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1 **Bacteriophage φMAM1, a Viunalikevirus, Is a Broad-Host-Range,**
2 **High-Efficiency Generalized Transducer That Infects**
3 **Environmental and Clinical Isolates of the Enterobacterial Genera**
4 **Serratia and Kluyvera**

5

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11 Running title: *Characterization of the ViI-like transducing phage φMAM1*

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13 transfer, oocydin A

14

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ABSTRACT

Members of the enterobacterial genus, *Serratia,* are ecologically widespread and some strains are opportunistic human pathogens. Bacteriophage φMAM1 was isolated on *Serratia plymuthica* A153, a biocontrol rhizosphere strain that produces the potently-bioactive antifungal and anticancer haterumalide, oocydin A. The φMAM1 phage is a generalized transducing phage that infects multiple environmental and clinical isolates of *Serratia spp.* and a rhizosphere strain of *Kluyvera cryorescens*. Electron microscopy allowed classification of φMAM1 in the family *Myoviridae*. Bacteriophage φMAM1 is virulent, uses capsular polysaccharides as receptor and can transduce chromosomal markers at frequencies of up to 7 x 10^{-6} transductants per p.f.u. We also demonstrated transduction of the complete 77-kb oocydin A gene cluster and heterogeneric transduction of a Type III Toxin-Antitoxin-encoding plasmid. These results support the notion of the potential ecological importance of transducing phages in the acquisition of genes by horizontal gene transfer. Phylogenetic analyses grouped φMAM1 within the ViI-like bacteriophages and genomic analyses revealed that the major differences between φMAM1 and other ViI-like phages arise in a region encoding the host-recognition determinants. Our results predict that the wider genus of ViI-like phages could be efficient transducing phages - with obvious implications for the ecology of horizontal gene transfer, bacterial functional genomics and synthetic biology.

46 **INTRODUCTION**

47 Bacteriophages (phages) are ubiquitous obligate viral parasites of bacteria that reproduce,
48 in concert with their hosts in diverse natural environments (1,2). Bacteriophage abundance
49 correlates with bacterial population densities in a given niche and the global phage
50 population has been estimated at over 10^{31} phages, reflecting over 10^{25} infections per
51 second (3,4). This intimate interaction between phages and their hosts results in rapid co-
52 evolution that has been observed under both natural environmental and laboratory
53 conditions (5-7).

54 Since discovery in the early 1900s, phages have been used as therapeutic agents in
55 human infections (8,9), but also as alternative biocontrol agents in agriculture, sewage
56 treatment and in the food industry (10-13). Furthermore, phage display technology has
57 proved useful in cancer studies and for the delivery of vaccines (12,13). Additionally,
58 transducing phages have been important tools in basic bacterial genetics, functional
59 genomics and synthetic biology applications (13-15).

60 Together with transformation and conjugation, transduction is one of the three main
61 classes of horizontal gene transfer (HGT) in bacteria. In generalized transduction, phages
62 accidentally package large fragments of host bacterial DNA instead of the viral genome.
63 The generated transducing particles adsorb normally and inject DNA into a recipient
64 bacterium. The injected bacterial DNA may integrate by homologous recombination into
65 the genome of the recipient host, resulting in a stable bacterial transductant (14,16).
66 Transducing phages have been used extensively in genetic manipulations, including
67 bacterial strain engineering, transposon mutagenesis and plasmid transfer (14,16).

68 Bacteria of the genus *Serratia* belong to the family *Enterobacteriaceae*. *Serratia* species
69 are widely distributed, being commonly found in water, soil, plants, animals and man;
70 some species are opportunistic human pathogens (17). Although *Serratia plymuthica*
71 strains have been isolated from diverse environments, they are mostly associated with

72 plants and are considered to be plant-growth promoting bacteria and excellent biocontrol
73 agents for many fungal and oomycete plant diseases (18). *Serratia plymuthica* A153 was
74 isolated from the rhizosphere of wheat (19) and produces several halogenated macrolides,
75 haterumalides (20). Haterumalides – also known as oocydins - were among the first
76 polyketides found to be synthesised by *Serratia* species (21) and they have been shown to
77 exhibit anti-cancer, antifungal, anti-oomycete and anti-hyperlipidemic properties (20-23).
78 Recently, we isolated and sequenced the new ViI-like *Serratia* phage, ϕMAM1 (24). Here,
79 we analyse the genome and report the morphological and biological characterization of
80 φMAM1, a generalized transducing phage that is able to infect many environmental and
81 clinical isolates from the *Serratia* and *Kluyvera* genera.
82

83 **MATERIALS AND METHODS**

84 **Bacterial strains, plasmids, phages, culture media and growth conditions**. Bacterial
85 strains and plasmids used in this study are listed in Tables 1 and A1. *Serratia, Kluyvera*
86 and their derivative strains were routinely grown at 30 ºC, unless otherwise indicated, in
87 Luria Broth (LB; 5 g yeast extract l^{-1},10 g Bacto tryptone l^{-1} and 5 g NaCl l^{-1}), Potato
88 Dextrose (24 g potato dextrose broth l^{-1}) or minimal medium (0.1%, w/v, $(NH_4)_2SO_4$, 0.41
89 mM $MgSO_4$, 0.2% (w/v) glucose, 40 mM K_2HPO_4, 14.7 mM KH_2PO_4, pH 6.9–7.1).
90 *Escherichia coli* strains were grown at 37 °C in LB. *Escherichia coli* DH5α was used for
91 gene cloning. Media for propagation of *E. coli* β2163 were supplemented with 300 µM 2,6-
92 diaminopimelic acid (DAPA). When appropriate, antibiotics were used at the following final
93 concentrations (in µg ml^{-1}): ampicillin, 100; kanamycin, 25 (*E. coli* strains) and 75 (*Serratia*
94 strains); streptomycin, 50; tetracycline, 10. Sucrose was added to a final concentration of
95 10% (w/v) when required to select derivatives that had undergone a second cross-over
96 event during marker exchange mutagenesis. Unless indicated, the growth temperature for

97 *S. plymuthica* A153, 30 ºC, was used for the phage incubations. Phages were stored at 4
98 ºC in phage buffer (10 mM Tris-HCl pH 7.4, 10 mM $MgSO_4$, 0.01% w/v gelatine) over a few
99 drops of $NaHCO_3$-saturated chloroform.

100 **φMAM1 phage isolation and phage lysate preparation.** Treated sewage effluent was
101 collected from the sewage treatment plant at Milton, Cambridge (United Kingdom) (24).
102 Briefly, a 10 ml sample of the effluent was filter sterilized. Then 500 µL of the sterilized
103 effluent was mixed with 200 µL of a *Serratia plymuthica* A153 overnight culture and 4 mL
104 of top LB-agar (LBA, 0.35% w/v agar) and poured as an overlay onto LBA plates (1.5%
105 w/v agar). Plates were incubated overnight at 30 ºC and single phage plaques were picked
106 with a sterile toothpick into 0.2 ml phage buffer and shaken with 20 µL of chloroform to kill
107 any bacteria. Phages obtained were plaque-purified three times. High-titre phage lysates
108 were then obtained as described Petty *et al.* (25). Phages were titrated by serial dilutions
109 in phage buffer and the phage titre determined in plaque-forming units (p.f.u.) per millilitre.

110 **φMAM1 genome sequencing**. Genomic DNA sequencing was performed using 454 DNA
111 pyrosequencing technology as described previously (24). The preparation of the 454
112 library was done by nebulisation using GS FLX Titanium Rapid Library Preparation Kit
113 following the manufacturer´s instructions (Roche, Cat. No. 05608228001). The shearing
114 was performed with nitrogen gas at a pressure of 30 psi (2.1 bar) for 1 min. The assembly
115 used 257,858 reads or 102.6 MB of raw data to give 650X coverage of the genome.

