

# How Broad Is Enough: The Host Range of Bacteriophages and Its Impact on the Agri-Food Sector

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## Abstract

Novel bacteriophages (phages) possessing a broad host range are consistently and routinely reported, yet there is presently no consensus on the definition of “broad host range.” As phages are increasingly being used in the development of methods for the detection and biocontrol of human pathogens, it is important to address the limitations associated with the host range. For instance, unanticipated host range breadth may result in the detection of nonpathogenic targets, thereby increasing the false-positive rate. Moreover, a broad host range is generally favored in biocontrol applications despite the risk of undesirable ancillary effects against nontarget species. Here, we discuss the research progress, applications, and implications of broad host range phages with a focus on tailed broad host range phages infecting human pathogens of concern in the Agri-Food sector.

**Keywords:** bacteriophages, host range, agri-food, food safety

## Introduction

THE WORLD'S FOOD supply is under considerable strain due to global population expansion. It is estimated that the yields of staple crops (e.g., maize, wheat, soy) will need to increase by ~25–70% by 2050 to meet demand.<sup>1</sup> Concomitant pressures on food production, processing, and distribution systems are expected to intensify the need for effective detection methods and control measures for existing or emergent foodborne microbiological hazards, including bacterial pathogens such as nontyphoidal *Salmonella enterica*, Verotoxin-producing *Escherichia coli* (VTEC), *Listeria monocytogenes*, and *Vibrio* spp.

Bacteriophages (phages), viral predators of bacteria, are the subject of intense research aimed at the development of detection methods and control measures directed at foodborne bacterial pathogens. Various commercially available phage preparations for the inactivation of foodborne pathogens exist.<sup>2</sup> For the control of *Salmonella*, PhageGuard S (Micareos, Wageningen, The Netherlands) and SalmoLyse<sup>®</sup> (Intralytix, Columbia, MD, USA)

are formulated for use on food products and pet food, respectively. EcoShield PX<sup>™</sup> (Intralytix) targets *E. coli*, including Shiga toxin producing *E. coli*, in carcasses and beef products. ShigaShield<sup>™</sup> (Intralytix) is validated for use against *Shigella* in ready-to-eat meat and poultry, fish, shellfish, fresh and processed produce, and dairy products. Lastly, for control of *Listeria*, ListShield<sup>™</sup> (Intralytix) and LISTEX P100 (Micareos) are validated for use against *L. monocytogenes* in ready-to-eat food products and food processing surfaces.

Compared with phage-based control measures, commercialized technologies employing phage-based bacterial detection are comparatively less popular in the Agri-Food sector. In North America, there is one commercially available phage-based technology for use on the Sample6 DETECT HT System,<sup>3</sup> which has been validated for environmental samples and finished products in the detection of *Listeria*, *Salmonella*, and *E. coli* (Microbiologique, Seattle, WA, USA).

Tailed phages represent ~90% of all phages<sup>4</sup> and they belong to the order *Caudovirales*, which consist solely of dsDNA phages. The order includes the families *Myoviridae*, *Siphoviridae*,

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*Podoviridae*, and *Ackermannviridae* and the recently introduced family *Herelleviridae*. *Myoviridae*, *Siphoviridae*, and *Podoviridae* are distinguished by distinct tail morphologies. Myoviruses possess long, contractile tails; Siphoviruses possess long, noncontractile tails; and Podoviruses have short tails.<sup>5</sup>

Most commonly, phages exhibit two life cycles (lytic or temperate), which serve different purposes and result in different outcomes for both the phage and its host. In the first stage of each lifestyle, phages adsorb to a host cell receptor via tail fibers (i.e., the recognition element of the phage). Host receptors include the lipopolysaccharide (LPS) layer of the Gram-negative bacterial outer membrane, flagella, pili, teichoic acids, or outer-membrane proteins.<sup>6,7</sup> The adsorption event is highly dependent on random collisions between phage and host, thereby allowing interaction of the tail fiber with the host receptor.<sup>7</sup>

Successful phage–host interaction determines the host range of a phage, that is, the range of bacterial hosts a phage can successfully infect.<sup>8</sup> Host range is critical in the selection of phage candidates for detection and control applications. Experimentally, host range determination is routinely performed for the initial characterization of novel phages.<sup>9,10</sup> Many authors have emphasized the importance of this parameter<sup>11–14</sup> and the discovery of novel phages with broad host range has been widely reported, notably in recent years<sup>13–16</sup> (Table 1).

