Characterization of a broad-host-range flagellum-dependent phage that mediates high-efficiency generalized transduction in, and between, *Serratia* and *Pantoea*

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A phage (Φ OT8) isolated on *Serratia* sp. ATCC 39006 was shown to be flagellum-dependent, and to mediate generalized transduction with high efficiency (up to 10^{-4} transductants per p.f.u.). Φ OT8 was shown to have a broad host range because it also infected a strain of *Pantoea agglomerans* isolated from the rhizosphere. Transduction of plasmid-borne antibiotic resistance between the two bacterial genera was demonstrated, consistent with purported ecological roles of phages in dissemination of genes between bacterial genera. *Serratia* sp. ATCC 39006 and *P. agglomerans* produce a number of interesting secondary metabolites that have potential applications in cancer therapy and biocontrol of fungal infections. Φ OT8 has utility as a powerful functional genomics tool in these bacteria.

INTRODUCTION

Bacteriophages are obligate intracellular parasites. However, the choice of host is usually considered to be specific. Generally, phages have been found to infect a single bacterial strain only, or related strains from the same species, in laboratory screens. There are several reasons for this. First, an appropriate receptor, which can be recognized by the corresponding phage-borne anti-receptor, must be expressed on the bacterial surface; the anti-receptor may be tail fibres or the base plate in the case of tailed phages. Next, the viral DNA must be injected into the host without significant host-controlled restriction. Finally, viral replication and virion assembly must occur before cell lysis; these steps can be actively targeted by abortive infection systems encoded by the bacterium. Some phages are so specific for subspecies and serotypes that phage sensitivity can be used to differentiate between closely related strains. This forms the basis of the phage typing system [for example, see Wentworth (1963) for an explanation of phage typing of the staphylococci]. Superficially, such apparent host specificity is counter-intuitive given the vast number of phages in the biosphere. At an estimated 10^{31} particles, phages are the most abundant biological entity on Earth, and outnumber bacteria 10:1 (Hendrix, 2002).

It is likely that any surface feature can serve as a phage receptor (Lindberg, 1973). Indeed, some membrane proteins were first identified through their ability to permit phage growth. For example, LamB (the maltose porin) binds phage λ , and TonA (renamed FhuA) binds phage T1 (see Heller, 1992 for further examples). In our experience, although anecdotal, LPS may be the most frequently used phage receptor in enteric bacteria. Alternative surface macromolecules, e.g. pili in the case of Φ CbK (Skerker & Shapiro, 2000), and flagella in the case of phage χ (Schade *et al.*, 1967), are known to permit phage infection.

Transduction, one of the three mechanisms of horizontal transfer of DNA in bacteria, drives adaptive evolution of bacterial hosts. Given that one model estimates $\sim 10^{14}$ transduction events per year in the Tampa Bay Estuary alone (Jiang & Paul, 1998), this mechanism is probably a very important source of genetic dissemination. In the laboratory, transduction protocols have been adapted to facilitate transposon mutagenesis, cosmid complementation and facile strain construction (Palva *et al.*, 1981; De Vries *et al.*, 1984), and thus they represent a significant tool for the genetic investigation of bacteria.

The generalized transducing phage described in this study was isolated on an unspiciated member of the genus *Serratia*: strain ATCC 39006 (*Serratia* 39006). Subsequent host-range analysis identified an additional host: a strain of *Pantoea agglomerans*. Both of these strains produce secondary metabolites that have potential biotechnological

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applications, and *Serratia* 39006 is established as a model organism for the investigation of the regulation of secondary metabolites.

Serratia 39006 produces the red pigment prodigiosin, as well as a carbapenem antibiotic. Prodigiosins, as prodiginines, have a diverse range of biological activities (Williamson *et al.*, 2006), but it is their anti-cancer activity that is currently being investigated in clinical trials (Nguyen *et al.*, 2007). The biosynthesis and regulation of prodigiosin have been studied in some detail (Thomson *et al.*, 2000; Williamson *et al.*, 2005; Slater *et al.*, 2003; Fineran *et al.*, 2005a, b, 2007), and some of this work has been facilitated by the facile construction of double and triple mutant strains using Φ OT8.

