[0005] The activity of *Sphingomonas* A1-III from the PL5 family was previously explored. A1-III is known to break polyM alginate down into disaccharides and trisaccharides. When expressed in two phage families, A1-III was shown to have activity against preformed biofilms of mucoid *Pseudomonas aeruginosa*. The Alg2A version of alginate lyase, which belongs to the PL7 family and is expressed by *Flavobacterium*, was also expressed. Alg2A had previously been shown to degrade both polyG and polyM and showed greater activity against *Pseudomonas aeruginosa* lawns than A1-III. In addition, Alg2A enhanced antibiotic treatment of *Pseudomonas aeruginosa* biofilms. Therefore, Alg2A was cloned and expressed from phages to compare its activity to that of A1-III. The data described herein indicate that Alg2A has a stronger degradation effect on *Pseudomonas aeruginosa* biofilms than A1-III.

[0006] Described herein are bacteriophages, compositions of bacteriophages, combinations of phages, and use of the same for medical and non-medical applications, including in the treatment of bacterial infections and illnesses.

[0007] In one aspect, the present disclosure describes a bacteriophage composition comprising one or more sequences selected from a sequence having at least 95% identity to the sequence of SEQ ID NO:20, at least 95% identity to the sequence of SEQ ID NO:21, at least 95% identity to the sequence of SEQ ID NO:36, or at least 95% identity to the sequence of SEQ ID NO:59. In one example, the present disclosure describes a bacteriophage engineered to express an exopolysaccharide (EPS) depolymerase.

[0008] In some examples, the EPS depolymerase is expressed from a nucleotide sequence selected from SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:36, or SEQ ID NO:59, or a sequence having at least 90% identity to the sequence of SEQ ID NO:20, at least 90% identity to the sequence of SEQ ID NO:21, at least 90% identity to the sequence of SEQ ID NO:22, at least 90% identity to the sequence of SEQ ID NO:23, at least 90% identity to the sequence of SEQ ID NO:24, at least 90% identity to the sequence of SEQ ID NO:25, at least 90% identity to the sequence of SEQ ID NO:36, or at least 90% identity to the sequence of SEQ ID NO:59.

[0009] In some examples, the EPS depolymerase is alginate lyase. In some examples, the alginate lyase comprises Alg2A or A1-III. In some examples, the alginate lyase comprises Alg2A. In some examples, the alginate lyase comprises A1-III.

[0010] In some examples, the bacteriophage shows improved host range.

[0011] In some examples, the bacteriophage belongs to the Genus *Phikmvvirus*. In some examples, the bacteriophage belongs to the Genus *Pakpunavirus*. In some examples, the bacteriophage belongs to the Genus *Bruynoghevirus*. In some examples, the bacteriophage belongs to the Genus *Pbunavirus*.

[0012] In some examples, the bacteriophage targets *Pseudomonas aeruginosa*. In some examples, the bacteriophage targets one or more of *Pseudomonas aeruginosa,* antibiotic-resistant *Pseudomonas aeruginosa,* and multiple antibiotic-resistant *Pseudomonas aeruginosa*. In some examples, the bacteriophage infects and kills one or more of *Pseudomonas aeruginosa,* antibiotic-resistant *Pseudomonas aeruginosa,* and multiple antibiotic-resistant *Pseudomonas aeruginosa*.

[0013] In some examples, the bacteriophage reduces biofilm mass.