116 **Genome annotation and bioinformatics.** Annotation of φMAM1 genome and
117 identification of tRNAs was performed as described previously (24). Putative bacteriocins
118 were identified using the web-based bacteriocin mining tool BAGEL2 (University of
119 Groningen, The Netherlands). Artemis software (Wellcome Trust Sanger Institute) was
120 used to visualize and annotate the φMAM1 genome. Genome comparison analyses were

121 performed employing the Artemis Comparison Tool and EMBOSS Stretcher (European
122 Bioinformatics Institute). The genomic organization and annotation of the seven previously
123 reported ViI-like enterobacterial bacteriophages (26) were used to determine the location
124 of ORF1 in φMAM1. The CGView Comparison Tool (27) was employed to visualize the
125 φMAM1 genome and to generate Figure 3. Multiple sequence alignments of phage
126 proteins were performed using ClustalW2 (European Bioinformatics Institute). To analyse
127 and identify motifs in promoter regions, 100 bp sequences upstream of the start codons
128 were extracted using extractUpStreamDNA (http://lfz.corefacility.ca/extractUpStreamDNA/)
129 and analyzed using the MEME Suite (28). Candidate late promoters in φMAM1 were
130 identified by using the T4 late promoters conserved consensus sequence (TATAAATA).
131 Rho-independent transcription terminators were identified by examining the secondary
132 structure of the DNA using ARNold (http://rna.igmors.u-psud.fr/toolbox/arnold/index.php).
133 The phylogenetic analyses were performed with the MEGA software v5.10 (29). The
134 φMAM1 GenBank submission file was generated using Sequin (NCBI). The complete
135 genome sequence of φMAM1 is available in GenBank under Accession Number
136 JX878496.

137 **Electron microscopy**

138 High-titre lysates for transmission electron microscopy were obtained as described by
139 Petty *et al.* (25) using 0.35% (w/v) LB agarose instead of 0.35% (w/v) LB agar overlays.
140 Twenty-five microlitres of high-titre φMAM1 phage lysates (≥ 3 x 10^{10} p.f.u. ml^{-1}) were
141 absorbed onto copper, 400 mesh grids with holey carbon support films (Agar Scientific,
142 Stansted, UK). Copper grids were discharged in a Quorum/Emitech K100X (Quorum,
143 Ringmer, UK) prior to use. After 1 min, excess phage suspension was removed with filter
144 paper and phage samples were negatively stained by placing the grids in 25 µl drops of
145 2% ammonium tugstate containing 0.1% trehalose (pH 7.0) or 2% aqueous uranyl acetate

146 (pH 4.0) for 1 min. The grids were then blotted on filter paper to remove the excess
147 solution and allowed to air-dry for 10 minutes. Phages were examined by transmission
148 electron microscopy, in the Multi-Imaging Centre (Department of Physiology, Development
149 and Neuroscience, University of Cambridge) using a FEI Tecnai G2 transmission electron
150 microscope (FEI, Oregon, USA). The accelerating voltage was 120.0 kV and images were
151 captured with an AMT XR60B digital camera running Deben software.

152 **Host range determination**

153 A collection of enterobacterial strains was assembled to test their sensibility to φMAM1.
154 Top agar overlays containing 4 ml of 0.35% (w/v) LBA and 200 µl of an overnight culture of
155 the bacteria strain to test were poured onto a solid LBA plate and allowed to set. A 10 µl
156 sample of serial dilution high-titre phage lysate of φMAM1 was spotted onto each bacteria
157 overlay and the plates were incubated overnight at 30 ºC or 37 ºC. Ten microlitres of
158 phage buffer were also added as negative dilution control. After incubation, each plate was
159 scored for lysis. Efficiency of plaquing (EOP) was determined by dividing the phage titre of
160 the φMAM1-sensitive strain by that of the wild type reference strain, *Serratia plymuthica*
161 A153. EOP assays were performed in triplicate.

162 **Transduction assays**

163 Phage lysates were prepared on bacterial strains carrying the desired mutation or plasmid.
164 Phages were initially tested for their ability to transduce the Tn-KRCPN1 transposon from
165 *oocJ*::Tn-KRCPN1 and *admK*::Tn-KRCPN1 mutations into wild type *S. plymuthica* A153.
166 The transduction protocol was optimized based on the incubation time, temperature,
167 multiplicity of infection (m.o.i.) and number of bacterial cells. An appropriate volume of a
168 high-titre transducing lysate was added to a 10 ml overnight culture of the recipient strain
169 (~1 x 10^{10} total bacterial cells) to give the desired m.o.i. The mixture was incubated at 30
170 ºC for 30 minutes (1 h for *Sma* 12), pelleted by centrifugation (4.000 g for 10 min at 4 ºC)

171 and washed twice with 10 ml of LB to remove any remaining lysate. The cells were
172 resuspended in 5 ml of LB and 100 µl aliquots of the cell mixture were spread onto LBA
173 plates containing the appropriate antibiotic and the plates were incubated overnight. When
174 the cell mixture was resuspended in small volumes (e.g. 300 µl of LB) and spread onto
175 LBA plates, we observed a reduction in the transduction efficiency due to phage
176 superinfection that caused lysis of transductant colonies. The transductants obtained were
177 streaked out three times prior to use to reduce or eliminate any bacteriophage carry-over.
178 Transductants were confirmed by screening the antibiotic resistant colonies for
179 coinheritance of a secondary phenotype of either antifungal and antibacterial properties,
180 auxotrophy or cellulose production (see supplementary information). During the
181 transduction assays, controls for spontaneous resistance to antibiotics were determined by
182 spreading 100 µl of the overnight culture onto LBA plates containing the relevant antibiotic.
183 A 100 µl volume of the high-titre lysate was also spread on non-LBA plates to confirm
184 lysate sterility. Transduction efficiency was defined as the number of transductants
185 obtained per p.f.u.

186 **One-step growth curve and burst size**

187 One step growth curve experiments were performed as described previously (25). Briefly,
188 overnight bacterial culture were adjusted to OD_{600} 0.02 in 25 ml LB in a 250 ml flask and
189 grown in a water bath at 30 ºC with orbital shaking (225 r.p.m.). At OD_{600} 0.1, 10 ml of the
190 bacterial culture were removed into a sterile tube and pelleted at 4.000 x g for 10 min at 4
191 ºC. Once the supernatant was removed, the pellet was resuspended in 10 ml of fresh LB
192 and phage samples were added at an m.o.i. of 0.001. The same amount of phages was
193 also added to 10 ml of LB, as a negative control. Phages were allowed to adsorb for 5 min
194 at room temperature without shaking and the supernatant containing any unadsorbed
195 phages was removed by centrifugation at 4.000 x g for 10 min at 4 ºC. Pellets were

196 resuspended in 10 ml LB, added to 15 ml LB in a 250 ml conical flask and the cultures
197 were grown in a shaking water bath as described previously. Every 10 min, samples were
198 taken and immediately titrated to determine the number of p.f.u. The final growth curve
199 represents the number of phages per initial infectious centre.

200 **Phage adsorption**

201 Phage adsorption experiments were carried out as described previously (25). Briefly, 10 ml
202 overnight cultures of the bacterial host, or non-host bacterial control (*Serratia marcescens*
203 Db11), were infected with φMAM1 at an m.o.i. of 0.01, mixed briefly and placed on a tube
204 roller at 30 ºC. A bacteria-free negative control was created by adding the same amount of
205 phage to 10 ml of LB. One hundred microlitre samples were removed every 5 min and
206 added to 900 µl of phage buffer and 30 µl of chloroform, mixed for 5 seconds and
207 centrifuged at 13.000 x g for 1 min. The number of unadsorbed φMAM1 phage particles
208 was determined by titrating the supernatant on 0.35% (w/v) LBA lawns, as described
209 previously. Phage adsorption to bacteria was expressed as the number of free φMAM1
210 particles per millilitre remaining in the supernatant, and expressed as a percentage of the
211 number of p.f.u. ml^{-1} in the bacteria free negative control.