The strain of *P. agglomerans*, 9Rz4, used in this study was isolated from the rhizosphere, and is reported to have antifungal activity against *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Verticillium dahliae* (Berg *et al.*, 2002). Given that these are pathogens of many plants of economic importance, *P. agglomerans* demands further investigation for its potential as a biocontrol agent. The commercial use of bacteria in biocontrol is well established: *Agrobacterium radiobacter* and *Streptomyces lydicus* are used as microbial fungicides, under the names Nogall (<http://www.bioglobal.com.tr/en/nogall>) and Actinovate (<http://www.naturalindustries.com/>), respectively.

METHODS

Bacterial strains, growth and media. Bacterial strains used in this study are listed in Tables 1 and 2. Overnight cultures of all strains were grown in Luria–Bertani broth (LB) (1^{-1} : 10 g tryptone, 5 g yeast extract and 5 g NaCl) at 30 °C, except *Citrobacter rodentium* and *Escherichia coli* strains, which were grown at 37 °C, and *Erwinia*

strains, which were incubated at 25 °C. Growth on solid medium used LB supplemented with 1.5 % agar (LBA). Minimal medium consisted of phosphate buffer (40 mM K_2HPO_4 , 15 mM KH_2PO_4 , pH 7.0), 0.2 % (w/v) $(NH_4)_2SO_4$, 0.2 % (w/v) sucrose and 0.1 M $MgSO_4$. Where required, kanamycin (Km) and spectinomycin (Sp) were added to a final concentration of 50 μ g ml^{-1} , tetracycline (Tc) was added to a final concentration of 10 μ g ml^{-1} and chloramphenicol (Cm) was added to a final concentration of 25 μ g ml^{-1} .

Phage isolation, titration and preparation of lysates. Phage were isolated from treated sewage effluent collected from Finham Sewage Treatment Works, Coventry, UK. A 10 ml filter-sterilized sample (0.2 μ m filter; Millipore) was added to 10 ml 2 $\times$ LB and 100 μ l of an overnight culture of *Serratia* 39006, and incubated with shaking at 30 °C. After 4 days, 1 ml samples were taken, and centrifuged at 13 000 g for 5 min. The supernatant was removed and 50 μ l $NaHCO_3$ -saturated chloroform was added. Serial dilutions of the supernatant were added to a top lawn of agar seeded with *Serratia* 39006, and incubated at 30 °C overnight. To purify phage, well-separated plaques were picked, resuspended in 50 μ l phage buffer [10 mM Tris/HCl, pH 7.4, 10 mM $MgSO_4$ and 0.01 % (w/v) gelatin], and serial dilutions were plated, as described below.

Phage lysates were produced by taking the agar from the top lawn of confluent lysis, and vortexing it with 4 ml phage buffer and 500 μ l $NaHCO_3$ -saturated chloroform. This was centrifuged at 2219 g for 20 min at 4 °C. The supernatant was collected, and 100 μ l $NaHCO_3$ -saturated chloroform was added. Phage lysates were stored at 4 °C. Titres were determined after serial dilution.

Host-range analysis. Sensitivity of bacterial strains to phage was tested on agar top lawns of bacteria containing 4 ml 0.35 % LBA and 200 μ l of an overnight culture of the test strain. A 10 μ l volume of a Φ OT8 lysate (at $\sim 10^{10}$ p.f.u. ml^{-1}) was spotted on the lawn, and plates were incubated overnight at 30 °C, before scoring for lysis.

