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Lysogeny and bacteriophage host range within the *Burkholderia cepacia* complex

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The *Burkholderia cepacia* complex comprises a group of nine closely related species that have emerged as life-threatening pulmonary pathogens in immunocompromised patients, particularly individuals with cystic fibrosis or chronic granulomatous disease. Attempts to explain the genomic plasticity, adaptability and virulence of the complex have paid little attention to bacteriophages, particularly the potential contribution of lysogenic conversion and transduction. In this study, lysogeny was observed in 10 of 20 representative strains of the *B. cepacia* complex. Three temperate phages and five lytic phages isolated from soils, river sediments or the plant rhizosphere were chosen for further study. Six phages exhibited T-even morphology and two were lambda-like. The host range of individual phages, when tested against 66 strains of the *B. cepacia* complex and a representative panel of other pseudomonads, was not species-specific within the *B. cepacia* complex and, in some phages, included *Burkholderia gladioli* and *Pseudomonas aeruginosa*. These new data indicate a potential role for phages of the *B. cepacia* complex in the evolution of these soil bacteria as pathogens of plants, humans and animals, and as novel therapeutic agents.

INTRODUCTION

In the last decade, bacteria previously identified as *Burkholderia cepacia sensu lato* have become recognized as important human pathogens, and particularly as a cause of life-threatening pulmonary infections in individuals with cystic fibrosis (CF) or chronic granulomatous disease (Govan *et al.*, 1996; LiPuma, 1998; Jones *et al.*, 2001; Mahenthiralingam *et al.*, 2002). Concurrently, polyphasic taxonomic approaches revealed that *B. cepacia sensu lato* comprises at least nine phylogenetically related but genomically distinct species (genomovars) (Vandamme *et al.*, 1997, 2003; Coenye *et al.*, 2001). Known as the *B. cepacia* complex, the group currently comprises *B. cepacia* (previously genomovar I), *Burkholderia multivorans* (genomovar II), '*Burkholderia cenocepacia*' (genomovar III), *Burkholderia stabilis* (genomovar IV), *Burkholderia vietnamiensis* (genomovar V), *B. cepacia* genomovar VI, *Burkholderia ambifaria* (genomovar VII), *Burkholderia anthina* (genomovar VIII) and *Burkholderia pyrrocinia* (genomovar IX). All species in the *B. cepacia* complex have been isolated from clinical specimens; however, the clinical significance of individual genomovars in human disease remains unclear. Approximately 90 % of *B. cepacia* complex isolates cultured from CF patients belong to *B. multivorans* and '*B. cenocepacia*' (Agodi *et al.*, 2001; LiPuma *et al.*, 2001; Mahenthiralingam *et al.*, 2002; Speert *et al.*, 2002; Vandamme *et al.*, 2003). These two species account for most episodes of epidemic spread in CF and non-CF patients (Holmes *et al.*, 1999; Mahenthiralingam *et al.*, 2002); '*B. cenocepacia*' is also the species most associated with the rapid pulmonary decline known as cepacia syndrome, and with post-transplant mortality (Aris *et al.*, 2001).

Most isolates of the *B. cepacia* complex exhibit high-level resistance to all major classes of antibiotics (Lewin *et al.*, 1993; Pitt *et al.*, 1996; Nzula *et al.*, 2002). The *B. cepacia* complex is also one of the few groups of bacteria to exhibit intrinsic resistance to cationic antimicrobial peptides (Hancock, 1997). Some strains are susceptible *in vitro* to ceftazidime and meropenem, arguably the most potent 'anti-cepacia' agents; however, the majority of strains, including the highly transmissible '*B. cenocepacia*' lineage ET12, are resistant to these agents (Lewin *et al.*, 1993; Nzula *et al.*, 2002).