Numerous methods for the isolation of naturally occurring broad host range phages have been reported<sup>17–19</sup>; and phages possessing engineered tail fibers to broaden host range have been synthesized.<sup>20</sup> However, the specific context with which broad host range phages are used must be considered carefully. For instance, a narrower host range may be more suitable for pathogen detection as the specificity of a detection assay may diminish with increasing host range.<sup>21</sup> On the other hand, a broader host range is highly desirable for biocontrol, as the

range of targeted bacterial pathogens is increased considerably, resulting in wider applications. A variety of phage-based approaches have been used for issues in plant and animal health, reviewed by Svircev et al.<sup>22</sup> Phages have also been used as inactivation agents for spoilage bacteria (e.g., *Pseudomonas*, *Clostridium*, and *Lactobacillus*) in food processing.<sup>23</sup> In this review, we discuss the studies, applications, and implications of broad host range phages, with a focus on tailed broad host range phages that infect bacterial pathogens of relevance in food quality, assurance, and processing.

### Phage Adsorption

The adsorption of a phage to a host cell generally initiates infection and is considered a three-step process comprising initial contact, reversible binding, and irreversible attachment.<sup>7</sup> Initial contact depends on random collisions between the phage and host, which may be influenced by the environment in which the two entities interact.<sup>24</sup> For instance, water can serve as a medium for the transport of bacteriophages in humid environments and facilitate access to target the host.<sup>25,26</sup> Phage transmission between distinct geographical sites may also be accomplished by animal vectors.<sup>27</sup> Heterogenous geographic distribution of bacterial host populations further influences global phage distribution.<sup>6</sup> The phage first undergoes reversible adsorption, wherein the phage can desorb from the host. Irreversible attachment is definitive and allows positioning of the phage for DNA ejection into the host cell.<sup>28</sup>

In Gram-negative bacteria, host receptors may be located on the LPS layer, flagella, pili, or outer membrane (proteins), but the LPS is generally considered the most common site of adsorption.<sup>6,7</sup> In Gram-positive bacteria, peptidoglycans and teichoic acids in the outer layer of the cell wall are often sites

TABLE 1. NOVEL BROAD HOST-RANGE PHAGES THAT INFECT FOODBORNE PATHOGENS REPORTED IN 2017–2020

Phage	Host	Test	Host range results	Refs.
STP4-a	<i>Salmonella</i> Typhimurium	Spot test	Infected 91/95 <i>Salmonella</i> strains and 0/3 non- <i>Salmonella</i> strains	14
vB_SPuM_SP116	<i>Salmonella</i> Pullorum	Spot test	Infected 27/37 <i>Salmonella</i> strains	15
SH6	<i>Shigella flexneri</i>	Spot test	Suspected lysis from without for 16/16 <i>Escherichia coli</i> O157:H7 strains, infected 9/19 other tested strains	97
SH7	<i>S. flexneri</i>	Spot test	Infected 9/17 <i>E. coli</i> O157:H7 strains, infected 11/19 other tested strains	97
SaFB14	<i>Salmonella</i> Enteritidis	Spot test	Infected 105/194 <i>Salmonella</i> strains	98
KFS-SE1	<i>Salmonella</i> Enteritidis	Spot test	Infected 6/14 <i>Salmonella</i> strains and 0/21 non- <i>Salmonella</i> strains	99
fmb-p1	<i>Salmonella</i> Typhimurium	Spot test	Infected 10/34 <i>Salmonella</i> strains and 0/12 non- <i>Salmonella</i> strains	100
SS3e	<i>Salmonella</i> Enteritidis	Spot test	Infected 481/482 <i>Salmonella</i> strains and 52/67 non- <i>Salmonella</i> strain	31
ZCSE2	<i>Salmonella</i> Enteritidis	Spot test	Infected 25/25 <i>Salmonella</i> strains	101
myPSH2311	Not specified	Spot test and EOP	Infected 58/80 pathogenic <i>E. coli</i> strains; EOP value = 34.07	102
ST32	<i>E. coli</i>	Spot test	Infected 10/73 <i>E. coli</i> strains ( $n=4$ pathogenic strains, $n=6$ non-pathogenic strains)	38
vB_ValP_IME271	<i>Vibrio</i> <i>alginolyticus</i>	Spot test	Infected 19/102 strains of <i>Vibrio</i> ( $n=12$ <i>V. alginolyticus</i> , $n=7$ strains of <i>Vibrio parahaemolyticus</i> )	32