Electron microscopy. The method for electron microscopy was adapted from the method described by Skerker & Shapiro (2000). Strain 4SCARA (100 μ l) and Φ OT8 (100 μ l; at $\sim 10^{10}$ p.f.u. ml^{-1}) were mixed, and incubated for 15 min. Samples were fixed with 200 μ l fixative (5 % glutaraldehyde in 50 mM cacodylate-HCl buffer,

Table 1. Bacterial strains and plasmids used in this study

Additional strains used in transduction experiments are described in Table 2.

Strain or plasmid	Genotype	Reference
Strains		
<i>Serratia</i> sp. ATCC 39006	Note: a <i>lac</i> derivative isolated in this laboratory was used throughout this study	Bycroft <i>et al.</i> (1987)
MCR2000	<i>smaR::cat</i> derivative of <i>Serratia</i> 39006	This study
4SCARA	<i>pigX::mini-Tn5Sm/Sp</i> , <i>carA::mini-Tn5lacZ1</i> derivative of <i>Serratia</i> 39006	Williamson <i>et al.</i> (2008)
OT8R 9	<i>flhC</i> derivative of <i>Serratia</i> 39006	This study
OT8R 11	<i>flhA</i> derivative of <i>Serratia</i> 39006	This study
POT8R 7	<i>motA</i> derivative of strain 9Rz4	This study
9Rz4	<i>P. agglomerans</i> wild-type strain isolated from the rhizosphere	Berg <i>et al.</i> (2002)
<i>E. coli</i> β 2163		Demarre <i>et al.</i> (2005)
SM10 λ pir		De Lorenzo <i>et al.</i> (1990)
Plasmids		
pQE80-L	Cloning vector	Qiagen
pflhAE	<i>Serratia</i> 39006 <i>flhAE</i> cloned into pQE80-L	This study
pDS1028	Delivery plasmid for mutagenesis	Smith (2005)
pUTmini-Tn5Sm/Sp	Delivery plasmid for mini-Tn5Sm/Sp	De Lorenzo <i>et al.</i> (1990)

Table 2. Transduction efficiency within, and between, *Serratia* 39006 and *P. agglomerans*

Strain	Transduced resistance marker	Co-inherited phenotype*	Transduction efficiency	m.o.i.	Source
Transduction within <i>Serratia</i> 39006					
AX1	Sp	Auxotrophy	4.9×10^{-4}		This study
AX4	Sp	Auxotrophy	2.2×10^{-4}		This study
AX11	Sp	Auxotrophy	1.8×10^{-4}		This study
AX13	Sp	Auxotrophy	2.1×10^{-4}		This study
AX21	Sp	Auxotrophy	1.3×10^{-4}		This study
MCP2L	Km	Pigment production	3.1×10^{-4}		This study
Lac-1 (pTROY9)	Tc		4.0×10^{-6}		This study
Transduction within <i>P. agglomerans</i>					
White 1	Km	Pigment production	1.4×10^{-4}	0.002	This study
White 2	Km	Pigment production	4.6×10^{-6}	0.05	This study
Aux 1	Km	Auxotrophy	7.4×10^{-5}	0.0002	This study
Aux 1	Km	Auxotrophy	8.3×10^{-5}	0.002	This study
Aux 2	Km	Auxotrophy	7.3×10^{-5}	0.05	This study
QS ⁻ A	Km	Lactone production	5.8×10^{-5}	0.001	This study
QS ⁻ A	Km	Lactone production	6.3×10^{-5}	0.01	This study
Intergeneric transduction					
pMUT13	Km		3.5×10^{-6}		E. T. Palva

*In all cases, there was 100 % co-inheritance of the relevant phenotype and antibiotic resistance.

pH 7.4) for 10 min at 25 °C. The cells and phage were collected by centrifugation, washed with 1 ml phage buffer, and resuspended in 100 µl phage buffer. Samples were stained with 1 % uranyl acetate, and examined using a Philips CM100 transmission electron microscope.

