A generalized transducing phage (ϕ IF3) for the genomically sequenced *Serratia marcescens* strain Db11: a tool for functional genomics of an opportunistic human pathogen

Nicola K. Petty,¹ Ian J. Foulds,¹ Elizabeth Pradel,^{2†} Jonathan J. Ewbank² and George P. C. Salmond¹

Correspondence
George P. C. Salmond
gpsc@mole.bio.cam.ac.uk

¹Department of Biochemistry, University of Cambridge, Building O, Tennis Court Road, Cambridge CB2 1QW, UK

²Centre d'Immunologie de Marseille Luminy, INSERM/CNRS/Université de la Méditerranée, Case 906, 13288 Marseille cedex 9, France

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A bacteriophage (ϕ IF3) capable of mediating generalized transduction in *Serratia marcescens* strain Db11 has been isolated and characterized. The genome of this *Serratia* strain has recently been sequenced and is likely to become the reference strain for *S. marcescens* researchers. ϕ IF3 is most likely a virulent phage, which can transduce markers at frequencies of 10^{-6} transductants per p.f.u. It has a lipopolysaccharide receptor and was determined to have a latent period of 50 min and a burst size of approximately 100 phages. The phage DNA was resistant to digestion with restriction enzymes. Electron microscopy showed ϕ IF3 to be a member of the family *Myoviridae*. This is the first report of a generalized transducing phage able to infect Db11 and this phage will be a valuable tool for functional genomic analysis of the pathogen host.

INTRODUCTION

The Gram-negative bacillus *Serratia marcescens* is a member of the *Enterobacteriaceae*. It is a broad-host-range pathogen, able to colonize a wide variety of ecological niches, and some strains can cause disease in plants, insects and animals (Grimont & Grimont, 1978). An opportunistic human pathogen and the aetiological agent of a range of diseases, it is an increasingly frequent cause of nosocomial infections, and represents a growing public health problem (Hejazi & Falkner, 1997). There is currently no single strain of *S. marcescens* most commonly used by researchers and comparatively little is known about its pathogenicity factors. Db11 is the first strain of *S. marcescens* to have its genome sequenced, and this will soon be fully annotated and published (http://www.sanger.ac.uk/Projects/S_marcescens/). Therefore, it is likely that Db11 will become a reference strain for all *S. marcescens* researchers.

S. marcescens strain Db11 is a non-pigmented, spontaneous streptomycin-resistant mutant of strain Db10, an insect pathogen isolated from *Drosophila melanogaster* (Flyg *et al.*, 1980). Db11 is used as a model pathogen to investigate innate immunity and pathogen–host interactions in the nematode *Caenorhabditis elegans* (Kurz *et al.*, 2003; Mallo *et al.*, 2002; Schulenburg & Ewbank, 2004) and the fruit fly

D. melanogaster (Kurz *et al.*, 2003; Lazzaro *et al.*, 2004). There are limited genetic tools currently available for the analysis of Db11, so there is a need to enhance the genetic tractability of this strain to realize fully the benefits of the genome sequence and the invertebrate infection models.

Transducing bacteriophages are useful genetic tools for the analysis of their hosts. Studies using generalized transduction remain important, even today in the post-genomic era (Hava & Camilli, 2001). Generalized transduction represents a simple way to perform a range of genetic manipulations, including moving mutations from one strain to another for fine-structure mapping and strain construction, plasmid transfer and transposon mutagenesis (Masters, 1985). To our knowledge, no transducing phages have yet been described for *S. marcescens* strain Db11. There is a transducing phage (ϕ 3M) available for other *S. marcescens* strains (Regue *et al.*, 1991) but this is not able to infect Db11.

Here we report the identification and characterization of ϕ IF3, a phage capable of generalized transduction in *S. marcescens* strain Db11.