212 **Transposon mutagenesis and isolation of phage-resistant mutants.**

213 Random transposon mutagenesis of *S. plymuthica* A153 using Tn-KRCPN1 was
214 performed as follows. In a biparental conjugal mating, 500 µl of overnight cultures *of E. coli*
215 β2163 (pKRCPN1) and *S. plymuthica* A153 were mixed, collected by centrifugation,
216 resuspended in 30 µl of fresh LB, and spotted on an LB agar plate supplemented with 300
217 µM DAPA. After overnight incubation at 30 °C, cells were scraped off the plate and
218 resuspended in 1 ml of LB. φMAM1-resistant mutants were selected by mixing 100 µl of
219 the resuspended mating patch with 200 µl phage lysate (≥ 1 x 10^{11} p.f.u. ml-1) and 4 ml of
220 0.35% (w/v) LBA containing 75 µg ml^{-1} kanamycin. The mixture was poured onto a solid

221 LBA plate, allowed to set and incubated overnight at 30 ºC. DAPA was not added to LBA
222 medium to allow counterselection of the *E. coli* donor. The insertion site of transposon Tn-
223 KRCPN1 in the φMAM1-resistant mutants was determined using random primed PCR
224 following the method described previously (30).

225 **Supplementary experimental procedures.** *In vitro* nucleic acid techniques, construction
226 of the A153 OocKmSm strain, phenotypic analysis of transductans and toxin-antitoxin
227 abortive infection assays are given as supplementary information.

228 RESULTS

229 Isolation of φMAM1

230 During environmental screening for new bacteriophages infecting clinical and
231 environmental isolates of *Serratia*, we isolated 17 new phages infecting the haterumalide-
232 producing strain, *Serratia plymuthica* A153 (24). Isolated, plaque purified phage lysates of
233 high titre were screened for their ability to transduce Tn-KRCPN1 chromosomal mutations
234 into the A153 wild type strain. Of the 17 new phages, φMAM1 was the only transducing
235 phage and was selected for further characterization.

236 φMAM1 forms plaques ranging between 6.6 ± 0.9 and 1.2 ± 0.3 mm in diameter when the
237 phages were titrated and plated in 0.2-0.8% agar overlays (Fig. A1). Within the tested
238 temperatures, ranging between 25 and 37 ºC, no differences in plaque formation were
239 observed (data not shown). φMAM1 viability was unaffected by the addition of chloroform
240 during storage in phage buffer at 4 ºC and phage lysates retained high viability during the
241 period of this study, 2 years (>65% of the initial titre).

242

243 φMAM1 morphology

244 Using transmission electron microscopy, the morphology of φMAM1 was determined.
245 φMAM1 has an icosahedral and isometric head measuring 90 ± 2 nm from flat face to flat

246 face. Its contractile tail measures 120 ± 2 nm long and 21 ± 2 nm in diameter when
247 extended, or 57 ± 2 nm long and 26 ± 2 nm in diameter when contracted (Fig. 1). The
248 φMAM1 tail consists of a 11 ± 1 nm neck with a collar, a tail tube surrounded by a
249 contractile sheath showing 24 transverse striations, a baseplate and a complex system of
250 tail spikes (Fig. 1). Based on its morphology and according to Ackermann´s (31)
251 classification, φMAM1 was grouped into the order *Caudovirales* within the *Myoviridae*
252 family, which comprises 25% of the tailed bacteriophages and includes the *Escherichia*
253 *coli* phage, T4. Morphologically, φMAM1 is very similar to *Salmonella* phages Vi01 (32),
254 φSH19 (33) and SFP10 (34), *Shigella* phage φSboM-AG3 (35), *E. coli* phages CBA120
255 (36) and PhaxI (37) and *Dickeya* phage LIMEstone1 (11). All these ViI-like enterobacterial
256 bacteriophages have been described recently as members of a newly proposed genus
257 within the *Myoviridae* family, "Viunalikevirus" (26). The adsorption "organelle" in
258 "Viunalikevirus" members shows two conformations consisting of "prongs" and "umbrella-
259 like" filaments with rounded tips, both associated with the baseplate (26). Both
260 conformations were observed in φMAM1 (Fig. 1A,B) and each of the "umbrella-like"
261 structures shows 4 short filaments attached to the baseplate by a stalk (Fig. 1B).
262

263 **Biological characterization of φMAM1**

264 We evaluated the ability of φMAM1 to lyse host bacteria in liquid cultures by adding
265 phages at different m.o.i. to *S. plymuthica* A153 cultures. φMAM1 infection caused a
266 reduction in growth rate followed by a cessation of bacterial growth 30 and 90 min after
267 phage addition, respectively. Bacterial lysis caused a decrease in the turbidity of the
268 culture two hours after phage addition (Fig. 2A). However, a resumption of growth was
269 observed in A153 after a prolonged incubation period (data not shown). This resumption
270 was associated with the emergence of phage resistant mutants, since A153 isolates from
271 late infected cultures showed φMAM1 resistance in agar lawn assays.

272 Phage adsorption assays confirmed that φMAM1 adsorbs rapidly to *S. plymuthica* A153,
273 with more than 95% of the phage particles adsorbed within the first 5 min. The number of
274 adsorbed phage particles reached an apparent maximum 30 min post-mixing (Fig. 2B) and
275 this time was chosen as the incubation time in the A153 transduction assays (see below).
276 To determine the burst size of φMAM1, one-step growth curves were carried out. φMAM1
277 showed a latent period of ~20 min and a rise period, where the phage particles were
278 released, of around 40 min. The average burst size was more than 300 phage particles
279 per initial infection centre (Fig. 2C).

280 **Host range of the virulent bacteriophage φMAM1**

281 Sensitivity to φMAM1 of a collection of 25 environmental and clinical isolates of *Serratia*
282 *marcescens* (*Sma*) (38) and 30 enterobacterial strains isolated from the rhizosphere of
283 agronomic crops (39) was investigated by spotting phage lysates onto bacterial lawns.
284 Bacterial clearing was observed on agar lawns of 17 *Sma* strains and in the
285 rhizobacterium, *Kluyvera cryorescens* 2Kr27 (Table 1; Fig. A1). The plaques formed in the
286 sensitive strains varied in size and were clear in most strains, but turbid in *Sma* 0006, *Sma*
287 1047, *Sma* 3127, *Sma* 3078V and *Sma* S6. However, subsequent phage titrations showed
288 no obvious plaque formation in *Sma* 1695 and *Sma* 2595. We showed that the observed
289 lysis in the latter two strains was not due to a bacteriocin present in the phage lysate (data
290 not shown). Further adsorption assays confirmed that φMAM1 adsorbs to *Sma* 1695 and
291 *Sma* 2595, but with low efficiencies (Fig. A2). For strains where φMAM1 was able to form
292 plaques, the efficiency of plaquing (EOP) was similar, except on *Sma* 365 (EOP of 6.1 x
293 10^{-3}), suggesting that there was no host controlled restriction system active against this
294 phage in most strains (Table 1).
295 As φMAM1 formed turbid plaques in several strains we used the protocol described by
296 Petty *et al.*, (25), to test for lysogeny in *Sma* 0006, *Sma* 1047, *Sma* 3127, *Sma* 3078V and

297 *Sma* S6 after φMAM1 exposure. The results were universally negative and it is reasonable
298 to assume that this is either a virulent phage or a temperate phage with a low frequency of
299 lysogeny.
300 Other enterobacterial strains belonging to *Dickeya, Escherichia, Pectobacterium,*
301 *Citrobacter, Photorhabdus, Yersinia, Salmonella*, *Pantoea* and *Enterobacter* genera were
302 also tested for susceptibility to φMAM1 infection. None of the strains tested showed signs
303 of φMAM1 sensitivity.
304

