fermentation. This approach can be used for other diverse bacteriophages to develop new NRTPs with desired inclusivity and exclusivity profiles with respect to host-range specificities.

SUMMARY

The present disclosure describes a bacteriophage lysogen genetically disrupted to the expression of one or more factors critical to native tail fiber production (examples -genes *s*, *u*, *u*', *r* and *s*'). This could be accomplished through any mutagenesis approach known to one skilled in the art, or through isolation of a tail fiber deficient phage remnant from natural sources. This genetically disrupted phage lysogen that lacks the ability to express functional tail fibers of its own without complementation is referred as a chassis or a "Bald" chassis. In one example, the disrupted lysogen has a mutation or a deletion in one or more of the genes selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes In another example, the disrupted lysogen contains a gene coding for a selectable marker that disrupts the expression of one or more of the genes. In yet another example, the disrupted lysogen comprises a nucleotide sequence of SEQ ID NO: 97.

Also disclosed herein are methods involving the expression of native, new tail fiber genes, or hybrid tail fibers in a lysogen genetically disrupted for native tail fiber expression. This could be accomplished using a packaging plasmid, secondary expression plasmid, genomic expression (phage lysogen or bacterial genome), or any other method known to one skilled in the art.

Therefore, in one aspect, the present disclosure refers to a bacteriophage tail fiber replacement platform comprising a lysogen from the family Myoviridae derived from the Punalikevirus genus (Myoviridae lysogen) that contains a genetic disruption that prevents the expression of one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen, wherein said one or more genes are selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes; and a complementary nucleic acid molecule comprising one or more genes selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes, wherein the complementary nucleic acid complements the genetic disruption of the Myoviridae lysogen whereby functional tail fibers are produced, wherein the one or more genes or the combination of genes on the complementary nucleic acid molecule

contain regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen such that the functional tail fibers that are produced are not native tail fibers.

In one example, the Myoviridae lysogen is derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one example, the Myoviridae lysogen is derived from the Punalikevirus genus. In another example, the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

In one example, the one or more genes or the combination of genes on the complementary nucleic acid molecule contain regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen such that the functional tail fibers that are produced are not native tail fibers. In such examples, the tail fibers that are produced are functional tail fibers that are not native to the initial bacteriophage lysogen. In another example, the one or more genes or the combination of genes contain regions that are not derived from P1 bacteriophage such that the functional tail fibers that are produced are not native P1 bacteriophage tail fibers.

In yet another example, the genetic disruption on the Myoviridae lysogen comprises one or more mutation in any of the one or more genes. In some examples, the Myoviridae lysogen may comprise one or more mutation in one of the one or more genes. In other examples, the Myoviridae lysogen may comprise one or more mutation in at least two of the one or more genes. In one example, the one or more mutation is in any one of the P1 bacteriophage genes s, s', u, u' and r, in any combination of genes s, s', u, u' and r, or homologs, orthologs, or analogs of genes s, s', u, u' and r.

In one example, the genetic disruption on the Myoviridae lysogen comprises one or more deletion in any one of the one or more genes. In some examples, the Myoviridae lysogen may comprise one or more deletion in one of the one or more genes. In other examples, the Myoviridae lysogen may comprise one or more deletion in at least two of the one or more genes. In certain examples, at least one deletion is a deletion of a complete coding sequence of one of the one or more genes. In other examples, any of the one or more deletions is a deletion of a complete coding sequence of one of the one or more genes. In one example, the one or more deletion is in any one of the P1 bacteriophage genes s, s', u, u' and r, in any combination of genes s, s', u, u' and r, or homologs, orthologs, or analogs of genes s, s', u, u' and r. In another example, the genetic disruption on the Myoviridae lysogen further comprises the insertion of a gene coding for a selectable marker that disrupts the expression of any one of the P1 bacteriophage genes. In one example, the Myoviridae lysogen comprises a nucleic acid sequence of SEQ ID NO: 97.

In one example, the complementary nucleic acid molecule is a plasmid molecule. In another example, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption. In yet another example, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption. In yet another example, the complementary nucleic acid molecule is integrated into a genome of a host bacteria cell that contains the Myoviridae lysogen.