METHODS

Bacterial strains and culture conditions. The bacterial strains used in this study are listed in Table 1. All *Serratia* strains were grown at 30 °C in Luria broth (LB) unless otherwise stated. For solid

[†]Present address: Equipe Inserm E0364, IBL, Institut Pasteur de Lille, BP447, 59021 Lille Cedex, France.

Table 1. Bacterial strains

Strain	Relevant characteristics	Source or reference
<i>Serratia</i> sp.		
ATCC 39006 Lac-A	Lac ⁻ derivative of ATCC 39006, pigmented	Laboratory stock
ATCC 39006 SCAR13	Laboratory-maintained strain, pigmented	Laboratory stock
<i>Serratia marcescens</i>		
1695	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
0006	Environmental isolate, non-pigmented	Aucken & Pitt (1998)
1047	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
0026	Environmental isolate, non-pigmented	Aucken & Pitt (1998)
4028	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
3078	Clinical isolate, pigmented	Aucken & Pitt (1998)
0038	Environmental isolate, pigmented	Aucken & Pitt (1998)
1024	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
0022	Environmental isolate, non-pigmented	Aucken & Pitt (1998)
3127V	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
0035	Environmental isolate, pigmented	Aucken & Pitt (1998)
3888	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
0028	Environmental isolate, non-pigmented	Aucken & Pitt (1998)
3255	Clinical isolate, pigmented	Aucken & Pitt (1998)
0040	Environmental isolate, pigmented	Aucken & Pitt (1998)
2595	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
0030	Environmental isolate, non-pigmented	Aucken & Pitt (1998)
3127	Clinical isolate, pigmented	Aucken & Pitt (1998)
3078V	Clinical isolate, non-pigmented	Aucken & Pitt (1998)
ATCC 274	Clinical isolate, pigmented	Regue <i>et al.</i> (1991)
S6	Clinical isolate, non-pigmented	Yang <i>et al.</i> (1990)
SM365	Laboratory maintained strain, pigmented	Sapriel <i>et al.</i> (2003)
2170	Laboratory maintained strain, pigmented	Palomar <i>et al.</i> (1990)
SS-1	Laboratory maintained strain, pigmented	Swift <i>et al.</i> (1993)
Db10	Wild-type, non-pigmented	Flyg <i>et al.</i> (1980)
Db11	Spontaneous Sm ^R mutant of Db10	Flyg <i>et al.</i> (1980)
Db10 JESM174	MiniTn5Sm/Sp causing unknown auxotrophy, Sm ^R	J. J. Ewbank & E. Pradel, unpublished
Db10 JESM175	MiniTn5Sm/Sp causing unknown auxotrophy, Sm ^R	J. J. Ewbank & E. Pradel, unpublished
Db10 JESM267	<i>swrA</i> ::miniTn5Sm/Sp, Swarm ⁻ , Sm ^R	J. J. Ewbank & E. Pradel, unpublished
Db10 JESM273	<i>entD</i> ::miniTn5Sm/Sp, Swarm ⁻ , Sm ^R	J. J. Ewbank & E. Pradel, unpublished
Db10 JESM270	<i>purL</i> ::miniTn5Sm/Sp, purine auxotroph, Sm ^R	J. J. Ewbank & E. Pradel, unpublished
Db10 JESM293	<i>aroC</i> ::miniTn5Sm/Sp, aromatic amino acid auxotroph, Sm ^R	J. J. Ewbank & E. Pradel, unpublished
Db11 20C2	<i>wzm</i> ::miniTn5Cm, no O-antigen LPS mutant, Cm ^R	Kurz <i>et al.</i> (2003)

medium 1.5% agar was used, and for the soft medium overlay (top agar) 0.35% agar was used, unless otherwise stated. When required, streptomycin was added to a final concentration of 100 µg ml⁻¹ for selection. Auxotrophic strains were identified on M9 glucose minimal medium agar as described by Sambrook *et al.* (1989), and swarm mutants identified on swarm agar (20 ml 50%, v/v, glycerol, 5 g peptone and 7.5 g agar per litre). Phage buffer was composed of 10 mM Tris/HCl pH 7.4, 10 mM MgSO₄ and 0.01%, w/v, gelatin. The chloroform used in this study was saturated with sodium hydrogen carbonate.