EOP, efficiency of plating.

of adsorption. Many Gram-negative host receptors have been identified and characterized, whereas Gram-positive bacteria have not been subjected to the same level of scrutiny and comparatively fewer Gram-positive phages have been reported to date.<sup>28</sup> To the best of our knowledge, there are currently no reports of phages whose host ranges span both Gram-negative and Gram-positive bacteria. Host range is largely dependent on the ability of phages to bind to either a single receptor (i.e., narrow host range) or multiple different receptors (i.e., broad host range). However, it is also known that adaptations in host receptor-binding proteins (RBPs) can be modulated to influence the range of bacterial hosts that phages can recognize.<sup>8</sup> For instance, mutations in the host RBP can lead to shifts in the host range of a particular phage. This has been documented with phage  $\lambda$ , which evolved the ability to utilize a different host receptor for infection, resulting in expansion of its host range.<sup>29</sup>

### The Definition of “Broad”

The definition of the term “broad” is an important source of misunderstanding in the context of phage host range. The term may be used to describe phages that can infect multiple strains of the same species<sup>14</sup> or *multiple species* or genera of bacteria, a property often referred to as polyvalence.<sup>30–32</sup> Numerous novel phages described as having a broad host range are shown in Table 1, and several recently discovered polyvalent phages, which are comparatively rare, are listed in Table 2. The host range of phages can show plasticity and is not necessarily a fixed property.<sup>8</sup> For example, Mapes et al.<sup>17</sup> showed that phage clones with an expanded host range were derived from 30 cycles of co-incubation of a lytic phage cocktail with *Pseudomonas aeruginosa*.

Moreover, there are currently no clear standards regarding the minimum number of strains that should be tested in host range analysis or the number of hosts that a phage must infect to be considered as having a “broad” host range.<sup>8</sup> For example, an analysis of 50 poultry-associated *Salmonella* strains by Brenner et al.<sup>33</sup> showed that two phages (SE13 and SE20) lysed all 50 strains, whereas phage SE19 lysed 44/50 and SE14 31/50. We previously found that *Salmonella* phage SI1 lysed 23 strains, phage SS4 22, SF1 20, and SS1 19.<sup>13</sup> Moreover, phage phiC119 lysed 75.75% of 25 *E. coli* O157:H7 strains tested.<sup>34</sup> All phages were deemed to possess broad host ranges. Indeed, there is no general consensus regarding minimum cutoff values for broad host infectivity, and testing against a limited numbers of strains may introduce a bias. Thus, the bacterial strains tested in determining host ranges, along with the context(s) with which the phages should be used, must be carefully considered in the reported studies.

The choice of the host range determination method is another potential confounding factor. Several methods for host range analysis have been reported in the literature. Classical spot testing (i.e., the direct plating of phages on an agar lawn of bacteria) requires interpretation of plaque turbidity to assess bacterial susceptibility to the phage. For instance, a scale that assigned ratings ranging from +4 for clear to +1 for turbid plaques was used to assess the infectivity of *Salmonella* phage by Fong et al.<sup>13</sup> However, such assessments are subject to observer bias and the titers of phages applied to the plates. Moreover, phage infectivity is dependent on the growth phase of the host (e.g., exponential, stationary phase) in spot testing<sup>35</sup> and