[0014] In another example, the present disclosure describes a bacteriophage composition comprising one or more bacteriophages that express an exopolysaccharide (EPS) depolymerase, wherein the one or more bacteriophages comprise a polynucleotide sequence selected from SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41; SEQ ID NO:42; SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:45; SEQ ID NO:46; SEQ ID NO:47; SEQ ID NO:48; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID NO:57; SEQ ID NO:58; SEQ ID NO:60; SEQ ID NO:61; SEQ ID NO:62; SEQ ID NO:63; SEQ ID NO:64; SEQ ID NO:65; SEQ ID NO:66; SEQ ID NO:67; SEQ ID NO:68; SEQ ID NO:69; SEQ ID NO:70; SEQ ID NO:71, SEQ ID NO:73; a polynucleotide sequence with at least 90% identity to SEQ ID NO:26; a polynucleotide sequence with at least 90% identity to SEQ ID NO:27; a polynucleotide sequence with at least 90% identity to SEQ ID NO:28; a polynucleotide sequence with at least 90% identity to SEQ ID NO:29; a polynucleotide sequence with at least 90% identity to SEQ ID NO:30; a polynucleotide sequence with at least 90% identity to SEQ ID NO:31; a polynucleotide sequence with at least 90% identity to SEQ ID NO:32; a polynucleotide sequence with at least 90% identity to SEQ ID NO:33; a polynucleotide sequence with at least 90% identity to SEQ ID NO:34; a polynucleotide sequence with at least 90% identity to SEQ ID NO:35; a polynucleotide sequence with at least 90% identity to SEQ ID NO:37; a polynucleotide sequence with at least 90% identity to SEQ ID NO:38; a polynucleotide sequence with at least 90% identity to SEQ ID NO:39; a polynucleotide sequence with at least 90% identity to SEQ ID NO:40; a polynucleotide sequence with at least 90% identity to SEQ ID NO: 41; a polynucleotide sequence with at least 90% identity to

SEQ ID NO:42; a polynucleotide sequence with at least 90% identity to SEQ ID NO:43; a polynucleotide sequence with at least 90% identity to SEQ ID NO:44; a polynucleotide

[0020] In some examples, the one or more bacteriophages of the composition infect and kill one or more of *Pseudomonas aeruginosa,* antibiotic-resistant *Pseudomonas aeruginosa,* and multiple antibiotic-resistant *Pseudomonas aeruginosa*.

[0021] In some examples, the compositions further comprise a storage medium for storage at room temperature or a temperature at or below 8ºC. In some examples, the composition is stored at a temperature ranging from -20ºC to 25ºC. In some examples, the composition is stored at 2ºC to 8ºC. In some examples, the composition is stored at room temperature. In some examples, the storage medium is for storage at 4ºC, 0ºC, -20ºC, or -80ºC.

[0022] In some examples, the storage medium comprises a cryoprotectant. In some examples, the cryoprotectant comprises glycerol. In some examples, the composition comprises between about 5% and about 50% glycerol. In some examples, the storage medium comprises about 20% glycerol. In some examples, the cryoprotectant comprises sucrose. In some examples, the composition comprises between about 5% and about 30% sucrose. In some examples, the composition comprises about 10% sucrose. In some examples, the cryoprotectant comprises dimethylsulfoxide (DMSO). In some examples, the DMSO is at a concentration of between 2% and 10%.

[0023] In some examples, the composition further comprises a pharmaceutically acceptable carrier, diluent, excipient or combinations thereof.

[0024] In some examples, the composition is a liquid, semi-liquid, solid, frozen, or lyophilized formulation.

[0025] In some examples, the composition comprises between 1×10^8 and 1×10^{12} PFU per milliliter of each bacteriophage.

[0026] In some examples, the one or more bacteriophages of the composition reduce biofilm mass.

[0027] In yet another aspect, the present disclosure describes a use of the bacteriophage composition as disclosed herein in the manufacture of a medicament for treating a *Pseudomonas aeruginosa* infection, wherein the composition is to be administered to a subject in

need thereof. In yet another example of the invention, described herein is a method for treating a *Pseudomonas aeruginosa* infection, comprising administering any of the compositions described herein to a subject in need thereof.

[0028] In some examples, the composition is administered at a dosage of at least 3 x 10^8 PFU of total bacteriophages per dose.

