HUMAN-SPECIFIC PHAGES INFECTING *ENTEROCOCCUS* HOST STRAIN MW47: ARE THEY RELIABLE MICROBIAL SOURCE TRACKING MARKERS?

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Running head: Reliability of MW47 phages for MST

Abstract

Aim: The aim of this study was to determine the morphological diversity and environmental survival of human-specific phages infecting *Enterococcus faecium* host strain MW47, to support their use as microbial source tracking (MST) markers.

Methods and Results: Twenty phages capable of infecting strain MW47 were propagated and their morphologies determined using transmission electron microscopy (TEM), which revealed that a heterogeneous group of phages was able to infect strain MW47. Three distinct morphologies from two different families (*Myoviridae* and *Siphoviridae*) were observed. *In situ* inactivation experiments were subsequently conducted to determine their environmental persistence.

Conclusion: The findings revealed a statistically significant link between morphology and the rate of inactivation, with phages belonging to the *Myoviridae* family demonstrating more rapid inactivation in comparison to those belonging to the *Siphoviridae* family.

Significance and Impact of Study: The results suggest that whilst *Enterococcus* MW47 phages appear to be a potentially valuable MST tools, significant variations in the persistence of the different phages mean that the approach should be used with caution, as this may adversely affect the reliability of the approach, especially when comparing MW47 phage levels or presence across different matrices (e.g. levels in sediments or shellfish). This highlights the importance of elucidating the ecological characteristics of newly proposed MST markers before they are used in full-scale MST investigations.

Keywords

Bacteriophages, Viruses, Diversity, Markers, Microbial Source Tracking, Enterococcus, TEM, Inactivation

1. Introduction

Faecal indicator organisms (FIO) are currently used to assess the hygienic quality of water and are present in the faeces of both human and non-human animals. Their detection offers no insight into the source(s) of environmental contamination, which would support a water quality monitoring strategy and provide more effective preventive measures. Many human waterborne pathogens are only found in human faeces, whereas important zoonotic disease agents (e.g. *Cryptosporidium*) are associated with specific non-human faecal sources (Soller *et al*., 2010). Determining the source of faecal pollution in raw drinking water provides valuable information on potential risks to human health. Microbial source tracking (MST) techniques, which make use of microbial populations that are specific to particular faecal sources, can support risk assessments by providing information on which pathogens are present in samples.

The field of MST has advanced rapidly during the past twenty five years and the available literature on possible techniques is extensive (Boehm *et al*., 2013). Many researchers have suggested that no single MST technique may be able to satisfy all requirements in all situations and instead a 'toolbox' approach combining several methods is needed (Gourmelon *et al*., 2010). This is due to a number of factors, such as differences in size, morphology, environmental resistance, shedding rate, inactivation rate and geographical distribution of different proposed markers and pathogens (Diston *et al*., 2012; Duran *et al*., 2003; Ebdon *et al*., 2012). Therefore, there remains a need for a greater understanding of the ecological characteristics of candidate MST markers (Vogel *et al*., 2007; Plummer *et al*., 2009; Gourmelon *et al*., 2010). For instance, studies have shown that enteric viruses survive longer in the environment than traditional bacterial faecal indicators, such as *Escherichia coli* (Nasser and Oman, 1999; Moce-Llivina *et al*., 2005). Bacteriophages (phages) have been proposed as alternative indicators that may better predict risks to human health associated with enteric viruses. The detection and enumeration of phages such as those infecting *Bacteroides* spp. and the subgroups of F-specific RNA coliphages have been successfully used to discriminate human from non-human faecal contamination of the environment (Jofre *et al*., 1986; Gourmelon *et al*., 2010; Ebdon *et al*., 2012). Ratios of somatic coliphages (non-specific indicators of faecal contamination) to phages of *Bacteroides thetaiotaomicron* GA17 (human-specific indicators of faecal contamination) have also been shown to provide a method for discriminating human and non-human samples (Muniesa *et al*., 2012). Another group of phages which have received growing attention in recent years are those of *Enterococcus* spp. (Bonilla *et al*., 2010; Santiago-Rodríguez *et al*., 2010; Purnell *et al*., 2011; Vijayavel *et al*., 2014; Verçosa and Moreira, 2016; Wangkahad *et al*., 2017). Initial investigations into the use of these phages have shown them to be promising MST candidates, with some displaying high specificity to particular human and non-human faecal

sources (Purnell *et al*., 2011; Santiago-Rodriquez *et al*., 2010; Santiago-Rodriquez *et al*., 2013).