Phage isolation. Phages were isolated from treated sewage effluent collected from the sewage treatment plant at Milton, Cambridge. A 10 ml sample of effluent was shaken vigorously with 500 µl chloroform for 1 min to kill any bacteria. Then 200 µl of this chloroform-treated effluent was mixed with 200 µl of a Db11 overnight culture and 4 ml top agar, and poured as an overlay onto solid agar plates.

Plates were incubated overnight and single phage plaques were picked with a sterile toothpick into 1 ml phage buffer and shaken with a few drops of chloroform.

Phage lysate preparation. Phages were titrated by serial dilution in phage buffer. A 10 µl volume of each dilution was added to 200 µl of an overnight culture of host bacteria and 4 ml top agar, and poured as an overlay onto a solid agar plate, then incubated at 30 °C overnight. Plates were inspected for plaques and the phage titre determined in p.f.u. ml⁻¹. Those plates showing confluent lysis (seen as a mosaic effect where the plaques have just merged) were identified for phage lysate preparation. The top agar was scraped off these plates, and the surface washed with 3 ml phage buffer. The wash was added to the harvested top agar, and vortexed vigorously with 500 µl chloroform for 2 min. After standing at room temperature for 30 min, the agar mix was centrifuged at 2220 g for 20 min at 4 °C. The supernatant was removed, then 100 µl chloroform was

added and vortexed briefly before storage at 4 °C. This process was repeated until high-titre lysates of around 10^9 – 10^{10} p.f.u. ml⁻¹ were obtained.

Host range determination. Top agar overlays containing 200 µl of the bacterial strain to be tested were poured onto LB solid agar (LBA) plates and allowed to set. A 10 µl sample of high-titre phage lysate was spotted onto each overlay, alongside a 10 µl phage buffer control, and the plates incubated overnight. The ability of the phage to produce localized clearing of the bacterial lawn was noted. Positive spot tests were followed up by titration to single plaques to confirm permissive hosts, rather than bacterial lysis caused by either bacteriocinogeny or lysis from without.

Transduction assay. Phages were tested for their ability to transduce the transposon from Db10 JESM175 (miniTn5Sm, auxotrophic) into wild-type Db10. Transduction was measured by the production of streptomycin-resistant (Sm^R) colonies and confirmed by co-inheritance of auxotrophy. One hundred microlitres of a high-titre phage lysate (propagated on strain Db10 JESM175) was added to 3 ml from an overnight culture of recipient wild-type strain (Db10), mixed for 5 s and left static at room temperature for 30 min. It was then incubated on a tube roller at 30 °C for 20 min, followed by centrifugation at 2220 g for 10 min at 4 °C. The supernatant was discarded and the pellet resuspended in 300 µl LB; 150 µl of the suspension was spread onto each of two LBA plates containing streptomycin. Plates were incubated, together with controls for spontaneous mutation to drug resistance and lysate contamination, at 30 °C for 24–48 h. Transduction was confirmed by screening Sm^R colonies for auxotrophy. Multiplicity of infection (m.o.i.), temperature and time of incubation for transduction were optimized.

To test for generalized transduction and determine transduction efficiencies, ϕ IF3, propagated on an appropriate donor host, was added to 10^8 c.f.u. ml⁻¹ of an overnight culture of wild-type Db10, at an m.o.i. of 0.1. All transduction steps were performed as above. Transduction was confirmed by screening Sm^R colonies for co-inheritance of a secondary phenotype of either auxotrophy or inability to swarm.

Electron microscopy. Carbon-coated, charge-discharged copper grids were placed on 5 µl drops of high-titre phage lysate for 30 s to 1 min, and washed briefly on water droplets three times before blotting dry and placing on 5 µl 2% phosphotungstic acid for the same length of time. Grids were then blotted to remove excess liquid and allowed to air dry before examination in a Philips CM100 transmission electron microscope.