305 **φMAM1 is a generalized transducing phage**

306 As described above, φMAM1 was the only A153 infecting phage able to transduce
307 mutations between A153 strains. No differences in the transduction efficiency were
308 observed at different temperatures (e.g. 25, 30 and 37 ºC; not shown) or using different
309 number of cells (e.g. 10^8, 10^9 and 10^{10} total bacterial cells) (Table 2). Transduction
310 efficiency was higher at an m.o.i of 0.01 and 0.1 (>10^{-6} transductants obtained per p.f.u).
311 However, the transduction efficiency decreased with higher m.o.i. probably due to φMAM1
312 superinfection killing of the transductants (Table 2). In fact, during the transduction assays,
313 the size of the transductants colonies after overnight incubation decreased as the m.o.i.
314 values increased and some phage "nibbling" was observed at the edges of these colonies.
315 Transduction efficiencies were determined in A153 and *Sma* 12 for several chromosomal
316 markers including mutations in the oocydin A biosynthetic genes *oocJ* and *oocU,* the
317 antibiotic biosynthetic genes *admH* and *admK* and the cellulose synthase regulator
318 encoding gene *bcsB*, among others. Transduction efficiencies between 1.1 x 10^{-6} and 4.2 x
319 10^{-6} were observed for the different chromosomal markers in the wild type reference strain
320 A153 (Table 3). The frequency ranged from 2.3 x 10^{-6} to 7.2 x 10^{-6} in *Sma* 12 (Table 3).
321 Transduction of a kanamycin derivative version of pECA1039 from *S. plymuthica* A153 to
322 *Sma* 12 and *Kluyvera cryorescens* 2Kr27 was also demonstrated. The plasmid pECA1039,

323 isolated from the Gram-negative bacterium *Pectobacterium atrosepticum* (40), encodes
324 the first known Type III Toxin-Antitoxin (TA) system, ToxIN, bifunctional as an abortive
325 infection system active against many phages. However φMAM1 was not aborted by the
326 ToxIN system. Although the plasmid transduction efficiencies were relatively high, these
327 were one order of magnitude lower than those obtained for the transduction of
328 chromosomal markers (Table 3).
329
330 **φMAM1 genome analysis**
331 φMAM1 has a dsDNA of 157,834 bp (98.5 x 10^6 Da) encoding 198 putative ORFs and
332 three tRNAs genes, tRNA-Pro (CCU), tRNA-Met (AUG) and tRNA-Cys (UGU) (Fig. 3;
333 Table A2) (24). Three different start codons, ATG (94.0%), GTG (3.5%) and TTG (2.5%),
334 were identified. The coding sequences of φMAM1 represent 91.6% of the genome with a
335 GC content of 52.5%. Based on BLASTP similarities and the presence of conserved
336 domains, a predicted function was assigned to 39.8% of the identified ORFs. Within the
337 60.2% of the genes encoding hypothetical proteins, 27.3% of them were unique to
338 φMAM1. Genome comparison analyses showed that the genome of φMAM1 is between
339 52.7 and 54.2% identical at the DNA level to the genomes of the seven sequenced ViI-like
340 phages and shares between 59.0 and 64.6% of their putative ORFs (Fig. 3-4; Table A2
341 and A3).
342 The highly homology observed between φMAM1 and ViI-like phages led us to investigate
343 their phylogenetic relationships. Thus, the amino acid sequences of a structural protein
344 (major capsid protein) and a non-structural protein (DNA polymerase) were chosen to
345 perform phylogenetic analyses. Previously, major capsid proteins and DNA polymerase
346 were often used as phylogenetic markers (26,34,41). As expected from the phage
347 morphology and the genomic comparison analyses, the phylogeny revealed that φMAM1 is
348 closely related to ViI-like phages (Fig. A3).

349

350 ***Transcription, regulatory sequences and translation***

351 The analysis of the promoter regions of the φMAM1 genes allowed us to identify the motif
352 TTCAATAA[N_{12}]TATtAT in the promoters of 27 φMAM1 genes (Fig. A4A). Most (81.4%) of
353 the φMAM1 genes containing this upstream consensus sequence encode hypothetical
354 proteins making it difficult to predict in which stage of the lytic cycle they may be
355 expressed. However, the motif shows similarity with the consensus sequence of the *E. coli*
356 RpoD-dependent promoters (TTGACA[$N_{15\text{-}18}$]TATAAT) (42) and to the conserved motif
357 identified in T4 early promoters (43) perhaps indicating that these genes are expressed in
358 early stages of the phage lytic cycle.
359 In general, late transcription is responsible for the synthesis of phage structural
360 components, besides other recombination and replication factors (43). Based on the
361 consensus sequence of T4 late promoters (TATAAATA) (44), we identified 16 putative late
362 promoters (Fig. A4B). Seven of them, including P_{MAM_038} (upstream of a tail spike head-
363 binding protein gene), P_{MAM_135} (baseplate hub subunit), P_{MAM_142} (Gp32 ssDNA binding
364 protein) and P_{MAM_143} (Gp19 baseplate tail tube) are expected to be late genes. Several
365 proteins have been described as involved in late transcription in T4-related phages and
366 φMAM1 encodes orthologs of all of them - including the transcription co-activator Gp33
367 (MAM_140), sigma factor Gp55 (MAM_108), sliding clamp protein Gp45 (MAM_078) and
368 the clamp-loader proteins Gp44 (MAM_079) and Gp62 (MAM_080) (43,44). Thirty six Rho-
369 independent terminators were identified in the genome of φMAM1, four of them predicted
370 to be on late transcripts (Fig. A4C).

371

372 ***Structural components***

373 Mass spectrometry analysis identified 41 structural proteins in Vi01 (32) and 39 in
374 LIMEstone1 (11), two ViI-like phages. However, the function of 33% (11) and 41% (32),

375 respectively, of the identified structural proteins is unknown. With the exception of 6 and 4
376 unknown structural proteins identified in Vi01 and LIMEstone1, respectively, orthologs for
377 the remaining structural proteins were found in φMAM1 (Table A2). However, genome
378 comparison analyses showed that major differences were observed in a 14.7-kb region
379 encoding the host-recognition elements, the tail spike proteins (TSPs) (Fig. 3-4; Table A2).
380 Other φMAM1 structural proteins are described in the supplementary material.
381 Most of the sequenced ViI-like phages encode four tail spike proteins (26) and the
382 presence of these TSPs has been confirmed by mass spectrometry analyses in Vi01 (32)
383 and LIMEstone1 (11). φMAM1 also encodes four putative TSPs, three of which
384 (MAM_031, MAM_034, MAM_037) contain a pectate lyase domain (Table A2). The TSP
385 MAM_031 is a 987 amino acid protein and BlastP analyses showed that its first 262 amino
386 acids are 45-49% identical (64% similar) to the N-terminal region of TSPs encoded by all
387 the seven sequenced ViI-like phages. However, the N-terminal regions of the other three
388 putative TSPs (MAM_034, MAM_037, MAM_038) are less conserved and appear to be
389 more specific to φMAM1. Thus, MAM_034 is 37-39% identical (53-55% similar) from amino
390 acid 23 to amino acid 114 to TSPs present in SFP10, CBA120 and PhaxI, whereas the
391 amino acids 25-211 of the N-terminal region of MAM_037 are 34% identical (54% similar)
392 to the TSP-1 of φSH19. Finally, the first 97 residues of the 602 amino acid TSP MAM_038
393 are 29% identical (46% similar) to the TSP Vi01_170c of the *Salmonella* phage Vi01
394 (Table A2). Interestingly, the BlastP analyses also showed that the C-terminal regions of
395 the four φMAM1 putative TSPs show similarity to several proteins of unknown function
396 encoded by *Serratia plymuthica* AS9 (45), *Serratia plymuthica* AS12 (46) and *S.*
397 *proteomaculas* 568 (47), bacterial strains closely related to the φMAM1 host, *S. plymuthica*
398 A153.
399
400 **Capsular antigen is the receptor for φMAM1**

