Xanthomonas bacteriophages: a review of their biology and biocontrol applications in agriculture

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Abstract
Phytopathogenic bacteria are economically important because they affect crop yields and threaten the livelihoods of farmers worldwide. The genus Xanthomonas is particularly significant because it is associated with some plant diseases that cause tremendous loss in yields of globally essential crops. Current management practices are ineffective, unsustainable and harmful to natural ecosystems. Bacteriophage (phage) biocontrol for plant disease management has been of particular interest from the early nineteenth century to date. Xanthomonas phage research for plant disease management continues to demonstrate promising results under laboratory and field conditions. AgriPhage has developed phage products for the control of Xanthomonas campestris pv. vesicatoria and Xanthomonas citri subsp. citri. These are causative agents for tomato, pepper spot and speck disease as well as citrus canker disease. Phage-mediated biocontrol is becoming a viable option because phages occur naturally and are safe for disease control and management. Thorough knowledge of biological characteristics of Xanthomonas phages is vital for developing effective biocontrol products. This review covers Xanthomonas phage research highlighting aspects of their ecology, biology and biocontrol applications.

Keywords: Taxonomy, Distribution, Isolation source, Host range, Life cycle, Phage efficacy

Background
The genus Xanthomonas is a well-studied group of plant-associated Gram-negative bacteria that belong to the family Xanthomonadaceae subclass Gammaproteobacteria [1]. An estimated 27 species is pathogenic to approximately 400 plants. These include but not limited to sugar cane, beans, cassava, cabbage, banana, citrus, tomatoes, pepper and rice [2]. The life cycle of Xanthomonas has two stages: epiphytic and endophytic [3]. The epiphytic stage starts once bacteria colonize the surfaces of a new plant using adhesion ligands such as bacteria surface polysaccharides [4], adhesion proteins [5], and type IV pili [6]. After colonization comes biofilm formation, which then protects the bacteria from environmental stress factors [7]. The endophytic stage is characterised by bacterial entry into plant tissue via lesions or stomata and eventual movement throughout the vascular system. The bacteria re-emerge onto the plant surfaces once their population reaches the threshold, transmission occurs to new hosts and the infection cycle repeats [3].

Although Xanthomonas species are well-studied, the genus remains responsible for many crop diseases that cause crop yield losses in economically important crops worldwide [2, 3]. The current management methods used to control Xanthomonas-associated diseases include de-budding, uprooting, burying and burning of infected plant tissues, sterilization of garden tools, and application of copper-based pesticides and antibiotics such as streptomycin.
The concerns raised about ineffective cultural practices, copper-based pesticide, antibiotic resistance problems, and environmental chemical contamination have piqued worldwide interest in Xanthomonas phage research and biocontrol application in agriculture.

Phages are viruses that infect and replicate in bacteria. Phage replication cycles include temperate and lytic pathways with the lytic pathway being the easier and more important pathway for employment in phage biocontrol. In the lytic pathway the phages bind to the surface of bacteria after which they inject their DNA and replicate inside the cell. This results in the production of phage progeny that lyse and kill the bacteria [11]. In the temperate pathway, once the phage has successfully bound and injected its DNA into the host, the phage may either stably integrate into the genome of the bacteria or enter into the lytic life cycle. Using temperate phages in phage biocontrol poses some disadvantages in that, once the phage inserts its genome into the bacterial DNA chromosome, the prophage is transmitted to daughter cells by horizontal gene transfer thereby providing undesirable genes that may aggravate bacterial disease, e.g. filamentous phage CTX Φ that encodes cholera toxin [12].

Historically, bacteriophage-based biocontrol specific for phytopathogen Xanthomonas dates back to the early nineteenth century, when a filtrate of decomposing cabbage stopped the spread of cabbage-rot disease caused by Xanthomonas campestris pv. campestris, [13]. Decades later, similar biocontrol success was reported with phage-containing lysates that inhibited bacterial spot disease in peach caused by Xanthomonas campestris pv. pruni [14, 15]. A number of phage applications have progressed from in-vitro experiments to field trials. These include studies on bacterial spot of tomato caused by Xanthomonas campestris pv. vesicatoria [16]; geranium bacterial blight caused by Xanthomonas campestris pv. pelargonii [17]; leaf blight of onion caused by Xanthomonas axonopodis pv. allii [18]; citrus canker and citrus bacterial spot caused by Xanthomonas axonopodis pv. citri and Xanthomonas axonopodis pv. citrumelo [19]; asiatic citrus canker caused by Xanthomonas axonopodis pv. citri [20] and Xanthomonas citri subsp. citri [21]; bacterial leaf blight of rice caused by Xanthomonas oryzae pv. oryzae [22, 23] and bacterial leaf blight of welsh onions caused by Xanthomonas axonopodis pv. allii [24]. Two Xanthomonas phage products manufactured by AgriPhage [25] have been shown to successfully control pathogens that cause tomato and pepper spot disease and citrus canker disease.

Owing to the growing interest in using Xanthomonas phages to control the genus Xanthomonas, this review emphasizes the taxonomy, ecology, biology and biocontrol applications.

Main text

Taxonomy of Xanthomonas phages

A total of 168 Xanthomonas phages described to date classify into orders: Caudovirales with 151 phages and Tubulavirales with 17 phages (Additional file 1). According to the International Committee on Taxonomy of Viruses (ICTV), Caudovirales contain 9 families [26] and Xanthomonas phages reported in literature or National Centre for Biotechnology Information (NCBI) database belong to 5 families namely: Podoviridae, Siphoviridae, Myoviridae, Autographiviridae, and Herelleviridae (Additional file 1). A total of 71 Xanthomonas phages belong to Myoviridae, 42 belong to Podoviridae, 17 belong to Siphoviridae, 3 belong to Autographiviridae and 1 member to Herelleviridae. Order Caudovirales possess tubal tails that can be either long and contractile (Myoviridae), long and non-contractile (Siphoviridae), or short and non-contractile (Podoviridae, Autographiviridae) [26–28]. The capsids of Caudovirales are non-enveloped, exhibit icosahedral symmetry with a typical diameter of 45 and 170 nm and encapsidate linear double-stranded genomes. Their genome length is between 39,980 and 384,670 nucleotides, carries between 40 and 592 open reading frames and has a guanine-cytosine (GC) content between 40 and 66% (Additional file 1). On the other hand, Tubulavirales consist of one family; Inoviridae. They are filamentous virions that possess helical symmetry and non-enveloped capsid (Additional file 1). The inovirus genomes are small, circular, single-stranded DNA molecules that range between 6000 and 8500 nucleotides. The genome encodes between 9 and 14 open reading frames and has a GC content between 57 and 60% (Additional file 1).

Ecology and host range

Ecology: geographical distribution, environmental isolation source, host bacteria and plant disease.

Geographical distribution

The geographical distribution of Xanthomonas phages spans parts of Asia, North America, South America, Europe, Zealandia and North Africa. The countries where the phages are isolated are summarized in Table 1. The Xanthomonas phages are distributed across the world depending on the pathogen that is present in that part of the world.

Ecology: environmental isolation source, host bacteria and plant disease

The environmental isolation source of Xanthomonas phages as well as bacterial host and plant disease are summarized in Table 2. These viruses establish infection in Xanthomonas pathovars responsible for a range of plant
Table 1  Country of isolation of *Xanthomonas* phages, their families and host strain/s they infect

| Country of isolation | *Xanthomonas* phage/s | Family                | Causative bacterium                      | Reference |
|----------------------|-----------------------|-----------------------|------------------------------------------|-----------|
| China                | Xop41                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [29]      |
| China                | Xoo-sp1, Xoo-sp2, Xoo-sp3, Xoo-sp4, Xoo-sp5, Xoo-sp6, Xoo-sp7, Xoo-sp8, Xoo-sp9, Xoo-sp10, Xoo-sp11, Xoo-sp12, Xoo-sp13, Xoo-sp14, Xoo-sp15 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [30]      |
| China                | X1, X2, X3, X4, X5    | Myoviridae            | *X. oryzae*                              | [31]      |
| China                | Xoo-sp14               | Myoviridae            | *X. oryzae pv. oryzae*                   | [32]      |
| China                | Xoo-sp13               | Myoviridae            | *X. oryzae pv. oryzae*                   | [33]      |
| China                | XM09                  | Inoviridae            | *X. oryzae pv. oryzicola*                | [34]      |
| Taiwan               | Xp10, Xp12, Xp20      | Siphoviridae          | *X. oryzae pv. oryzae*                   | [35]      |
| Taiwan               | φXc10                 | Autographiviridae     | *X. citri pv. glycinus, *X. campestris pv. campestris, *X. campestris pv. citri* | [36]      |
| Korea                | P8L, P27L, P30L, P59L, P73L | Siphoviridae          | *X. oryzae pv. oryzae*                   | [22]      |
| Korea                | P4L, P4M, P6M, P6M1, P14M, P14M1, P18M, P23M1, P33M, P37L, P37M, P37M1, P41M, P43M, P45M, P47M, P48M, P60M, P61M, P62M, P66M, P68M, P70M, P71L, P72M | Siphoviridae          | *X. oryzae pv. oryzae*                   | [22]      |
| Japan                | XacN1                 | Myoviridae            | *X. citri*                               | [37]      |
| Viet Nam             | Phage Xaa_vB_φ31      | Autographiviridae     | *X. euvesicatoria pv. allii XaaBL11      | [38]      |
| Philippines          | XPP1                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPP2                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPP3                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPP4                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPP6                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPP8                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPP9                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPV1                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPV2                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| Philippines          | XPV3                  | Myoviridae            | *X. oryzae pv. oryzae*                   | [39]      |
| India                | φXOF1                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | φXOF2                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | φXOF3                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | φXOF4                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | φXOT1                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | φXOT2                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | φXOM1                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | φXOM2                 | Siphoviridae          | *X. oryzae pv. oryzae*                   | [23]      |
| India                | Xcc9SH3               | Siphoviridae          | *X. campestris pv. campestris*           | [40]      |
| India                | Xcc3SH, Xcc6SH3, Xcc7SH3, Xcc8SH3, Xcc95H3, Xcc14SH3, JPS-xcc-3_P1, JPS-xcc-4_P1, JPS-xcc-7_P1, NBL-xcc-7_P1, NBL-xcc-4_P1, NBL-xcc-7_P1, NBL-xcc-3_P1, NBL-xcc-9_P1, NFS-xcc-9_P1, GRW-xcc-9_P1, NFS-xcc-9_P2, NBL-xcc-9_P2, GRW-xcc-10_P1, NFS-xcc-10_P1, NBL-xcc-10_P1, GRW-xcc-14_P1, NFS-xcc-14_P1, NBL-xcc-14_P1, GRW-xcc-17_P1, NFS-xcc-17_P1, NBL-xcc-17_P1, GRW-xcc-19_P1, NFS-xcc-19_P1, NBL-xcc-19_P1 | Siphoviridae          | *X. campestris pv. campestris* | [40] |
| India                | Xap-1, Xap-2, Xap-3, Xap-4, Xap-5 | n/a                  | *X. axonopodis pv. punicae*              | [41]      |
| USA                  | T7-like podophage Pagan | Autographiviridae     | *Xanthomonas* sp., rice isolate ATCC PTA-13101 | [42]      |
| USA                  | Cf2                   | Inoviridae            | *X. citri pv. citri*                     | [43]      |
| USA                  | Phage River Rider     | Podoviridae           | *X. fragariae*                          | [44]      |
| Mexico               | Xaf13                 | Inoviridae            | *X. vesicatoria*                        | [45]      |
diseases including but not limited to bacterial leaf blight, black rot, bacterial leaf spot and citrus canker (Table 2). The majority of Xanthomonas phages are isolated from infected plant phyllosphere and rhizosphere, while others are isolated from compost, sewage and water (irrigation, pond, freshwater lakes and rivers) (Table 2).