In one example, the Myoviridae lysogen contains a further disruption in one or more packaging genes that are involved in packaging the genome of the Myoviridae lysogen. Thereby the one or more packaging genes that are involved in packaging the genome of the Myoviridae lysogen are rendered non-functional. In one example, the one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in the plasmid molecule. In another example, the one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in a reporter or therapeutic expression plasmid that is separate from the plasmid molecule.

In another aspect, the present disclosure also refers to a bacterial cell line comprising a lysogen derived from P1 bacteriophge that comprises a nucleic acid sequence of SEQ ID NO: 97.

In yet another aspect, the present disclosure further refers to a method for producing bacteriophage particles or non-replicating transduction particles (NRTPs) with non-native tail fibers comprising, providing a bacteria cell containing the bacteriophage tail fiber replacement platform as disclosed herein, wherein the complementary nucleic acid molecule comprises a gene that encodes a tail fiber structural protein with one or more regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen, and providing conditions to the bacterial cell that induces a lytic phase of the Myoviridae lysogen to produce bacteriophage particles with non-native tail fibers.

In one example, the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacteriophages within the order Caudovirales. In one example, the tail fiber variable region is obtained from bacteriophages within the family Myoviridae. In another example, the tail fiber variable region is obtained from bacteriophages within a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one example, the genus is Punalikevirus.

from a second tail fiber structural protein encoding gene from a different source to generate a chimeric tail fiber structural protein encoding gene; providing the chimeric tail fiber structural protein encoding gene as the complementary nucleic acid molecule in a bacteria cell; expressing the chimeric tail fiber structural protein gene in a bacteria cell that contains the bacteriophage tail fiber replacement platform as disclosed herein; and providing conditions to the bacteria cell that induces a lytic phase of the Myoviridae lysogen to generate the engineered bacteriophage or NRTP.

Also disclosed herein is a method of generating an engineered bacteriophage or a non-replicative transduction particle (NRTP) that displays a bacteria host cell specificity that differs from the bacteria host cell specificity displayed by a native bacteriophage comprising, fusing a first nucleotide sequence derived from one tail fiber structural protein encoding gene with a second nucleotide sequence derived from a second tail fiber structural protein encoding gene from a different source to generate a chimeric tail fiber structural protein encoding gene; and expressing the chimeric tail fiber structural protein gene in a bacteria cell that contains the bacteriophage tail fiber replacement platform of the present invention; and providing conditions to the bacteria cell that induces a lytic phase of the Myoviridae lysogen to generate the engineered bacteriophage or NRTP.

In one example, the Myoviridae lysogen is derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one example, the Myoviridae lysogen is derived from the Punalikevirus genus. In another example, the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

In one example, the first nucleotide sequence is derived from the Myoviridae lysogen that contains the genetic disruption. In another example, the first nucleotide sequence comprises the s_c region of tail fiber gene s from P1 bacteriophage. In yet another example, the first nucleotide sequence comprises a sequence with at least 90% sequence identity with the nucleotide sequence that encodes amino acid sequence 1-250 of SEQ ID NO: 53. In yet another example, the first nucleotide sequence comprises a sequence with at least 90% sequence identity with the nucleotide sequence that encodes amino acid sequence 1-500 of SEQ ID NO: 53. In one example, the first nucleotide sequences comprises the s_c region derived from plasmid p15B (as described in Ikeda et al., J. Mol. Biol., 1970, 50: 457-470) or from a P1-like type 11 bacteriophage in *Klebsiella pneumoniae* (*Kpn*).

In another aspect, the present disclosure refers to a method of generating engineered bacteriophages or non-replicative transduction particles (NRTPs) having multiple types of tail fibers from a single bacteria cell line, wherein at least one of the tail fiber type is a non-native tail fiber comprising, providing a bacteria cell containing the bacteriophage tail fiber replacement platform as disclosed herein, wherein the complementary nucleic acid molecule comprises at least two receptor binding

regions from tail fiber structural protein encoding genes that are fused to one or more tail interacting regions from tail fiber structural protein encoding genes, wherein at least one of the receptor binding regions is derived from a different source than the source of one of the tail interacting region; and providing conditions to the bacterial cell that induces a lytic phase of the lysogen to produce bacteriophages or NRTPs having multiple types of tail fibers.

In one example, the Myoviridae lysogen is derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one example, the Myoviridae lysogen is derived from the Punalikevirus genus. In another example, the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

In one example, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption. In another example, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption. In yet another example, the complementary nucleic acid molecule is integrated into the genome of the bacteria cell. In yet another example, the complementary nucleic acid molecule is integrated into a plasmid molecule.