All bacteria in the *B. cepacia* complex have large genomes (mean size approx. 8 Mbp), comprising multiple replicons that may contribute to genomic plasticity (Lessie *et al.*, 1996; Wigley & Burton 2000; Parke & Gurian-Sherman, 2001; Mahenthiralingam *et al.*, 2002). Ironically, the *B. cepacia* complex could be considered as both friend and foe, as some strains are highly effective as biopesticides in the control of plant fungal diseases and in bioremediation of contaminated

Abbreviations: CF, cystic fibrosis; NBYE, nutrient broth with yeast extract.

soils (Holmes *et al.*, 1998; Parke & Gurian-Sherman, 2001). These dual roles raise important medical, agricultural and ecological issues (Govan *et al.*, 1996, 2000; Govan & Vandamme, 1998; Environmental Protection Agency, 2002), including the significance of horizontal gene transfer in assessment of the risk to humans of using these bacteria as biopesticides or in bioremediation (Holmes *et al.*, 1998; LiPuma & Mahenthiralingam, 1999; Govan *et al.*, 2000; Parke & Gurian-Sherman, 2001).

Attempts to explain the genomic plasticity, adaptability and virulence of the *B. cepacia* complex have paid little attention to the potential contribution of bacteriophages. In many pathogens, these bacterial viruses are recognized as important contributors to virulence, in the form of bacterial lysogens, or as vectors in horizontal gene transfer. Interest in the use of phage-induced bacterial lysis for therapeutic purposes was widespread in the 1920s, but declined with the arrival of the antibiotic era. However, as antibiotic resistance increasingly threatens standard therapies against bacterial infections, there is renewed interest in the antimicrobial properties of these highly specific agents (Pirisi, 2000; Sulakvelidze *et al.*, 2001).

Little is known of the phages of the *B. cepacia* complex. Early reports on the '*B. cepacia*' phages CP1 (Cihlar *et al.*, 1978) and CP 75 (Matsumoto *et al.*, 1986) predate the revision of *B. cepacia* taxonomy. However, a recent report from our laboratories described two transducing bacteriophages, NS1 and NS2, whose host range included the five genomovars (I–V) that were known at the time, and also extended to *Pseudomonas aeruginosa* (Nzula *et al.*, 2000). Lytic phages with an interspecies host range within the *B. cepacia* complex have also been reported in association with soil-borne strains of '*B. cenocepacia*' (LiPuma *et al.*, 2000). These results suggest that lysogenic conversion and transduction could play a role in the evolution of species of the *B. cepacia* complex as human pathogens, and indicate the need for further studies on the host range and properties of phages associated with the *B. cepacia* complex and related bacteria.

In this study, we investigated the prevalence of lysogeny within the nine current species of the *B. cepacia* complex and isolated lytic phages from natural habitats of these bacteria, including the plant rhizosphere. Our results show that the host range of the phage panel includes seven genomovars and, in the case of individual phages, is not genomovar-specific.

METHODS

Bacterial strains. The 66 strains of the *B. cepacia* complex used in this study are listed in Table 1. The collection comprised environmental and clinical isolates belonging to genomovars I–V that were included in the *B. cepacia* strain panel (Mahenthiralingam *et al.*, 2000a), and isolates representing the recently identified genomovars VI–IX (Coenye *et al.*, 2001). Four isolates identified as *Burkholderia ubonensis*, a putative tenth genomovar of the *B. cepacia* complex (Vermis *et al.*, 2002), were also included. Isolates were identified using *recA* RFLPs, whole-cell protein electrophoresis and DNA–DNA hybridization (Mahenthiralingam *et al.*, 2000b; Coenye *et al.*, 2001). In addition, 55 strains of related pseudomonad species were screened as potential phage hosts: *P. aeruginosa* (30 strains), *Stenotrophomonas maltophilia* ($n = 11$), *Burkholderia caledonica* ($n = 1$), *Burkholderia gladioli* ($n = 2$), *Comamonas acidovorans* ($n = 2$), *Pseudomonas fluorescens* ($n = 2$), *Pseudomonas mendocina* ($n = 1$), *Pseudomonas stutzeri* ($n = 2$), *Pseudomonas putida* ($n = 1$), *Pseudomonas testosteroni* ($n = 1$), *Pseudomonas syringae* pv. *tabaci* ($n = 1$) and *Ralstonia pickettii* ($n = 1$). Clonal relationships were excluded by PFGE fingerprinting using a Bio-Rad CHEF Mapper PFGE system (Butler *et al.*, 1995).

Media. Bacteria were grown in nutrient broth with 0·5 % yeast extract (NBYE) at 37 °C in a shaking incubator. Soft overlay agar for phage experiments comprised Luria–Bertani (LB) broth with 0·3 % bacteriological agar (Difco). The nutrient agar used was Columbia agar base (39 g l^{-1}; Oxoid).