**Host range**

Phages with a narrow host range infect one or few of the same bacteria strains, broad host range phages infect multiple strains of the same bacteria, and polyvalent phages infect several species or unrelated genera [77, 78]. A total of 148 Xanthomonas phages described in literature have a narrow, broad or polyvalent host range. Of these 52 have a narrow and 88 have a broad host range. The remaining 8 have a polyvalent host range. The lytic activity of phages with a narrow host range is between 13 and 57% while those with a broad range is between 60 and 100% (Table 3).

The polyvalent Xanthomonas phage Pg125, is lytic to multiple strains from 25 species within the genus Xanthomonas [69]. Others in this category include phage Xcu-P1, Xcu-P3, Xve-P1, and Xca-P1 which are lytic to Xanthomonas campestris pathovars (Table 3). The varied host ranges demonstrated by Xanthomonas phages imply that these lytic viruses can offer viable plant disease management alternatives. The high level of host specificity minimizes the risk of phage attack on beneficial bacteria [50].

**Biology: physiological parameters**

**Incubation temperature, storage temperature, storage media**

Incubation temperature Xanthomonas phages can maintain their viability over a wide incubation temperature range. For example, Xanthomonas phaseoli phages (1, 20, 22, ΦPS, ΦSD, ΦSL, ΦRS, Φ56, Φ112, Pg60) remain viable between 2 and 28°C [74]; Xanthomonas pruni phages (Xp3-A and Xp3-I) and Xanthomonas oryzae phages (Xp12 and ΦXOF4) between 20 and 50°C [15, 23, 81] and Xanthomonas euvesicatoria phages (Kφ1- Kφ15) between 35 and 70°C [50].
## Table 2: Ecology of selected Xanthomonas phages: environmental source of isolation, host bacteria and plant disease

| phage/s        | Environmental source                  | Host bacterium (pv.) | Plant disease                       | Plant                  | Reference |
|----------------|---------------------------------------|----------------------|-------------------------------------|------------------------|-----------|
| Xop411         | Xoo infected leaves                   | *X. oryzae*         | Bacterial leaf blight               | Rice                   | [29]      |
| Xp12           | Xoo infected paddy water              | *X. oryzae*         | Bacterial leaf blight               | Rice                   | [67]      |
| P4L, P4M, P6M, P6M1, P14M, P14M1, P18M, P23M1, P33M, P37L, P37M, P37M1, P41M, P43M, P45M, P47M, P50M, P53M, P54M, P57M, P58M, P60M, P61M, P62M, P66M, P68M, P70M, P71L, P72M, P8L, P27L, P30L, P59L, P73L | Xoo infected paddy water | X. oryzae (pv. oryzae) | Bacterial leaf blight | Rice | [22]      |
| XPP1-XPP9, XPV1-XPV3 | Xoo infected paddy water & soil                         | *X. oryzae*         | Bacterial leaf blight               | Rice                   | [39]      |
| X1, X2, X3, X4, X5  | Xoo infected leaves                                    | *X. oryzae*         | Bacterial leaf blight               | Rice                   | [31]      |
| φXOF1-φXOF4, φXOT1-φXOT2, φXOM1-φXOM2 | Xoo infected leaves                      | *X. oryzae*         | Bacterial leaf blight               | Rice                   | [23]      |
| Xoo-sp1, Xoo-sp2, Xoo-sp3, Xoo-sp4, Xoo-sp5, Xoo-sp6, Xoo-sp7, Xoo-sp8, Xoo-sp9, Xoo-sp10, Xoo-sp11, Xoo-sp12, Xoo-sp13, Xoo-sp14, Xoo-sp15 | Xoo infected paddy water       | *X. oryzae*         | Bacterial leaf blight               | Rice | [30]      |
| Xf              | Xoo infected leaves                                    | *X. oryzae*         | Bacterial leaf blight               | Rice                   | [68]      |
| Xcc3SH, Xcc6SH3, Xcc7SH3, Xcc9SH3, Xcc14SH3, JPS-xcc-3_P1, JPS-xcc-4_P1, JPS-xcc-7_P1, NBL-xcc-7_P1, NBL-xcc-3_P1, NBL-xcc-9_P1, NBS-xcc-9_P1, GRW-xcc-9_P1, NBS-xcc-9_P2, NBL-xcc-9_P2, GRW-xcc-10_P1, NBS-xcc-10_P1, GRW-xcc-17_P1, NBS-xcc-17_P1, GRW-xcc-19_P1, NBS-xcc-19_P1, NBL-xcc-19_P1 | Xcc infected soil and leaves, river water | *X. campestris* | Bacterial leaf blight | Crucifers; cabbage, cauliflower, brasicca | [40] |
| Xc18H1         | Xcc infected soil                                    | *X. campestris*     | Bacterial leaf blight               | Crucifers; turnip, cabbage, swede | [69]      |
| Xcc φ1         | Xcc infected soil                                    | *X. campestris*     | Black rot                           | Crucifers; broccoli, cabbage, swede, radish | [70]      |
| XTP1           | Xcc infected soil                                    | *X. campestris*     | Black rot                           | Crucifers; cabbage     | [71]      |
| XcaP1          | Xcc infected leaves                                  | *X. campestris*     | Black rot                           | Crucifiers; cabbage    | [72]      |
| XC2            | Xcc infected leaves                                  | *X. campestris*     | Black rot                           | Crucifer, cauliflower  | [47]      |
| DB 1           | Xcc infected soil                                    | *X. campestris*     | Black rot                           | Crucifer, cabbage      | [49]      |
| XcuP3          | Xc sox infected fruit                               | *X. campestris*     | Bacterial leaf spot                 | Pumpkin                | [72]      |
| XcuP1          | Xc infected leaves                                   | *X. campestris*     | Bacterial leaf spot                 | Zucchini                | [72]      |
| XholP1         | Xo infected leaves                                   | *X. campestris*     | Bacterial leaf spot                 | Sorghum                 | [72]      |
| Xp3-l          | Xp infected soil                                     | *X. pruni*          | Bacterial leaf spot                 | Peach                  | [15]      |
| Xanthomonas phage/s | Environmental source | Host bacterium | Plant disease | Plant | Reference |
|--------------------|----------------------|----------------|--------------|-------|-----------|
| Xp3-A              | Xp infected soil     | X. pruni      | Bacterial leaf spot | Peach | [15]      |
| XprP1              | Xpr infected stem    | X. campestris pv. pruni | Bacterial leaf spot | Plum  | [72]      |
| XmaP1              | Xma infected leaves  | X. campestris pv. malvacearum | Bacterial blight | Cotton | [72]      |
| XveP1              | Xve infected leaves  | X. campestris pv. vesicatoria | Bacterial leaf spot | Goosberry | [72] |
| Kϕ1, Kϕ2, Kϕ3, Kϕ4, Kϕ5, Kϕ6, Kϕ7, Kϕ8, Kϕ9, Kϕ15 | Xe infected leaves, stems, fruits, soil, seeds & irrigation water | X. euvesicatoria | Bacterial leaf spot | Pepper | [50]      |
| Phages I to XX     | Xtr infected grains  | X. trilfolii  | Wheat disease | Wheat  | [73]      |
| X. phage 1 & X. phage 2 | Xax infected leaves | X. axonopodis | Bacterial leaf spot | Pepper  | [66]      |
| Xap-1, Xap-2, Xap-3, Xap-4, Xap-5 | Pond water | X. axonopodis pv. punicae | Bacterial leaf blight | Pomegranate  | [41]      |
| 1, 20, 22, ΦPS, ΦSD, ΦSL, ΦRS, ΦS6, Φ112, Pg60 | Sewage, compost, Xp infected soil, seed & dry bean straw | X. phaseoli | Common blight of beans | Beans  | [74]      |
| Pg176, Pg177, Pg181 | Xp infected soil     | X. phaseoli   | Common blight of beans | Beans  | [75]      |
| Xanthomonas Siphophage Samson | Sewage         | X. sp. strain ATCC PTA-13101 | Bacterial leaf blight | Rice  | [76]      |
| Xanthomonas phage pagan | Fresh water   | X. sp. strain ATCC PTA-13101 | Bacterial leaf blight | Rice  | [42]      |
| Xanthomonas phage XaciN1 | Xci infected soil | X. citri | Asian citrus canker | Orange | [37]      |
| BP6XC-1, BP10, BP20, BP22 | Xcj infected soil | X. campestris pv. juglandis | Walnut blight | Walnut  | [51]      |
| P1-P26             | Xp infected soil, leaves & fruit | X. arboricola pv. juglandis | Walnut blight | Walnut  | [52]      |
| XaF1 3             | Xve infected soil   | X. vesicatoria | Bacterial leaf spot | Pepper  | [45]      |