Phage adsorption. Overnight cultures (10 ml) of bacterial host, or non-host bacterial control, were infected with ϕ IF3 at an m.o.i. of 0.01. The same amount of phage was added to an LB-only control. The bacteria and phage were mixed briefly and samples were removed immediately from each, for the 0 min reading, before placing on a tube roller at 30 °C. Samples (100 µl) were removed every 5–10 min for a total of 50 min and added to 900 µl phage buffer and 30 µl chloroform, mixed for 5 s then centrifuged at 13 000 g for 5 min. The supernatant was removed and titrated as described above to determine the number of p.f.u. Absorption of phage to bacteria was measured by the number of p.f.u. ml⁻¹ remaining in the supernatant, and expressed as a percentage of the number of p.f.u. ml⁻¹ in the 'no bacteria' control.

Growth curve and phage infection. Bacterial growth was determined by measuring optical density using a Unicam HeZios spectrophotometer at a wavelength of 600 nm (OD₆₀₀) and cuvettes with a 1 cm path length. Overnight cultures were diluted to OD₆₀₀ 0.02 in 50 ml prewarmed LB, in 500 ml conical flasks, and incubated in a shaking water bath at 30 °C, 260 r.p.m. Samples (1 ml) were removed every 30 min and bacterial growth measured. Phage

was added to three flasks when the cells were in early exponential phase (OD₆₀₀ 0.1), and the same volume of phage buffer added to the three control flasks. The m.o.i. was varied to determine its effect on bacterial growth. Samples were measured every 10–30 min as necessary.

One-step growth curve and burst size. The one-step growth experiment was adapted from that devised by Ellis and Delbrück in 1939, and described in Hayes (1970). A bacterial growth curve was set up as above. At OD₆₀₀ 0.1, the culture was removed from the shaker, and 10 ml was transferred to a tube and centrifuged at 2220 g for 10 min at 4 °C. The supernatant was removed and pellet resuspended in 10 ml LB, before adding phage at an m.o.i. of 0.001. Phage were also added to an LB-only control, and an uninfected bacterial culture acted as a 'no phage' control. Assay and control tubes were left static at room temperature for 4 min to allow the phage to adsorb to the bacteria. The samples were then centrifuged as before, the supernatants removed and the pellets resuspended in 10 ml LB. Each 10 ml suspension was then added to a separate 250 ml conical flask containing 15 ml LB and returned to the shaking water bath for incubation. Samples were removed every 10 min and immediately serially diluted and titrated as described above to determine the number of p.f.u. and hence the number of infectious centres. Unadsorbed phage were also titrated as described above for phage adsorption. Results were expressed as the ratio of p.f.u. per infected cell.

DNA isolation and characterization. ϕ IF3 DNA was isolated from high-titre phage lysates obtained from liquid infection (no impurities from agar) using the Phase Lock Gel (PLG) kit from Eppendorf, following the protocol in the manual and using 1.5 ml Light PLG tubes. Once isolated, the DNA was submitted to digestion with a range of restriction enzymes for 2 h, and digestion monitored by electrophoresis on a 0.7% agarose gel.

Test for lysogeny. Bacteria from the centre of ϕ IF3 spot tests on a Db11 lawn were streaked to single colonies, and isolated colonies restreaked three times on LBA plates. Twenty colonies were picked from different plates of the third streak, for overnight cultures. Each culture was tested for the spontaneous release of phage by spotting 10 µl of each supernatant onto a seeded top agar lawn of Db11, along with a ϕ IF3 control. Cultures made from the colonies were tested for immunity to ϕ IF3 by spotting 10 µl onto top agar lawns made from each overnight culture, alongside the appropriate supernatant. Plates were incubated overnight and phage infection identified by zones of clearance.