[0029] In some examples, the method further comprises administration of an antibiotic. In some examples, the antibiotic is selected from the group consisting of fluoroquinolone, carbapenem, aminoglycoside, ansamycin, cephalosporin, penicillin, beta

lactam, beta lactamase inhibitor, folate pathway inhibitor, fucidane, glycopeptide, glycylcycline, lincosamide, lipopeptide, macrolide, quinolone, oxazolidinone, phenicol phosphonic acid, streptogramin, tetracycline, sulfonamide, imipenem, meropenem, amikacin, ciprofloxacin, levofloxacin, tobramycin, azithromycin, aztreonam, colistin, inhaled tobramycin, inhaled aztreonam, and inhaled colistin.

[0030] In some examples, the method further comprises administration of one or more CFTR modulators. In examples, the CFTR modulator may be selected from, but is not necessarily limited to, ivacaftor, lumacaftor/ivacaftor, tezacaftor/ivacaftor, elexacaftor/tezacaftor/ivacaftor, or any combination thereof.

[0031] In some examples, the bacterial infection has become resistant to one or more antibiotics selected from a fluoroquinolone, carbapenem, aminoglycoside, ansamycin, cephalosporin, penicillin, beta lactam, beta lactamase inhibitor, folate pathway inhibitor, fucidane, glycopeptide, glycylcycline, lincosamide, lipopeptide, macrolide, quinolone, oxazolidinone, phenicol phosphonic acid, streptogramin, tetracycline, sulfonamide, imipenem, meropenem, amikacin, ciprofloxacin, levofloxacin, tobramycin, azithromycin, aztreonam, colistin, inhaled tobramycin, inhaled aztreonam, and inhaled colistin.

[0032] In some examples, the bacteriophage composition is administered via inhalation. In some examples, the bacteriophage composition is administered via nebulization. In some examples, the bacteriophage composition is administered intravenously.

[0033] In some examples, the bacteriophage composition is administered at least once a day. In some examples, the bacteriophage composition is administered for at least one day.

[0034] In some examples, the subject is human.

[0035] In some examples, the subject suffers from cystic fibrosis (CF). In some examples, the subject suffers from non-cystic fibrosis bronchiectasis (NCFB).

[0036] In yet another aspect, the present disclosure describes an assay for determining alginate lyase activity of an engineered bacteriophage, comprising administering an effective

amount of any of the engineered bacteriophages described herein to a *Pseudomonas aeruginosa* biofilm and determining reduction in biofilm mass.

[0037] In yet another aspect, the present disclosure describes a use of the bacteriophage or bacteriophage composition as disclosed herein in the manufacture of a medicament for treating a *Pseudomonas aeruginosa* infection comprising that the bacteriophage or bacteriophage composition is to be administered to a selected subject having a *Pseudomonas aeruginosa* infection, thereby treating the *Pseudomonas aeruginosa* infection. In yet another example, the present disclosure describes a method for treating a bacterial infection comprising: (a) selecting a subject having a bacterial infection, and (b) administering to the subject an effective amount of any of the bacteriophages described

[0045] In some examples, the bacteriophage composition is administered via inhalation. In some examples, the bacteriophage composition is administered via nebulization.

[0046] In some examples, the bacteriophage composition is administered at least once a day. In some examples, the bacteriophage composition is administered for at least one day.

[0047] In some examples, the subject is human.

[0048] In yet another aspect, the present disclosure describes a method for making an engineered bacteriophage, comprising providing a bacteriophage and incorporating a payload into the bacteriophage.

[0049] In some examples, the EPS depolymerase is alginate lyase. In some examples, the alginate lyase comprises Alg2A or A1-III. In some examples, the alginate lyase comprises Alg2A. In some examples, the alginate lyase comprises A1-III.

[0050] In some examples, the bacteriophage belongs to the Genus *Phikmvvirus*. In some examples, the bacteriophage belongs to the Genus *Pakpunavirus*. In some examples, the bacteriophage belongs to the Genus *Bruynoghevirus*. In some examples, the bacteriophage belongs to the Genus *Pbunavirus*.

[0051] In yet another aspect, the present disclosure describes a kit comprising any of the bacteriophage compositions described herein, and instructions for using the same.

[0052] In some examples, the kit further comprises an antibiotic.