X, Xanthomonas; pv, Pathovar; sp., species; Xanthomonas oryzae pv. oryzae; Xcc, Xanthomonas campestris pv. campestris; Xau, Xanthomonas campestris pv. cucurbita; Xho, Xanthomonas campestris pv. holcicola; Xp, Xanthomonas pruni; Xpr, Xanthomonas campestris pv. pruni; Xma, Xanthomonas campestris pv. malvacearum; Xve, Xanthomonas campestris pv. vesicatoria; Xeu, Xanthomonas euvesicatoria; Xtr, Xanthomonas trifolii; Xax, Xanthomonas axonopodis; Xp, Xanthomonas phaseoli; Xci, Xanthomonas citri; Xcj, Xanthomonas campestris pv. juglandis; Xaj, Xanthomonas arboricola pv. juglandis; Xve, Xanthomonas vesicatoria
Table 3  Host range of *Xanthomonas* phages

| Host range | Phage                       | Bacteria strain used | Number bacteria strains | Lysed bacteria strains | % lytic activity | Reference |
|------------|-----------------------------|----------------------|-------------------------|------------------------|------------------|-----------|
| Narrow     | *X. vesicatoria* phage (chilli derived) | *X. vesicatoria*     | 8                       | 4                      | 50               | [79]      |
| Narrow     | *X. vesicatoria* phage (datura derived) | *X. vesicatoria*     | 8                       | 1                      | 13               | [79]      |
| Narrow     | XC2                         | *X. campestris pv. campestris* | 10                      | 5                      | 50               | [47]      |
| Broad      | Xoo-sp1                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp2                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp3                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp4                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp5                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp6                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp7                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp8                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp9                     | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp10                    | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp11                    | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp12                    | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp13                    | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp14                    | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Xoo-sp15                    | *X. oryzae pv. oryzae* | 10                      | 9                      | 90               | [30]      |
| Broad      | Kp1                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp2                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp3                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp4                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp5                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp6                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp7                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp8                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp9                         | *X. euvesicatoria*    | 59                      | 59                     | 100              | [50]      |
| Broad      | Kp15                        | *X. euvesicatoria*    | 59                      | 47                     | 80               | [50]      |
| Broad      | Xma-P1                      | *X. pv. malvacearum*  | 8                       | 8                      | 100              | [72]      |
| Broad      | Xho-P1                      | *X. campestris pv. holcicola* | 4               | 4                      | 100              | [72]      |
| Broad      | Xpr-P1                      | *X. campestris pv. pruni* | 6               | 6                      | 100              | [72]      |
| Broad      | OP_{2}                      | *X. oryzae pv. oryzae* | 82                      | 78                     | 95               | [80]      |
| Broad      | OP_{1+2}                    | *X. oryzae pv. oryzae* | 82                      | 75                     | 91               | [80]      |
| Narrow     | OP_{1}                      | *X. oryzae pv. oryzae* | 82                      | 46                     | 56               | [80]      |
| Narrow     | OP_{1+3}                    | *X. oryzae pv. oryzae* | 82                      | 20                     | 24               | [80]      |
| Broad      | ϕXOF1                       | *X. oryzae pv. oryzae* | 6                       | 4                      | 67               | [23]      |
| Broad      | ϕXOF2                       | *X. oryzae pv. oryzae* | 6                       | 4                      | 67               | [23]      |
| Broad      | ϕXOF3                       | *X. oryzae pv. oryzae* | 6                       | 5                      | 83               | [23]      |
| Broad      | ϕXOF4                       | *X. oryzae pv. oryzae* | 6                       | 6                      | 100              | [23]      |
| Narrow     | ϕXOT1                       | *X. oryzae pv. oryzae* | 6                       | 3                      | 50               | [23]      |
| Narrow     | ϕXOT2                       | *X. oryzae pv. oryzae* | 6                       | 3                      | 50               | [23]      |
| Narrow     | ϕXOM1                       | *X. oryzae pv. oryzae* | 6                       | 3                      | 50               | [23]      |
| Narrow     | ϕXOM2                       | *X. oryzae pv. oryzae* | 6                       | 3                      | 50               | [23]      |
| Broad      | X1                          | *X. oryzae pv. oryzae* | 23                      | 15                     | 65               | [31]      |
| Broad      | X2                          | *X. oryzae pv. oryzae* | 23                      | 21                     | 91               | [31]      |
| Broad      | X3                          | *X. oryzae pv. oryzae* | 23                      | 22                     | 96               | [31]      |
| Broad      | X4                          | *X. oryzae pv. oryzae* | 23                      | 21                     | 91               | [31]      |
| Broad      | X5                          | *X. oryzae pv. oryzae* | 23                      | 14                     | 61               | [31]      |
| Host range | Phage   | Bacteria strain used          | Number bacteria strains | Lysed bacteria strains | % lytic activity | Reference |
|------------|---------|-------------------------------|-------------------------|------------------------|------------------|-----------|
| Broad      | P4L     | X. oryzae pv. oryzae          | 47                      | 33                     | 70               | [22]      |
| Broad      | P4M     | X. oryzae pv. oryzae          | 47                      | 46                     | 98               | [22]      |
| Broad      | P6M     | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P6M1    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P8L     | X. oryzae pv. oryzae          | 47                      | 36                     | 77               | [22]      |
| Broad      | P14M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P14M1   | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P18M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P23M1   | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P27L    | X. oryzae pv. oryzae          | 47                      | 33                     | 70               | [22]      |
| Broad      | P30L    | X. oryzae pv. oryzae          | 47                      | 31                     | 66               | [22]      |
| Broad      | P33M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P37L    | X. oryzae pv. oryzae          | 47                      | 33                     | 70               | [22]      |
| Broad      | P37M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P37M1   | X. oryzae pv. oryzae          | 47                      | 46                     | 98               | [22]      |
| Broad      | P41M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P43M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P45M    | X. oryzae pv. oryzae          | 47                      | 33                     | 70               | [22]      |
| Broad      | P47M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P50M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P53M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P54M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P57M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P58M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P59L    | X. oryzae pv. oryzae          | 47                      | 31                     | 66               | [22]      |
| Broad      | P60M    | X. oryzae pv. oryzae          | 47                      | 28                     | 60               | [22]      |
| Broad      | P61M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P62M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P66M    | X. oryzae pv. oryzae          | 47                      | 46                     | 98               | [22]      |
| Broad      | P68M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P70M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Narrow     | P71L    | X. oryzae pv. oryzae          | 47                      | 27                     | 57               | [22]      |
| Broad      | P72M    | X. oryzae pv. oryzae          | 47                      | 47                     | 100              | [22]      |
| Broad      | P73L    | X. oryzae pv. oryzae          | 47                      | 46                     | 98               | [22]      |
| Narrow     | Xcc3SH  | X. campestris pv. campestris  | 17                      | 6                      | 35               | [40]      |
| Narrow     | Xcc75H  | X. campestris pv. campestris  | 17                      | 5                      | 29               | [40]      |
| Narrow     | Xcc6SH  | X. campestris pv. campestris  | 17                      | 7                      | 41               | [40]      |
| Narrow     | Xcc8SH  | X. campestris pv. campestris  | 17                      | 4                      | 24               | [40]      |
| Narrow     | Xcc9LK  | X. campestris pv. campestris  | 17                      | 5                      | 29               | [40]      |
| Broad      | Xcc9SH3 | X. campestris pv. campestris  | 17                      | 17                     | 100              | [40]      |
| Narrow     | Xcc14SH | X. campestris pv. campestris  | 17                      | 7                      | 41               | [40]      |
| Narrow     | JPS-xcc-3_P1 | X. campestris pv. campestris | 17                      | 6                      | 35               | [40]      |
| Narrow     | JPS-xcc-4_P1 | X. campestris pv. campestris | 17                      | 6                      | 35               | [40]      |
| Narrow     | JPS-xcc-7_P1 | X. campestris pv. campestris | 17                      | 6                      | 35               | [40]      |
| Narrow     | NBL-xcc-7_P1 | X. campestris pv. campestris | 17                      | 6                      | 35               | [40]      |
| Narrow     | NBL-xcc-4_P1 | X. campestris pv. campestris | 17                      | 4                      | 24               | [40]      |
| Narrow     | NBL-xcc-7_P1 | X. campestris pv. campestris | 17                      | 5                      | 29               | [40]      |
| Narrow     | NBL-xcc-3_P1 | X. campestris pv. campestris | 17                      | 3                      | 18               | [40]      |
| Host range | Phage          | Bacteria strain used          | Number bacteria strains | Lysed bacteria strains | % lytic activity | Reference |
|------------|----------------|------------------------------|-------------------------|------------------------|------------------|-----------|
| Narrow     | NBL-xcc-9_P1   | *X. campestris* pv. *campestris* | 17                      | 8                      | 47               | [40]      |
| Narrow     | NFS-xcc-9_P1   | *X. campestris* pv. *campestris* | 17                      | 6                      | 35               | [40]      |
| Narrow     | GRW-xcc-9_P1   | *X. campestris* pv. *campestris* | 17                      | 3                      | 18               | [40]      |
| Narrow     | NFS-xcc-9_P2   | *X. campestris* pv. *campestris* | 17                      | 5                      | 29               | [40]      |
| Narrow     | NBL-xcc-9_P2   | *X. campestris* pv. *campestris* | 17                      | 7                      | 41               | [40]      |
| Narrow     | GRW-xcc-10_P1  | *X. campestris* pv. *campestris* | 17                      | 7                      | 41               | [40]      |
| Narrow     | NFS-xcc-10_P1  | *X. campestris* pv. *campestris* | 17                      | 3                      | 18               | [40]      |
| Narrow     | NBL-xcc-10_P1  | *X. campestris* pv. *campestris* | 17                      | 5                      | 29               | [40]      |
| Narrow     | GRW-xcc-14_P1  | *X. campestris* pv. *campestris* | 17                      | 8                      | 47               | [40]      |
| Narrow     | NFS-xcc-14_P1  | *X. campestris* pv. *campestris* | 17                      | 12                     | 71               | [40]      |
| Narrow     | NBL-xcc-14_P1  | *X. campestris* pv. *campestris* | 17                      | 7                      | 41               | [40]      |
| Narrow     | GRW-xcc-17_P1  | *X. campestris* pv. *campestris* | 17                      | 9                      | 53               | [40]      |
| Narrow     | NFS-xcc-17_P1  | *X. campestris* pv. *campestris* | 17                      | 3                      | 18               | [40]      |
| Narrow     | NBL-xcc-17_P1  | *X. campestris* pv. *campestris* | 17                      | 5                      | 29               | [40]      |
| Narrow     | GRW-xcc-19_P1  | *X. campestris* pv. *campestris* | 17                      | 8                      | 47               | [40]      |
| Narrow     | NFS-xcc-19_P1  | *X. campestris* pv. *campestris* | 17                      | 12                     | 71               | [40]      |
| Narrow     | NBL-xcc-19_P1  | *X. campestris* pv. *campestris* | 17                      | 7                      | 41               | [40]      |
| Broad      | Pg60           | *X. phaseoli*                | 16                      | 15                     | 94               | [69]      |
| Broad      | Pg176          | *X. phaseoli*                | 16                      | 14                     | 88               | [69]      |
| Narrow     | Pg177          | *X. phaseoli*                | 16                      | 7                      | 44               | [69]      |
| Narrow     | Pg181          | *X. phaseoli*                | 16                      | 9                      | 56               | [69]      |
| Broad      | P1             | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P2             | *X. arboricora* pv. *juglandis* | 16                      | 13                     | 81               | [52]      |
| Broad      | P3             | *X. arboricora* pv. *juglandis* | 16                      | 12                     | 75               | [52]      |
| Broad      | P4             | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P5             | *X. arboricora* pv. *juglandis* | 16                      | 13                     | 81               | [52]      |
| Broad      | P6             | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P7             | *X. arboricora* pv. *juglandis* | 16                      | 10                     | 63               | [52]      |
| Broad      | P8             | *X. arboricora* pv. *juglandis* | 16                      | 12                     | 75               | [52]      |
| Broad      | P9             | *X. arboricora* pv. *juglandis* | 16                      | 11                     | 69               | [52]      |
| Broad      | P10            | *X. arboricora* pv. *juglandis* | 16                      | 12                     | 75               | [52]      |
| Broad      | P11            | *X. arboricora* pv. *juglandis* | 16                      | 12                     | 75               | [52]      |
| Broad      | P12            | *X. arboricora* pv. *juglandis* | 16                      | 11                     | 69               | [52]      |
| Broad      | P13            | *X. arboricora* pv. *juglandis* | 16                      | 11                     | 69               | [52]      |
| Broad      | P14            | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P15            | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P16            | *X. arboricora* pv. *juglandis* | 16                      | 12                     | 75               | [52]      |
| Broad      | P17            | *X. arboricora* pv. *juglandis* | 16                      | 12                     | 75               | [52]      |
| Broad      | P18            | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P19            | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P20            | *X. arboricora* pv. *juglandis* | 16                      | 14                     | 88               | [52]      |
| Broad      | P21            | *X. arboricora* pv. *juglandis* | 16                      | 11                     | 69               | [52]      |
| Broad      | P22            | *X. arboricora* pv. *juglandis* | 16                      | 12                     | 75               | [52]      |
| Narrow     | P23            | *X. arboricora* pv. *juglandis* | 16                      | 5                      | 31               | [52]      |
| Narrow     | P24            | *X. arboricora* pv. *juglandis* | 16                      | 5                      | 31               | [52]      |
| Narrow     | P25            | *X. arboricora* pv. *juglandis* | 16                      | 7                      | 44               | [52]      |
| Narrow     | P26            | *X. arboricora* pv. *juglandis* | 16                      | 5                      | 31               | [52]      |
| Narrow     | φ5A            | *X. axonopodis* pv. *allii*   | 12                      | 5                      | 42               | [24]      |
The storage temperature of *Xanthomonas* phages differs between strains. The initial titer of 4 × 10⁷ pfu/ml of phage Kφ1, is maintained for 6 months when stored at +4 °C in nutrient broth, compared to storage at +20 °C where it declines to 2 × 10⁷ pfu/ml within the same period [82]. Similarly, the lytic activity of *Xanthomonas trifolii* phages is maintained for a month at +4 °C in phosphate buffer, pH 7 [73]. On the contrary, *Xanthomonas arboricora* phages (P6, P11, P15, P16, P20) survive poorly at +4 °C in double distilled water during a one-year storage period. The initial phage titer (1 × 10⁸ pfu/ml) drops drastically to 1 × 10³ pfu/ml. The same phages decline to 8 × 10⁴ pfu/ml when maintained at −34 °C in the same media [52]. Therefore, *Xanthomonas* phages are maintained longer when stored at +4 °C in nutrient broth. The appropriate storage conditions for different phages should be determined in order to ensure longevity of their effectiveness during storage and prior to biocontrol applications [83].