401 Group 1 capsular polysaccharides (CPS) and lipopolysaccharide (LPS) O-antigen
402 biosynthetic gene clusters of *Enterobacteriaceae* have important common features. In fact,
403 research on the biosynthesis of LPS O-antigens has been extrapolated to understand the
404 assembly of CPS (48). Thus, both CPS and LPS O-antigen gene clusters share many
405 genes and they are located between the *galF* and *gnd* genes in strains belonging to
406 *Escherichia*, *Salmonella*, *Shigella* and *Enterobacter* genera (49-52). However, it is the
407 presence of the *wza, wzb* and *wzc* genes that differentiates these CPS and LPS loci (48).
408 All the A153 phage resistance mutations mapped in a 17.6-kb region between *galF* and
409 *gnd* (Fig. A5). The 17.6-kb region consists of 13 genes predicted to form an operon (Fig.
410 A5) and, consequently, the insertion of the Tn-KRCPN1 transposon may cause polar
411 effects in the expression of the downstream genes. In this region, *wza*, *wzb* and *wzc*
412 genes are involved in the synthesis, export and regulation of CPS (48,53). The hexose-1-
413 phosphate transferase encoding gene (*wbaP*) was proposed to be involved in the initiation
414 of the capsular polysaccharide synthesis (48). The polysaccharide can be extended by the
415 action of glycosyltransferases, four of which are encoded by genes in this region. The
416 region also contains the gene *wzx*, encoding a flippase required for the translocation of the
417 CPS biosynthetic units across the inner plasma membrane (53). Not surprisingly, the
418 region also encodes a hypothetical protein that is 31% identical (49% similar) to the C-
419 terminal region of the TSP MAM_034 protein.
420 During the characterization of the phage resistant mutants, we noted that they exhibited a
421 "rough" colony morphology characteristic of some CPS defective mutants and they
422 showed the same antifungal activity as the parental A153 strain (Fig. A5). Adsorption of
423 φMAM1 to all the phage-resistant mutants was abolished, confirming the role of CPS as
424 receptors (Fig. 2B).
425

426 **Mobilization of the oocydin A gene cluster by transduction**

427 The abundance and diversity of secondary metabolite gene clusters in some bacterial
428 strains, and their irregular distribution, raised the possibility of horizontal transfer of such
429 gene clusters between bacterial strains (54). Recently, we identified a 77-kb gene cluster
430 involved in the biosynthesis of the haterumalide, oocydin A (*ooc*) (30). Given the high
431 transduction efficiency and its large genome size, we decided to use φMAM1 as a genetic
432 tool for mobilizing the *ooc* gene cluster. Thus, we constructed the strain *S. plymuthica*
433 A153 OocKmSm containing a kanamycin resistance and a streptomycin resistance
434 cassette up- and downstream of the *ooc* gene cluster, respectively. The OocKmSm strain
435 was the donor strain used to transduce the *ooc* gene cluster into the non-oocydin A
436 producing strain OocEV, containing in-frame deletions in *oocE* and *oocV*. Among the
437 transductants, 17.7 % and 9.5 % of the streptomycin and kanamycin resistant colonies
438 showed restoration of the oocydin A production, respectively (Table 4; Fig. 5). The *ooc*
439 gene cluster was transduced into OocEV with a frequency of 1.9 x 10^{-7} and the production
440 of the haterumalide was fully restored in the kanamycin and streptomycin doubly resistant
441 colonies (Table 4). We also tried to transduce the *ooc* gene cluster into the other φMAM1
442 sensitive strains. However, no transductants were obtained with these strains presumably
443 due to insufficient DNA homology between the donor (A153) and the recipient strains to
444 enable homologous recombination.

445 **DISCUSSION**

446 Horizontal gene transfer (HGT) is a powerful source of genomic diversity and contributes
447 actively to the rapid evolution of prokaryotes (55). In fact, it has been estimated that up to
448 80% of known bacterial and archaeal genomes may be affected by HGT (56). From an
449 ecological perspective, the acquired genes may represent an adaptive advantage for the
450 microorganisms under certain environmental conditions (57,58). Transduction has been
451 described in natural environments such as sewage, fresh and sea water and on plant

452 leaves reflecting its importance in microbial ecology and in the evolution of microbial
453 populations (14,56).
454 Several *Serratia* transducing bacteriophages have been identified previously (25,59,60).
455 However, to our knowledge, φMAM1 is the first transducing phage described for *Serratia*
456 *plymuthica*. Compared with other enterobacterial phages (25,61,62), the high transduction
457 efficiency mediated by φMAM1, together with its large genome (it can transduce around
458 3% of the A153 host genome) and its wide host range, makes this new phage an
459 extraordinary tool for bacterial genetics. Additionally, given the DNA homology between
460 φMAM1 and the other sequenced ViI-like phages (Table A3; Fig. 3-4), it remains possible
461 that all these "Viunalikevirus" bacteriophages will be highly efficient transducers.
462 Consistent with this notion, *Salmonella* phage Vi01 was shown to be a transducing phage
463 twenty years ago (63).
464 We have shown recently that the oocydin A gene cluster is present in four plant associated
465 bacteria belonging to *Serratia* and *Dickeya* genera (30). The fact that the genomic context
466 of the *ooc* gene cluster in three of these bacteria is different, together with the identification
467 of sequences similar to a bacteriophage P4 integrase bordering the cluster in one of the
468 strains, suggests that the gene cluster was previously acquired by HGT (30). High
469 bacterial density has been associated with elevated transduction rates (64), perhaps
470 indicating that the rhizosphere may be a niche conducive to higher transduction
471 frequencies. In agreement with this idea, we have found that several *Serratia* strains
472 isolated from the rhizosphere of the same crop are able to produce oocydin A (M.A. Matilla
473 and G.P.C. Salmond, unpublished data). This observation indicates that the *ooc* gene
474 cluster is more widespread than perhaps expected and it is possible that it has been
475 transferred between rhizosphere strains by transduction.
476 φMAM1 contains three tRNA genes located together in a cluster. Although tRNA-Met is
477 conserved within most the sequenced ViI-like phages, thus far tRNA-Pro and tRNA-Cys

478 genes are unique to φMAM1. The function of phage tRNA is still an open debate but it has
479 been suggested that a positive correlation exists between the tRNA distribution and phage
480 codon usage (65). Although this correlation is absent in φMAM1 (data not shown), phage
481 tRNAs have been associated with overcoming differences in the GC content between the
482 phages and their hosts (66). Interestingly, the G+C content of the φMAM1 tRNA genes
483 (56.2%) and the genome of its host *S. plymuthica* A153 (55.9%) (24) are similar.
484 Many reports have reported promiscuity in enterobacterial phages (34,35,60,67,68) and
485 φMAM1 is another noteworthy example. φMAM1 infects 68% of the *Serratia marcescens*
486 strains tested and a rhizobacterial strain from the *Kluyvera* genus. φMAM1 sensitive
487 strains include 7 environmental and 10 clinical isolates, including the model bacterium
488 *Sma* 365 and the carbapenem-resistant, *Sma* S6. Additionally, the C-terminal region of the
489 TSPs from φMAM1 show similarity to several hypothetical proteins encoded by strains
490 such as *Serratia proteamaculans* (*Spro*) 568, a rhizobacterial strain with a genome highly
491 similar to that of A153 (47). TSP proteins are involved in the recognition of host receptor(s)
492 and the analysis of their C-terminal regions has been predicted as a rational approach for
493 the identification of new phage hosts (26,69,70). Strain *Spro* 568 shows interesting
494 biocontrol properties including plant growth promotion and production of exoenzymes (47),
495 however its genome does not contain genetic information for the synthesis of oocydin A.
496 We were able to transduce the complete, functional *ooc* gene cluster back into the non-
497 oocydin A producer strain OocEV at high efficiency (Table 4, Fig. 5). Thus, φMAM1 might
498 prove an effective tool for mobilizing the *ooc* gene cluster into non-oocydin producers,
499 thereby engineering synthetic derivative strains with enhanced biocontrol properties,
500 perhaps mimicking a process that is likely to be occurring naturally in the environment.
501 Mechanisms of bacterial defence against bacteriophages include the mutation of host
502 receptors, restriction of the incoming phage DNA, abortive infection (i.e. through toxin-
503 antitoxin systems) and CRISPR-Cas systems (71). Toxin-antitoxin (TA) systems are