[0053] In some examples, the kit further comprises a means of administering the bacteriophage or bacteriophage composition. In some examples, the means comprises a syringe, a transdermal patch, a slow-release device, a spray, a nebulizer, an inhaler, or a respirator. In some examples, the slow-release device comprises a mini-osmotic pump.

[0054] In some examples, the kit further comprises a second bacteriophage or bacteriophage composition.

[0055] In yet another example, the present disclosure describes a bacteriophage composition comprising one or more bacteriophages engineered to express an exopolysaccharide (EPS) depolymerase, wherein the one or more bacteriophages belong to the Genus *Phikmvvirus*, *Pakpunavirus*, *Bruynoghevirus*, and/or *Pbunavirus.*

WHAT IS CLAIMED IS:

1. A bacteriophage composition comprising one or more sequence selected from a sequence having at least 95% identity to the sequence of SEQ ID NO:20, at least 95% identity to the sequence of SEQ ID NO:21, at least 90% identity to the sequence of SEQ ID NO:22, at least 95% identity to the sequence of SEQ ID NO:36, or at least 95% identity to the sequence of SEQ ID NO:59.

2. The bacteriophage composition of claim 1, wherein the sequence encodes A1-III.

3. The bacteriophage composition of claim 1 or 2, wherein the bacteriophage shows improved host range and/or reduces biofilm mass.

4. The bacteriophage composition of any of claims 1 to 3, wherein the bacteriophage belongs to the Genus *Phikmvvirus*, *Pakpunavirus*, *Bruynoghevirus*, *Nankokuvirus*, *Litunavirus*, *Luzseptimavirus,* or *Pbunavirus*.

5. The bacteriophage composition of claim 2, wherein the bacteriophage targets one or more of *Pseudomonas aeruginosa,* antibiotic-resistant *Pseudomonas aeruginosa,* and multiple antibiotic-resistant *Pseudomonas aeruginosa*.

6. The bacteriophage composition of claim 1, wherein one or more of the bacteriophages are engineered, and/or one or more of the bacteriophages are naturally occurring phages.

7. The bacteriophage composition of any one of claims 1 to 6, further comprising a storage medium for storage at room temperature or a temperature at or below 8ºC, wherein the storage medium comprises a cryoprotectant for storage below 0ºC.

8. The bacteriophage composition of claim 6 or 7, wherein the composition is stored at a temperature ranging from -80ºC to 25ºC.

9. The bacteriophage composition of claim 7, wherein the cryoprotectant comprises between 5% and 50% glycerol.

10. The bacteriophage composition of any of claims 5 to 9, wherein the composition further comprises a pharmaceutically acceptable carrier, diluent, excipient or combinations thereof.

11. The bacteriophage composition of claim 7, wherein the cryoprotectant comprises between 5% and 30% sucrose.

12. The bacteriophage composition of claim 7, wherein the cryoprotectant comprises DMSO at a concentration of between 2% and 10%.

13. The bacteriophage composition of any of claims 5 to 12, wherein the composition is a liquid, semi-liquid, solid, frozen, or lyophilized formulation.

14. The bacteriophage composition of any of claims 5 to 13, wherein the composition comprises between 1×10^8 and 1×10^{12} PFU per milliliter of each bacteriophage.

15. Use of the bacteriophage composition of any of claims 5 to 14 in the manufacture of a medicament for treating a *Pseudomonas aeruginosa* infection, wherein the composition is to be administered to a subject in need thereof.

16. The use of claim 15, wherein the composition is to be administered at a dosage of at least 3×10^8 PFU of total bacteriophages per dose.

17. The use of claim 15, further comprising that an antibiotic selected from the group consisting of fluoroquinolone, carbapenem, aminoglycoside, ansamycin, cephalosporin, penicillin, beta lactam, beta lactamase inhibitor, folate pathway inhibitor, fucidane, glycopeptide, glycylcycline, lincosamide, lipopeptide, macrolide, quinolone, oxazolidinone, phenicol phosphonic acid, streptogramin, tetracycline, sulfonamide, imipenem, meropenem, amikacin, ciprofloxacin, levofloxacin, tobramycin, azithromycin, aztreonam, colistin, inhaled tobramycin, inhaled aztreonam, and inhaled colistin is to be administered.