**Storage media, ionic strength and pH** Phage viability is dependent on the storage media, ionic strength and pH and these have to be optimal to ensure phage longevity. Different types of storage media have been investigated to understand their effects on phage viability. SM buffer is a mixture of sodium chloride (100 mM), magnesium sulphate (10 mM), tris-HCL (50 mM, pH 7.5) and gelatin (0.01%). In addition to SM buffer is nutrient broth, water/chloroform (H₂O-CHCl₃) and nutrient broth/chloroform (NB-CHCl₃) combinations [52]. The initial phage titer (1 × 10¹⁰ pfu/ml) of *Xanthomonas arboricora* phages drops to 1 × 10⁶ pfu/ml in SM buffer and to 1 × 10⁵ pfu/ml in nutrient broth and water/chloroform during a one-year period at +4 °C. In addition, phage titers decline further down to 1 × 10⁴ pfu/ml under nutrient/chloroform combination [52]. In other studies, nutrient broth and SM buffer are favorable storage media for phage viability at +4 °C for long-term storage. For example, the initial titer, 8 × 10¹⁰ pfu/ml of phage Kφ1 declines slightly to 8 × 10⁹ pfu/ml in nutrient broth and SM buffer at +4 °C during a three-week storage period [82]. Further decline in phage titer of 3 × 10⁹ pfu/ml is detected in sterile tap water and 10 mM magnesium sulphate while in distilled water the titers sharply fall to 3 × 10⁷ pfu/ml at the same storage temperature and period [82]. Therefore, SM buffer is a better medium for phage survival than nutrient broth.

### Table 3 (continued)

| Host range | Phage | Bacteria strain used | Number bacteria strains | Lysed bacteria strains | % lytic activity | Reference |
|------------|-------|----------------------|-------------------------|----------------------|-----------------|-----------|
| Narrow     | φ5B   | *X. axonopodis* pv. *allii* | 12                      | 5                    | 42              | [24]      |
| Broad      | φ6    | *X. axonopodis* pv. *allii* | 12                      | 9                    | 75              | [24]      |
| Narrow     | φ7A   | *X. axonopodis* pv. *allii* | 12                      | 7                    | 58              | [24]      |
| Narrow     | φ7B   | *X. axonopodis* pv. *allii* | 12                      | 7                    | 58              | [24]      |
| Narrow     | φ14   | *X. axonopodis* pv. *allii* | 12                      | 6                    | 50              | [24]      |
| Broad      | φ16   | *X. axonopodis* pv. *allii* | 12                      | 11                   | 92              | [24]      |
| Broad      | φ17A  | *X. axonopodis* pv. *allii* | 12                      | 11                   | 92              | [24]      |
| Broad      | φ17B  | *X. axonopodis* pv. *allii* | 12                      | 9                    | 75              | [24]      |
| Broad      | φ31   | *X. axonopodis* pv. *allii* | 12                      | 12                   | 100             | [24]      |
| Polyvalent | Kg125 | *Xanthomonas* strains  | 52                      | 52                   | 100             | [69]      |
| Polyvalent | Xcu-P1| *X. campestris* pv. *cucurbitae*, *X. campestris* pv. *dieffembachiae*, *X. campestris* pv. *holcicola* | 38                      | 26                   | 68              | [72]      |
| Polyvalent | Xcu-P3| *X. campestris* pv. *cucurbitae*, *X. campestris* pv. *holcicola* | 38                      | 17                   | 45              | [72]      |
| Polyvalent | Xve-P1| *X. campestris* pv. *pruni*, *X. campestris* pv. *vesicatoria* | 38                      | 9                    | 24              | [72]      |
| Polyvalent | Xca-P1| *X. campestris* pv. *campestris*, *X. campestris* pv. *pruni* | 38                      | 15                   | 39              | [72]      |
| Polyvalent | Xhol-P1| *X. campestris* pv. *cucurbitae*, *X. campestris* pv. *holcicola* | 38                      | 15                   | 39              | [72]      |
| Polyvalent | Xma-P1| *X. campestris* pv. *cucurbitae*, *X. campestris* pv. *malvacearum* | 38                      | 14                   | 37              | [72]      |
| Polyvalent | Xpr-P1| *X. campestris* pv. *holcicola*, *X. campestris* pv. *pruni* | 38                      | 15                   | 39              | [72]      |

**X Xanthomonas; pv pathovar**
broth, tap water, magnesium sulphate, water/chloroform and nutrient broth/chloroform combinations [52]. The right storage media type will preserve the structural integrity of the phage and retain their infectivity during long-term storage [83].