Transposon mutagenesis, and selection of phage-resistant mutants. Equal volumes of overnight cultures of donor and recipient cells were mixed, and 30 µl samples were spotted on non-selective LBA for overnight incubation at 30 °C. The mating patch was scraped into 1 ml LB, and then washed, resuspended in 100 µl LB, and spread on selective agar plates. The recipient strain, donor strain and antibiotic selection used for isolation of *Serratia* 39006 auxotrophic mutants were MCR2000, SM10 λ pir (pUTmini-Tn5Sm/Sp), and Sp and Cm, respectively. Auxotrophic requirements were identified using the plate pool method of Holliday (1956). For phage-resistant mutants, 50 µl of the resuspended mating patch was mixed with 200 µl phage and mixed into a top lawn of 4 ml 0.35 % LBA. The recipient strain, donor strain and antibiotic selection used, respectively, were MCA54, BW20767 (pDS1028), Cm (note: minimal medium was used instead of LBA) for *Serratia* 39006 phage-resistant mutants; and *P. agglomerans*, *E. coli* β 2163 (pNRW124), Km for *P. agglomerans* phage-resistant mutants. Diaminopimelic acid was added to all media, at a final concentration of 300 µM, for growth of β 2163.

Motility assays. Motility was assayed using tryptone swarm agar [0.3 % (w/v) Bacto agar (Becton Dickinson), 1 % (w/v) Bacto tryptone (Becton Dickinson) and 0.5 % (w/v) NaCl]. A 4 µl volume of an overnight bacterial culture, diluted to an OD₆₀₀ of 0.2, was spotted onto the agar, and incubated overnight at 25 °C. The diameter of the halo was measured after 16 h.

Cloning of *flhAE*, and mapping transposon mutants. *flhAE* was amplified from *Serratia* 39006 using primers σ TE64 (ATGAGCTCTA-AATAAATAAGAGAGATATGACTGATGGCTAATCTGG) and σ TE65 (TAAAGCTTGAAGTAGACGTAGCACTTTGTC). Following digestion with *SacI* and *HindIII*, and purification, the fragment was

cloned into pQE80 digested with the same enzymes, using standard molecular techniques (Sambrook *et al.*, 1989). The genes disrupted in phage-resistant mutants were defined using the random prime PCR protocol described by Fineran *et al.* (2005b).

Adsorption assays. Adsorption of Φ OT8 to *Serratia* 39006 was carried out by using protocols described by Petty *et al.* (2006) for phage infecting *Serratia marcescens* Db11.

Generalized transduction. Phage lysates were prepared, as described above, on bacterial strains carrying the desired mutation or plasmid. Overnight cultures (10 ml) of the recipient strain were centrifuged at 6000 g for 10 min, and the cells were resuspended in 1 ml LB. The frequency of spontaneous resistance to antibiotics was determined by spreading 100 µl of the cell suspension on LBA containing the relevant antibiotic. An appropriate volume of the transducing lysate was added to the remaining cells to give the desired m.o.i., and it was then mixed, and incubated for 1 h at 30 °C. A 100 µl volume was spread on agar plates containing the relevant antibiotic to select for the acquisition of the antibiotic-resistance gene. Transductants were then screened for the relevant co-inherited phenotype (auxotrophy, pigmentation or lactone production). A 100 µl volume of the phage lysate was also spread on non-selective agar plates to confirm sterility.

Test for lysogeny. Φ OT8 was tested for lysogeny by using the methods described by Petty *et al.* (2006).

Analysis of phage proteins. A high-titre phage lysate was treated with DNase I (1 µg ml⁻¹) and RNase A (1 µg ml⁻¹) at room temperature for 30 min. NaCl was added to a final concentration of 1 M, and this was followed by incubation on ice for 1 h, and centrifugation at 11 000 g for 10 min at 4 °C. PEG 8000 (Sigma) was added to a final concentration of 10 % (w/v), followed by incubation on ice for 1 h. The precipitated phage preparation was centrifuged at 11 000 g for 10 min at 4 °C, and the supernatant was discarded. The pellet was resuspended in 2 ml phage buffer. A 2 ml volume of

NaHCO₃-treated chloroform was added, and the mixture was vortexed, and centrifuged at 3000 g for 15 min at 4 °C. CsCl (0.75 g ml⁻¹) was dissolved in the supernatant, and then the supernatant was centrifuged at 35 000 g for 24 h at 4 °C, using an SW50.1 rotor. The ultracentrifuge tube was pierced with a hypodermic syringe, and the white band of phage particles was removed. It was then transferred to dialysis tubing, and dialysed in 5 l 50 mM Tris/HCl (pH 8.0) overnight.