RESULTS AND DISCUSSION

Isolation of bacteriophage

Thirty-five phages were isolated from treated sewage effluent and designated ϕ IF1–35. Eight of them were screened for transduction ability and, of these, only ϕ IF3 was able to transduce the test transposon from a *S. marcescens* Db10-derived mutant. ϕ IF3 was further characterized.

The plaque size of ϕ IF3 was found to be highly dependent on the concentration of agar in the top agar overlay, the strain infected and the incubation conditions. The plaque size was largest in 0.35% agar and could be easily seen at 0.5–2 mm diameter (data not shown). This agar concentration was chosen for routine use, as lower concentrations did not solidify properly. At higher concentrations of agar, smaller plaques were produced which were difficult to see, and at the

commonly used concentration of 0.8 % agar, plaques were barely visible and could easily be overlooked.

Host range

Thirty-nine *Serratia* strains, including two *Serratia* ATCC 39006 derivatives and 24 non-Db10 *S. marcescens* strains (listed in Table 1), were tested for susceptibility to ϕ IF3 infection. Some clearing was seen on lawns of 24 of the strains. However, subsequent titrations confirmed that ϕ IF3 was able to produce plaques on only Db10/Db11 strains, and therefore the clearing seen on other *Serratia* strains must have been due to either bacteriocinogeny or lysis from without. We found evidence that Db10/11 might produce bacteriocins, as culture supernatants produced clearance of some bacterial lawns (data not shown). There was no evidence of plaque-forming prophages in these strains (data not shown). Other Gram-negative bacteria were also tested for susceptibility to infection with ϕ IF3: *Escherichia coli* DH5 α , *Erwinia carotovora* subsp. *carotovora* ATCC 39048, *Erwinia carotovora* subsp. *atroseptica* 1043, *Pseudomonas aeruginosa* PA01 and *Citrobacter rodentium* DBS100. The Gram-positive *Staphylococcus aureus* was also tested. None of these strains showed any signs of plaque formation.

ϕ IF3 was able to infect all Db10/11 strains used in this study, except Db11 20C2 (Fig. 1). This strain is a lipopolysaccharide (LPS) mutant, containing a transposon insertion in a homologue of *wzm* that encodes an O-antigen transporter, and has a detectable difference in its LPS structure (Kurz *et al.*, 2003). The O-antigen of LPS is very important for full virulence of *S. marcescens* (Kurz *et al.*, 2003; Palomar *et al.*,

1993). These results suggest that LPS is the receptor for ϕ IF3. Adsorption studies suggest that ϕ IF3 is unable to adsorb effectively to Db11 20C2, the LPS mutant (data not shown). These adsorption data support the findings of the host-range studies and indicate that LPS is the receptor for ϕ IF3. Only two (ϕ IF1 and ϕ IF16) out of 25 Db11 phages tested were able to infect Db11 20C2 (data not shown), thereby showing that 92 % of the new phages tested use LPS as their receptor.

Characterization of ϕ IF3

The morphology of ϕ IF3 was determined by transmission electron microscopy. Four morphological types of ϕ IF3 were seen, as shown in Fig. 2(a–d). The most likely explanation for the different morphologies of what is clearly the same phage is that the virion is unstable and many phages can be seen at various stages of degradation. The phage was also more commonly seen with a contracted tail than with the non-contracted form. The morphology of the intact virion (Fig. 2e) showed that ϕ IF3 had an icosahedral head of 100 nm diameter, a 12 nm long neck region, and a long contractile tail of 290 nm with a 20 nm diameter. Based on its morphology, ϕ IF3 can be classified into the order *Caudovirales* and family *Myoviridae*, according to the Ackermann classification (Ackermann, 2003). There was evidence of tail fibres and a possible base plate observed on only a few phages with non-contracted tails.

