher bacterial lysis and consequent release of components of bacteria along with toxins leading to triggering the immune response [105,106]. To overcome this error, the phages were engineered as lysis deficient or non replicative mutants [26]. To continue, in the attempt to change lytic phage into non-lytic phage, P954, a *Staphylococcus aureus* temperate bacteriophage was modified with the help of homologous recombination for inactivating coding of gene for endolysin that causes lysis of the bacterial cell [107].

4.5. A process to engineer bacteriophage for creating agents against microorganisms which are DNA sequence specific:

There are chances that the bacteriophages may be processed via engineering in order to cause bacterial death based on genetic makeup of them leading to more accurate antibacterial activity [26].Likewise, Bikard et al. [6] and Citorik et al. [8] studied development of antimicrobials of which, range of action may be programmed against sequences of DNA which are specific, with ability to kill purely those bacteria that have targeted DNA, in a way that the DNA that encodes virulence or resistance to antibiotics.

4.6. A science of bacteriophage engineering to detect and diagnose bacteria:

Interestingly, bacteriophage engineering is useful in bacterial detection and diagnostics. To support this idea, Loessner et al. [108] proposed easy, rapid as well as sensitive method with help of engineered bacteriophage for detecting the presence of *Listeria monocytogenes* in the food which is contaminated. In addition, the mycobacteriophages which were engineered identified *Mycobacterium tuberculosis* by means of passing the reporter genes in the cells [109].

4.7. Delivery of drugs by bacteriophage engineering:

The phages may be adapted to deliver the drug at targeted site in not only prokaryotes but also eukaryotes along with cancer cells [26].

4.8. Delivery of drugs to treat cancer:

For cancer therapy, the drug delivery system which is dependent on application of both genetically modified as well as chemically altered Fuse5-ZZ bacteriopahges (filament like) were studied [110].

4.9. Antibody delivery:

The filamentous phages were engineered in order to mediate delivery of antibody to the brain for detecting Alzheimer's disease [111].

5. Phages for vaccine development:

Phage engineering has vital role in the development of vaccines. With the help of it for displaying different antigens on surfaces of phages, vaccines may be produced which are comfortable to veterinary or medical requirements such as outbreaks that are new [26]. Likewise, multiple antigens may be included in an effective HIV vaccine, and should produce widely neutralizing antibodies [112,113]. The recombinant FMDV-T4 vaccine was found as efficient in the assays of mouse as a model providing 100% guarding opposition to FMDVO serotype subsequent to subcutaneous or oral immunization [114].

5.1. Phage engineering for material science:

With combination of techniques of genetic engineering and phage display, phages were used to construct novel nano structured materials which were having different applications including energy generation as well as storage [115,116,117], tissue regeneration [118,119,120] and biosensing [121,122,123]. Further, the M13 phages which were engineered genetically have been made for assembling and arranging quantum dots [124], building of not only films but also liquid crystals [125,126,127], as well as fabricating nanorings [128], and micro as well as nanofibers [129]. For displaying peptides that bind to gold along with attraction for ions of cobalt on its protein present on the coat which are major, M13 phage was engineered first time [130].

5.2. Genomic stability with refactoring:

The stability with respect to evolution of engineered T7 phage for infecting encapsulated *E.coli* through production of a endosialidase that degrade capsule which acts as exoenzyme was studied *in vitro* [84]. Even if the phages which are engineered permitted replication in strain which was encapsulated, advantage provided through endosialidase synthesis was shared by means of wild type and non-producing bacteriophages which were cheater, that could speedily surpass the viruses in a competitive situation that are engineered owing to their higher fitness [28]. Though these investigations indicate fragility of ongoing efforts of synthetic biology, systems based on bacteriophages can be excellent platform in order to know the constraints presented on the man-made genetic circuits through evolution and inform designs of future [28].

6. Future Perspective:

The parts of bacteriophage can be initially constructed including head, collar, tail, tail fiber, and spikes artificially by use of genetic engineering and protein synthesis technologies available in the world.

7. Conclusion:

The variant bacteriophages can be constructed in the laboratory by genetic engineering. We could engineer pahge with respect to genetics but not able to artificially produce whole assembly of it in the lab.

Authors' contributions:

SK: Developed an idea. BS: Wrote manuscript. RK: verified the data.

Competing interest:

Authors declare that no competing interest exists among them.

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Abbreviations: dsDNA-Double stranded DNA, ssDNA-Single stranded DNA, MOI-Multiplicity of Infection, CRISPR: Clustered regularly. interspaced short palindromic repeats.