Isolation of lysogenic phages. Temperate phages were assayed and maintained as described previously (Nzula *et al.*, 2000). Lysogeny was investigated using the following method: the 20 strains designated in Table 1 with the symbol † were prepared as saline suspensions (approx. 10^6 c.f.u. ml^{-1}), inoculated onto tryptone soy agar (TSA; Oxoid) using a multipoint inoculator and incubated at 30 °C for 6 h. Bacterial growth was inverted over chloroform vapour for 15 min and then allowed to air-dry for 15 min. Soft agar overlays (2·5 ml), inoculated with 100 µl exponential-phase culture of each of the 20 strains, were layered over the original bacterial growth and allowed to set; the plates were incubated overnight at 37 °C. Phage plaques were identified in the overlay in the proximity of the original inoculum and used to prepare single-plaque preparations as follows: an agar plug containing a single phage plaque was removed using a sterile glass pipette, transferred to 10 ml phage buffer (10 mM Tris/HCl, pH 8·0; 10 mM $MgCl_2$), vortexed for 30 s, centrifuged at 3000 ***g*** for 30 min and filtered (pore size 0·2 µm; Millipore).

Isolation of lytic environmental phage. The natural habitats of the *B. cepacia* complex include soils, river sediments and plants, particularly the plant rhizosphere (Fisher *et al.*, 1993; Butler *et al.*, 1995; Parke & Gurian-Sherman, 2001). Therefore, 20 samples of soil, river sediment and rhizosphere (soil plus root material) were collected. The presence of phage was then investigated using a modification of the phage enrichment technique described by Weiss *et al.* (1994), as follows: approximately 10 g sample was suspended in 15 ml LB broth and dispersed by shaking in an orbital incubator for 30 min at 30 °C. After removal of soil particles by centrifugation (4000 ***g*** for 20 min), the supernatant was filter-sterilized (pore size 0·2 µm; Acrodisc) and 1 ml aliquots were added to 15 sterile tubes. To each extract was added 25 µl exponential-phase culture from one of 15 propagating strains chosen to represent the genomovars of the *B. cepacia* complex strain panel (Mahenthiralingam *et al.*, 2000a), and the contents were incubated at 37 °C overnight. The bacterial cells were removed by centrifugation (4000 ***g*** for 30 min), the supernatant was membrane-filtered as before and 10 µl filtrate was spotted onto single-layer lawns of the propagating strain. Phage plaques were identified after overnight incubation at 37 °C, and single-plaque stocks were prepared as described in the previous section.

High-titre phage preparations. High-titre phage preparations were prepared as follows: 100 µl single-plaque preparation, containing approximately 10^5 p.f.u. ml^{-1}, was added to 2·5 ml soft nutrient agar, previously seeded with 100 µl exponential-phase culture of the propagating strain. The mixture was then overlaid on nutrient agar and allowed to set. After 18 h at 37 °C, overlays showing semi-confluent lysis were transferred into 10 ml phage buffer. The lysate was then vortexed and centrifuged at 3200 ***g*** for 30 min and the supernatant was membrane-filtered. Phage titres were determined, as p.f.u. ml^{-1}, by

incorporating 100 µl host bacteria (exponential-phase NBYE culture) and 100 µl phage stock in 2·5 ml soft agar overlay, and lytic plaques were enumerated after 18 h incubation at 37 °C. Stock preparations were maintained at 4 °C.

Host range of phages. Stock phage preparations were diluted in phage buffer to approximately 10^8 p.f.u. ml^{-1} against the propagating strain, and 10 µl was spotted onto single-layer lawns (prepared from exponential-phase NBYE cultures) of potential host bacteria. Lytic activity was recorded after 24 h at 37 °C on a scale ranging from $<$ 10 plaques (+) to confluent lysis (+++) (Table 1).

Electron microscopy. Stock phage preparations (approx. 10^8 p.f.u. ml^{-1}) were centrifuged at 100 000 ***g*** for 1 h. Phage pellets were resuspended in 1 M ammonium acetate, negatively stained with 2 % (w/v) potassium phosphotungstate solution (pH 7·0) and examined with a Hitachi model HU-12A transmission electron microscope.