TABLE 2. ISOLATED POLYVALENT PHAGES THAT INFECT MULTIPLE GENERA OF FOODBORNE BACTERIAL PATHOGENS

Phage	Genus host range	Refs.
vB_EcoS_AKFV <sub>33</sub>	VTEC <i>Salmonella enterica</i>	39
SS3e	<i>S. enterica</i> <sup>a</sup> <i>Escherichia coli</i> <i>Shigella sonnei</i> <i>Enterobacter cloacae</i> <i>Serratia marcescens</i>	31
EcS1	<i>Shigella</i> spp. <i>S. enterica</i> <i>E. coli</i>	103
SH6 SH7	<i>Shigella flexneri</i> <i>Shigella dysenteriae</i> <i>E. coli</i> (including O157:H7) <i>S. enterica</i> <i>Citrobacter freundii</i>	97
phiE142	<i>S. enterica</i> <i>E. coli</i> O157:H7	12
JHP	<i>P. aeruginosa</i> <sup>a</sup> <i>E. coli</i> <i>S. enterica</i> <i>Campylobacter jejuni</i> <i>Acinetobacter baumannii</i> <i>Proteus mirabilis</i>	104
vB_PcaM_CBB	<i>Pectobacterium carotovorum</i> <sup>a</sup> <i>Erwinia mallotivora</i> <i>Cronobacter</i>	105
phi92	<i>E. coli</i> <sup>a</sup> <i>S. enterica</i>	106
Bo-21 Av-05 Av-06 AV-08	<i>E. coli</i> <sup>a</sup> <i>S. enterica</i> <sup>a</sup>	107
phi PVP-SE1	<i>S. enterica</i> <sup>a</sup> <i>E. coli</i>	108

<sup>a</sup>Isolation host strain (where specified).  
VTEC, verotoxin-producing *Escherichia coli*.

molecules of bacterial origin (e.g., quorum-sensing molecules) may be transferred in the spotting lysate, potentially affecting phage–host interactions on the agar lawn.<sup>36</sup> The agar-based method has also been prone to discrepancies; some phages form poor, or no, plaques on agar plates, and plaque results are semi-quantitative at best.<sup>37</sup>

Adaptations to this classic test have been used, such as the plating of additional dilutions in spot tests<sup>38</sup> to determine the presence of lysis from without (host lysis without productive infection), and microplate assays that involve host–phage co-culture and *post hoc* inspection of turbidity.<sup>39</sup>

Efficiency of plating (EOP) is another widely used host range metric, wherein the productive infection is determined from the number of plaques obtained from a dilution series applied to a variety of hosts.<sup>40</sup> The EOP (average plaque-forming units (PFU) on target bacteria/average PFU on host bacteria) is then calculated, where an EOP equal to or less than 0.001 is deemed inefficient.<sup>40</sup> Brenner et al. proposed a

derived metric known as relative host efficiency (RHE) to account for initial phage titer variations that may be overlooked in EOP analyses. In this scenario, RHE is a logarithmic scoring metric that calculates the difference between the maximum phage titer and the phage spot dilution that results in visible plaques on EOP analyses.<sup>33</sup>

Specific applications for phages should be carefully considered, and the panel of bacterial strains and method of choice rationalized. Given the diversity and complexity of test methods used to evaluate host range, the term “broad” is often a source of confusion and leads to inevitable misinterpretation.

### Broad Host-Range Phages in Pathogen Detection

A plethora of methods have been developed for the detection of bacterial pathogens in the agri-food sector, including traditional culture-based (e.g., employing selective growth media), molecular (e.g., polymerase chain reaction [PCR], pulsed field gel electrophoresis [PFGE]), sequence-based (e.g., whole genome sequencing [WGS]), and immunological (e.g., Enzyme-Linked Immunosorbent Assay) methods.<sup>21</sup> Methods based on PFGE are rapidly being replaced by WGS for outbreak investigation and source tracking.<sup>41</sup> Each method comes with its own advantages and disadvantages, such as high cost, lack of sensitivity, and lack of specificity.<sup>42</sup> Conventional culture-based methods remain in common use for the routine detection of bacterial pathogens<sup>21</sup> due to their relatively low cost, although sensitivity of detection is low and analyses are time-consuming.