A potential limitation of certain phage-based MST techniques, particularly those which involve the detection of a wide range of phages (such as somatic coliphages) can be the differential survival rates of the various phages that are capable of infecting the host strain. Phages exhibiting different morphologies have been shown also to differ with respect to their survival rates (and hence abundance) in the non-gut environment, which can hinder the interpretation of MST (Muniesa *et al*., 1999). Previous studies have also shown that the predominant phages observed in wastewaters and other waterbodies recently contaminated by faeces may differ markedly from those observed in waterbodies exposed to environmental factors such as UV light, desiccation, and elevated temperatures and those that have undergone drinking water treatment processes (Dee and Fogleman, 1992; Lasobras *et al*., 1997; Muniesa *et al*., 1999). In order to provide consistent source tracking information throughout the water cycle, candidate MST markers must demonstrate consistent responses to environmental stressors (Diston *et al*., 2012), which are more likely among homogenous groups of phages that demonstrate consistent ecological behaviour and environmental survival characteristics (Queralt *et al*., 2003). For this reason it is important to assess the morphological homogeneity of phages infecting host strains prior to their widespread uses as MST tools.

Enterococcus phages have been examined by transmission electron microscopy (TEM). According to Ackerman (2007), the majority of these have been demonstrated to belong to tailed phage families, predominantly the family *Siphoviridae*. These phages have shown varying host ranges. Whilst some phages only appear to be able to infect their original hosts,

others appear to be capable of infecting all *Enterococcus* species (Paisano *et al*., 2004; Ramírez *et al*., 2006; Son *et al*., 2010; Vinodkumar *et al*., 2011). Although the majority of *Enterococcus* phages observed are members of the *Siphoviridae* family, authors have also reported tail-less phages that are capable of infecting such strains (Bachrach *et al*., 2003; Fard *et al*., 2010). Santiago-Rodríguez *et al*. (2010) proposed phages infecting an *Enterococcus faecalis* host strain as potential surrogates of enteric viruses in studies of recreational waters as they were more resistant to primary and tertiary wastewater treatments and their survival abilities in fresh and marine waters were comparable to somatic coliphages. The data indicated that various phage populations were able to infect the *E. faecalis* host strain. Initial *in vitro* survival experiments also demonstrated that the phages shared similar survival characteristics (Santiago-Rodriquez *et al*., 2010; 2013). The authors observed the presence of both non-tailed and tailed phages, though their findings suggested that the majority of these phages belonged to the *Siphoviridae* family.

Purnell *et al.* (2011) isolated an *Enterococcus faecium* host strain (MW47) that demonstrated high specificity to human faecal sources. Phages of MW47 were detected in both raw and treated wastewaters and have not to date been detected in non-human faeces. Whilst the specificity of these phages indicates their strong potential as MST markers, the degree of morphological diversity and their survival characteristics have not been explored. This study elucidates ecological characteristics of these phages that are essential to assessing suitabilities as MST tools. The study described here presents new knowledge of the morphological diversity and survival characteristics of human-specific phages capable of infecting *E. faecium* host strain MW47.

2. Materials and Methods

2.1 Phage enumeration

Phages infecting *E. faecium* (MW47) were enumerated using previously described double agar-layer and spot test plaque assay methods (Adams, 1959; Jofre *et al*., 1986; Purnell *et al*., 2011). Tryptone soya broth (TSB) (Oxoid, Fisher Scientific, UK) was used as the growth medium for host strain MW47. Tryptone soya agar (TSA) was used as the solid medium for all double agar-layer and spot test plaque assays. The concentrations of agar in top and bottom layers used were the same as those reported elsewhere (ISO 10705/2) (Anon, 2001). Briefly, when performing double agar-layer assays, 1 ml of each sample was added to 1 ml of exponentially growing host strain, and 2.5 ml of semi-solid agar (TSAss). The resulting suspension was mixed briefly using a Whirlimixer™ (Fisher Scientific, UK) and poured onto previously prepared TSA (Oxoid, Fisher Scientific, UK) in 90 mm diameter Petri-plates. Once the top layer had solidified, plates were inverted and incubated at 37 ^{o}C (±2 ^{o}C) for 18-24 h. To perform spot test assays, double agar-layer plates were prepared as described above, with 1 ml of exponentially growing host strain and 2.5 ml of TSAss without addition of sample and were poured onto previously prepared 90 mm TSA agar-layer Petri-plates. Once solidified, 10 µl of phage lysates and dilutions thereof (1:10) were spotted onto the top agar, being careful to label each spot, so that they could be identified following incubation. The drops were left to air dry before plates were inverted and incubated at 37 ^{o}C (±2 ^{o}C) for 18-24 h. Following incubation, circular 'zones of lysis' in the confluent lawn were expressed as Plaque Forming Units (PFU) per ml of sample.