Because of the apparent instability of ϕ IF3 seen in electron micrographs and a rapid reduction of titre by 10^2 p.f.u. in 6 weeks observed in phage stocks, the effect of different

Fig. 1. Sensitivity of wild-type and LPS mutant to ϕ IF3 and ϕ IF1: phage spot tests on a lawn of Db11 (left) and Db11 20C2 (LPS mutant, right). Dilutions of ϕ IF3 are on the left of each plate, and dilutions of ϕ IF1 are on the right of each plate. ϕ IF3 formed clear plaques on Db11 but was unable to infect Db11 20C2. ϕ IF1 formed turbid plaques on both Db11 and Db11 20C2.

Fig. 2. Electron micrographs of ϕ IF3 negatively stained with phosphotungstic acid. (a) Intact virion with shortened sheath and exposed central tube (this is most likely the tail in contracted form); (b) intact virion; (c) virion with lysed head and non-contracted tail; (d) virion with lysed head and elongated tail with shortened sheath; (e) intact virion with tail fibres (arrows). (a)–(c) are taken from the same micrograph and therefore to the same scale. Bars, 200 nm.

storage buffer components on the stability of ϕ IF3 was assessed. The addition or omission of MgSO_4 , gelatin, MgCl_2 , NaCl or CaCl_2 to 10 mM Tris (pH 7.4) had no effect on the titre of ϕ IF3 over time (data not shown). To test long-term storage, a lysate of 1×10^8 p.f.u. ml^{-1} was dispensed into aliquots and stored under different conditions: at 4 °C with and without chloroform, and at –20 °C and –80 °C in either 15 % or 50 % glycerol. ϕ IF3 appeared to be much more stable when kept at –20 °C in 15 % glycerol than at 4 °C or –80 °C, as it showed no loss of viability when titrated after 15 weeks, compared to a loss of 95–99.8 % of viable phages under the other conditions. Storage in 50 % glycerol appeared to have an adverse effect on ϕ IF3, with >99.9 % loss of viable phages. Viability of ϕ IF3 was not affected by the presence or absence of chloroform.

The nucleic acid was isolated from high-titre ϕ IF3 lysates, to try to determine the nature and size of the genome, but it proved refractory to digestion with various restriction enzymes (data not shown). Enzymes tested included *Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, *Bfa*I and *Alu*I, which were shown to cut a pUC19 control but not ϕ IF3 DNA. It was

therefore not possible to cut the ϕ IF3 genome with this selection of restriction enzymes. A plasmid control added to the ϕ IF3 DNA sample was digested with the enzymes, showing that there was no enzyme inhibitor in the phage DNA extract. We presume that ϕ IF3 DNA is highly modified. Experiments with pulsed-field gel electrophoresis suggest that the genome of this phage is greater than 250 kb in size (Ana Toribio, personal communication).

Although ϕ IF3 plaques appeared slightly turbid, none of the colonies tested that survived phage infection showed signs of lysogeny (after restreaking isolated colonies to reduce the risk of phage carry over). Therefore we think it reasonable to assume that ϕ IF3 is a virulent phage, or if it is temperate then it has a low frequency of lysogeny.

Biological properties of ϕ IF3

Phage were added at different m.o.i. to Db11 in early exponential growth phase to assess the effect of phage titre on bacterial growth rate (Fig. 3). Infection with phage at an m.o.i. of 0.1 produced a slight reduction of Db11 growth

Fig. 3. Db11 growth curves in the presence and absence of ϕ IF3. The graph shows a representative experiment of four separate growth curves. Each point is the mean of three samples, $\pm$ SD. The arrow indicates the point of addition of ϕ IF3. Phage was added at different m.o.i. to Db11 in the early exponential growth phase (OD_{600} 0.1) as described in Methods.

