Article ID 240203009

Techniques for Engineering Synthetic Phages

J. Sai Prasad[1], P. Jayamma[2], S. Nagalakshmi[3] and R. Aruna[4]

[1]Assistant professor, Department of Food Safety and Quality Assurance, College of Food Science and Technology, Rudrur, Niambad, Telangana

[2]Assistant professor, Department of Food Safety and Quality Assurance, College of Food Science and Technology, ANGRAU, Pulivendula, A.P.

[3]Assistant professor (Biotechnology), Assistant professor, Dr. Y.S.R.H.U, College of Horticulture, parvathipuram,

[4]Assistant professor, Department of Food Safety and Quality Assurance, College of Food Science and Technology, ANGRAU, Pulivendula, A.P.

*Corresponding Author: p.jayamma@angrau.ac.in

The specificity of phages means that they can target bacterial strains precisely; however, because a single phage type is unlikely to target all strains within a given species, cocktails combining various phages are often necessary to be broadly applicable for treating the wide range of bacteria that can cause clinical infections. Obtaining regulatory approval for the therapeutic applications of such cocktails can be challenging because of the significant diversity of phages in terms of structure, life cycle, and genome organization. Like certain antibiotics, phages can cause rapid and massive bacterial lysis and the subsequent release of cell wall components (e.g., lipopolysaccharides [LPS]), which can induce adverse immune responses in the human host. Bacteria frequently live in biofilm communities surrounded by extracellular polymeric substances (EPS), which can act as a barrier to phage penetration. Furthermore, as bacteria evolve, they can develop resistance mechanisms to avoid phage infection. By genetically engineering phages, it may be possible to overcome many of these limitations.

Techniques for Engineering Synthetic Phages

Homologous Recombination

One of the most commonly used and well-established methods for engineering phage genomes is homologous recombination in their bacterial hosts, which can occur between two homologous DNA sequences as short as 23 bp.

Homologous recombination is a naturally occurring phenomenon. It enables cells to recombine heterologous DNA introduced into cells with their own genomic DNA when both sequences share regions of homology. This mechanism can also be co-opted to incorporate foreign genes into a phage genome.

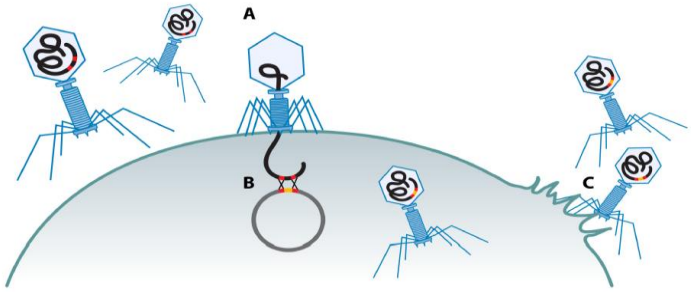

Fig. 1 Homologous Recombination

Bacteriophage Recombineering of Electroporated DNA

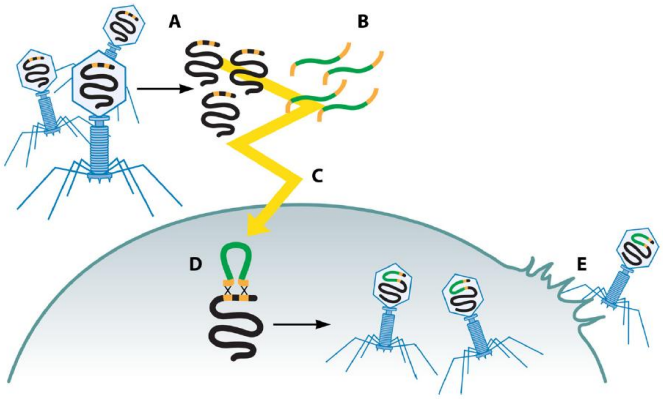

Fig. 2 Bacteriophage Recombineering of Electroporated DNA

Another frequently used strategy for the engineering of phage genomes is bacteriophage recombineering of electroporated DNA (BRED). This technique was first applied by Marinelli *et al.* to modify mycobacteriophages and has since been expanded to modify phages that target bacterial hosts other than mycobacteria for which recombineering systems are available, such as *Escherichia coli* and *Salmonella enterica*. BRED can be used to delete, insert, and replace genes, as well as to create point mutations in phage genomes. It consists of coelectroporating the

recombineering substrates, i.e., phage DNA and double stranded DNA (dsDNA), into electrocompetent bacterial cells carrying a plasmid that encodes proteins promoting high levels of homologous recombination, such as the RecE/RecT-like proteins.

In Vivo Recombineering

The *in vivo* recombineering method uses phage _ as a tool for the engineering of other, less well-studied *E. coli* phages. Briefly, *E. coli* cells carrying a defective prophage and the *pL* operon are infected with the phage to be engineered at a multiplicity of infection (MOI) of 1 to 3 and allowed to adsorb for 15 min. The *pL* operon, which is involved in general and site-specific recombination, is under the control of a temperature-sensitive repressor.

Following phage infection, the red recombination functions are induced by heating the mid-log-phase bacterial culture to 42°C. At this point, the cells are electroporated with the dsDNA or single-stranded DNA (ssDNA) substrate.