2.2 Phage isolation, purification and concentration

Samples (n=5) of untreated municipal wastewater were collected for isolation of phages capable of infecting *E. faecium* MW47 from the influent of a biological wastewater treatment works (WWTW) situated in South East England, UK (population equivalent 37,327). Well-

152 distributed plaques enumerated by the double agar-layer method were picked at random for
153 isolation to avoid plaque morphology bias with the intention of characterising twenty phages
154 from the host bacterium MW47. Phages were numbered MW47- 1 to MW47- 20 for all
155 subsequent analyses.
156
157 All phages were purified and concentrated by a plate propagation technique that was
158 modified from previously described methods used by Carey-Smith *et al*. (2006) and Fard *et*
159 *al*. (2010). In brief, cores of agar containing plaques were picked using sterile glass Pasteur
160 pipettes and suspended in 200 µl of phage buffer (19.5 mmol Na_2HPO_4, 22 mmol KH_2PO_4,
161 85.5 mmol NaCl, 1 mmol $MgSO_4$, 0.1 mmol $CaCl_2$) in microcentrifuge tubes (Fisher
162 Scientific, UK). The phage suspensions were incubated at 4^{o}C overnight to allow diffusion of
163 phage into the buffer. The phage suspensions and dilutions were retested with the double
164 agar-layer method to purify and confirm the presence of phage. This was repeated three times
165 to obtain purified phages. Once purified, 5 ml of phage buffer solution was added to plates
166 with near complete lysis of the host bacterium and left at room temperature for 1 h. The
167 plates were swirled regularly. The liquid and top agar-layer were then scraped into 50 ml
168 centrifuge tubes (Fisher Scientific, UK), mixed briefly using a Whirlimixer™, and left at
169 room temperature for an additional 30 min. Bacterial debris and top agar-layer were removed
170 from the suspension by centrifugation at 3000 *g* for 20 min. The supernatant was then filtered
171 through a 0.22 µm polyvinylidene diflouride membrane syringe filter unit and stored in light
172 tight glass bottle at 4 ^{o}C in the dark (for not longer than seven days). The titre of the
173 suspension was determined by testing ten-fold dilutions (10^{-1} to10^{-8}) using the spot test plaque
174 assay. The process was repeated until a minimum titre of 10^{8} PFU ml^{-1} was achieved for all
175 phage suspensions. Plaque diameters were measured using a Vernier Caliper (Fisher
176 Scientific, UK).

177

178 **2.3 Transmission electron microscopy (TEM)**

179 All 20 propagated phage were examined by TEM to determine the phage morphologies. In
180 order to view phage under the TEM, the phage suspensions were negatively stained. Uranyl
181 acetate (UA) stain (pH 4-4.5) was used to stain the phage suspensions. One drop (10 µl) of
182 previously prepared high-titre phage suspension was applied to a 200 mesh Formvar/Carbon
183 copper electron microscope grid (Agar Scientific, UK). After 2 min, excess suspension was
184 removed with Whatman No.1 filter paper (Whatman, UK). One drop (10 µl) of UA stain (1
185 % w/v, previously filtered through a 0.22 µm filter unit) was then applied to the grid for 1
186 min. Excess stain was removed again using a new Whatman No. 1 filter paper, and the grid
187 was then left to dry. The grid was kept in a labelled Petri-plate (55 mm) prior to viewing
188 under the TEM (Hitachi-7100) at 100 kV.