The effect of ionic strength (salt concentration in liquid media) and pH on phage viability has been studied for a few Xanthomonas phages. Xp12 and Cf, lytic activity is maintained in distilled water or 0.1 M phosphate buffer, pH 7.0. However, the ability of these phages to lyse bacterial cells is prevented when they are stored in normal saline (0.9% sodium chloride) or 0.1 M citrate phosphate buffer, pH 7.0 [67, 84]. The optimal pH of Xanthomonas phages is between 5 and 11, with a number of phages being stable in acidic conditions such as pH 4 [23, 67, 82, 85].

Ultraviolet irradiation and chloroform resistance The phyllosphere is a hostile environment and many factors such as ultraviolet (UV) irradiation prevent phage persistence and survivability [86]. As with all phages, Xanthomonas phages are inactivated by UV light. Formulations that increase phage survival consist of milk, corn and sucrose, minimizing UV-induced damages that result from the production of thymine dimers [82, 87, 88]. Chloroform treatment during isolation and enrichment process is used to release phage and kill host bacteria [89]. With the exception of Xf and Cf, many Xanthomonas phages are resistant to chloroform treatment because they lack a lipid envelope that surrounds the capsid. The organic solvent disrupts lipid membranes and inactivates the phage [23, 50, 52, 74, 82, 90]. The ability to resist chloroform denaturation makes non-enveloped Xanthomonas phages easy to isolate, culture and maintained for long-term storage [88].

Biology: life cycle, replication parameters and molecular mechanisms

Life cycle
Generally, clear plaques on a bacterial lawn could suggest that phages may have lytic life cycles, while turbid plaques represent temperate life cycles [91]. Xanthomonas phages produce both lytic and turbid plaques (Table 4). The latter outcome is due to the absence of bacterial host lysis resulting from phage genome integration into host bacteria chromosomes, causing latent infection [27]. Genome integration is facilitated by host XerC/D recombinases that mediate site-specific recombination of the phage genome into a 15 base-pair dif locus of the bacterial genome [93, 98]. Unlike lytic phages, temperate phages are not suitable for use as biocontrol agents due to their ability to cause lysogenic conversion, induction of superinfection immunity and increased risk of horizontal gene transfer [83].

During adsorption, Xanthomonas phages bind to different bacteria host cell surface receptors [99]. The adsorption of phage ΦL7 onto Xanthomonas campestris pv. campestris requires binding to a complex receptor consisting of lipopolysaccharide and a secondary protein on the outer membrane.

Other filamentous phages such as Cf use the host pili (pilR) to bind to Xanthomonas campestris pv. citri [94, 100]. The phage then penetrates using chaperon proteins such as, TonB, ExbB, and ExbD1 encoded by operon, tonB–exbB–exbD1–exbD2 [101, 102]. The host bacteria are lysed by peptidoglycan glycohydrolase, which is located in the phage tail [103].

Replication parameters
The replication of phages is studied using the one-step growth experiment which measures the latent period and burst size of a phage on a specific bacterium. These are essential parameters in the description of phage properties. The latent period is the period between initial phage adsorption to a host cell to lysis and release of progeny viruses [91]. Xanthomonas phages have short latent periods ranging from 20 to 45 min to moderate periods, 60 to 90 min (Table 5). Very long latent periods ranging from 120 to 210 min occur for P125, Xoo-sp2, Xp12 (Siphoriviridae) and XTP (Myoviridae) (Table 5). The burst sizes range from 4.6 to 350 virions per infected cell (pfu/cell), with P125 showing the lowest burst size (4.6 pfu/cell) and Xoo-sp2 with the highest burst size (350 pfu/cell) (Table 5).

The multiplicity of infection (MOI) of reported Xanthomonas phages lie between 0.001 to 1, with the lowest observed for phage X2 at 0.001, and highest for X4, X5 and XTP1 at 1 (Table 5). It has been reported that phages with short latent period and high burst size have more efficient replication cycles [105]. Also, the optimal temperature and incubation time are essential parameters during phage adsorption. These conditions range between 22 and 30°C, while incubation times are between 5 and 30 min for Xanthomonas phages (Table 5).

Molecular mechanisms
Phage-bacterial infection induces molecular changes that include DNA methylation, phosphorylation and transcription. DNA methylation is well-studied in phage Xp12 [81]. Upon infection in Xanthomonas oryzae pv. oryzae, Xp12 induces biosynthesis of an unusual base, 5-methylcytosine, that replaces all cytosine residues in the DNA of Xp12 [81]. The rest of the bases; adenine,
thymine, and guanine, remain unaltered [67, 81]. DNA methylation confers unique physical and chemical properties upon Xp12 DNA i.e., acquisition of a low buoyant density and high melting temperature, compared to typical DNA [106]. The Xp12 phage-infected bacterial cells produce an enzyme deoxycytidylate methyltransferase,
that catalyzes the direct methylation of deoxycytidine monophosphate (dCMP) to 5-methylcytosine, in the presence of tetrahydrofolic acid [107, 108].

Modification of phosphorylation occurs during \textit{Xanthomonas} phage infection. When Xp12 infects \textit{Xanthomonas oryzae pv. oryzae}, phosphorylation of three proteins is induced. The phosphorylated proteins 28 kDa, 28.5 kDa and 45 kDa in size are present only on infected cells. This type of molecular modification is suggestive of the existence of a phage specific regulatory mechanism involved during phage infection [109].

Transcriptional modifications are initiated upon phage-bacterial infection. In phage Xp10, infecting \textit{Xanthomonas oryzae pv. oryzae} displays complete loss of transcription activity due deactivation of host RNA polymerase resulting from dissociation of the δ subunit from the host core RNA polymerase [110]. Later studies show that Xp10 reverts the transcription process by encoding an anti-termination factor p7 that allows formation of RNA transcripts by host RNA polymerase [111].

### Biocontrol applications of \textit{Xanthomonas} phages

This section explores several approaches where \textit{Xanthomonas} phages are employed as biocontrol agents to manage \textit{Xanthomonas} species in either greenhouse or field conditions. These methods have been successful at either inhibiting \textit{Xanthomonas} growth or reducing disease severity. These include, but are not limited to use of monophages or cocktail treatments, phage mixtures with non-pathogenic or with pathogenic bacteria, phage combinations with antibiotics or plant inducers, UV-protectants and phage mutants [16, 21, 24, 30, 88, 112, 113].

To date, two \textit{Xanthomonas} phage-based products are commercially available for the biocontrol of tomato, pepper spot and citrus canker [25]. The earliest evidence of \textit{Xanthomonas} phage application was published in the early nineteenth century by Mallmann & Hemstreet [13], who determined that filtrate from decomposing cabbage applied to rotting cabbage inhibits the growth of \textit{Xanthomonas campestris pv. campestris} in infected tissue. Since then, other forms of phage mixtures have been investigated.

Civerolo [114] applied crude lysates of lytic phage cocktail (Xp3-A and Xp3-I) on peach seedling foliage, 1–2 h before infection with \textit{Xanthomonas pruni} under greenhouse conditions. Only 6–8% of leaves were infected, and the disease significantly reduced to 17–31% compared with 96% recorded on the water-treated control plants. In addition, application of either Xp3-A or Xp3-I mixed with \textit{Xanthomonas pruni} and applied immediately before pathogen inoculation resulted in a 51–54% decrease of bacterial spot symptoms in peach seedlings under similar environmental settings. Therefore, the use of the phage