Phage protein samples (40 µl) were mixed with 10 µl 5× loading buffer [50 mM Tris/HCl (pH 6.8), 50 % glycerol, 1 % bromophenol blue, 5 mM EDTA], boiled for 4 min, and loaded onto an SDS-PAGE gel (3.3 % stacking gel; 13–23 % gradient resolving gel).

N-terminal sequencing of proteins was carried out by the Protein and Nucleic Acid Chemistry Facility in the Department of Biochemistry, University of Cambridge, Cambridge, UK.

Purification of phage DNA, and restriction digests. Phage DNA was purified from lysates made by substituting agarose for agar in the top lawns. Restriction digest reactions contained 500 ng DNA, and were done, as directed by the manufacturer, using 2 µl enzyme (New England Biolabs). Following a 16 h incubation at 37 °C, samples were run on a 1 % agarose gel.

RESULTS AND DISCUSSION

The host range of ΦOT8

ΦOT8 was isolated by enrichment using treated sewage effluent. Clear plaques with a diameter of 1–2 mm were seen in 0.3 % agar top lawns.

To investigate the host range of ΦOT8, a collection of 21 clinical and environmental *S. marcescens* isolates (described in detail by Harris *et al.*, 2004) were tested for phage sensitivity by spot test. Also tested were the following enteric strains studied in this laboratory: *Erwinia carotovora* subsp. *atroseptica* SCRI 1043, *Erwinia carotovora* subsp. *carotovora* MH1000, *Erwinia carotovora* subsp. *carotovora* MS102, *Yersinia enterocolitica*, *E. coli* TG1, *E. coli* DH1, *C. rodentium* ICC169 and *S. marcescens* Db11. None of the strains was found to be phage-sensitive (A. Harris, personal communication; I. Foulds, personal communication). Subsequently, 29 strains isolated from the rhizosphere by Berg *et al.* (2002) were also tested for ΦOT8

sensitivity. One strain, 9Rz4, was susceptible to phage infection, as determined by using spot tests. Strain 9Rz4 was identified as *P. agglomerans* by fatty acid methyl ester analysis (Berg *et al.*, 2002) and 16S rDNA sequencing (data not shown), and is referred to as *P. agglomerans* hereafter. Phage sensitivity of *P. agglomerans* was confirmed by a full titration to isolated plaques with ΦOT8. While plaques were very small and often difficult to see, the efficiency of plaquing on *P. agglomerans* was the same as that on *Serratia* 39006, and this suggested that there was no host-controlled restriction (data not shown).

ΦOT8 is flagellum-dependent

All *Serratia* 39006 mutants that were spontaneously resistant to ΦOT8 were non-motile. This suggested that the receptor for ΦOT8 was the flagellum. Indeed, both *Serratia* 39006 and *P. agglomerans* are motile (data not shown). We tried to show flagellum dependence via transmission electron microscopy, as described in Methods. However, unambiguous evidence of direct binding of the phage to the flagellum was not obtained, even when using the hyperflagellated *Serratia* 39006 strain 4SCARA (Table 1). The electron micrographs (Fig. 1) showed that ΦOT8 is a member of the *Siphoviridae* family. ΦOT8 had an icosahedral head, with a diameter of 97 nm. Head size correlates with genome size (Leffers & Rao, 1996). Therefore, by comparison with phages of a similar size (e.g. ΦIF3; Petty *et al.*, 2006), the results suggest a genome size of approximately 250 kb for ΦOT8. The tail was flexible, and there was no evidence of it being contractile. A base plate was attached to the distal end of the tail.