Phage DNA extraction and RFLP profiling. In preparation for DNA extraction, high-titre phage stocks (containing at least 10^{10} p.f.u. ml^{-1}) were prepared using soft agar overlays, as described above. DNA was extracted from 10 ml phage stock using the Wizard Lambda preparation DNA purification system in conjunction with the Vac-Man laboratory vacuum manifold (both from Promega). Extracted DNA was eluted in sterile distilled water and stored at −20 °C. DNA quality was assessed on an E-gel pre-cast 0·8 % agarose gel (Invitrogen Life Technologies). In cases where DNA was not of sufficient quality for DNA restriction, purification was performed using the PCR protocol from the QIAquick gel extraction kit (Qiagen). Purified DNA was eluted in 30 µl elution buffer and stored at 4 °C. To determine genome size and to confirm that the phages were different from one another, approximately 1 µg DNA was restricted using 10 U *Hin*dIII (Promega), incubated for 3 h at 37 °C and visualized on 0·6 % 0·5× TBE agarose gel alongside 1 µl Ready-Load Lambda DNA/*Hin*dIII fragments (Invitrogen Life Technologies).

RESULTS

Lysogeny

Of the 20 strains of the *B. cepacia* complex that were investigated, 10 strains [ATCC 25416^T and ATCC 17759 (genomovar I); C3161^T, C1576, C1962 and C3163 (*B. multivorans*); J2315^T, C3166 and C3170 ('*B. cenocepacia*'); and C3174 (*B. stabilis*)] were found to be lysogenized. These provided 14 temperate phages (DK1–DK4 and MM1–MM10) for further study.

Isolation of environmental phages

Five virulent phages, JB1, JB3, JB5, RL1c and RL2, were isolated from soils and from the rhizosphere of various plants (Table 2). Most positive samples included decayed plant material collected from moist environments, but phages were also isolated from dry soils.

Host range of *B. cepacia* complex phages NS1, NS2 and newly isolated phages

To confirm that distinct phages were being accumulated and investigated, we determined the host range of the 19 phages against a preliminary bacterial panel comprising the 20 isolates of the *B. cepacia* complex that were used in the lysogeny screen, and also the *Hin*dIII RFLP profile (and hence an approximate genome size; Table 2). With the exception of phages JB3, DK2 and DK3, the genomes of the *B. cepacia* complex phages were within the range 40–48 kbp (Table 2). If several phages shared the same host range and RFLP profile, only one phage was used for further study. An exception was made for phages RL1c and RL1t, which shared the same host range and RFLP profile but produced different plaque morphologies: clear plaques associated with virulent phage (RL1c) or turbid, temperate phage plaques (RL1t). Similar host ranges and RFLP profiles were observed with the temperate phages DK2 and DK3, which had respectively been isolated from '*B. cenocepacia*' C3166 and *B. stabilis* C3174. As previously observed for NS1 and NS2, none of the newly identified phages was inactivated by treatment with chloroform. In addition, no evidence of bacteriocin activity was found during the search for *B. cepacia* complex phages.

The host range of phages NS1, NS2 and eight novel phages (JB1, JB3, JB5, DK1, DK3, RL1c, RL1t and RL2) was then determined against an enlarged panel of 66 isolates of the *B. cepacia* complex and 55 isolates representing other pseudomonads. The host range of individual phages included multiple species of the *B. cepacia* complex. Collectively, the host range of the phage panel included seven of the presently recognized *B. cepacia* genomovar species; no phage activity was detected against the single representatives of *B. cepacia* genomovar VI or *B. ambifaria* (Table 1). However, within each *B. cepacia* species, there was wide variation in susceptibility to an individual phage. *B. multivorans* appeared to be least susceptible to phages investigated in this study: of nine *B. multivorans* strains examined as potential phage hosts, only strain C2775 showed susceptibility. As observed previously for NS1 and NS2, the host range of some of the phages was not restricted to the *B. cepacia* complex. *P. aeruginosa* strains C1546 and J2852 were susceptible to phage JB3, and *B. gladioli* strain C3654 was susceptible to phages NS2, DK1, RL1c, RL1t and JB5.