18. The use of any of claims 15 to 17, wherein the use further comprises that one or more CFTR modulators selected from ivacaftor; lumacaftor and ivacaftor;

tezacaftor and ivacaftor; elexacaftor, tezacaftor, and ivacaftor; or any other combination thereof is to be administered.

19. The use of any of claims 15 to 18, wherein the *Pseudomonas aeruginosa* infection has become resistant to one or more antibiotics selected from a fluoroquinolone, carbapenem, aminoglycoside, ansamycin, cephalosporin, penicillin, beta lactam, beta lactamase inhibitor, folate pathway inhibitor, fucidane, glycopeptide, glycylcycline, lincosamide, lipopeptide, macrolide, quinolone, oxazolidinone, phenicol phosphonic acid, streptogramin, tetracycline, sulfonamide, imipenem, meropenem, amikacin, ciprofloxacin, levofloxacin, tobramycin, azithromycin, aztreonam, colistin, inhaled tobramycin, inhaled aztreonam, and inhaled colistin.

20. The use of any of claims 15 to 19, wherein the bacteriophage composition is to be administered via pulmonary delivery, via inhalation, nebulization, intranasally and/or intravenously.

21. The use of any of claims 15 to 20, wherein the bacteriophage composition is to be administered at least once a day and/or for at least one day.

22. The use of any of claims 15 to 21, wherein the subject is human.

23. The use of any of claims 15 to 22, wherein the subject suffers from cystic fibrosis (CF) or non-cystic fibrosis bronchiectasis (NCFB).

24. The use of any of claims 15 to 23, wherein the subject was previously treated with one or more antibiotics.

25. An assay for determining alginate lyase activity of an engineered bacteriophage, comprising administering an effective amount of the engineered bacteriophage of any of claims 1 to 14 to a *Pseudomonas aeruginosa* biofilm and determining reduction in biofilm mass.

26. Use of the bacteriophage or bacteriophage composition of claims 1 to 14 in the manufacture of a medicament for treating a *Pseudomonas aeruginosa* infection

comprising that the bacteriophage or bacteriophage composition is to be administered to a selected subject having a *Pseudomonas aeruginosa* infection, thereby treating the *Pseudomonas aeruginosa* infection.

27. The use of claim 26, wherein the bacterial infection is characterized by a biofilm.

28. A method for making an engineered bacteriophage, comprising providing a bacteriophage and incorporating a payload into the bacteriophage.

29. A kit comprising a bacteriophage composition of any one of claims 1 to 14, and instructions for using the same.

30. The kit of claim 29, further comprising an antibiotic.

31. The kit of claim 29 or 30, wherein the kit further comprises one or more CFTR modulators selected from ivacaftor; lumacaftor and ivacaftor; tezacaftor and ivacaftor; elexacaftor, tezacaftor, and ivacaftor; or any other combination thereof.

32. The kit of any of claims 29 to 31, further comprising a means of administering the bacteriophage or bacteriophage composition selected from a syringe, a transdermal patch, a slow-release device, a spray, a nebulizer, an inhaler, or a respirator.

33. The kit of claim 32, wherein the slow-release device comprises a mini-osmotic pump.

34. The kit of any one of claims 29 to 33, further comprising a second bacteriophage or bacteriophage composition.

35. The bacteriophage of claim 1, comprising one or more sequences selected from a sequence having at least 96% identity to the sequence of SEQ ID NO:20, at least 96% identity to the sequence of SEQ ID NO:21, at least 96% identity to the sequence of SEQ ID NO:36, or at least 96% identity to the sequence of SEQ ID NO:59.