189

190 **2.4 Assessment of host range**

191 The phages isolated from *E. faecium* strain MW47 were tested for their abilities to infect
192 other species of the genus *Enterococcus* (including *E. asini*, *E. casseliflavus*, *E. durans*, *E.*
193 *faecalis*, *E. gallinarum*, *E. hirae*, *E. mundti*, *E. pseudoavium*, *E. saccharolyticus*, and *E.*
194 *sulfureus*), and their abilities to infect two other bacterial host strains from genetically
195 different genera, GB-124 (*Bacteroides*) and WG5 (*E. coli*). Host range provides further
196 information on phage diversity and was determined by spot tests of dilutions (10^{-1} to 10^{-8}) of
197 each high titre phage stock suspension on a lawn of each host bacterium. Spot tests were
198 performed in triplicate and reported as mean PFU ml^{-1}.

199

200 **2.5 *In situ* inactivation investigation**

River water samples (100ml fresh-water) were collected from the Bevern Stream (a tributary of the River Ouse, UK). The samples were immediately analysed for temperature, pH level, conductivity, salinity, dissolved oxygen, and turbidity using a handheld multi-parameter probe (Aquaread Ltd, UK). *In situ* inactivation experiments were then set up by spiking surface water samples with isolated phages (10^5 PFU ml^{-1}) and placing them into individual dialysis tubes (cut-off 14 kDa), which were then sealed and positioned in the stream at a depth of 15-20 cm below the surface. The site was chosen as being representative of streams found in the catchment in terms of flow characteristics, land use, and underlying geology. The *in situ* inactivation experiments were performed in duplicate using phages belonging to the three distinct morphologies isolated. Phages were enumerated in duplicate, immediately after spiking (T_0), and after one (T_{24}), two (T_{48}), five (T_{120}), eight (T_{192}) and nine (T_{216}) days. Results were reported as mean PFU per ml of sample.

2.6 Statistical analysis

Statistical tests were performed using the statistical package IBM SPSS Statistics 20.0, with the significance level set at 5%. Inactivation is presented as the decrease in Log10 units (Log N_0 / N_t) of phage numbers of each phage type (MW47-1, -2, -5, -6, -10, -15) before (N_0) and after (N_t) inactivation processes. Presenting the data in this way allows for comparison with similar studies of inactivation of bacteriophages used in MST. Anderson-Darling normality tests were performed and indicated that data were not-normally distributed. Therefore, non-parametric tests were selected to determine if there were significant differences between the number of phages detected (Wilcoxon signed-rank test), inactivation of phages from different families (Mann-Whitney test) and inactivation of phages with different capsid morphologies (Mann-Whitney test). The P-value and the test conducted are presented in the text where appropriate.

3. Results

3.1 Phage Isolation

In total, 20 single distinct plaques were successfully picked and purified from double agar-layers of host strain MW47 infected with phages from raw (untreated) municipal wastewater obtained from the influent of a wastewater treatment works. All plaques were successfully propagated to a high titre (at least 10^8 PFU ml^{-1}) in accordance with the plate propagation method described in section 2.2. To determine the resulting titre, phage lysates were tested in duplicate using spot test plaque assays. The final twenty phage cultures demonstrated titres of between 5.0 x 10^8 and 1.1 x 10^{10} PFU ml^{-1}. During phage isolation and propagation, it was evident that different phages produced plaques of varying size. For example, phages MW47-4, -9, -10, -15 and -19 produced particularly small 'pin-hole' sized plaques (<0.2 mm diameter). Although these plaques were small, they were still clear and readable. MW47-15 produced the smallest plaques, with a mean diameter of 0.18 mm (*n*=10) and MW47-7 produced the largest plaques with a mean diameter of 1.74 mm (*n*=10). A number of the phage isolates producing larger plaques (>1 mm diameter) also exhibited a halo formation around the plaque.

3.2 Phage morphology

In order to explore the degree of morphological diversity of the isolated phages, all 20 high-titre phage cultures were viewed using TEM. TEM phage micrographs of the three distinct phage morphologies observed are presented in Figure 1.