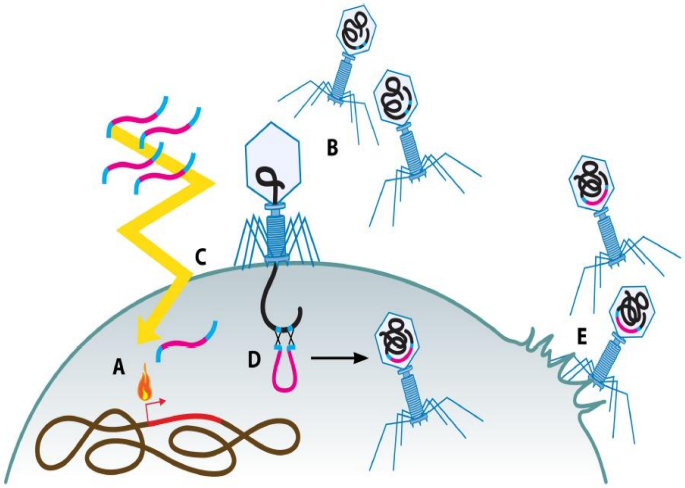

Fig.3 *In Vivo* Recombineering

CRISPR-Cas-Mediated Genome Engineering

Clustered regularly interspaced short palindromic repeats (CRISPR) in combination with *cas* (CRISPR-associated) genes form an "adaptive" immune system in bacteria and archaea, protecting microbial cells from invading foreign DNA, such as DNA delivered by invading phage genomes.

The CRISPR-Cas systems consist of two main components: the Cas proteins, which work as the catalytic core of the system and are responsible for cleaving DNA, and the CRISPR locus, which functions as the genetic memory that directs catalytic activity against foreign DNA. CRISPR loci are typically composed of several noncontiguous direct repeats separated by short stretches of variable DNA sequences, called spacers, acquired from extrachromosomal elements. CRISPR-Cas systems are currently divided into three major types (I, II, and III) characterized by distinct sets of *cas* genes, with a further division into several subtypes.

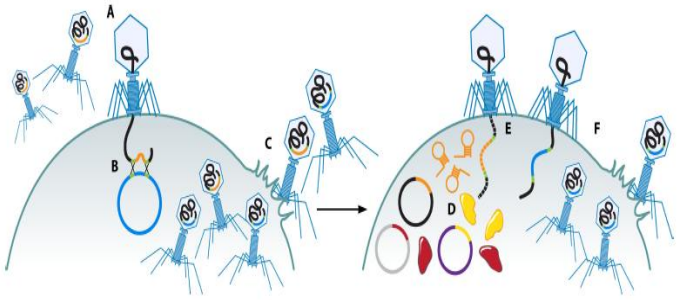

FIG 4 CRISPR-Cas-mediated phage engineering. Upon phage infection, homologous recombination occurs between phage DNA (A) and plasmid DNA (B), such that a phage gene (in orange) is deleted. The resulting phage population is mixed (phages containing fragments in blue or orange) (C), but by using the CRISPR-Cas system (single guide RNA [sgRNA] is shown in orange and Cas proteins in red and yellow, encoded on separate plasmids) to target the gene retained in the wild-type particles (D), it is possible to counterselect the wild-type phage population (fragment in orange in genome) (E) and to retain the recombinant version (phage containing the blue-colored fragment) (F).

Fig. 4 CRISPR-Cas-Mediated Genome Engineering

The mode of action of CRISPR-Cas systems comprises three main processes, namely, CRISPR adaptation, RNA biogenesis, and CRISPR-Cas interference, which are further reviewed by Westra *et al.* and Makarova *et al.* Recently, Kiro *et al.* described a method to enhance the engineering of the T7 phage genome by using the type I-E CRISPR-Cas system.

Whole-Genome Synthesis and Assembly from Synthetic Oligonucleotides

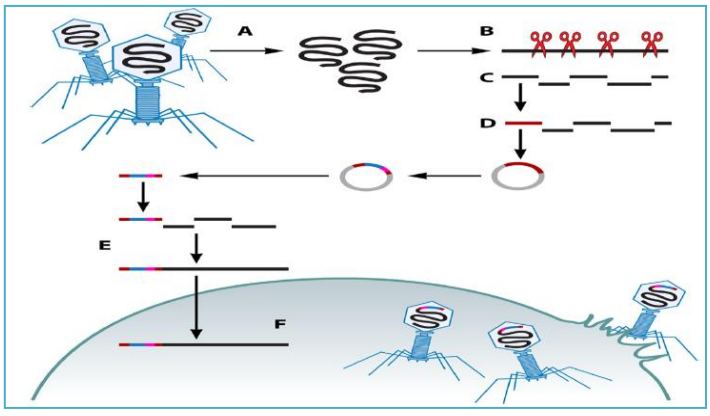

Fig. 5. Whole-Genome Synthesis and Assembly from Synthetic Oligonucleotides

Complete phage genomes can also be assembled from synthetic oligonucleotides *in vitro*. The synthesized oligonucleotides were gel purified, phosphorylated, annealed, and assembled *in vitro* by polymerase cycling assembly (PCA).

The full-length genome was amplified by PCR, digested with a restriction enzyme, gel purified, and circularized by ligation. The assembled genome was then electroporated into *E. coli*, followed by plating to check for phage plaques.

References

Briers, Y., Walmagh, M., Puyenbroeck, V., V., Cornelissen, A., Cenens, W., Aertsen, A., Oliveira, H., Azeredo, J., Verween, G., Pirnay, J. P., Miller, S., Volckaert, G. and Lavigne, R., 2014, Engineered endolysin-based "Artilysins" to combat multidrug-resistant Gram-negative pathogens. *mBio.,* 5(4): e01379-14.

Pires, D. P., Cleto, S., Sillankorva, S., Azeredo, J. and Lu, T. K., 2016, Genetically engineered phages: a review of advances over the last decade. *Microbiol. Mol. Biol. Rev.,* 80: 523–543.

* * * * * * * *