To identify the receptor of ΦOT8, phage-resistant, transposon-insertion derivatives of *Serratia* 39006 and *P. agglomerans* mutant banks were selected. Transposon-insertion sites were determined by sequencing the transposon–chromosome boundaries (Table 1). Many structural components of the flagellum are represented in this list, though the impact of most mutations is likely to be more dramatic than they appear, due to polar effects on polycistronic gene clusters. In contrast, a functional MotA, the gene for which is mutated in strain POT8R 7,

Fig. 1. Transmission electron micrographs of ΦOT8 stained with uranyl acetate. Left: the phages were identified as *Siphoviridae* because of their flexible, non-contractile tails. Right: a base plate and tail fibres are evident; a flagellum runs horizontally across the micrograph. Bar, 500 nm.

is not necessary for flagella biosynthesis in *Salmonella typhimurium* (Enomoto, 1966), and the *Erwinia carotovora* subsp. *carotovora* *motA* mutant remained peritrichously flagellated (Hossain *et al.*, 2005). *motA* mutants have been termed 'paralysed' because, despite the presence of flagella, they are non-motile. The fact that the *motA* mutant is phage-resistant in *P. agglomerans* may demonstrate the need for a functional flagellum or a chemotactic response for phage infection. This is reminiscent of phage χ , which is a flagellatropic coliphage. Phage χ first binds to the flagellum, but motility is important for the translocation of the phage along the filament to its ultimate receptor at the cell surface (Schade *et al.*, 1967).

The *flhA* mutant, which is deficient in flagellum export (Ghelardi *et al.*, 2002), had the dual phenotype of phage resistance and non-motility, as predicted. This mutant was chosen for genetic complementation tests because, by comparison with gene organization in other genomes, the gene is predicted not to be part of a large operon. The *Serratia* 39006 *flhAE* locus has been sequenced (GenBank accession no. FN396602). The phage sensitivity and motility phenotypes were complemented *in trans* using a plasmid construct that overexpressed *flhAE*. Phage sensitivity of the complemented strain was fully restored: mean (SD) phage titres (p.f.u. ml⁻¹) were 2.5×10^9 (4.6×10^8) for *Serratia* 39006 (pQE80) (wild-type), 0 (0) for OT8R 11 (pQE80) (*flhA* mutant), and 3.7×10^9 (1.3×10^9) for OT8R 11 (pflhAE) (complemented strain). Motility was restored to ~50% of the wild-type level: mean (SD) halo diameters (mm) were 38 (1) were for *Serratia* 39006 (pQE80), 5 (0) for OT8R 11 (pQE80), and 17 (2) for OT8R 11 (pflhAE). The discrepancy in the halo diameter values for the wild-type strain and the complemented strain may be due to copy number effects leading to aberrant regulation of the *flhAE* genes.

If Φ OT8 depends on the flagellum for adsorption to the bacterium, adsorption to a non-flagellated strain should be abolished, as has been shown for the *Erwinia* flagellatropic phage, Φ AT1 (Evans *et al.*, 2009). As shown in Fig. 2, this was also true for Φ OT8. In this case, OT8R 19 was chosen as the non-flagellated strain, as *flhC* (part of the flagella 'master' operon) is mutated in this strain.

Φ OT8 is a generalized transducing phage

In a crude screen for novel generalized transducing phages, Φ OT8 was tested and found to be capable of mediating transduction of auxotrophic markers between *Serratia* 39006 strains. In order to optimize the experimental conditions, the effect of m.o.i. on transduction efficiency (defined as the number of transductants per p.f.u.) was determined. For the following m.o.i., the number of transductants and transduction efficiency, respectively, were obtained. m.o.i. 0.001: 4900, 4.9×10^{-4} ; m.o.i. 0.01: 15 000, 1.5×10^{-4} ; m.o.i. 0.1: 60 000, 6.0×10^{-5} . The results showed that as more phage were used, the absolute number of transductant colonies recovered also increased.