rate, followed 4.5 h later by stationary phase. This observation is in agreement with data showing that infections of Db11 in later exponential phase do not have much impact on the observed growth rate (data not shown). This is presumably because the process of the lytic cycle is not dramatic enough, though in both cases high-titre lysates of 10^8 – 10^9 p.f.u. ml⁻¹ were obtained despite high optical densities at the end of the experiment. Following ϕ IF3 infection at an m.o.i. of 1, there was immediate cessation of growth of Db11, then after 20 min, lysis was observed as a decrease in OD_{600} over 2 h. However, growth restarted after this time and the same growth rate was observed as for the Db11 control. The reasons for this regrowth are not immediately obvious, but it is unlikely to be due to the rapid emergence of phage-resistant mutants. Infection with an m.o.i. of 3 had an immediate effect on Db11, with only slight increase in growth for 20 min post-infection, followed by rapid and complete lysis within 3 h, producing a phage lysate of 2.1×10^8 p.f.u. ml⁻¹. These results show that bacterial lysis occurs more quickly with a higher m.o.i., as expected with the higher number of cells initially infected, but also that, under these conditions, there is a rapid return to bacterial growth if insufficient phage are added. Infection of Db11 earlier in exponential phase has the same effect as increasing m.o.i., that of causing quicker lysis (data not shown). This is likely to be due to the lower overall number of bacteria.

As shown in Fig. 4, ϕ IF3 adsorbs rapidly to Db11, with 90 % adsorbed within 5 min and 97 % adsorbed in 10 min. A standard adsorption time of 20 min was chosen to ensure that the maximum amount of phage had adsorbed, but reducing the time needed for the transduction assay. The phage was also able to adsorb to *S. marcescens* 3888, and an unrelated Gram-negative bacterium, *Citrobacter rodentium*. However transduction assays showed that it was unable to infect these strains (data not shown).

Fig. 4. Adsorption of ϕ IF3 to Db11. Representative graph showing results of three separate experiments. Adsorption was carried out at an m.o.i. of 0.01, and the supernatant was titrated at various time points to determine the amount of phage unadsorbed. Ninety per cent of ϕ IF3 adsorbed rapidly to Db11 over the first 5 min, increasing at a slower rate to 97 % after 10 min post-infection.

One-step growth curves were performed and a representative experiment is shown in Fig. 5. From these data, a latent period of 50 min was observed for ϕ IF3 infection of Db11, followed by a rise period of approximately 40 min, and a burst size of around 100 phages per infected cell.

Transduction

To obtain the optimal frequency of transduction, the effect of m.o.i., temperature and the addition of divalent cations was assessed. The frequency of transduction was not affected by temperature (as shown in Table 2) although some superinfection by wild-type phage, causing lysis of transductants, was observed as 'nibbled' edges of colonies at 37 °C. Therefore, to reduce killing of host cells, without significantly extending the incubation time of plates by

Fig. 5. One-step growth curve of Db11 infection with ϕ IF3 at an m.o.i. of 0.001. Representative graph of three separate experiments with reproducible results. A latent period of 50 min is seen before the phage numbers start to increase, followed by a growth period of approx. 40 min. The burst size is approx. 100 p.f.u. per infected cell.

Table 2. Frequency of transduction of miniTn5 transposon from Db10 JESM175 to Db10 using ϕ IF3, at different temperatures and m.o.i.

Transduction frequency is shown as transductants per p.f.u.

Incubation temp. (°C)	Multiplicity of infection (m.o.i.)				
	10 ⁸ Db10 cells			10 ⁹ Db10 cells	
	0.1	1	10	0.1	1
25	2.9×10^{-6}	3.1×10^{-6}	$<1 \times 10^{-9}$	1.2×10^{-6}	4.4×10^{-8}
30	2.4×10^{-6}	4.2×10^{-6}	$<1 \times 10^{-9}$	1.9×10^{-6}	5.6×10^{-8}
37	2.6×10^{-6}	5.3×10^{-6}	$<1 \times 10^{-9}$	1.5×10^{-6}	Not tested