The TEM data revealed all phages have helical tails and thus belong to the order *Caudovirales*. Of the 20 phages viewed by TEM, 18 (90%) had simple non-contractile tails,

placing them in the *Siphoviridae* family (Table 1). The remaining two phages (10%), MW47-10 and -15, exhibited tails with a contractile sheath. This tail structure identified these phages as belonging to the *Myoviridae* family. Within the 18 recognised *Siphoviridae* phages, two distinctive capsid morphologies were apparent. Twelve of the phage possessed icosahedral capsids, whereas six possessed elongated icosahedral capsids. Both recognised *Myoviridae* phages possessed icosahedral capsids that appeared to be much larger than the capsids observed for *Siphoviridae* phages.

3.3 Assessment of host range

Assessment of host range is an important additional investigation because for these phages to be useful for MST they should be highly specific to their bacterial host (origin). The host ranges of MW47 phages were determined using 11 *Enterococcus* type strains (Table 2), an *E. coli* host strain WG5 and a *Bacteroides* host strain GB124. As expected, no phages were shown to be capable of infecting either the *E.coli* WG5 or the human-specific *Bacteroides* GB124. Fifteen of the phage isolates were unable to infect any other *Enterococcus* host type strains (Table 2). Interestingly, no phages of *E. faecium* MW47 were able to infect *E. faecium* type strain DSM 20477. These results provide further evidence that these phages have very narrow host ranges.

Phages MW47-9, -10, and -20 displayed broader host ranges (Table 2). Phages MW47-9 and -10 were also capable of infecting the *E. faecalis* type strain DSM 20478, and MW47-20 was able to infect *E. asini* DSM 11492, but the phage numbers detected were low (1.0×10^3 PFU ml^{-1}). Phages MW47-8, -15 and -20 displayed the broadest host ranges and were able to infect type strains *E. faecalis* and *E. asini*. Although some phages exhibited broader host ranges, the number of phages detected on the alternative *Enterococcus* host strains was

significantly lower than that recorded on host strain MW47 (Wilcoxon signed ranks test, $P<0.05$). Phages MW47-8, -9, -10, -15, and -20, represent a morphologically diverse group that includes phage from the families *Siphoviridae* and *Myoviridae*, which display both icosahedral and elongated icosahedral capsids. These results indicate variation in host ranges that may be linked to the diversity of phage morphology. *Enterococcus faecalis* is commonly found in human faeces and in raw wastewater. However, *E. asini* is reported to be found exclusively in donkey faeces (de Vaux *et al*., 1998). In southern England, *E. asini* is less likely to be encountered in inland surface waters. Therefore, it is probable that the primary host of the phages MW47-8, -15 and -20 is *E. faecalis*.

3.4 Phage survival

The results demonstrated that phages isolated from MW47 have diverse morphologies. Previous investigations have shown that the inactivation characteristics of phages appear to vary with regard to phage morphology (Muniesa *et al*., 1999). Although it was not practically feasible to perform natural *in situ* survival experiments on all 20 phages isolated, an investigation into natural survival was successfully conducted on a representative subset of six phages that showed the greatest morphological diversity. The aim of this investigation was to determine whether these phages exhibit varying inactivation rates in surface water as this could impact the future use of host strain MW47 as an MST marker. Four phages belonging to the *Siphoviridae* family - two with icosahedral capsids and two with elongated icosahedral capsids (MW47-1, -2, -5 and -6)- were selected for investigation, alongside both isolated phages of the *Myoviridae* family (MW47-10 and -15). A summary of the observed physico-chemical composition of the Bevern Stream used in these experiments is presented in Table 3. The results of the inactivation experiments for phages MW47-1, -2, -5, -6, -10 and -15 are presented in Figure 2, which demonstrates that limited inactivation of the six phages

was observed during the first three days of the experiment. From day three onwards, the *Myoviridae* phage MW47-10 was inactivated to the greatest degree (3.4 log), whereas all other phages (MW47-1,-2,-5,-6 and -15) demonstrated between 1.8 and 2.4 log reductions. It may be important to note that the water temperature during the first three days at the time of sampling was on average 3.1^{o}C lower than on days four to nine (Table 3). Statistical analysis supported observations that the inactivation rates of *Siphoviridae* and *Myoviridae* phages ($P<0.05$; Mann-Whitney) were significantly different, with greater persistence exhibited by *Siphoviridae* phages. Statistical analysis also revealed that differences between phages with different capsid morphology were not significant ($P=>0.05$; Mann- Whitney).