Electron microscopy

In accordance with previous studies on phages NS1 and NS2 (Nzula *et al.*, 2000), electron microscopy revealed the novel phages JB1, JB5, DK2/DK3, RL1c/RL1t and RL2 to be morphologically T-even-like phages, with hexagonal heads and contractile tails of variable length. In contrast, phages JB3 and DK1 were lambda-like, with hexagonal heads and flexuous, non-contractile tails.

DISCUSSION

This study confirmed that lysogeny is relatively common in isolates of the *B. cepacia* complex and demonstrated the presence of virulent *B. cepacia* complex phages in the natural habitats of these bacteria. In agreement with previous observations of phages NS1 and NS2 (Nzula *et al.*, 2000), the host range of the newly isolated phages was not genomovar-specific and, in some phages (for example JB1), it included the majority of *B. cepacia* complex species. This broad host range, which in some phages extended to the

Table 1. Host range of bacteriophages within the *B. cepacia* complex

−, Lack of sensitivity to phage; +, < 10 plaques at phage inoculation site; ++, >10 plaques at phage inoculation site; +++, confluent lysis at phage inoculation site.

Strain	JB1	JB3	JB5	DK1	DK3	NS1	NS2	RL1t/RL1c	RL2
B. cepacia									
ATCC 25416^T (= LMG 1222^T)*†	−	−	−	−	−	−	−	−	−
C2970 (= LMG 17997)	+	−	+	−	−	+	+	+++	−
C3159 (= LMG 18821)†	−	−	−	−	−	−	−	−	−
ATCC 17759 (= LMG 2161)*†	−	−	−	−	+++	−	−	−	−
B. multivorans									
C2775	−	+	−	+	+	+	+	+++	−
C3161^T (= LMG 13010^T)*†	−	−	−	−	−	−	−	−	−
C3162 (= LMG 18825)†	−	−	−	−	−	−	−	−	−
C3163 (= LMG 18824)*†	−	−	−	−	−	−	−	−	−
C3164 (= LMG 18823)†	−	−	−	−	−	−	−	−	−
ATCC 17616 (= LMG 17588)	−	−	−	−	−	−	−	−	−
C1962 (= LMG 16665)*†	−	−	−	−	−	−	−	−	−
C1576 (= LMG 16660)*†	−	−	−	−	−	−	−	−	−
C3160 (= LMG 18822)†	−	−	−	−	−	−	−	−	−
'*B. cenocepacia*'									
J415 (= LMG 16654)	−	−	−	+	−	−	+	−	+++
J2315^T (= LMG 16656^T)*†	−	−	−	−	−	−	+++	−	−
C1394 (= LMG 16659)	−	−	−	−	+	−	+	−	++
J2956	+++	+++	+++	−	+++	−	−	+	+++
C2836	−	−	+	−	−	+	+	−	++
C3165 (= LMG 18826)	−	−	−	−	−	−	+	−	−
C3166 (= LMG 18863)*†	−	−	−	−	−	+	+	−	−
C3167 (= LMG 18827)†	−	−	−	−	−	−	−	−	−
C3168 (= LMG 18828)†	−	−	−	−	−	−	−	−	−
C3169 (= LMG 18829)	+++	+++	++	+++	+++	++	++	−	+++
C3170 (= LMG 18830)*	−	−	−	−	−	+	+	−	+++
ATCC 17765 (= LMG 18832)	++	−	+	+++	−	−	+	+	−
B. stabilis									
C3171^T (= LMG 14294^T)	−	−	−	−	−	+	−	+++	−
C3172 (= LMG 18870)	+	−	−	−	−	−	−	−	−
C3173 (= LMG 18888)†	−	−	−	−	+	−	−	−	−
C3174 (= LMG 14086)*†	−	−	−	−	−	−	−	−	−
B. vietnamiensis									
C2978 (= LMG 16232)†	−	−	−	−	−	+	+	+++	−
C3175 (= LMG 18835)†	−	−	+	−	−	−	−	−	−
C3176 (= LMG 18836)†	−	−	−	−	−	−	+	−	−
C3177^T (= LMG 10929)†	+++	−	+++	+	+++	−	−	+	+++
Genomovar VI									
E12	−	−	−	−	−	−	−	−	−
B. ambifaria									
J2742^T (= LMG 19182^T)	−	−	−	−	−	−	−	−	−
B. anthina									
J2552 (= LMG 16670)	−	−	−	−	−	−	−	−	−
J2553	−	−	−	−	−	−	−	−	−
J2863 (= LMG 20980)	−	−	−	−	−	−	−	−	−
J2927	−	−	−	−	−	−	−	−	−
J2928	−	−	−	−	−	−	−	−	−
J2941	−	−	−	−	−	−	−	−	−
J2943	−	−	−	−	−	−	−	−	−
J2944	−	−	−	−	−	−	−	−	−

(continued overleaf)

Table 1. cont.