incubating at 25 °C, 30 °C was chosen as the temperature for transduction experiments. At an m.o.i. of 0.1 with 10⁹ cells, frequencies were 10²-fold higher than for an m.o.i. of 1, but the same as for m.o.i. 0.1 and 1 at 10⁸ cells. No transductants were seen at an m.o.i. of 10. The reduced frequency of transduction with higher numbers of phage is probably due to either lysis from without or superinfection by wild-type phage. An m.o.i. of 0.1 with 10⁸ cells was chosen for routine generalized transduction studies. The addition of divalent cations (10 mM MgSO₄, 10 mM MgCl₂ or 10 mM CaCl₂) had no effect on transduction frequency (data not shown). ϕ IF3 was able to mediate transduction of markers from six different donor strains carrying transposons with a Sm^R marker, conferring a phenotype of auxotrophy or loss of ability to swarm, into Db10. This proved that ϕ IF3 is a generalized transducing phage. The frequencies of transduction did vary slightly from 1×10^{-6} to 6×10^{-6} transductants per p.f.u. (Table 3) for the different markers transduced, but this is probably not significant.

Conclusions

ϕ IF3 is a novel transducing phage, capable of generalized transduction in *S. marcescens* Db11. ϕ IF3 has an icosahedral head and a long contractile tail, and belongs to the order *Caudovirales* and family *Myoviridae*. It appears to be fairly unstable as shown by electron microscopy studies and a

10²-fold reduction in p.f.u. over 6 weeks in standard phage stocks at 4 °C. However the regular facile propagation of this phage is sufficient to maintain it, and storage at –20 °C in 15 % glycerol appears to maintain its viability for much longer.

Only *S. marcescens* Db10/11 strains have been found to be hosts for ϕ IF3 and the phage forms clear plaques of 0.5–2 mm diameter in 0.35 % top agar on lawns of susceptible strains. LPS has been shown to be its receptor. LPS is a common phage receptor, with 92 % of the new Db11 phages tested unable to infect the O-antigen deficient mutant.

Infection of Db11 liquid cultures in early exponential phase with an m.o.i. of more than 1 is required for rapid and complete lysis of bacterial cells. ϕ IF3 rapidly adsorbs to Db11, with 90 % adsorption within 10 min. It has a latent period of infection of 50 min and a burst size of approximately 100 phages per infected cell.

The generalized nature of ϕ IF3 transduction was demonstrated by the transduction of six different markers from different transposon-insertion mutant strains. Frequencies of transduction were similar for all markers at around 10^{-6} transductants per p.f.u., a reasonably efficient transduction frequency. The DNA of ϕ IF3 was resistant to digestion by restriction enzymes, suggesting extensive modification.

Table 3. ϕ IF3-mediated transduction of different markers into Db10

The number of transductants was determined by the number of Sm^R colonies (which were confirmed as true transductants by co-inheritance of the second phenotype, either an auxotrophy or inability to swarm). Transduction frequencies are expressed as the number of transductants per p.f.u. and are the means of five experiments.

Donor strain	Relevant marker	Phenotype	Mean frequency of transduction	Standard deviation
Db10 JESM267	<i>swrA</i> ::TnSm	Swarm [–]	3×10^{-6}	2×10^{-6}
Db10 JESM273	<i>entD</i> ::TnSm	Swarm [–]	4×10^{-6}	2×10^{-6}
Db10 JESM174	MiniTn5Sm	Unknown auxotrophy	3×10^{-6}	1×10^{-6}
Db10 JESM175	MiniTn5Sm	Unknown auxotrophy	6×10^{-6}	4×10^{-6}
Db10 JESM270	<i>purL</i> ::TnSm	Purine auxotroph	4×10^{-6}	3×10^{-6}
Db10 JESM293	<i>aroC</i> ::TnSm	Aromatic amino acid auxotroph	1×10^{-6}	1×10^{-6}

ϕ IF3 is a useful tool for the molecular and genetic analysis of *S. marcescens* strain Db11, and is already being used for extensive strain constructions in this laboratory.

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