The remarkable host specificity of phages has been exploited in the development of alternative detection methods. Phage production for the purposes of bacterial detection is relatively inexpensive and is easily scaled up for commercial purposes.<sup>43</sup> Further, the rapid replication and short infection cycle of lytic phages enable rapid detection of bacterial pathogens.<sup>21</sup> Lastly but importantly, phages only multiply in viable (i.e., living) host cells and false-positive rates derived from phage-based detection methods tend to be lower than with other methods such as PCR.<sup>43</sup> The variety of available phage-based detection tools may be separated into three broad categories based on: (1) detection of components from lysed bacterial cells; (2) phage amplification; and (3) detection with phage components (e.g., tail fibers).<sup>21</sup>

The host range of phages suitable for pathogen detection must be carefully considered to ensure accurate targeting and exclusion of nontarget bacteria. The host range must be sufficiently broad to effectively include all members of a particular species or genus. For instance, an *E. coli* O157:H7-specific phage used for the purposes of detection should ideally be able to detect all strains of *E. coli* O157:H7. However, broad host-range phages may also detect generic *E. coli*, resulting in high false-positive rates. Phages suitable for the detection of multiple variants or serotypes, for example nontyphoidal *Salmonella* or VTEC, need to be thoroughly screened against an exhaustive panel of strains. In addition, they should be screened against a wide array of nontarget hosts, primarily from closely related variants or species, to lessen the potential of false-positives. Several recent studies have shown promise in the phage-based detection of pathogens in the agri-food sector. Hoang et al.<sup>44</sup> developed a colorimetric method by using recombinant broad

host-range phages (IP008 and IP052) to detect *E. coli* in lettuce and mustard greens. Using a panel of 60 *E. coli* strains and 11 nontarget strains, IP052 and IP008 infected 33% of *E. coli* in combination, whereas none of the 11 nontarget strains were susceptible. The reported detection limit was as low as 4 CFU/g, which was considerably lower than detection limits reported in apple juice<sup>45</sup> and beef.<sup>46</sup> More recently, Meile et al.<sup>47</sup> introduced a nanoluciferase protein (NLuc) into a panel of three *Listeria* phages with varying host ranges: a broad host-range phage (A511) and narrow-range serovar 1/2- and serovar 4b/6a-specific phages (A006 and A500, respectively). It was found that broad host-range NLuc-based phage A511 (A511::nluc) could detect one CFU of *L. monocytogenes* in artificially contaminated milk, meat cold cuts, and lettuce in less than 24 h, without false-positive or false-negative results. Serovar differentiation was subsequently achieved by A006::nluc and A500::nluc. These results illustrate how a two-pronged approach utilizing both broad host-range (i.e., species-specific) and narrow host-range (i.e., serovar-specific) phages used in tandem can both detect and delineate variants of *L. monocytogenes* in a variety of food products.<sup>47</sup>

### Broad Host-Range Phages in Biocontrol

The mitigation of foodborne bacterial pathogens that threaten human health during food processing can be accomplished by using chemical (e.g., sodium hypochlorite), thermal (e.g., pasteurization) or nonthermal (e.g., high pressure pasteurization) approaches. In parallel with detection methods, these mitigation strategies suffer from inherent limitations to different extents, including variable inactivation rates, concomitant destruction of nutrients, and adverse effects on sensory properties, resulting in diminished consumer acceptance.<sup>48–50</sup>

The majority of research addressing the use of phages for the control of bacterial pathogens has focused on their application as biocontrol agents in animal husbandry<sup>51–53</sup> and in food products, including agricultural commodities and processed products.<sup>13,54–57</sup> Phages are well suited to food biocontrol applications, because they are: (1) self-replicating and self-limiting (i.e., increase and decrease in titer with bacterial infection and decline, respectively); (2) specific to target bacterial hosts and are usually incapable of crossing genus and species barriers (see exceptions in Table 2); (3) ubiquitously distributed in nature; and (4) can easily be scaled up for commercial applications.<sup>54</sup> Human feeding trials and safety assessments support the view that they are safe for consumption.<sup>58,59</sup> However, thorough screening and identification of key characteristics are essential for selection of phage for food applications. These include: (1) a strictly lytic life cycle; (2) absence of genes encoding for antimicrobial resistance (AMR) and/or virulence traits; (3) stability under a variety of storage or treatment-related conditions (e.g., pH up/downshifts, temperature fluctuations, sanitizers), as foods are often preserved by the application of multiple hurdles (i.e., techniques for pathogen inactivation applied in tandem); and (4) broad host range against target pathogens.<sup>2,13,60</sup>