36. The bacteriophage of claim 1, comprising one or more sequences selected from a sequence having at least 97% identity to the sequence of SEQ ID NO:20, at least 97% identity to the sequence of SEQ ID NO:21, at least 97% identity to the sequence of SEQ ID NO:36, or at least 97% identity to the sequence of SEQ ID NO:59.

37. The bacteriophage of claim 1, comprising one or more sequences selected from a sequence having at least 98% identity to the sequence of SEQ ID NO:20, at least 98% identity to the sequence of SEQ ID NO:21, at least 98% identity to the sequence of SEQ ID NO:36, or at least 98% identity to the sequence of SEQ ID NO:59.

38. The bacteriophage of claim 1, comprising one or more sequences selected from a sequence having at least 99% identity to the sequence of SEQ ID NO:20, at least 99% identity to the sequence of SEQ ID NO:21, at least 99% identity to the sequence of SEQ ID NO:36, or at least 99% identity to the sequence of SEQ ID NO:59.

39. The bacteriophage of claim 1, comprising one or more sequences selected from SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:36, or SEQ ID NO:59.

40. The bacteriophage of claim 1, comprising two or more sequences selected from a sequence having at least 95% identity to the sequence of SEQ ID NO:20, at least 95% identity to the sequence of SEQ ID NO:21, at least 95% identity to the sequence of SEQ ID NO:36, or at least 95% identity to the sequence of SEQ ID NO:59.

41. The bacteriophage of claim 40, comprising one or more sequences selected from a sequence having at least 96% identity to the sequence of SEQ ID NO:20, at least 96% identity to the sequence of SEQ ID NO:21, at least 96% identity to the sequence of SEQ ID NO:36, or at least 96% identity to the sequence of SEQ ID NO:59.

42. The bacteriophage of claim 40, comprising one or more sequences selected from a sequence having at least 97% identity to the sequence of SEQ ID NO:20, at least 97% identity to the sequence of SEQ ID NO:21, at least 97% identity to the sequence of SEQ ID NO:36, or at least 97% identity to the sequence of SEQ ID NO:59.

43. The bacteriophage of claim 40, comprising one or more sequences selected from a sequence having at least 98% identity to the sequence of SEQ ID NO:20, at least 98% identity to the sequence of SEQ ID NO:21, at least 98% identity to the sequence of SEQ ID NO:36, or at least 98% identity to the sequence of SEQ ID NO:59.

44. The bacteriophage of claim 40, comprising one or more sequences selected from a sequence having at least 99% identity to the sequence of SEQ ID NO:20, at least 99% identity to the sequence of SEQ ID NO:21, at least 99% identity to the sequence of SEQ ID NO:36, or at least 99% identity to the sequence of SEQ ID NO:59.

45. The bacteriophage of claim 1, comprising two or more sequences selected from a sequence having at least 96% identity to the sequence of SEQ ID NO:20, at least 96% identity to the sequence of SEQ ID NO:21, at least 96% identity to the sequence of SEQ ID NO:36, or at least 96% identity to the sequence of SEQ ID NO:59.

46. The bacteriophage of claim 1, comprising two or more sequences selected from a sequence having at least 97% identity to the sequence of SEQ ID NO:20, at least 97% identity to the sequence of SEQ ID NO:21, at least 97% identity to the sequence of SEQ ID NO:36, or at least 97% identity to the sequence of SEQ ID NO:59.

47. The bacteriophage of claim 1, comprising two or more sequences selected from a sequence having at least 98% identity to the sequence of SEQ ID NO:20, at least 98% identity to the sequence of SEQ ID NO:21, at least 98% identity to the

sequence of SEQ ID NO:36, or at least 98% identity to the sequence of SEQ ID NO:59.

48. The bacteriophage of claim 1, comprising two or more sequences selected from a sequence having at least 99% identity to the sequence of SEQ ID NO:20, at least 99% identity to the sequence of SEQ ID NO:21, at least 99% identity to the sequence of SEQ ID NO:36, or at least 99% identity to the sequence of SEQ ID NO:59.

49. The bacteriophage of claim 1, comprising two or more sequences selected from SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:36, or SEQ ID NO:59.