| Phage | Host Bacterium | Family | Latent Period (Min) | Burst size (pfu/cell) | MOI | Phage Adsorption Temperature Time (min) | Reference |
|-------|----------------|--------|---------------------|----------------------|-----|----------------------------------------|-----------|
| Cp1   | X. axonopodis pv. citri | Siphoviridae | 60                  | 20                   | 1   | 28°C 10                                | [92]       |
| Cp2   | X. axonopodis pv. citri | Podoviridae | 90                  | 100                  | 1   | 28°C 10                                | [92]       |
| P5    | X. axonopodis pv. citri | Myoviridae | n/a                 | 60%                  | n/a | 25°C 20                                | [83]       |
| Xp3-A | X. pruni         | Myoviridae | n/a                 | 30–45                | 0.1 | 27°C 20                                | [15]       |
| Xp3-I | X. pruni         | Myoviridae | 60–75               | 176–256              | 0.1 | 27°C 20                                | [15]       |
| Kp1   | X. euvesicatoria | Myoviridae | 20                  | 75+/-4               | 0.1 | 27°C 5                                 | [50]       |
| Kp8   | X. euvesicatoria | Myoviridae | 30                  | 74+/-22              | 0.1 | 27°C 5                                 | [50]       |
| Kp15  | X. euvesicatoria | Myoviridae | 30                  | 70+/-11              | 0.1 | 27°C 5                                 | [50]       |
| Xoo-sp2 | X. oryzae pv. oryzae | Siphoviridae | 180                  | 350                  | 0.1 | 28°C 10                                | [30]       |
| Xp12  | X. oryzae pv. oryzae | Siphoviridae | 140                  | 35                   | 0.1 | 28°C                                  | [81]       |
| X1    | X. oryzae pv. oryzae | Myoviridae | 20                  | 88                   | 0   | 30°C 15                                | [31]       |
| X2    | X. oryzae pv. oryzae | Myoviridae | 20                  | 88                   | 0.001 | 30°C 15                              | [31]       |
| X3    | X. oryzae pv. oryzae | Myoviridae | 40                  | 50                   | 0.01 | 30°C 15                                | [31]       |
| X4    | X. oryzae pv. oryzae | Myoviridae | 20                  | 75                   | 1   | 30°C 15                                | [31]       |
| X5    | X. oryzae pv. oryzae | Myoviridae | 20                  | 100                  | 1   | 30°C 15                                | [31]       |
| qXDF4 | X. oryzae pv. oryzae | Siphoviridae | 20–30               | 1.8 × 10⁷ pfu/ml     | 0.1 | 28°C 10                                | [23]       |
| XTP1  | X. campestris pv. campestris | Myoviridae | 120                 | 30–35                | 1   | 30°C 15                                | [71]       |
| X. phaseoli phage | X. phaseoli | Siphoviridae | 30–45               | 40                   | n/a | 22°C 25                                | [104]      |
| P125  | Xanthomonas sp. | Siphoviridae | 210                 | 4.6                  | n/a | 27°C 30                                | [69]       |

\textit{X. Xanthomonas; sp., species; (n/a) not available in literature; min, minutes; MOI, multiplicity of infection; pfu, plaque forming unit; %, percentage; ml, millimeters}
cocktail significantly reduced disease severity better than single phage-pathogen mixture. This could be due to the synergy between the replication characteristics of both phages in the cocktail i.e. the latent period of Xp3-A and Xp3-1 is 30–45 min and 60–75 min, whereas the burst size is 42–49 and 176–256 pfu/cell [114].

Some studies disagree with the evidence that supports the benefits provided by cocktail phage biocontrol of Xanthomonas associated diseases. In a recent study [24], spray application of a purified phage cocktail made up of three phages (ϕ16, ϕ17A, ϕ31) failed to inhibit the growth Xanthomonas axonopodis pv. allii, the causative agent of bacterial leaf blight of welsh onions. The cocktail treatment reduced infection of onion leaves to 43.3%, while a monophage phage treatment consisting ϕ31 reduced to 26.6% compared to the untreated, infected control leaves at 67.5% at 9 days after inoculation. Phage ϕ31, family Autographiviridae, had the broadest spectrum and lysed 12 out of 12 Xanthomonas axonopodis pv. allii strains, a trait that may contribute to its biological efficacy [24].

In another study [23], the phage ϕXOF4 inhibited the growth of Xanthomonas oryzae pv. oryzae that causes bacterial leaf blight. The seedlings treated with ϕXOF4 at a titer of $1 \times 10^8$ pfu/ml showed no symptoms compared to 73% of the untreated group. Phage ϕXOF4, Siphoviridae, exhibited a broad host range where it lysed 6 out of 6 Xanthomonas oryzae pv. oryzae strains and had a short latent period between 20 and 30 min and a burst size that yields to the titer $1.8 \times 10^7$ pfu/ml. There is preference for cocktail phages because of their ability to effectively control pathogenic strains and delay the emergence of resistant strains [115, 116]; however, studies [23, 24] support the evidence that monophage treatment can be effective at disease reduction or elimination.

Applications of premixed phage-pathogen suspensions are further demonstrated by Dong [30], who observed low treatment outcomes in rice plants treated with Xoo-sp2 and Xanthomonas oryzae pv. oryzae suspension. The average lesion length in treated plants was 13.31 ± 1.69 cm compared to two control groups treated in sterile water (20.83 ± 2.43 cm) or skimmed milk (19.29 ± 2.07 cm). Phage Xoo-sp2 (Siphoviridae) had a broad host range where it lysed 9 out of 10 Xanthomonas oryzae pv. oryzae strains and had a latent period of 180 min and burst size of 350 pfu/cell. Although the authors considered only Xoo-sp2 out of the 15 phages, a phage cocktail should have been considered to improve biocontrol efficacy since the remaining phages displayed equally a broad host range where they lysed 9 out of 10 of the same strains.

Alternative control approaches using non-pathogenic bacteria and phage suspensions are demonstrated by Nagai [112]. The combination of non-pathogenic Xanthomonas strain (npX, AXCBl201) and phage (pXS, XcpSFC211) was sprayed on broccoli plants before inoculation of Xanthomonas campestris pv. campestris. The npX-pXS mixture significantly reduced disease severity to 18.9% compared with 86.2% by pXS alone and 93.7% of water-treated control plants in greenhouse settings. Field trials showed a decrease in disease severity albeit lower than the results from the greenhouse experiments. The npX-pXS mixture reduced the symptoms by 74% compared to 98% of water treated control plants or 86% of copper treated plants [112].

Integration of Xanthomonas phages with antimicrobials or UV-protectants has been explored as a disease management option. Borah [117] found that the combination of phage (XMP-1) and antibiotic (streptomycin) suppressed leaf spot of mungbean caused by Xanthomonas axonopodis pv. vignae radiate to 4% compared with 68% of the untreated seedlings. Moreover, seed germination increased to 86% in comparison to 75% in the untreated group. Furthermore, Balogh [88] applied formulated phages on tomato plants infected with bacterial spot incited by Xanthomonas campestris pv. vesicatoria. The phages were mixed with either 0.5% pregelatinized corn-flour (PCF), casecrete NH-400 with 0.25% PCF, or 0.75% powdered skim milk with 0.5% sucrose. Phage treatment improved plant yield by 62% (skim milk), 51% (Casecrete), and 30% (PCF) compared to unformulated phages at 1% in greenhouse experiments. Under field experiments, phage treatment increased plant yield by 18% (skim milk), 32% (casecrete) and 23% (PCF) compared to unformulated phages at 14%. Therefore, skim milk gave better results in greenhouse experiments while casecrete performed better in the field. Similarly, Tewfike and Shi maa [66] found that formulated phages in skim milk controlled better bacterial halo blight symptoms of pepper caused by Xanthomonas axonopodis than with corn flour by 20.5 and 18.3% in the greenhouse and 19.5 and 32.2% in field conditions.

Some studies have shown that unformulated phages can control better plant diseases. Balogh [19] applied unformulated phages to citrus leaves infected with asiat ic citrus canker and recorded an average of 59% reduction in disease severity in five greenhouse experiments. The same phage mixture in skim milk was not effective at controlling disease under similar environmental settings. In nursery experiments, unformulated phage treatment also reduced disease, but was less effective than copper-mancozeb, a chemical bactericide. Moreover, mixing the unformulated phages with copper-mancozeb achieved comparable results to unformulated phages alone [19]. Therefore different field settings (greenhouse, open field and nursery beds) should be considered
during biocontrol studies because there is a possibility that phage efficacy depends on the field settings.

Plant inducers successfully control plant diseases, and therefore form an integral part of disease management practices. The application of mixtures of phages in skim milk/sucrose with Acibenzolar-S-methyl (ASM), a plant inducer, decrease the bacterial spot of tomato caused by Xanthomonas campestris pv. vesicatoria under field conditions. The fruit yield of the formulated phage/ASM mixture was 67.9% compared to 60.8% of untreated control when applied twice biweekly in the first year [113]. Equally, Ibrahim [21] applied mixtures containing ASM and phages in skim milk/sucrose on citrus leaves for 4 days triweekly before inoculation of Xanthomonas citri subsp. citri, causative agent of asiatic citrus canker. Disease severity was reduced to 18.3% compared to 75.2% of the untreated control under greenhouse conditions. This observation agrees with results from field experiments where ASM/phages in skim milk/sucrose reduced disease to 12.5%, compared to 70.2% of the untreated control. When ASM was applied alone in the soil by drenching method, the disease was reduced to 38.2%, compared to 74.3% of the water-treated group after spraying 7 times triweekly before pathogen inoculation.

Mutated phages in formulations provide modest protection against plant disease compared with unformulated phages. The h-mutant phage mixtures (PMh; P4L, P43M, P23M1) in skim milk reduced bacterial blight disease of rice incited by Xanthomonas oryzae pv. oryzae to 18.1%, and wild type phage mixtures (PM; P4L, P43M, and P23M1) in the same formulation reduced the disease to 19.2%, compared to 39.1% of the untreated group. The mixtures were sprayed three times within an interval of 10 days. These tailed phages belong to the family Myoviridae and possess broad host range properties. Phage P4L lysed 33 out of 47, while P43M and P23M1 lysed 47 out of 47 Xanthomonas oryzae pv. oryzae strains [22]. Treatment with tecloftalam wettable powder, an agrochemical, demonstrated better results, with the disease symptoms reduced to 5% [22]. Therefore integration of tecloftalam wettable powder in plant protection could be a promising strategy for managing bacterial blight disease. On the contrary, agrochemicals have proved to be less effective than phages in controlling plant diseases. In a two-year greenhouse experiment, formulated phage DB1 in skim milk demonstrated improved black rot control by 71.1% while copper-based pesticide by 59.1%. Thus black rot caused by Xanthomonas campestris pv. campestris on cabbage seedlings can be successfully controlled by phage application [49].