Cocktails consisting of mixtures of phage strains have traditionally been used to achieve a broad host range for biocontrol applications.<sup>51,61,62</sup> However, the formulation of effective cocktails is further complicated by the need to

exhaustively screen against all potential targets; therefore, the metrics used to define “broad host-range” (i.e., the test method used and the strains tested) must be made clear and interpreted in the context of the approach. This is especially important when considering practical applications. Islam et al.<sup>63</sup> incorporated three broadly lytic *Salmonella* phages in a biocontrol cocktail, including phages LPSTLL and LPST94, which lysed 41 of 41 *Salmonella* strains, and phage LPST153, which lysed 31. The cocktail was effective against both a *Salmonella* Typhimurium strain alone and in combination with an *Salmonella* Enteritidis strain in milk and on chicken breast, reducing the density to below the detectable limit (<1 CFU/100  $\mu$ L) at multiplicities of infection (MOIs) of 10,000 and 1000. Phage SE13 isolated by Fong et al.<sup>13</sup> was utilized in a cocktail comprising phages  $\Phi$ 3,  $\Phi$ 6, Felix01, and HER20, which could lyse at least 35 of 43 *S. enterica* strains.<sup>64</sup> The five-phage cocktail reduced *Salmonella* Enteritidis and *Salmonella* Javiana populations by >3 log CFU/g on cantaloupe and lettuce.<sup>64</sup> In a separate study performed with a novel broad host-range phage termed PE37, Son et al.<sup>65</sup> reported that 100% of *E. coli* O157:H7 ( $n=37$ ) and *E. coli* O26 strains ( $n=12$ ) were lysed. In addition, PE37 infected 16 of 25 extended spectrum  $\beta$ -lactamase (ESBL)-producing *E. coli* isolates belonging to 10 different O-antigen groups. After 24 h of treatment, PE37 reduced *E. coli* O157:H7 in raw beef by 2.3 log CFU/piece at MOI = 100. Similarly, reductions of 1.5 log CFU/piece of raw beef were observed when a cocktail of three *E. coli* strains (*E. coli* O157:H7 and ESBL-producing *E. coli* [EBL66 and EBL116]) were treated with PE37.<sup>65</sup> Yu et al.<sup>66</sup> showed that a cocktail containing polyvalent phages PER01 and PER02 was significantly more effective for the control of  $\beta$ -lactam-resistant *E. coli* NDM-1 in sludge than a narrow host-range cocktail formulated with phages MER01 and MER02. Because polyvalent phages were able to utilize alternative production hosts to increase phage titer, target bacteria were suppressed by 2.4 log CFU/mL, compared with 1.5 log CFU/mL when the narrow host-range cocktail was used. These results also demonstrated the potential of using benign hosts to propagate phage for commercial use, leading to enhanced safety and decreased capital and operating costs.<sup>66</sup> More recently, it was shown that polyvalent phage PS5 inhibited *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *E. coli* O157:H7 *in vitro* and in a variety of foods (raw chicken skin, raw sliced beef, fresh lettuce, whole fat pasteurized milk, and whole egg). This was the first report of successful pathogen control in food matrices using polyvalent phages.<sup>67</sup>