4. Discussion

The results from this study present an important and timely insight into the diversity and environmental survival of human-specific phages capable of infecting *Enterococcus* host MW47. Transmission electron microscope (TEM) analysis revealed that a heterogeneous group of phages were able to infect host strain MW47. Three distinct morphologies were observed from two different families (*Myoviridae* and *Siphoviridae*). This highlights that phages infecting this enterococcal host strain appear to be morphologically more heterogeneous than F-RNA phages and those infecting *Bacteroides* spp. (Queralt *et al*., 2003). According to previous inactivation studies that focused on the role that somatic coliphage morphology plays in wastewater and faecally-polluted surface waters in which a wide variety of phages exists, *Myoviridae* phages are often initially shown to be the predominant family (Muniesa *et al*., 1999). However, following wastewater treatment in engineered systems or inactivation in the natural environment, these proportions have been shown to change, with *Siphoviridae* phages becoming dominant (Lasobras *et al*., 1997; Muniesa *et al*., 1999). The results reported here support the findings of other studies that have

shown phages of the *Siphoviridae* family to demonstrate greater resistance to inactivation than other phage families (P=<0.05, Mann-Whitney), including *Myoviridae* (Duran *et al*., 2003). *Siphoviridae* may be more resistant to inactivation because they can incorporate into the host genome (lysogenic) and may be released by lysogenic bacteria, whereas *Myoviridae* are primarily lytic (lysis causing) (Muniesa *et al*., 1999). There also may be differences in the ability of the phages to form plaques, because of some phages going into the prophage state (latent). The differences observed in the morphology of phages capable of infecting *Enterococcus* strain MW47 do have implication for their potential use in MST studies and it is these differences that prompted the investigation into the inactivation of phage of different morphologies capable of infecting host strain MW47 that is reported here.

A significantly statistical link was established between inactivation rate and phage morphology. In comparison with phages of the family *Siphoviridae*, MW47-10 and MW47-15 (both members of the *Myoviridae* family) demonstrated more rapid inactivation. The results of this study suggest that some cautions should be exercised in interpreting *Enterococcus* phage-lysis results from MST studies, especially when different sample types are being compared because observed variance in phage counts could be the result of a disparity in inactivation rates rather than actual differences in contamination level. Whilst only phages capable of infecting host strain MW47 were assessed in this study, it may be that other *Enterococcus* phages that infect host strains of importance to MST also comprise heterogeneous families. Further investigation of these phages is warranted. This study has demonstrated that, whereas *Enterococcus* phages continue to demonstrate potential as a valuable MST tool, they are heterogeneous and demonstrate varying inactivation rates in the environment. This issue needs to be considered when interpreting findings of MST studies that use phage-lysis of host-specific *Enterococcus* spp. as a way to identify sources of faecal

351 contamination, particularly when samples from different environmental matrices are being
352 compared. It also highlights the importance of gaining a better understanding of the
353 ecological characteristics of newly proposed markers. In conclusion, the research outlined
354 here indicates that whilst there may be certain benefits to using *Enterococcus* phages over
355 FRNA or *Bacteroides* phages for MST purposes (simpler growth requirements, lower cost
356 media, and higher resistance to wastewater treatment), the use of FRNA and *Bacteroides*
357 phage for MST studies may still offer a more credible source of information on pollution
358 sources because of the significantly lower heterogeneity of these phage groups.
359

360 **5. Acknowledgements**

361 The authors would like to acknowledge Southern Water for providing access to Scaynes Hill
362 wastewater treatment works for sample collection.
363

364 **6. Conflict of interest**

365 No conflict of interest declared.
366

367 **7. References**

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Tables

Table 1. Morphology of phages isolated from MW47

Phage ID (MW47-)	Capsid morphology	Family
1	Icosahedral	*Siphoviridae*
2	Elongated icosahedral	*Siphoviridae*
3	Icosahedral	*Siphoviridae*
4	Icosahedral	*Siphoviridae*
5	Elongated icosahedral	*Siphoviridae*
6	Icosahedral	*Siphoviridae*
7	Elongated icosahedral	*Siphoviridae*
8	Elongated icosahedral	*Siphoviridae*
9	Elongated icosahedral	*Siphoviridae*
10	Icosahedral	*Myoviridae*
11	Icosahedral	*Siphoviridae*
12	Icosahedral	*Siphoviridae*
13	Icosahedral	*Siphoviridae*
14	Icosahedral	*Siphoviridae*
15	Icosahedral	*Myoviridae*
16	Elongated icosahedral	*Siphoviridae*
17	Icosahedral	*Siphoviridae*
18	Icosahedral	*Siphoviridae*
19	Icosahedral	*Siphoviridae*
20	Icosahedral	*Siphoviridae*