504 generally comprised of two genes encoding a toxin component and its cognate antitoxin
505 (72). TA loci are widespread in bacteria and they have been found in plasmids and
506 bacterial chromosomes, strongly implicating HGT in the dissemination of these systems
507 (72,73). In 2009, we identified and characterised the first Type III protein-RNA TA system,
508 encoded by the plasmid pECA1039 of the phytopathogen, *Pectobacterium atrosepticum*
509 (40). Using φMAM1, transduction of pECA1039-km3 between different species of *Serratia*
510 and between two bacterial genera was demonstrated (Table 3). Given the importance of
511 anti-viral abortive infection systems in the population biology of bacteria that are constantly
512 assaulted by viral parasites, these observations reinforce the notion that phages are key
513 players in the dissemination of important genetic information - and therefore in adaptive
514 bacterial evolution in changing ecological niches. Interestingly, neither the *P. atrosepticum*
515 TA system nor the Type III TA system encoded by the insect pathogenic bacterium
516 *Photorhabdus luminescens* (72) were able to protect *S. plymuthica* A153 from φMAM1
517 infection (not shown) suggesting that this phage evolved the capacity to naturally
518 circumvent the abortive infection system. However, another phage defensive
519 mechanism(s) may be present in the φMAM1 sensitive strains *Sma* 1695 and *Sma* 2595
520 since no plaque formation was observed in these two strains.
521 The recognition of host receptors by ViI-like phages is dependent on tail spikes proteins
522 (TSP) instead of T4-like tail fibers (26). Based on the crystal structure of the C-terminal
523 TSP of the *Salmonella* phage Det7 (69), a phage morphologically similar to φMAM1,
524 several authors have proposed a role for the TSP regions. Thus, the N-terminal region
525 may bind to the phage base plate transferring the signal to the tail structure after phage
526 adsorption whereas the C-terminal region is responsible of the recognition of the host
527 receptor (26,36,69). As observed in other ViI-like phages (26), the C-terminal region of the
528 TSPs from φMAM1 contains domains with enzymatic activity. These enzymatic domains
529 are required for phage infection and it has been proposed that they serve to separate the

530 phage from cell debris after bacterial lysis (69). TSPs from the ViI-like *Salmonella* phages
531 Vi01 (32) and Det7 (69) contain an acetyl transferase and an O-antigen binding domain,
532 respectively, responsible for the recognition of the exopolysaccharide capsule and LPS as
533 receptors. TSP-2 of the phage φSH19 contains a pectate lyase domain and it was
534 suggested that it may have a role in the cleavage of glycosidic bonds (33). In agreement
535 with these observations, capsular antigen appears to be the receptor for φMAM1 and,
536 interestingly, three of its four TSPs contain a putative pectate lyase domain. Recently,
537 capsular polysaccharides also have been proposed to be the receptor in the ViI-like
538 *Klebsiella* phage o507-KN2-1 (74). Future synthetic biology approaches will involve
539 engineering of highly efficient transducing phages with promiscuous host ranges through
540 exploitation of structural knowledge that underpins the exquisite molecular specificity seen
541 in these phage-host interactions.
542

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551

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768 isolates. Antimicrob. Agents Chemother. **34**:755-758.

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782 **Table 1: φMAM1 host range**

Strain	Relevant characteristics	Source or reference	EOP[a]	Morphology	Average plaque size (mm)[d]
Serratia plymuthica A153	Rhizosphere isolate, oocydin A$^+$	19	1	Clear	1.5
Serratia marcescens 1695	Clinical isolate, non-pigmented	42	-[b]	Turbid	-
S. marcescens 0006	Environmental isolate, non-pigmented	42	0.6[c]	Turbid	0.1
S. marcescens 1047	Clinical isolate, non-pigmented	42	0.5[c]	Turbid	0.3
S. marcescens 0026	Environmental isolate, non-pigmented	42	1.2	Clear	0.7
S. marcescens 3078	Clinical isolate, pigmented	42	1.2	Clear	1
S. marcescens 0038	Environmental isolate, pigmented	42	0.3	Clear	1
S. marcescens 3127	Clinical isolate, non-pigmented	42	0.3	Turbid	0.5
S. marcescens 0035	Environmental isolate, pigmented	42	0.1	Clear	0.5
S. marcescens 12	Clinical isolate, non-pigmented	42	0.3	Clear	0.1
S. marcescens 0040	Environmental isolate, pigmented	42	1.4	Clear	1.5
S. marcescens 2595	Clinical isolate, non-pigmented	42	-[b]	Turbid	-
S. marcescens 3127	Clinical isolate, pigmented	42	0.6	Clear	1
S. marcescens 3078V	Spontaneous non-pigmented variant of *Sma* 3078	42	0.9	Turbid	0.8
S. marcescens ATCC 274	Clinical isolate, pigmented	66	0.5	Clear	0.5
S. marcescens S6	Clinical isolate, non-pigmented	76	0.6	Turbid	0.8
S. marcescens 365	Lab stock, pigmented	L. Debarbieux	6.1×10^{-3}	Clear	0.1
S. marcescens 2170	Lab stock, pigmented	T. Watanabe	0.9	Clear	1
Kluyvera cryorescens 2Kr27	Rhizosphere isolate	39	0.4	Clear	0.3

783 [a]EOP was determined by dividing the phage titre of the φMAM1-sensitive strain by that of the wild type
784 reference strain, *Serratia plymuthica* A153.

785 [b]No plaques observed. Adsorption assays confirmed that φMAM1 adsorbs to *Sma* 1695 and *Sma* 2595.

786 [c]No phage plaques were observed at 30 ºC. Phage plaques arose at 37 ºC in *Sma* 0006 and *Sma* 1047.

787 [d]0.35% (w/v) LB-agar lawns.

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795 **Table 2: Frequency of transduction of φMAM1**

Number of A153 cells	m.o.i.	Transduction efficiency[a,b]	Standard deviation[b]
1×10^{10}	10	$< 1.0 \times 10^{-11}$	-
	1	3.4×10^{-7}	4.1×10^{-8}
	0.1	2.7×10^{-6}	2.5×10^{-7}
	0.01	3.3×10^{-6}	6.7×10^{-7}
1×10^{9}	10	$< 1.0 \times 10^{-10}$	-
	1	3.0×10^{-7}	1.8×10^{-8}
	0.1	4.5×10^{-6}	3.0×10^{-7}
	0.01	5.8×10^{-6}	9.7×10^{-7}
1×10^{8}	10	$< 1.0 \times 10^{-9}$	-
	1	2.8×10^{-7}	5.9×10^{-8}
	0.1	7.4×10^{-6}	1.2×10^{-6}
	0.01	6.8×10^{-6}	0.9×10^{-6}

796 [a]Efficiency of transduction, defined as the number of transductants per p.f.u. obtained carrying the *oocJ*::Tn-

797 KRCPN1 marker in the A153 recipient.

798 [b]Mean and standard deviation of three independent experiments are shown.

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813 **Table 3: φMAM1 transduction efficiency of different markers within *Serratia***

814 ***plymuthica* A153, *Serratia marcescens* 12 and *Kluyvera cryorescens* 2Kr27.**

Donor strain	Relevant marker/plasmid	Recipient strain	Phenotype	Transduction efficiency[a,b,c]	Standard deviation[b]
Transduction within *Serratia plymuthica* A153					
VN1	*admH*::Km	A153	Antibiotic$^-$	1.8×10^{-6}	2.5×10^{-7}
VN2	*admK*::Km	A153	Antibiotic$^-$	1.1×10^{-6}	1.8×10^{-7}
MMnO13	*oocJ*::Km	A153	Oocydin A$^-$	2.7×10^{-6}	2.5×10^{-7}
MMnO15	*oocU*::Km	A153	Oocydin A$^-$	4.2×10^{-6}	2.3×10^{-7}
OocKmSm	Km,Sm	A153	Oocydin A$^+$	2.1×10^{-7}	3.1×10^{-8}
BcsB	*bcsB*::Sm	A153	Cellulose$^-$	1.3×10^{-6}	1.1×10^{-7}
A153	pECA1039-km3	A153	ToxIN$^+$	2.5×10^{-7}	7.4×10^{-8}
Transduction within *Serratia marcescens* 12					
Sma12I	*luxI*::Km	*Sma* 12	Lactone$^-$	5.3×10^{-6}	1.3×10^{-7}
Dho	*narX*::Km	*Sma* 12	Pyrimidine auxotroph	6.6×10^{-6}	2.7×10^{-7}
Cps	*cps*::km	*Sma* 12	Uracil auxotroph	2.3×10^{-6}	6.9×10^{-8}
Sma12S	*luxS*::Km	*Sma* 12	Autoinducer-2$^-$	7.2×10^{-6}	8.2×10^{-7}
Intergeneric transduction					
A153	pECA1039-km3	2Kr27	ToxIN$^+$	2.7×10^{-7}	2.6×10^{-8}
A153	pECA1039-km3	*Sma* 12	ToxIN$^+$	4.8×10^{-8}	9.6×10^{-9}

815 [a]The number of transductants showing co-inheritance of the antibiotic resistance and the listed phenotype.