## Implications of Broad Host-Range Phages

### Host–phage coevolution

Although many regard adsorption as the defining event in phage infection and the most important host range determinant, several additional factors influence phage infectivity. Sustained selective pressure can lead to the development of bacterial resistance to phage infection, with important consequences for host range.<sup>68–71</sup> The prospect of phage resistance is primarily managed by the formulation of biocontrol preparations with multiple phages, preferably from different families or with variable receptors.<sup>72</sup> Here, an understanding of host cell defenses and their role in the development of resistance can support strategies intended to minimize this

risk. Selective pressures induced by food components, environmental stresses, and transferable resistance in nontarget species may also contribute to resistance.<sup>73</sup> Bacteria have evolved diverse defense strategies, often described as a molecular arms race, to evade phage infection and/or lysis, which are broadly categorized as “innate” bacterial immunity (e.g., adsorption inhibition, restriction modification, etc.) and “adaptive” immunity (e.g., CRISPR/Cas9). Both can induce phage-associated costs, notably limits on the number of suitable hosts for infection in populations where host immunity to infection is highly prevalent.<sup>74</sup> Previously, we have observed that mutation of the surface LPS receptor in *Salmonella* Enteritidis induced resistance to infection by phage SII.<sup>68</sup> Diminished expression of the LPS O1 antigen to resist phage infection has also been seen in *Vibrio cholera*.<sup>75</sup> Some phages also possess anti-host defenses to overcome host immunity, for example, encoded anti-CRISPR (Acr) proteins that overcome bacterial CRISPR-Cas systems.<sup>76</sup> Hence, multiple phage- and host factors that contribute to host range limitation and expansion<sup>74</sup> must be considered in the selection of phages for specific applications.

### Spread of clinically relevant genes

Phages are a major driver of bacterial evolution and do so by transferring genes through specialized or generalized transduction. Some genes may have an advantageous effect on the host (e.g., AMR genes, virulence, etc.), which, in turn, promote phage survival and dissemination. The presence of such clinically relevant genes (e.g., encoding for AMR, virulence, etc.) has been well documented in both lytic and temperate phages. Shousha et al.<sup>77</sup> found that 24.7% of 243 phages isolated from poultry transduced antibiotic resistance into *E. coli*. Several  $\beta$ -lactam resistance genes (*bla*<sub>CMY</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub>, and *bla*<sub>OXA</sub>) in *E. coli* phages isolated from wastewater were transduced in *E. coli* strains, including VTEC.<sup>78</sup> Colomer-Lluch et al.<sup>79</sup> detected  $\beta$ -lactam resistance genes *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, and *mecA* by qPCR in phages sourced from river and sewage water samples. The AMR genes such as *qnrS* and *bla*<sub>SHV</sub> were identified in the phage DNA fraction of environmental water samples, reaching up to 4 log<sub>10</sub> copy number/mL.<sup>80</sup> Further, it is well known that several virulence determinants in *Salmonella* Typhimurium and VTEC are associated with prophages (i.e., temperate phages).<sup>81,82</sup>

An obvious prerequisite for the dissemination of clinically relevant genes (e.g., AMR and virulence factor-encoding genes) is the recognition of the donor and recipient cells by phages. Inevitably, wide-scale dissemination of such genes is amplified as the host range broadens. Given that the host ranges of some phages span great taxonomic distances (Table 2), this may promote the evolution and emergence of increasingly virulent pathogens.<sup>83</sup> Although relatively uncommon, the transduction of clinically significant genes between distant hosts may carry profound evolutionary consequences.<sup>83</sup> An analysis of 2239 phage sequences provided strong statistical evidence for the intergeneric dissemination of antibiotic-resistant genes *bla*<sub>CTX-M</sub>, *mel*, and *tetM* between several hosts such as *E. coli*, *Salmonella*, *Shigella*, *Streptococcus*, and *Bacillus*.<sup>84</sup> These observations suggest that broad host-range phages play a role in the spread of antibiotic resistance between species and genera.

Intergeneric transduction events have been engineered in the laboratory, where the host range was increased by designing hybrid particles of the *E. coli* T7 phage with various tail and tail fiber proteins of other phages.<sup>85</sup> The T7 phage was able to transduce the target DNA (including an antibiotic resistance marker) into novel hosts, such as *Klebsiella*, *Salmonella*, *Shigella*, and *Enterobacter*.<sup>85</sup> Collectively, these results confirm the necessity to screen candidate phages for biocontrol applications to ensure the absence of such genes. Further, it is important to thoroughly assess the host ranges of novel phages (ideally with multiple methods such that error is minimized), their host receptors and to include taxonomically distinct bacteria in such analyses.