In one example, fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase and in the presence of multiple cix recombination sites. In one example, fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase or a homolog, ortholog, or paralog of the cin recombinase and in the presence of multiple associated recombination sites. In one example, the two or more distinct tail fiber structural protein encoding genes are expressed in an operon. In another example, the two or more distinct tail fiber structural protein encoding genes are expressed from independent promoters. In yet another example, the two or more distinct tail fiber structural protein encoding genes are expressed from independent genomic locations.

In another example, the present disclosure describes a bacterial cell packaging system for packaging a reporter or therapeutic nucleic acid molecule into a non-replicative transduction particle (NRTP) for introduction into a cell, the packaging system comprising, a host bacteria cell; a bacteriophage lysogen that contains a genetic disruption that prevents the expression of one or more genes that are critical for the production of native tail fibers, wherein said one or more genes encode a tail fiber structural protein responsible for binding a receptor, a chaperone needed for folding of one or more regions of a tail fiber structural protein, a protein required for attaching the tail fiber structural protein to the tail structure, or any combination of these genes; and also comprising a first bacteriophage gene that contains a non-functional packaging initiation site sequence, wherein the non-functional packaging initiation site

In another aspect, the present disclosure refers to a method of generating Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs that are able to recognize and transduce bacteria cells for diagnostic or therapeutic use comprising, providing a bacteria cell containing the bacteriophage tail fiber replacement platform as described herein, wherein the complementary nucleic acid molecule comprises tail fiber genes non-native to the Myovridae lysogen and derived partially or entirely from either Myoviridae, Siphoviridae (as represented in SEQ ID Nos: 5, 10, 32, 33), or Podoviridae tail fiber sequences; and providing conditions to the bacterial cell that induces a lytic phase of the lysogen to produce Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs capable of recognizing and transducing bacteria previously refractory to a native Myoviridae bacteriophage or Myoviridae-family derived NRTP particles for diagnostic or therapeutic use.

In one example, the Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs are derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one example, the Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs are derived from the Punalikevirus genus. In another example, the Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

FIG. 1 is a diagram showing the tail-fiber swapping platform.

FIG. 2 Diagram of natural P1 tail fiber genomic region. Gene *r* encodes a tail fiber accessory protein putatively involved in attaching tail fibers to the tail structure. The *cin* gene encodes a sequence specific recombinase, which flips the DNA between the Cin recombination sites (called cix sites) enabling expression of two distinct tail fibers from a single piece of DNA, sites are denoted as circles in diagram. Genes *u* and *u*' encode distinct tail fiber accessory proteins, which are thought to act as chaperones to fold the binding domain of the S and S' tail fiber proteins, respectively (binding domains encoded by s_v and s_v' coding sequences, respectively). The conserved region of both S and S' tail fibers proteins are encoded by the s_c

CLAIMS

1. A bacteriophage tail fiber replacement platform comprising:
   - a lysogen from the family Myoviridae derived from the Punalikevirus genus (Myoviridae lysogen) that contains a genetic disruption that prevents the expression of one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen, wherein said one or more genes are selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes; and
   - a complementary nucleic acid molecule comprising one or more genes selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes, wherein the complementary nucleic acid complements the genetic disruption of the Myoviridae lysogen whereby functional tail fibers are produced,

   wherein the one or more genes or the combination of genes on the complementary nucleic acid molecule contain regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen such that the functional tail fibers that are produced are not native tail fibers.

2. The platform of claim 1, wherein the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

3. The platform of claim 2, wherein the one or more genes or the combination of genes contain regions that are not derived from P1 bacteriophage such that the functional tail fibers that are produced are not native P1 bacteriophage tail fibers.

4. The platform of any one of claims 1 to 3, wherein the complementary nucleic acid molecule is a plasmid molecule; or

   wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption; or

   wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption; or

wherein the complementary nucleic acid molecule is integrated into a genome of a host bacteria cell that contains the Myoviridae lysogen.

5. The platform of any one of claims 1 to 3, wherein the Myoviridae lysogen contains a further disruption in one or more packaging genes that are involved in packaging the genome of the Myoviridae lysogen.