Fig. 2. Adsorption of Φ OT8 to *Serratia* 39006 derivatives was measured by determining the levels of phage present in culture supernatants. There was no adsorption of phage to the *flhC* mutant OT8R 19 (■). In contrast, the parental strain MCA54 (▲) adsorbed phage rapidly, with 97% of the phage being removed from the culture supernatant by 30 min. ●, Negative control (LB only).

However, a drop in transduction efficiency was observed, probably due to superinfection killing of transductants. The transduction efficiency mediated by Φ OT8 was very high compared with that of many other transducing phages. For example, the recently described transducing phages Φ CR1 and Φ IF3 have optimal transduction efficiencies of 10^{-8} and 10^{-6} transductants per p.f.u., respectively (Petty *et al.*, 2006, 2007). The large genome size of Φ OT8 may contribute to the high efficiency of transduction, since it permits the packaging of large amounts of bacterial DNA in transducing particles.

Transduction efficiencies were determined for various markers in *Serratia* 39006 and *P. agglomerans*, and they demonstrated the generalized nature of the transduction (Table 2). The efficiencies recorded for *P. agglomerans* were approximately an order of magnitude lower than those obtained for *Serratia* 39006; this may reflect inherent variation in the transduction of different markers rather than an underlying difference in phage replication within the two strains. However, marked differences are sometimes seen with broad-host-range transducing phages. For example, three transducing phages that each infect both *Streptococcus thermophilus* and *Lactococcus lactis* displayed transduction efficiencies that were four orders of magnitude lower in *L. lactis* compared with those in *S. thermophilus*, and this was explained by the weak absorption of the phage to *L. lactis* (Ammann *et al.*, 2008). The lower transduction efficiencies seen in *P. agglomerans* compared with *Serratia* 39006 may be a consequence of less avid adsorption of Φ OT8 to *P. agglomerans*.

Transduction of a plasmid from *P. agglomerans* to *Serratia* 39006 was also demonstrated. A slightly lower transduction efficiency was seen for plasmid transduction compared

with that for transduction of chromosomal markers; this is consistent with results obtained for other transducing phages (Petty *et al.*, 2007). In the plasmid-transduction experiment conducted here, an antibiotic-resistance gene was moved between the two bacterial genera. Phages are well known for their ability to mediate horizontal acquisition of DNA by bacteria, and this most strikingly manifests itself as lysogenic conversion (Canchaya *et al.*, 2004). However, there are wider ecological implications, and incorporation of new DNA into a bacterial host may have more subtle effects than the acquisition of toxin genes. The results presented here underscore similar findings in Gram-positive strains that have been published recently (Ammann *et al.*, 2008; Chen & Novick, 2009).

ΦOT8 is virulent

While *Serratia* 39006 colonies were recovered after ΦOT8 exposure (that is, they were either lysogens immune to superinfection, or they were phage-resistant), phage particles were not detected in the culture supernatants following overnight growth. Therefore, the colonies were probably spontaneous phage-resistant mutants rather than superinfection-resistant lysogens. Plaques obtained with ΦOT8 were always found to be clear. Thus, there was no evidence that ΦOT8 is a temperate phage.

Analysis of phage proteins and DNA

To analyse the structural proteome of ΦOT8, a purified phage preparation was run on an SDS-PAGE gel. In total, 13 bands were seen (Fig. 3). The two most prominent bands, with sizes of approximately 24 and 31 kDa, were excised and submitted for N-terminal sequencing. However, the sequences obtained (SXDLSLSRFX for the 24 kDa protein, and SLGETLAQEI for the 31 kDa protein) were not similar to any sequences in the BLAST database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). This result emphasizes the fact that comparatively little sequence data have been obtained for phages (Lima-Mendez *et al.*, 2007).