#### Future research considerations

Clearly, caution must be applied to the interpretation of data from phage host-range analysis. Assignment of this designation to novel phage isolates relies on careful selection of both methods and analytical conditions. Moreover, the host range should not be represented as a percentage (i.e., the percentage of hosts infected), as this may artificially enhance the actual host range. For biocontrol applications, the selection of experimental strains must reflect the anticipated host diversity in the target system, and include both significant (e.g., outbreak isolates) and closely related strains and/or serotypes. When considering phage candidates for detection, additional “non-target” bacterial strains should be included in the validation assays as controls.

At minimum, phages used for biocontrol should not increase the pathogenicity or virulence of the target pathogen. Genes encoding for AMR and virulence should be absent, and in certain applications, genes encoding for lysogeny should also be absent to guarantee lysis. A variety of bioinformatic tools that require little technical expertise, yet accurately predict the putative functions of open reading frames, such as RAST, NCBI BLASTp, and others, are available for this purpose.<sup>86–89</sup>

False-positive results due to the detection of both target and nontarget hosts by excessively broad host-range phages is one of the main challenges associated with their use for detection. This can be avoided by conducting host-range analysis using a panel of nontarget bacteria. On the other hand, a broad host range is warranted for the purposes of biocontrol, as the primary goal is to inactivate bacterial pathogens. As the number of target pathogens increases, safety and confidence in the agri-food sector is also enhanced. A number of studies have emphasized the efficacy of both single phages<sup>13,31,38</sup> and phage cocktails (i.e., formulations comprising several phages)<sup>52,63,72</sup> for pathogen inactivation. Phage cocktails are generally formulated with several phages, usually with broad and overlapping host ranges, primarily to minimize or delay the risk of bacterial resistance.<sup>90,91</sup> This strategy is intended to ensure continued efficacy in the event that a host develops resistance to one or more of the phages.<sup>90</sup> Ideally, phages should be from different families, as it is known that receptor preference varies across phage families,<sup>9</sup> and cocktails should contain a restricted number of strains to avoid the possibility of antagonism.<sup>92</sup> Further, alternating use of multiple cocktails can further delay bacterial resistance.<sup>93</sup> Interestingly, synergy among pha-

ges has also been observed, where the host range of a cocktail is actually greater than the sum of the host range for the individual phage components.<sup>94–96</sup>

#### Conclusions

We have presented an overview of broad host-range phages and the implications of their use in the agri-food sector. Although numerous broad host-range phages are being discovered, the scientific community has yet to decide on a standard for the definition of “broad host range.” We propose the criteria for selecting appropriate bacterial strains to conduct host-range analyses, and high-quality, in-depth assays to appropriately gauge the host range. Depending on the application, the host range should include as many relevant strains and species as possible, which may differ depending on the target pathogen. When screening phages for biocontrol purposes, researchers may consider including strains of high clinical importance (i.e., most commonly implicated in outbreaks, high mortality). Previously, we screened 45 *Salmonella* phages against 61 strains of *S. enterica* representing diverse serotypes that were commonly implicated in foodborne outbreaks and that were antimicrobial resistant, and we found this to be suitable in gauging host range.<sup>9</sup> Other considerations may be to include non-target microorganisms (e.g., commensal microorganisms) to ensure the absence of cross-infectivity. Clearly, from an application-based perspective, the implications of broad host range differ depending on intended use and may determine the success or failure of detection or biocontrol objectives. Special attention should be paid to host evolutionary processes, which may reduce the host range of phages in the long run, and the dissemination of clinically significant genes across diverse bacterial populations.

#### Author Disclosure Statement

No competing financial interests exist.

#### Funding Information

This work was supported by the British Columbia Ministry of Agriculture and Lands (BCMAL) grants URACP19-210 and URACP19-211, and an Agriculture and Agri-Food Canada start-up grant.

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