816 Transduction efficiency is expressed as the number of transductants per p.f.u.

817 [b]Mean and standard deviation of three independent experiments are shown.

818 [c]Transduction experiments were performed using 10^{10} cells with φMAM1 at an m.o.i. of 0.1.

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829 **Table 4: Transduction efficiency of the 77-kbp oocydin A gene cluster by φMAM1.**

Donor strain	Relevant Marker	Recipient strain[a]	Antibiotic used[b]	Transduction efficiency	Standard deviation	Restoration of oocydin A production in transductants (%)
OocKmSm	Km,Sm	OocEV	Km	1.2×10^{-6}	4.7×10^{-7}	17.7 ± 4.8
OocKmSm	Km,Sm	OocEV	Sm	2.1×10^{-6}	4.2×10^{-7}	9.5 ± 2.0
OocKmSm	Km,Sm	OocEV	Km,Sm	1.9×10^{-7}	1.7×10^{-8}	100 ± 0

830 [a]OocEV is a markerless, double in-frame deletion mutant defective in the genes *oocE* and *oocV* (Table A1).

831 [b]Antibiotic used in the selection plate. Km, kanamycin; Sm, streptomycin.

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853 **FIGURE AND FIGURE LEGENDS**

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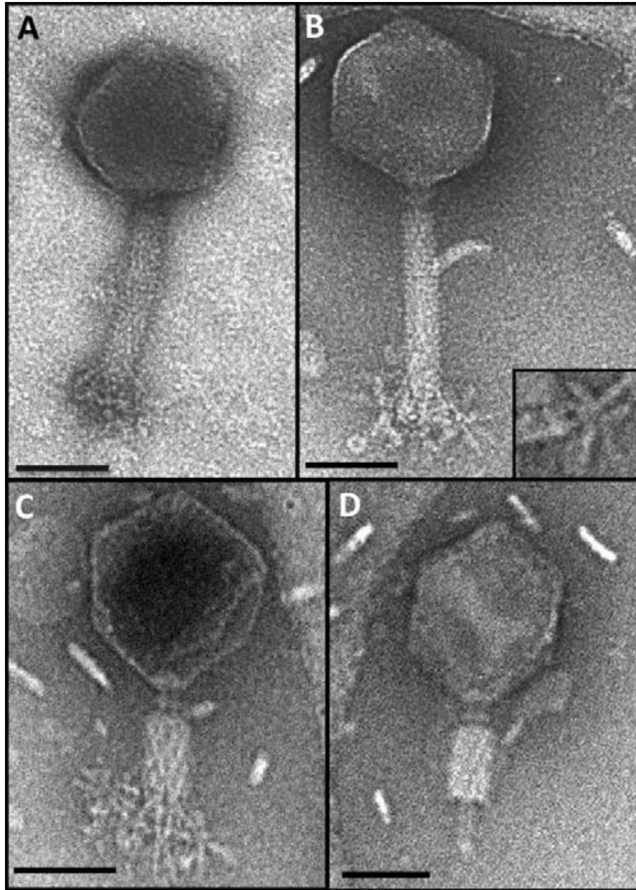

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856 **Fig. 1. Transmission electron micrographs of phage φMAM1. A**, Intact virion with
857 folded "prong-like" structures. **B**, Intact virion with unfolded "prong-like" structures. A detail
858 of an unfolded structure with the four tail-spike proteins (TSP) attached to the baseplate is
859 also shown. **C**, Intact virion with contracted tail, deformed head and TSP structures. **D**,
860 φMAM1 phage particle lacking the adsorption structures and with the central tube
861 exposed. φMAM1 particles were stained with uranyl acetate (A) or ammonium tungstate
862 (B-D) as described in materials and methods. Bars, 50 nm.

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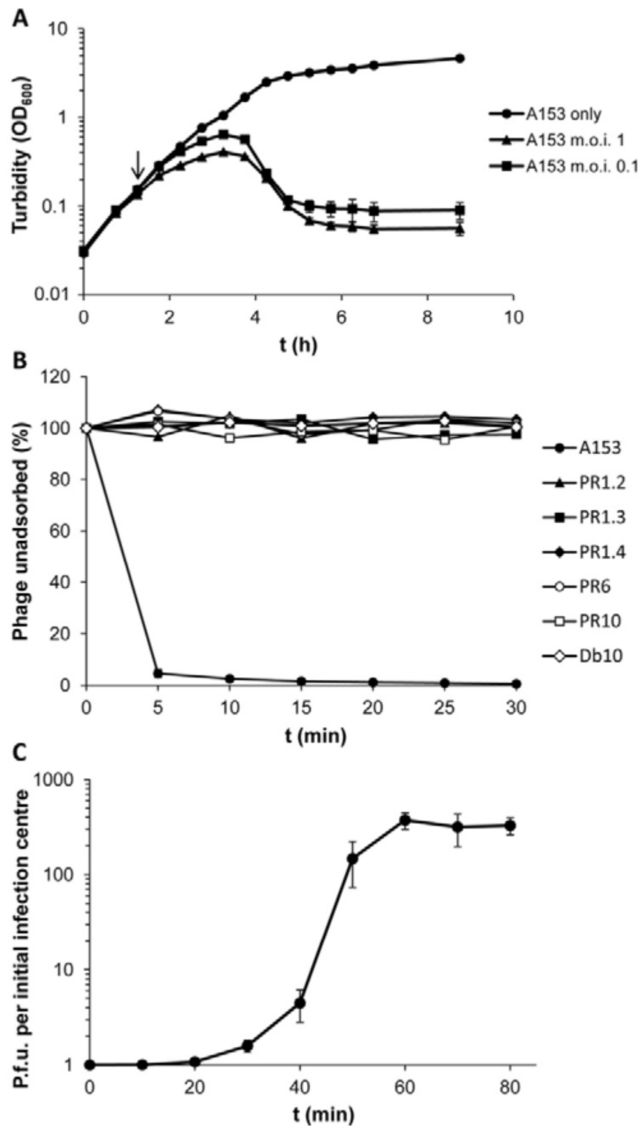

866 **Fig 2. Biological characterization of φMAM1. A**, *Serratia plymuthica* A153 growth
867 curves in the presence and absence of φMAM1. Phage was added at an m.o.i. of 0.1 and
868 1 to A153 in the early exponential phase of growth as described Petty *et al*., (25). The
869 arrow indicates the point of addition of φMAM1. ***B**, Adsorption of* φMAM1 to A153 and
870 A153 phage resistant (PR) mutants. The φMAM1 insensitive strain *Serratia marcescens*
871 Db10 was used as negative control for the adsorption. Description of the strains and the
872 location of the transposon insertion points are shown in Table A1 and Fig. A5,
873 respectively. **C**, One-step growth curve of φMAM1. In all cases, *error bars* represent the
874 *standard deviations (n=3).*