Unformulated mutants reduce disease severity in infected plants. Flaherty [16] applied a mixture of host range mutant phages on tomato seedlings infected with Xanthomonas campestris pv. vesicatoria and symptoms of bacterial spot of tomato reduced to 0.9% compared to 40.5% of the untreated in the greenhouse. It increased the total weight of extra-large fruit by 14.9 and 24.2% in 1997 and 1998, respectively. Similarly, the severity of geranium bacterial blight declined when unformulated phage mutant mixtures were applied daily by foliar sprays on potted and seedling geraniums in greenhouse conditions [17].

Biofilm degradation is essential for the control of bacterial pathogenicity. The phage X3 causes 53% degradation of exopolysaccharide production and 43% biofilm degradation caused by Xanthomonas oryzae pv. oryzae that causes bacterial blight of rice [31]. When phage X3 was sprayed on rice plant foliage and seeds before pathogen inoculation, the plants improved by 83.1 and 95.4%. The phage X3 did not perform well when applied after pathogen inoculation, with results recorded between 28.9 and 73.9% [31]. Phage X3, family Myoviridae, had the broadest host range, lysed 22 out of the 23 Xanthomonas oryzae pv. oryzae strains tested and had the most extended latent period of 40 min with a burst size of 50 pfu/cell [31]. Likewise, infection of XacF1 (Inoviridae), a temperate phage, pathogenic to Xanthomonas axonopodis pv. citri, causing asiac citrus canker, inhibits xanthan production, a component of extracellular polysaccharide that exacerbates the disease. The lesions on leaves sprayed with XacF1 reduced to 1 mm in width compared to 6.5 mm in untreated leaves. Therefore, the reduction in xanthan production caused by XacF1 phage reduces disease symptoms [20].

The frequency of phage spray and contact time on plant surfaces are factors investigated to improve the efficacy of phage applications. Lang [18] showed that multiple applications, i.e. biweekly or weekly applications of phages, effectively reduce symptoms of leaf blight of onion caused by Xanthomonas axonopodis pv. allii to 50%. Similar results were obtained when copper hydroxide-mancozeb was sprayed weekly on onion plants. Furthermore, biweekly application of Acibenzolar-S-methyl and phages reduced the disease by up to 50%. Hence, biweekly spray schedules are a promising strategy for sustainable control of leaf blight of onion.

Successful control of plant diseases is directly linked to the contact time of phages on plant surfaces. Gašić [82] successfully controlled bacterial pepper spot caused by Xanthomonas euvesicatoria by allowing a long contact time of phage Kφ1 (Myoviridae) on plant leaves. The longest time of phage contact was 2 h before and 15 min after pathogen inoculation. This resulted in an average lesion number of 157, 213, and 189 compared to 332, 422, and 567 of the untreated control in three greenhouse experiments. The contact time experiments were further
tested on copper hydroxide mixed with Kφ1. At a contact
time of 26 h before pathogen inoculation, a significant
reduction in average lesion number was observed with
scores of 63, 41, and 66 compared to 332, 422, and 567 of
the untreated control. Thus longer contact time of phage
Kφ1 on plant surfaces allows effective control of pepper
bacterial spot. There is a direct relationship between the
timing of phage application and the efficacy of disease
control. Evening applications of phage on foliage achieve
better disease control since this period minimizes phage
exposure to UV irradiation and extends phage longevity [88]. Phage Kφ1 had the broadest host range where it
lysed 59 out of 59 Xanthomonas euvesicatoria strains
[50] and had a latent period and burst size of 20 min and
75 phage particles per infected cell respectively. Its mul-
tiplication and broad lytic abilities may contribute to its
success at managing pepper bacterial spot.

The study of phage lysins as alternative biocontrol for
Xanthomonas phytopathogens is rarely reported. One
study has shown that phage lysozyme, Lys411, encoded
by the genome of Xanthomonas oryzae phage, φXo411,
can lyse Xanthomonas strains, making the protein a can-
didate with potential to control plant diseases caused by
Xanthomonas [118].

One of the limitations faced by plant-based phage
application is the hostile environment of the phyllo-
sphere, where phages degrade rapidly due to desiccation
or UV light. Phage formulations demonstrate protective
benefits that enhance phage longevity and antibacte-
rial activity [19, 88]; however, not all phages are effective
in UV protectants [19]. Although, leaf surfaces of some
plants do support phage multiplications, others do not;
and this could potentially have adverse effects on the effi-
cacy of a biocontrol product. Balogh [119] found that two
Xanthomonas perforans phages (φXv3–21 and φXp06–
02) multiplied and maintained populations on tomato
leaf surface but did not achieve the same level of multi-
plication on grapefruit leaves. More research is needed
to understand plant compounds involved and the mecha-
nisms involved in this plant-phage interaction.

Conclusion
Several Xanthomonas phages are evaluated for their
potential as biocontrol agents against Xanthomonas
species. So far, most of these belong to order Cau-
dovirales and are lytic to a broad range of host strains.
They are isolated from diverse ecosystems and distrib-
uted across the globe depending on the presence of
the pathogen they infect. Their structural integrity and
functionality in in vitro conditions is maintained under
optimal growth and storage conditions. Pathogenesis
of Xanthomonas phages in bacteria induce molecular
alterations that may have regulatory functions impor-
tant during their life cycle. Although few studies have
focused on this aspect of biology, more research is
needed to understand their life cycle.

From their first discovery in filtrates to applica-
tions as phage/pathogen suspensions, or in combina-

tion with other antimicrobials or with UV-protectants
or as cocktail/monophage treatments, phages have
proved to be promising alternatives to agrochemicals
and antibiotics. They can reduce disease severity or
inhibit bacteria growth in diverse field settings. So far,
two Xanthomonas phage-based biocontrol products are
commercially available for plant disease control. As the
transition into commercial products continues, more
studies are needed to tap into the many unexploited
potentials of Xanthomonas phages for a range of Xan-
thonas related plant diseases.

Abbreviations
ICTV: International Committee on Taxonomy of Viruses; nm: Nanometer; DNA:
Deoxyribonucleic acid; GC: Guanine-Cytosine; ORF: Open Reading Frame; nts:
Nucleotides; %: Percentage; pH: Potential of Hydrogen; NB: Nutrient broth;
H2O: Water; CHCl3: Chloroform; M: Molarity; UV: Ultraviolet light; PFU: Plaque
Forming Units; MOI: Multiplicity of Infection; dCMP: Deoxycytidine monophos-
phate; kDa: Kilodalton; min: Minutes.

Supplementary Information
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Additional file 1: Table S1 Taxonomic classification, genomic proper-
ties and host bacteria of Xanthomonas phages. Description of data:
Xanthomonas phages of order Caudovirales and Tubulavirales, their mor-
phological and genomic properties and host bacteria.

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RN, conceptualized, designed the framework, wrote and proof read the
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1. Jun SR, Sims GE, Wu GA, Kim SH. Whole-proteome phylogeny of prokaryotes by feature frequency profiles: an alignment-free method with optimal feature resolution. PNAS. 2010;107(1):133–8.

2. Ryan RP, Vorhölter FJ, Potnis N, Jones JB, Van Sluys MA, Bogdanove AJ, et al. Mechanisms and responses of Xanthomonas axonopodis pv. citri to acibenzolar-S-methyl. J. Bacteriol. 2007;189:853–62.

3. Pradhan BB, Ranjan M, Chatterjee S. XadM, a novel adhesin of Xanthomonas euvesicula pv. oryzae. Microbiol. Resour. Announc. 2019;8(27):e00366–18.

4. Garlick BD, Osorio DA. Novel bacteriophages infecting different strains of Xanthomonas axonopodis. J. Gen. Virol. 2018;99(10):1453–62.

5. Dong Z, Xing S, Liu J, Wang X, Sun M, et al. Identification and characterization of a novel phage Xoo-sp2 that infects Xanthomonas oryzae pv. Oryzae. J. Gen. Virol. 2018;99(10):1453–62.

6. Oyungyemi SO, Chen J, Zhang M, Wang L, Mazum MML, Yan C, et al. Identification and characterization of five new OP2-related Myoviridae bacteriophages infecting different strains of Xanthomonas oryzae pv. Oryzae. J. Plant Pathol. 2019;101:263–73.

7. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/MT9399492. Accessed 20 May 2021.

8. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/MN047793. Accessed 10 Mar 2021.

9. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/KN074793. Accessed 10 Mar 2021.

10. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/KY853667. Accessed 10 Mar 2021.

11. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/MF375456. Accessed 10 Mar 2021.

12. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/KY853667. Accessed 10 Mar 2021.

13. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/MF375456. Accessed 10 Mar 2021.

14. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/KY853667. Accessed 10 Mar 2021.

15. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/MF375456. Accessed 10 Mar 2021.

16. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/KY853667. Accessed 10 Mar 2021.

17. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nucleotide/MF375456. Accessed 10 Mar 2021.
40. Renu BMS, Singh UB, Sahu U, Nagrare DT, Sahu PK. Characterization of lytic bacteriophages XCC0536, XCC0433 and XCC0081 infecting Xanthomonas campestris pv. Campestris. J. Plant Pathol. 2017;99(1):47–60.

41. Harshitha KN, Manoranjitham SK, Somasundaram E, Rajendran L, Githinji HI, Chen J, Suchashri R, Kaur S, Magesh S, Vaidyanathan R, et al. Characterization of Xanthomonas campestris pv. campestris phage. Microb. Resour. Announc. 2019;8(3):e01381–19.