6. The platform of claim 5, wherein the complementary nucleic acid molecule is a plasmid molecule and wherein one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in said plasmid molecule; or

wherein the complementary nucleic acid molecule is a plasmid molecule and wherein one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in a reporter or therapeutic expression plasmid that is separate from said plasmid molecule.

7. A bacterial cell line comprising a lysogen derived from P1 bacteriophage that comprises a nucleic acid sequence of SEQ ID NO: 97.

8. A method for producing bacteriophage particles with non-native tail fibers comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 6, wherein the complementary nucleic acid molecule comprises a gene that encodes a tail fiber structural protein with one or more regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen; and
- providing conditions to the bacterial cell that induces a lytic phase of the Myoviridae lysogen to produce bacteriophage particles with non-native tail fibers.

9. The method of claim 8, wherein the complementary nucleic acid molecule further comprises a gene that encodes a chaperone protein.

10. The method of claim 8, wherein the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacterial genomes encoding phage tail-like particles or structures or from nucleic acid delivery particles.

11. A method of producing non-replicative transduction particles (NRTPs) with non-native tail fibers comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 6, wherein the Myoviridae lysogen comprises a further disruption in one or more packaging genes that are involved in packaging the

Myoviridae lysogen genome, and wherein the complementary nucleic acid comprises a gene encoding the tail fiber structural protein that contains one or more regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen; and wherein the bacteria cell further comprises a reporter plasmid that comprises the one or more packaging genes that are disrupted in the Myoviridae lysogen; and

- providing conditions to the bacterial cell that induces a lytic phase of the Myoviridae lysogen to produce NRTPs with non-native tail fibers.

12. The method of 11, wherein the complementary nucleic acid molecule further comprises a gene that encodes a chaperone protein.

13. The method of claim 11, wherein the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacterial genomes encoding non-replicative virus derived structures or from nucleic acid delivery particles.

14. A method of generating an engineered bacteriophage or a non-replicative transduction particle (NRTP) that displays a bacteria host cell specificity that differs from the bacteria host cell specificity displayed by a native bacteriophage comprising:

- fusing a first nucleotide sequence from one tail fiber structural protein encoding gene with a second nucleotide sequence from a second tail fiber structural protein encoding gene from a different source to generate a chimeric tail fiber structural protein encoding gene;
- providing the chimeric tail fiber structural protein encoding gene as the complementary nucleic acid molecule in a bacteria cell;
- expressing the chimeric tail fiber structural protein gene in the bacteria cell that contains the bacteriophage tail fiber replacement platform of any one of claims 1 to 6; and
- providing conditions to the bacteria cell that induces a lytic phase of the Myoviridae lysogen to generate the engineered bacteriophage or NRTP.

15. A method of generating engineered bacteriophages or non-replicative transduction particles (NRTPs) having multiple types of tail fibers from a single bacteria cell line, wherein at least one of the tail fiber type is a non-native tail fiber comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 6, wherein the complementary nucleic acid molecule

comprises at least two receptor binding regions from tail fiber structural protein encoding genes that are fused to one or more tail interacting regions from tail fiber structural protein encoding genes, wherein at least one of the receptor binding regions is derived from a different source than the source of one of the tail interacting region; and

- providing conditions to the bacterial cell that induces a lytic phase of the lysogen to produce bacteriophages or NRTPs having multiple types of tail fibers.

16. The method of claim 15, wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption; or wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption; or wherein the complementary nucleic acid molecule is integrated into the genome of the bacteria cell; or wherein the complementary nucleic acid molecule is integrated into a plasmid molecule.

17. The method of any one of claims 15 to 16, wherein fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase and in the presence of multiple cix recombination sites; or

wherein fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase or a homolog, ortholog, or paralog of the cin recombinase and in the presence of multiple associated recombination sites.

18. A method of generating Myoviridae-family bacteriophages or Myoviridae-family derived non-replicative transduction particles (NRTPs) that are able to recognize and transduce bacteria cells for diagnostic or therapeutic use comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 6, wherein the complementary nucleic acid molecule comprises tail fiber genes non-native to the Myovridae lysogen and derived partially or entirely from either Myoviridae, Siphoviridae, or Podoviridae tail fiber sequences; and
- providing conditions to the bacterial cell that induces a lytic phase of the lysogen to produce Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs capable of recognizing and transducing bacteria previously refractory to a native Myoviridae bacteriophage or Myoviridae-family derived NRTP particles for diagnostic or therapeutic use.