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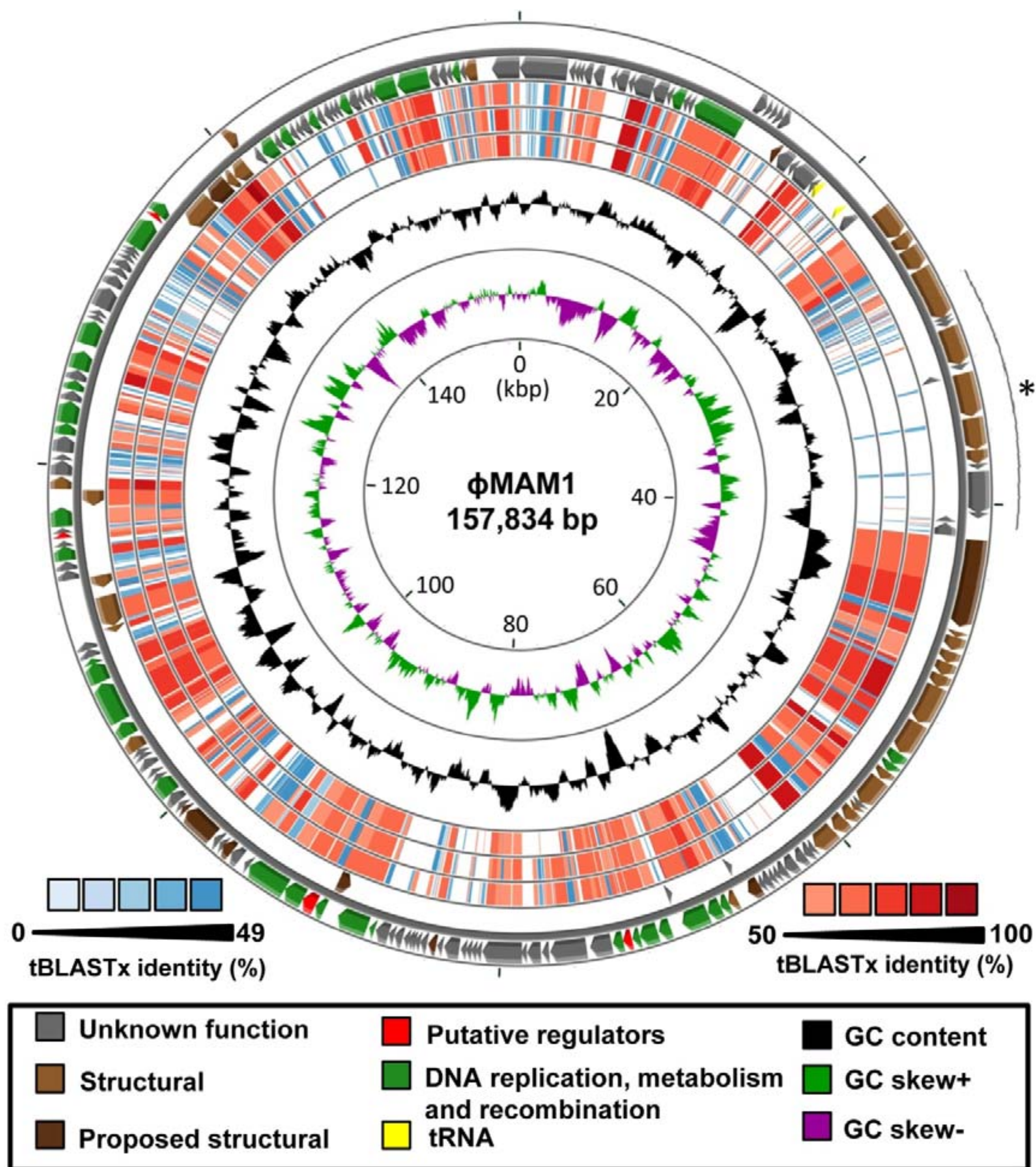

876 **Fig. 3: Genome map of phage φMAM1.** The physical locations by strand of all ORFs are
877 shown on the outer ring. Colour code representing the functional category of each gene is
878 given where possible. The proposed structural genes (dark brown) are based on previous
879 mass spectrometry analyses (11,32). The region encoding the host-recognition
880 determinants, the tail spike proteins, is indicated with an asterisk. The three bluish and
881 reddish internal rings represent the tBLASTx results against the ViI-like phages Vi01 (outer
882 ring), φSboM-AG3 (middle ring) and LIMEstone1 (inner ring). GC content and GC skew

883 analysis are also shown. The genome map was generated using the CGView Comparison
884 Tool (27).

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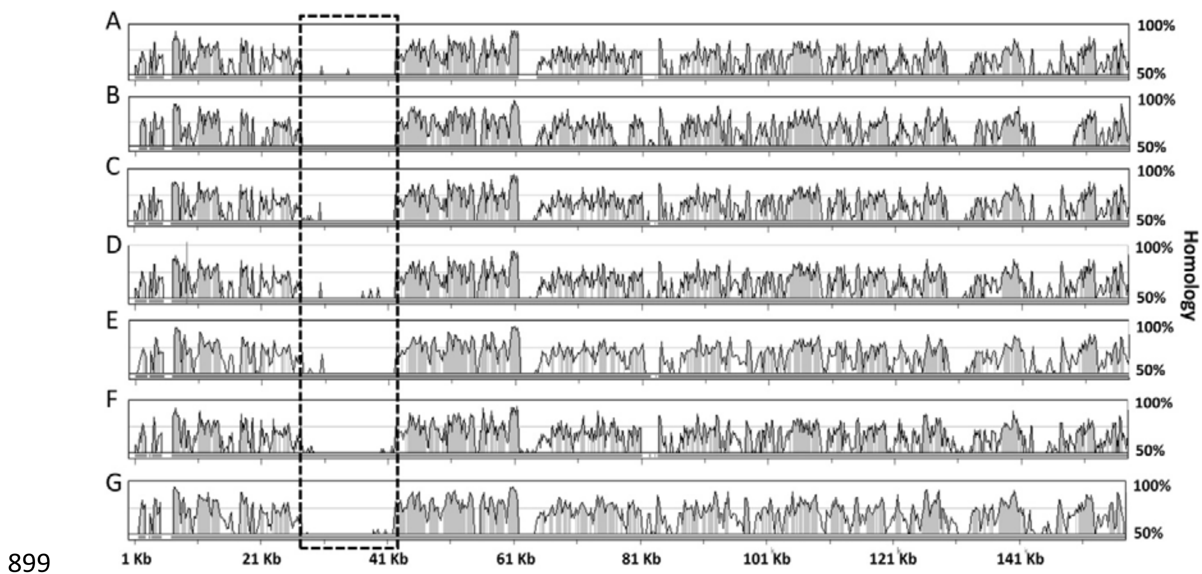

900 **Fig. 4. DNA homology between the genome of φMAM1 and the genomes of seven**
901 **sequenced ViI-like phages.** The alignments represent the percentage of DNA homology
902 between the genome of φMAM1 and those of PhaxI (**A**; 37), LIMEstone1 (**B**; 11), CBA120
903 (**C**; 36), φSH19 (**D**; 33), SFP10 (**E**; 34), Vi01 (**F**; 32), and φSboM-AG3 (**G**; 35). The regions
904 encoding the host-recognition determinants, the tail spike proteins, are highlighted.
905 Alignments were performed using wgVISTA (75).

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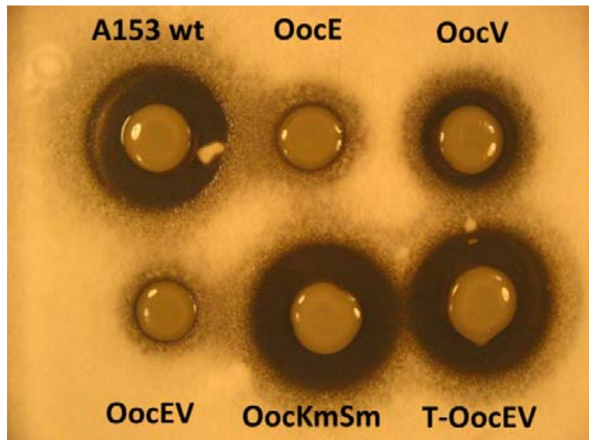

916 **Fig. 5. Mobilization of the oocydin A gene cluster into the non-producing *Serratia***
917 ***plymuthica* A153 OocEV mutant through transduction mediated by phage φMAM1.**
918 Production of oocydin A, shown as the ability of inhibiting the growth of the plant
919 pathogenic fungi *Verticillium dahliae*, in several A153 derivative strains. The bioassays
920 were performed as described previously (30). The assays were repeated at least five
921 times, and representative results are shown. Bacterial strains used are listed in Table A1.
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