We attempted to produce restriction enzyme digests of purified phage genomic DNA. However, ΦOT8 genomic DNA was resistant to cleavage by restriction enzymes *Bam*HI, *Eco*RI, *Eco*RV and *Hind*III, even after incubation for 16 h (data not shown). Inhibition of bacterial restriction endonucleases by phage proteins has been observed, notably in phage P1 infections when DarA and DarB are co-injected with the phage DNA into the bacterial cell (Iida *et al.*, 1987). To exclude the possibility that inhibitors were contaminating the DNA preparations, phage λ DNA was mixed with ΦOT8 DNA prior to incubation with *Hind*III. Complete digestion of λ DNA was seen, discounting the possibility that an inhibitor was present (data not shown). Apart from inhibition, a number of anti-restriction strategies are known to be employed by phage; these strategies include elimination of recognition sequences from the genome, and incorporation of

Fig. 3. A purified phage preparation was analysed by SDS-PAGE (middle lane). Molecular mass markers (kDa) are shown in the outside lanes. The two dominant bands (arrows) were N-terminally sequenced (see text).

modified bases (Tock & Dryden, 2005). However, it seems unlikely that the genome has no recognition sites for four different endonucleases, especially given the proposed large genome size. Thus, it seems most plausible that the genome of ΦOT8 contains chemically modified, atypical nucleotide bases; perhaps this is a feature that contributes to the broad host range of this phage.

Conclusions

The finding that ΦOT8 is flagellum-dependent was surprising because the flagellum is not often used in this way. Indeed, Petty *et al.* found that 92 % of newly isolated *Serratia* phages use LPS as their receptor (Petty *et al.*, 2006), and this is consistent with results obtained for phages isolated on other enteric species, in this laboratory (Petty *et al.*, 2007; data not shown).

Notwithstanding the well-known broad-host-range phages such as Mu and P1 (Harshey, 1988; Yarmolinsky & Sternberg, 1988), phage host range is popularly thought to be narrow; this view is enshrined in, and strengthened by, the phage-typing system for classifying bacteria (reviewed by Pitt & Gaston, 1995). Despite a 1998 study specifically assessing the prevalence of broad-host-range phages (Jensen *et al.*, 1998), and recent examples of such phages in Gram-positive genera (Ammann *et al.*, 2008; Chen & Novick, 2009), the consensus remains that phage

infection tends to be species and strain specific. However, other examples of broad-host-range phages have been reported (for example, see Gill *et al.*, 2003), and such a stringent model of phage–bacterium interactions fails to explain the abundance of phages in the environment. Neither of the bacterial strains used in this study has been genomically sequenced, and the *P. agglomerans* strain has not been characterized in detail. Therefore it is difficult to quantify how similar the strains are. However, they are both Gram-negative enterobacteria that were isolated from the soil: a salt marsh in the USA, in the case of *Serratia* 39006; and the rhizosphere of oilseed rape in Germany, in the case of *P. agglomerans*.

Identification of phage susceptibility is usually based on plaque formation; this is a demanding test. However, we have shown here that plaques are not always easily observed even when a bacterial host permits viral replication: the plaques may be intermittently too small to see under the conditions employed. Since the lack of a positive result in crude phage spot tests does not necessarily indicate complete phage resistance, a more reliable method to identify alternative bacterial hosts might result in the reclassification of many so-called narrow-host-range phages.

Furthermore, in terms of horizontal dispersal of DNA, lysis of a bacterium is not required for transduction to occur. For example, coliphage P1 is able to adsorb to, and inject DNA into, *Myxococcus xanthus* cells, thus forming transductants, but viral replication is not seen within this species (Kaiser & Dworkin, 1975). Taken together, this means that inter-species and intergeneric transduction is likely to be more prevalent than we currently believe, particularly since phage-mediated acquisition of DNA is already reckoned to occur on a very large scale (Jiang & Paul, 1998).

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