Table 2. Host range of phages capable of infecting enterococcal host strain MW47

	***Enterococcus* type strains (DSM No.)**										
ID	*asini* (20681)	*cassel.* (20680)	*durans* (20633)	*faecalis* (20478)	*faecium* (20477)	*gallin.* (24841)	*hirae* (20160)	*mundti* (4838)	*pseudo.* (5632)	*sacchar.* (20726)	*sulphur.* (6905)
1	N[a]	N	N	N	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	N	N	N	N
3	N	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N	N
5	N	N	N	N	N	N	N	N	N	N	N
6	N	N	N	N	N	N	N	N	N	N	N
7	N	N	N	N	N	N	N	N	N	N	N
8	$6.1x10^7$	N	N	$5.4x10^6$	N	N	N	N	N	N	N
9	N	N	N	$1.1x10^7$	N	N	N	N	N	N	N
10	N	N	N	$1.7x10^6$	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N	N
12	N	N	N	N	N	N	N	N	N	N	N
13	N	N	N	N	N	N	N	N	N	N	N
14	N	N	N	N	N	N	N	N	N	N	N
15	$4.7x10^8$	N	N	$3.7x10^8$	N	N	N	N	N	N	N
16	N	N	N	N	N	N	N	N	N	N	N
17	N	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N	N	N
19	N	N	N	N	N	N	N	N	N	N	N
20	$1.0x10^3$	N	N	N	N	N	N	N	N	N	N

[a]N= Phage lysis not detected; DSM= *Deutsche Sammlung* von Mikroorganismen

538 **Table 3.** Physico-chemical parameters

	Day						Mean
	1	2	3	5	8	9	
Temperature (^{o}C)	11.7	10.3	11.9	12.4	14.3	16.7	13.5
Turbidity (NTU)	31.2	36.7	24.0	22.1	30.9	29.8	29.4
pH	7.9	8.1	7.8	8.0	8.0	8.0	8.0
DO (mg/l)	9.0	10.9	9.3	8.9	9.8	8.4	9.4
EC (uS/cm)	553.0	472.0	599.0	629.0	744.0	712.0	637.9
TDS (mg/l)	359.0	306.0	389.0	408.0	483.0	462.0	414.0
SAL (ppt)	0.3	0.2	0.3	0.3	0.4	0.4	0.3

539 ^{o}C = degrees Celsius, NTU = Nephelometric Turbidity Unit, mg/l = milligrams per litre,
540 uS/cm = microsiemens per centimetre, ppt= part per thousand.
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574 **Figures**

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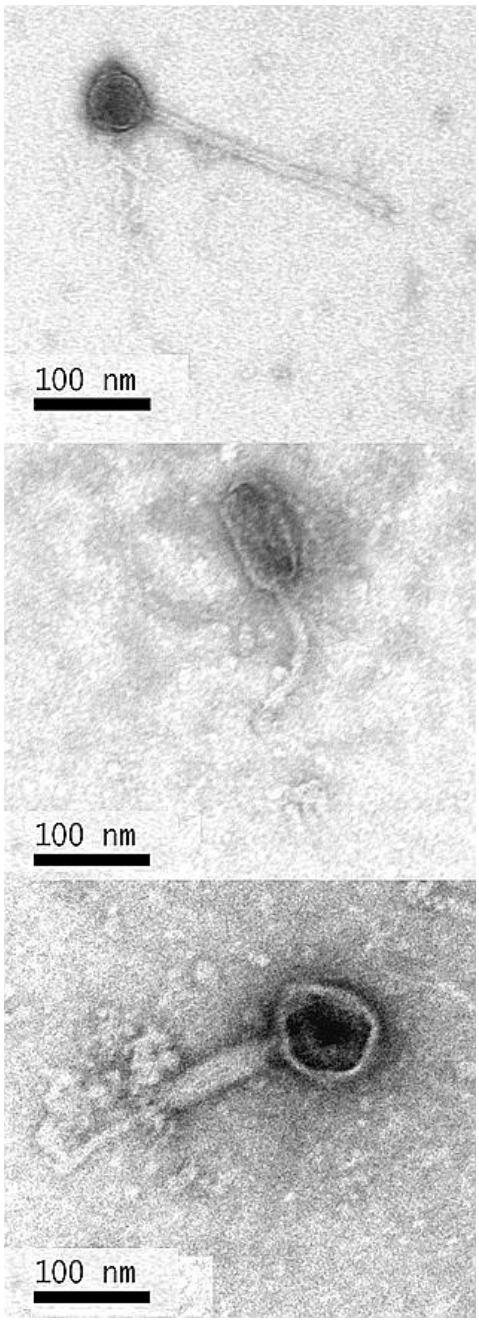