Strain	JB1	JB3	JB5	DK1	DK3	NS1	NS2	RL1t/RL1c	RL2
J2945	–	–	–	–	–	–	–	–	–
J2946	–	–	–	–	–	–	–	–	–
J2949	–	–	–	–	–	–	–	–	–
J2950	–	–	–	–	–	–	–	–	–
J2951	+++	–	+++	–	–	+	–	–	+++
J2862	–	–	–	–	–	–	–	–	+++
C1658 (= LMG 20982)	+++	+	+++	–	–	–	–	–	–
C1765 (= LMG 20983)	+++	+	++	–	–	+	–	++	–
B. pyrrocinnia									
J2536	–	–	–	–	–	–	–	–	–
J2542	–	–	–	–	–	–	–	–	–
C1469	–	–	–	–	–	–	–	–	–
C3909	++	–	++	–	–	–	–	–	–
C3918	+	–	+	+	–	+	–	–	+++
C3928	–	–	–	–	–	–	–	–	–
C3930	+++	–	+++	–	–	+++	–	–	++
C3993	+++	–	+++	–	–	–	–	–	++
C3995	+++	–	+++	–	–	–	–	–	++
C3997	+	–	–	–	–	–	–	–	–
B. ubonensis									
E26	–	–	–	–	–	–	–	–	++
E27	–	–	–	–	–	–	–	–	–
E571	–	–	–	–	–	–	–	–	–
E551	+	++	–	–	–	–	–	–	–
Genomovar unclassifiable									
J2540 (= LMG 16672)	–	–	–	–	–	+	+	–	–

*Lysogenic strains.

†Strains used in lysogeny experiments.

Table 2. Phage genome size, plaque morphology and source

Phage	Genome size (bp)	Source	Plaque morphology*
JB1	40 000	Soil, the Caribbean	Turbid plaques, variable size (0·5–1 mm in diameter)
JB3	34 000	Plant rhizosphere, UK	Clear plaques (1 mm in diameter)
JB5	40 000	Plant rhizosphere, UK	Turbid plaques, variable size (0·5–1 mm in diameter)
DK1	45 900	Lysogeny	Turbid plaques, variable size (0·5–1 mm in diameter)
DK2/DK3	29 300	Lysogeny	Turbid plaques (1 mm in diameter)
NS1	48 000†	Lysogeny	Turbid plaques (0·5–1 mm in diameter)
NS2	48 000†	Lysogeny	Semi-clear plaques, variable size (0·5–1 mm in diameter)
RL1c/RL1t	44 300	Plant rhizosphere, UK	RL1c, clear plaques (1 mm in diameter); RL1t, turbid plaques (1 mm in diameter)
RL2	40 000	Pond sediment, UK	Semi-clear plaques, variable size (0·5–1 mm in diameter)

*Plaque morphology on propagating strains (i.e. strain J2956 for phages JB1, JB3 and JB5; strain C3169 for phages DK1, DK3, NS2 and RL2; strain C2978 for phages RL1c/RL1t; strain C3166 for phage NS1).

†Data from Nzula *et al.* (2000).

related pseudomonads *P. aeruginosa* and *B. gladioli*, is interesting. With the exception of unusual phages such as the plasmid-like 'phasmid' P4 (Gutmann *et al.*, 1990), the host range of most phages is species-specific. Lack of bacteriocin activity in our study could be explained by the techniques and conditions used and the low prevalence of bacteriocinogeny in *B. cepacia sensu lato* (Govan & Harris, 1985).

A broad host range for *B. cepacia* complex phages could contribute to the genomic plasticity of these bacteria, and their evolution from highly metabolically active soil saprophytes to plant and human pathogens and, recently, also animal pathogens (Berriatua *et al.*, 2001). Lysogenic conversion and transduction are important processes by which chromosomal host genes can be acquired and exchanged between bacteria. We have previously demonstrated *in vitro* transfer of antibiotic-resistance genes between *B. vietnamensis* strains by phages NS1 and NS2 (Nzula *et al.*, 2000).