42. Sahu U, Nagrale DT, Sahu PK, Singh UB. Characterization of bacteriophages infecting Xanthomonas campestris pv. campestris. Plant Pathol. 2017;99(1):47–60.

43. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MT161388. Accessed 10 Mar 2021.

44. Miller M, Deiulio A, Holland C, Douthitt C, McMahon J, Wiersma-Mosthoom. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MA725231. Accessed 10 Mar 2021.

45. da Silva FP, Xavier AD, Bruckner FP, de Rezende RR, Vidigal PMP, Orynbayev AT, Dzhalilov FSU, Ignatov AN. Improved efficacy of a bacteriophage infecting Xanthomonas campestris pv. campestris. J. Plant Pathol. 2017;99(1):47–60.

46. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MA461279. Accessed 10 Mar 2021.

47. Karthikeyan G. Characterization of bacteriophages infecting Xanthomonas campestris pv. campestris. J. Bacteriol. 1994;176(11):3354–9.

48. Romero-Suarez S, Jordan B, Heinemann JA. Isolation and characterization of bacteriophages infecting Xanthomonas campestris pv. campestris. J. Bacteriol. 1999;171(11):3534–9.

49. Sutton MD, Katznelson H, Quadding C. A bacteriophage that attacks numerous phytopathogenic Xanthomonas species. Can J Microbiol. 1958;4(5):493–7.

50. Papaianni M, Cuomo P, Fulgione A, Albanese D, Gallo M, Paris D, et al. Bacteriophages promote metabolic changes in bacteria biofilm. Microorganisms. 2020;8(4):480.

51. Weiss BD, Capage MA, Kessel M, Benson SA. Isolation and characterization of a generalized transducing phage for Xanthomonas campestris pv. Campestris. J. Bacteriol. 1994;176(11):3534–9.

52. Alippa AM. Host range and particle morphology of some bacteriophages affecting pathogens of Xanthomonas campestris. Microbiologia. 1989;5(1):35–43.

53. James N, Roslycky EB. Specificty of bacteriavirus for Xanthomonas trifolii. Can J Microbiol. 1956;2(1):6–11.

54. Vidavé AK, Schuster ML. Characterization of Xanthomonas phaseolii bacteriophages. J Virol. 1969;4(3):300–8.

55. Sutton MD, Wallen VR. Phage types of Xanthomonas phaseolii isolated from beans. Can J Bot. 1967;45(2):267–80.

56. Clark S, Le T, Moreland R, Liu M, Gonzalez CF, Gill JJ, et al. Complete genome sequence of Xanthomonas phaseoli siphophage Samson. Microbiol. Resour. Announc. 2019;8(42):e01097–19.

57. Ackermann HW, DuBow MS. "Phage multiplication". In: viruses of prokaryotes: general properties of bacteriophages, Ackermann HW, Mol Biol. 1968a;34:373–5.

58. Kuo TT, Huang TC, Teng MH. 5-Methylcytosine replacing cytosine in the deoxyribonucleic acid of a bacteriophage for Xanthomonas oryzae. J Mol Biol. 1968a;34:373–5.

59. Vidavé AK, Schuster ML. Characterization of Xanthomonas phaseoli bacteriophages. J Virol. 1969;4(3):300–8.

60. Mathew J, Patel PN. Host specific bacteriophages of Xanthomonas vesicatoria. J Phytopathol. 1979;94:3–7.

61. Nakayinga T, Thiara B, Katabazi A, Nakayinga T. Factors affecting survival of bacteriophage on tomato leaf surfaces. Appl Environ Microbiol. 2007;73(6):1704–11.
87. Wommack KE, Hill RT, Muller TA, Colwell RR. Effects of sunlight on bacteriophage viability and structure. Appl Environ Microbiol. 1996;62(4):1336–41.
88. Balogh B, Jones JB, Momol MT, Olson SM, Obradovic A, King P, et al. Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. Plant Dis. 2003;87(8):949–54.
89. Schisler DA, Slininger PJ. Micobial selection strategies that enhance the likelihood of developing commercial biological control products. J Ind Microbiol Biotechnol. 1997;19:172–9.
90. Dai I, Chow TY, Liao HJ, Chen ZY, Chiang KS. Nucleotide sequences involved in the neolysogenic insertion of filamentous phage CF16-v1 into the Xanthomonas campestris pv. Citri chromosome. Virology. 1988;167(2):613–20.
91. Cloke MR, Kropinski A. Bacteriophages: Methods and Protocols. Volume 1: Isolation, characterization, and interactions. 1st ed. USA: Humana press, Springer Protocols, 2009.
92. Ahmad AA, Ogawa M, Kawaasaki T, Fujie M, Yamada T. Characterization of bacteriophages cp1 and cp2, the strain-typing agents for Xanthomonas axonopodis pv. Citri. Appl. Environ. Microbiol. 2014b;80(1):77–85.
93. Dai H, Tsay SH, Kuo TT, Lin YH, Wu WC. Neolysogenization of Xanthomonas campestris pv. infected with filamentous phage Cf16. Virology. 1987;156(3):313–20.
94. Kuo TT, Lin YH, Huang CM, Chang SF, Dai H, Feng TY. The lysogenic cycle of the filamentous phage Cfl from Xanthomonas campestris pv. Citri. Virology. 1987;156(2):305–12.
95. Lee CN, Lin JW, Weng SF, Tseng TT. Genomic characterization of the intron-containing T7-like phage phiL7 of Xanthomonas campestris. Appl Environ Microbiol. 2009;75(24):7828–37.
96. Okabe N, Goto M. Bacteriophages of plant pathogens. Annu Rev Phytopathol. 1997;1: Isolation, characterization, and interactions. 1st ed. USA: Humana press, Springer Protocols, 2009.
97. Ehrlich M, Ehrlich K, Mayo JA. Unusual properties of the DNA from Xanthomonas phase XP-12 in which 5-methylcytosine completely replaces deoxycytidylic acid in Xanthomonas oryzae pv. Oryzae. J Gen. Microbiol. 2006;162(2):567–72.
98. Inoue Y, Matsuura T, Ohara T, Azegami K. Bacteriophage OP1, lytic for Xanthomonas oryzae pv. Oryzae RNA polymerase of Xanthomonas oryzae pv. Oryzae. J Gen Plant Pathol. 2006b;72:111–8.
99. Kuo TT, Chow TY, Liao HJ, Chen ZY, Chiang KS. Nucleotide sequences involved in the neolysogenic insertion of filamentous phage CF16-v1 into the Xanthomonas campestris pv. Citri chromosome. Virology. 1988;167(2):613–20.
100. Balogh B, Nga NTT, Jones JB. Relative level of bacteriophage multiplication in vitro or in phyllosphere may not predict in planta efficacy for controlling bacterial leaf spot on tomato caused by Xanthomonas perforans. Front Microbiol. 2018;9:2176.
101. Lee CN, Lin JW, Chow TY, Tseng YH, Weng SF. A novel lysosome from Xanthomonas axonopodis pv. Xvgoenoidae and chemicals. J. Mycol. Plant Pathol. 2000;30:99–21.
102. National Center for Biotechnology Information. Bethesda Maryland. 2016. https://www.ncbi.nlm.nih.gov/nuccore/LR743529. Accessed 8 Mar 2021.
103. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/LR743528. Accessed 8 Mar 2021.
104. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/LR743531. Accessed 8 Mar 2021.
105. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/LR743529. Accessed 8 Mar 2021.
106. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/LR743528. Accessed 8 Mar 2021.
107. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/LR743531. Accessed 8 Mar 2021.
108. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/LR743529. Accessed 8 Mar 2021.
109. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/LR743528. Accessed 8 Mar 2021.
110. Lin S-H, Liu J-S, Yang B-C, Kuo T-T. Disassociation of sigma subunit from RNA polymerase of Xanthomonas oryzae pv. Oryzae by phage Xp10 infection. FEBS. Microbiol. Lett. 1998;162(1):9–15.
111. Zenkin N, Severinov K, Yuzenkov Y. Bacteriophage Xp10 anti-termination factor p7 induces forward translocation by host RNA polymerase. Nucleic Acids Res. 2015;43(13):6299–308.
112. Nagai H, Miyake N, Kato S, Maekawa D, Inoue Y, Yosikawa Y. Improved control of black rot of broccoli caused by Xanthomonas campestris pv. Campestris using a bacteriophage and a nonpathogenic Xanthomonas sp. strain. J. Gen. Plant Pathol. 2017;83:375–81.
113. Obradovic A, Jones JB, Momol MT, Balogh B, Olson SM. Management of tomato bacterial spot in the field by foliar applications of bacteriophages and SAR inducers. Plant Dis. 2004;88(7):736–40.
133. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/JN022534. Accessed 10 Mar 2021.
134. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MH059633. Accessed 10 Mar 2021.
135. Ghei OK, Eisenstark A, To CM, Consigli RA. Structure and composition of Xanthomonas pruni bacteriophage. J. Gen. Virol. 1968;3:133–6.
136. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MH206183. Accessed 10 Mar 2021.
137. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MH218848. Accessed 10 Mar 2021.
138. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MH206184. Accessed 10 Mar 2021.
139. National Center for Biotechnology Information. Bethesda Maryland. 2021. https://www.ncbi.nlm.nih.gov/nuccore/MN263053. Accessed 10 Mar 2021.
140. Lin NT, You BY, Huang CY, Kuo CW, Wen FS, Yang JS, et al. Characterization of two novel filamentous phages of Xanthomonas: J. Gen. Virol. 1994;75(9):2543–7.
141. Tseng YH, Lo MC, Lin KC, Pan CC, Chang RY. Characterization of filamentous bacteriophage ΦLF from Xanthomonas campestris pv. Campestris. J. Gen. Virol. 1990;71(8):1881–4.

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