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577 **Figure 1.** Examples of the three major morphologies observed by transmission electron

578 microscopy (TEM). Top - MW47-1 (*Siphoviridae* with icosahedral capsid); middle - MW47-

579 5 (*Siphoviridae* with elongated icosahedral capsid); and bottom - MW47-10 (*Myoviridae* with

580 icosahedral capsid).

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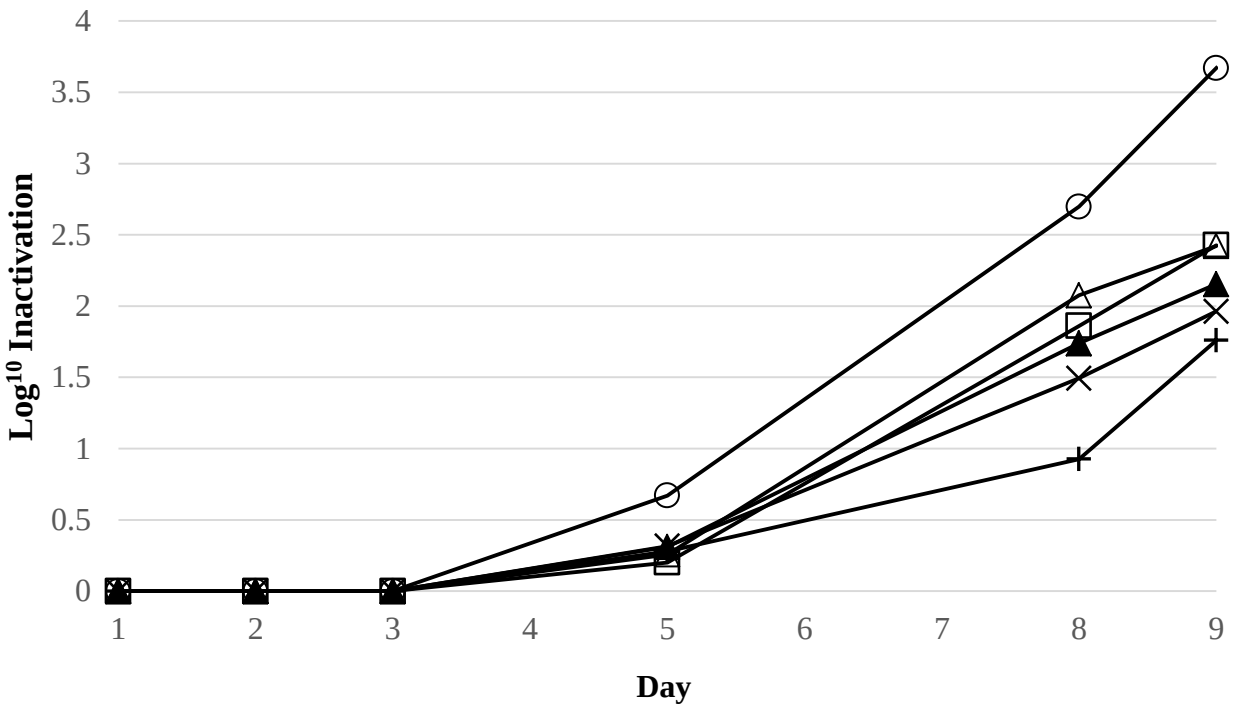

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583 **Figure 2.** Log_{10} inactivation of the six phage morphologies: (□) MW47-1, (▲) MW47-2, (+)
584 MW47-5, (X) MW47-6, (O) MW47-10, (Δ) MW47-15

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