As reported for *Shigella flexneri* (Allison & Verma, 2000), prophages may contribute to O-antigen modification in the *B. cepacia* complex (Kenna *et al.*, 2003), and to the role of *B. cepacia* complex LPS as a potent virulence determinant (Shaw *et al.*, 1995; Hughes *et al.*, 1997). Opportunities for transduction and lysogenic conversion would exist not only in natural environments shared by various *B. cepacia* complex species and related bacteria, but also in CF airway secretions, where mixed infections are frequent and bacterial populations can reach densities in excess of 10^9 c.f.u. ml^{-1}. Based on a close taxonomic relationship and shared insertion sequences and environmental habitats, we were particularly keen to test the phage panel against isolates of *Burkholderia pseudomallei*, the causative agent of melioidosis and a potential agent for bioterrorism (Mack & Titball, 1998). In collaboration with Dr Ty Pitt (PHLS, Colindale, London, UK), only phage NS2 was found to be active, lysing 13 of 40 *B. pseudomallei* strains tested (unpublished results). The potential importance of broad-host-range phages such as NS2 is also suggested by recent reports of *B. pseudomallei* infection in CF patients (including coinfection with *B. cepacia*) following travel to Thailand, where melioidosis is endemic (Schulin & Steinmetz, 2001; Visca *et al.*, 2001).

Further studies are required to determine the transducing potential and other biological properties (e.g. nucleic acid content and bacterial receptors) of the *B. cepacia* complex phages that were accumulated in this study. Meanwhile, several preliminary observations merit comment. The shared host range and RFLP profiles of the temperate phages DK2 and DK3, respectively isolated from strains of '*B. cenocepacia*' and *B. stabilis*, suggest that integration of the same phage can occur in different species of the *B. cepacia* complex. While the presence of multiple prophages within a single strain is common in *P. aeruginosa* (Holloway *et al.*, 1960), we found no evidence of polylysogeny during our investigation of *B. cepacia* complex phages. The variable plaque morphology exhibited by the environmental phages RL1c and RL1t is interesting and, to our knowledge, has not been reported previously for *B. cepacia* complex phages.

The relative lack of susceptibility of *B. multivorans* to the phage panel was interesting. Taken together, *B. multivorans* and '*B. cenocepacia*' account for almost 90 % of clinical isolates of the *B. cepacia* complex; however, in contrast to '*B. cenocepacia*', *B. multivorans* is rarely isolated from natural environments (Bevivino *et al.*, 2002; authors' unpublished data). In our study, of nine *B. multivorans* strains tested, only one (C2775) exhibited phage susceptibility. However, this resistance may be misleading, as a recent study in our laboratories identified a novel *B. cepacia* complex phage (RU2) from soil, which plates on *B. multivorans* C3164 and also on three other *B. cepacia* complex isolates that are resistant to the primary phage panel (Table 1), namely J2552 and J2553 (both *B. anthina*) and E571 (*B. ubonensis*).

Whole-genome sequencing of bacteria provides increasing evidence for widespread exchange of chromosomal genes and other extrachromosomal elements, mediated by phages. Thus, analyses following the recent sequencing and annotation of '*B. cenocepacia*' J2315^T (= LMG 16656^T = NCTC 13227^T; http://www.sanger.ac.uk/Projects/B_cepacia/) are keenly awaited. In relation to the issue of multiple lysogeny in the *B. cepacia* complex, we performed a BLAST search of the provisional J2315^T genome sequence and found evidence of a single prophage. If confirmed, this would be an interesting result as, in this study and in a more extensive search for lysogeny in J2315^T, we isolated only one temperate phage, DK4 (authors' unpublished data). The availability of broad-host-range phages complements the panel of *B. cepacia* complex strains (Mahenthiralingam *et al.*, 2000a) and should facilitate future research on these highly adaptable and increasingly important bacteria. Furthermore, in addition to the established therapeutic use of lytic phages, phage-encoded lytic enzymes may provide novel therapeutic agents against *B. cepacia* complex infections (Schuch *et al.*, 2002), for which there are few antibiotic options at present (Nzula *et al.*, 2002).

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